

## Research Article



## Seasonal Variation in the Microscopic Anatomy of Gonads and Gonadosomatic Index of *Clupisoma garua*

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**Abstract** | Seasonal variation in the gonads of *Clupisoma garua* was studied from the river Indus and its tributaries in Khyber Pakhtunkhwa and Northern Punjab, Pakistan. A total of 48 fishes of both sexes (n= 12 per season; 9 female and 3 male) were collected in different seasons of 2016-17. Preparatory phase of reproductive cycle was observed in spring with having thick tunica albuginea and rapid spermatogenesis in testes while appearance of cortical alveoli or yolk vesicle in cortex of cytoplasm in ovaries. Gonads attain maximum size and weight in spawning phase during summer. Free oozing of spermatozoa in testes and ovaries packed with fully grown eggs are the distinct features of this phase. The highest values of Gonadosomatic Index (GSI) for male and female in summer coincided with the histological structure of the gonads where they are in their spawning phase. Present study revealed that *C. garua* breed once in a year during summer season and this information will be helpful in culturing of this economically important catfish in Pakistan.

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### Introduction

Freshwater catfish *Clupisoma garua*, commonly known as bacha, garua-bacha, garua-bachcha widely distributed in different countries of Indian subcontinent e.g. Pakistan, India and Bangladesh (Gupta and Banerjee, 2016). Good taste and having very less intramuscular bones makes it a popular table fish, also known as a popular game fish. It is a bottom and marginal dweller mainly inhabits large rivers and reservoirs and also been reported from stagnant

impoundments (Chondar, 1999). This species inhabits fluviatile habitats in larger rivers with a sandy or muddy bottom. To understand fish reproduction, it is essential to consider the fact that information on size at maturity, spawning season, fecundity and sex ratio is fundamental for accurate stock assessments (Caddy and Mahon, 1995). Environmental extremes faced by fishes take them from 'feast' during high water to 'famine' during low water when competition for resources becomes intense (Arrington et al., 2006). When resources are plentiful, many fishes will store fat

energy subcutaneously, in the abdominal cavity, liver and muscles. These lipid stores can then be utilized not only to survive a period during which resources are scarce but also to build up gonads in preparation for the upcoming rainy season (Brito and Bazzoli, 2003). Fish has the capability to store energy in the liver and other tissues to meet the requirements of spawning (Liao and Chang, 2011). Individuals of the same size caught at the same time are found to have wide range of fecundity. This may be due to the fact that some eggs may have been released by some individuals in different stages of gonad maturation. Variation in fecundity may also be due to the existence of varied mixture of age classes (Saliu et al., 2007).

Fish is an important source of animal protein, the understanding of the biology of different economically important fish species is highly required. *C. garua* present in the upper and lower stream of river Indus and its tributaries in Khyber Pakhtunkhwa and Northern Punjab, Pakistan. Overfishing is a major threat due to the high market demand. There is a need to understand the reproductive biology of this species to overcome this problem by rearing it in confined areas. There is limited published data available on the life history of this economically important fish. In view of above said issues the present research was designed to study the seasonal variation in the gonad histology of *C. garua* in relation to its gonadosomatic index.

## Materials and Methods

A total of 48 mature samples were collected from different areas of the river Indus and its tributaries from Khyber Pakhtunkhwa and northern Punjab during different seasons of the year (2016-17). Samples were transferred to the Histology laboratory, PMAS- Arid Agriculture University Rawalpindi for further analysis. Gonads from three male and nine females per season were collected for the histological analysis. Before sampling for microscopy, the growth parameters viz. total body weight and total body length of experimental animals were measured. After giving a distilled water wash the fish were dissected to obtain gonads and the gonad weight was measured to calculate Gonado-somatic Index values.

To study the histology of the gonads the tissues were obtained from the anterior, middle and posterior portion of the ovaries and testes. The tissues were

fixed in Bouin's solution and processed for paraffin embedding technique (Bancroft and Steven, 1990). Rotary microtome was used to obtain 5-7µm thick sections of tissues. Tissues were placed onto glass slides and stretched using albumin and distilled water solution. The prepared slides were stained by hematoxylin and eosin for the study of seasonal variation in the histology of gonads. The stained slides were mounted with cover slips by using Canada balsam and were analyzed under Meiji-MT 4300 H (Japan) light microscope.

To determine the Gonado-somatic index the body weight of each sample and their respective gonad weight was recorded and GSI was calculated by using the following formula:

$$GSI = \text{Weight of the gonads} / \text{Weight of the fish} \times 100$$

## Results and Discussion

The mean body weight of male recorded was 119.16±14.62 and 86 ± 4.16 g in spring and summer season and their gonad weight was observed 0.14±0.01 and 2.26±0.37 g respectively. A significant difference (P<0.05) was observed during both seasons in gonad weight. Similar trend was found in female where the average body weight was observed 114.14±8.69 and 163.80±29.83 g and their respective gonad weight was 0.84±0.26 and 11.16±4.34 g in spring and summer. In accordance with the obtained body weight the gonadosomatic index values were found higher in both male and female in summer indicative of peak on the onset of spawning activity (Table 1). In autumn and winter, the mean body weight in male and female was non-significantly different and the gonads were found in regressed form.

### Histology of testis

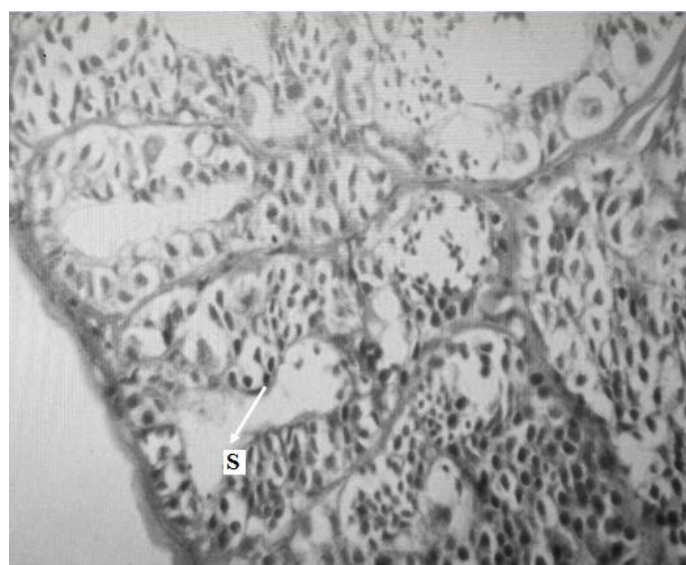
The annual cycle in *C. garua* testes consists of spent, resting, preparatory and spawning phase. The histology of the testis in present study revealed preparatory phase during spring season with small testicular lobules and packed interlobular spaces with dense stroma which consists of loose connective tissue, food vessels and interstitial cells with thick tunica albuginea. The thin testicular and lobular wall and the presence of the spermatozoa were the characteristics of preparatory phase also called as spermatogenic phase (Figure 1). Reduction in size of testes due to free oozing of spermatozoa was observed

in summer indicating spawning season in *C. garua*. Thin testicular and lobular wall were observed and some empty lobules are also present (Figure 2).

**Table 1:** Seasonal variations in the mean body weight (g), Gonads weight (g) and Gonadosomatic index of *C. garua*.

Group	Sex	Season	Mean± SE	P-Value
BW	Male	Spring	119.67 ± 14.62	0.09 <sup>NS</sup>
		Summer	86 ± 4.16	
	Female	Spring	114.14 ± 8.69	0.09 <sup>NS</sup>
		Summer	163.80 ± 29.83	
GW	Male	Spring	0.14 ± 0.01	0.005 <sup>s</sup>
		Summer	2.26 ± 0.37	
	Female	Spring	0.85 ± 0.26	0.017 <sup>s</sup>
		Summer	11.16 ± 4.34	
GSI	Male	Spring	0.11 ± 0.01	0.003 <sup>s</sup>
		Summer	2.68 ± 0.39	
	Female	Spring	0.66 ± 0.18	0.023 <sup>s</sup>
		Summer	6.75 ± 2.72	

\*BW (Body weight); GW (Gonad weight); GSI (Gonadosomatic index); NS (non-significant); S (significant).

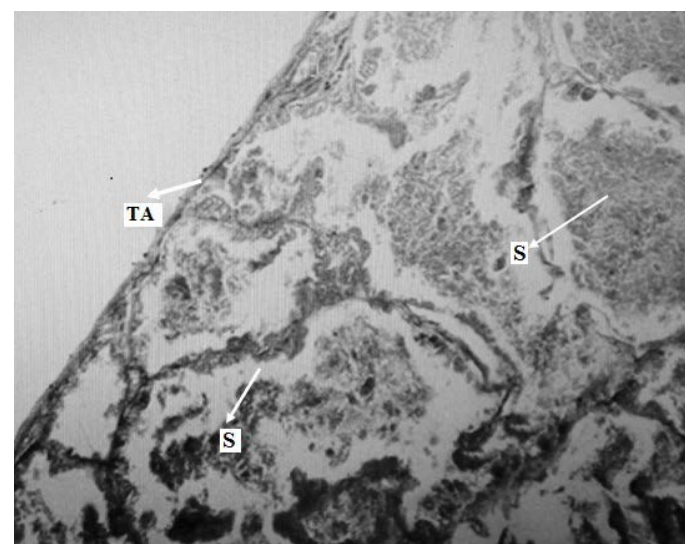


**Figure 1:** Preparatory Phase in the testis of *Clupisoma garua* during spring season. S: Spermatozoa.

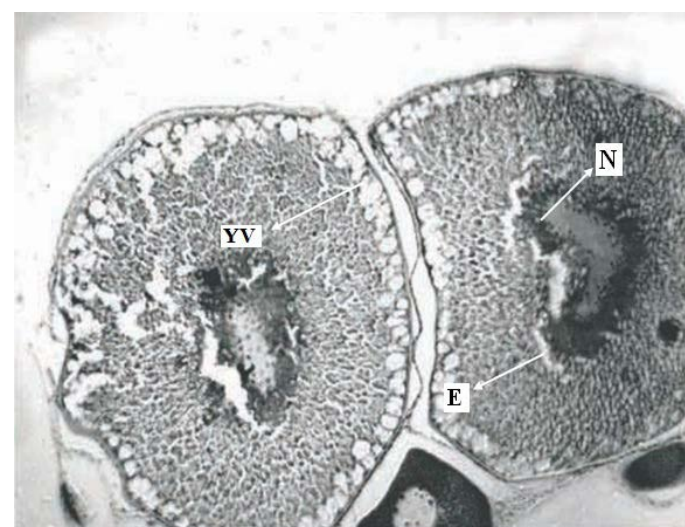
### Histology of ovaries

Two ovarian phases were marked in *C. garua* on the basis of histology and development of oocyte during spring and summer season. The growth of oocyte, several large folds of nuclear membrane and evagination appearance of nuclear membrane revealed the preparatory phase during spring season. Due to evagination the surface area of nucleus was increased and nucleo-cytoplasmic exchange of molecules to nuclear membrane was observed. Extruded nucleoli lose their consistency due to distributed material in

ooplasm and yolk vesicles in the cortex of cytoplasm (Figure 3). In spawning phase during summer ovaries attained maximum size and weight. The eggs were fully grown and completely pack the yolk mass. Cortical alveoli layer was clearly observed adjacent to the egg envelop and nucleus become indistinct (Figure 4). In accordance to histological study of gonads the GSI values of both gonads i.e. testes and ovaries were recorded lowest in spring and higher values were obtained in summer season (Table 1).



**Figure 2:** Spawning phase in the testis of *C. garua* during summer season. S: Spermatozoa, TA: Tunica albuginea.

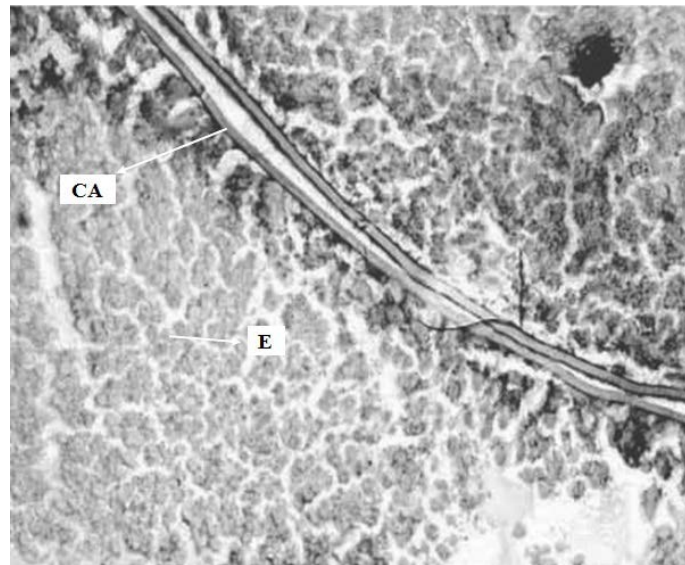


**Figure 3:** Preparatory phase in the ovary *C. garua* during spring season. N: Nucleus, E: Evagination in nucleus, YV: Yolk vesicles.

Linear increase in gonad weight with the increase in body weight in *C. garua* was observed during different seasons and developmental stages of gonads. The obtained results of increased body and gonad weight during spawning stage was in accordance with different studies (Bahuguna and Khatri, 2009; Offem et al., 2008; Mohan, 2005; Hussain et al., 2003).



During autumn and winter, season degenerated gonads showed the post spawning or spent and resting stage respectively. In agreement with the study on *Shizothorax palgeostomis* (Pasha et al., 2016) and *Labio rohita* (Lone, 2009) significant difference in gonadosomatic index (GSI) values of *C. garua* was observed during preparatory and spawning phase.



**Figure 4:** Spawning phase in the ovary of *C. garua* during summer season. E: Eggs, CA: Cortical Alveoli.

Histological studies of the testis show rapid spermatogenesis and thick tunica albuginea in preparatory phase supported the findings of El-Gohary, 2001 in *Oreochromis niloticus*. While in ovaries the growth of oocyte, several large folds of tunica albuginea and evagination in the nuclear membrane was observed. Cortical alveoli or yolk vesicles appearance in cortex of cytoplasm converted into large spherical yolk filled globules almost covering the entire ooplasm agreed with the results of Gaber, 2000 in *Bagrus bagrus* and Van Oordt et al., 1987 in *Clarius gariepinus*.

Thin testicular and lobular walls, free oozing of spermatozoa and observation of some empty lobules in the testis of *C. garua* were the characteristics of spawning phase. Similar results were reported by Nakaghi et al., 2003 in *Colossoma macropomum* and Arenas et al., 1995 in *Gambusia affinis*. In ovaries the eggs are fully grown with completely packed yolk mass. Cortical alveoli layer is clearly observed adjacent to the egg envelop agreed with the previous findings in *Bagrus* and in *Tilapia noliticus* by Gaber, 2000 and Dougbag et al., 1988 respectively.

## Conclusions and Recommendations

The microscopic anatomy of gonads and gonadosomatic index of *C. garua* revealed the preparatory and spawning phase in spring and summer season respectively. Both male and female of studied species become sexually dynamic in summer season and spawn once in the year. This information would be helpful in understanding the reproductive pattern of this economically important fish species and benefited to commercial farmer for its production in confined area.

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## Statement of conflict of interest

The authors declared that there is no conflict of interest.

## Author's Contributions

Riaz Hussain Pasha and Akram Khan Niazi provided technical support for laboratory analysis, write up of the manuscript and expertise in identification of different developmental stages in tissues. Anjum-Zubair M performed write up of the manuscript and supervised the research. Imran Ullah, Adnan Ali, Arslan Emmanuel and Sana Batool did the sampling, transportation and laboratory analysis of the fish data.

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