



Histomorphological changes in gonads of *Clupisoma naziri* during spring and summer season

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Abstract

Present study was conducted to analyse the effect of breeding and non-breeding seasons on histomorphological changes in gonads of *Clupisoma naziri*. In Khyber Pakhtunkhwa and northern Punjab, Pakistan, 43 mature fish of both sexes were gathered from the Indus River and its tributaries. In the non-breeding season (spring), both testis and ovaries went through a preparatory phase, with thick tunica albuginea and fast spermatogenesis in testes and the formation of cortical alveoli or yolk vesicles in the cortex of the cytoplasm in ovaries. The testicular lobules were loaded with spermatozoa and some spermatogenic cells during breeding season (summer), while the ovaries were filled with mature ovarian follicles. The breeding season is marked by spermatozoa leaking freely from testes and ovaries brimming with completely mature eggs. The gonadosomatic (GSI) results are consistent with the histomorphological results of gonads, as their values peak during breeding season and drop during non-breeding season. *C. naziri* breeds once a year during the summer season, according to the current study, and this information will aid aqua breeders in the culturing of this commercially significant catfish in Pakistan.

Keywords: *Clupisoma naziri*, Gonads, Breeding season, Non-breeding season, Gonadosomatic index.

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1. INTRODUCTION

With the increase in human population the demand of food is also increasing. To overcome this food demand, we need to reproduce the food animals artificially or naturally in confined areas or in the wild. Aquaculture and fisheries have experienced twelve times increase in last three decades in food fish production, making the fish industry one of the fastest growing food industries. Fish is an important source of animal protein. To overcome the shortage of food and to get high level of fish production; understanding of the biology of different economically important fish species is highly required. Although a lot of research has been done on fish life but still there is a need of improvement in many aspects regarding to economically

important local species. Reproductive behaviour in fishes is one of the most important factors which helps in their management, reproduction and conservation.

To understand fish reproduction and proper stock assessment, the information on size at maturity, spawning season, fecundity and sex ratio is very essential². Fishes experience environmental extremes that range from 'feast' at high water to 'famine' during low water, when resource competition is severe¹¹. Many fishes store fat energy subcutaneously, in the abdominal cavity, liver and muscles during plentiful resources to utilize them not only for survive a scarce resource period but also to build up gonads in preparation for the breeding season¹².

Individuals of similar size captured at the same time had a wide range in fecundity. This could be because some eggs were discharged at different phases of gonad development by different individuals. Variation in fecundity could also be attributable to the presence of a diverse mix of age groups⁷. The changes in gonadosomatic index (GSI) due to maturation cycle in gonads is a good sign of growth as well as it provides a valuable information of gonad's condition and reproductive staging¹³.

Clupisoma naziri is regarded as one of the tastiest fish of Pakistan. *Clupisoma naziri* was first described from the river Indus¹⁴. It ranges from river Swat system up to river Jhelum and some tributaries of river Indus in Pakistan and Azad Kashmir. This economically important fish species has high demand due to its taste and is widely captured from the upper and lower water stream of river Indus and its tributaries leading to its decline in natural water. To beat the decline in his population there is a need to understand the reproductive biology of that species so it can culture in captivity. Due to the limited data availability on the reproductive biology of this valuable fish species, the present research was designed with the objective of histomorphological study in the gonads of male and female *C. naziri* during non-breeding and breeding season. A recent study conducted on liver of *Luciobarbus bocagei* indicates that the degree of liver histopathological alterations served as a reliable predictor of water quality since it showed variations in the types of contaminants in various seasons and sample periods¹⁵.

2. MATERIALS AND METHODS

A total of 48 mature *Clupisoma naziri* of both sexes were collected from the river Indus and its tributaries in Khyber Pakhtunkhwa, Pakistan. The sampling was done during non-breeding (spring) and breeding season (summer) in the year 2016-17. The fish were transported to the aquaculture and fisheries laboratory, Department of Zoology, PMAS- Arid Agriculture University Rawalpindi for histomorphological studies. The male and female individuals were separated, and body parameters were measured viz., body weight and gonad weight.

For histological studies the male and female gonads were collected. A small incision was made on the surface of each gonad and sections of tissues from the anterior, middle or posterior portion were collected and placed in saline solution for the removal of external debris. The tissue sections were fixed in Bouin's solution for 24 hours followed by dehydration of the samples with gradual immersion in ascending series of alcohol and cleared by xylene. Embedded in paraffin wax and sectioned by rotary microtome, at 5µm thick sections. Fixation and staining were done as described by Bancroft & Stevens (1990) with Haematoxylin and Eosin for recording the seasonal morphological changes in the gonads under light microscope (Meiji-MT 4300 H, Japan). To check the morphological changes in the gonads during non-breeding and breeding season the gonadosomatic index (GSI) of the collected samples were calculated with following formula

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

3. RESULTS AND DISCUSSIONS

The results of the present study revealed that the gonads of the *Clupisoma naziri* showed histomorphological changes during spring and summer season.

Testes

During the spring season the weight of the testes was observed $0.14 \pm 0.026\text{g}$ and it reached to the maximum size of $3.33 \pm 0.57\text{g}$ in summer (Table. 1). The histological study revealed that in spring season the

interlobular spaces were packed with loose connective tissues and food vessels which is the clear sign of preparatory phase in the gonads of a catfish. Leydig cells were also present with thick tunica albuginea. Inactive spermatogonia were seen at higher magnification. At the end of this phase the weight of the testis increased with the increase in lobular size and lobular wall of the testes i.e., tunica albuginea becoming thin and the interlobular space began to reduce (Fig. 1). While in summer the gonads attained full size and have maximum weight showing the sign of spawning phase. In testis the seminiferous tubules contained spermatozoa and interstitial connective tissues were also visible. The color of the testis became whitish during spawning season. The thin testicular and lobular walls are the characteristics of this phase. However, some empty lobules were also present. (Fig. 2). The gonadosomatic index (GSI) results were compared to the findings of a histological analysis of male gonads. During spring season, the GSI values was observed 0.11 ± 0.03 and it increases significantly ($P > 0.05$) to its peak value 3.71 ± 0.54 in summer.

Ovaries

The minimum average weight of the ovaries was recorded 2.79 ± 1.81 g in spring and the maximum average gonad weight was observed 5.61 ± 4.89 g in summer (Table. 1). The histological study shows that the wall of the ovary which is made up of vascular collagenous tissues appeared thick and the ovarian follicles were in previtellogenic stage and contained basophilic cytoplasm in spring season. The weight of the ovaries increases with the increase in oocyte size. The evagination appeared in the nuclear membrane which increases the surface area of nucleus. Due to distributed material in ooplasm the extruded nucleoli lose their consistency. The minute vacuole appears in the cortex of cytoplasm (cortical alveoli or yolk vesicles) which later on changes into large spherical yolk filled globules covering the entire ooplasm (Fig.3) showing the sign of preparatory phase of ovaries in catfishes. During summer season, the ovarian follicles were determined by the presence of large yolk vesicles. The eggs were fully grown and completely filled the yolk vesicle determining the spawning stage. Cortical alveoli layer adjacent to the egg envelop were clearly visible and the nucleus became indistinct (Fig.4). The results of the gonadosomatic index (GSI) were matched with the obtained results of histological study of ovaries in *Clupisoma naziri*. The mean gonadosomatic indexes value of ovaries was observed 2.43 ± 0.99 in spring which significantly ($P > 0.05$) increases up to 3.71 ± 2.97 in summer (Table. 1).

The testis of the catfish situated in posterior part of the abdominal cavity. They are paired, elongated organs having fringes along the entire length and surrounded by thick wall "tunica albuginea" which is composed of connective tissues. The seminiferous lobules contained the spermatogenic cells which are further composed of interstitial connective tissues containing the leydig cells. The catfish *Clupisoma naziri* had the similar testicular structure of American channel catfishes⁶. The maturation cycle and seasonal variation in gonadosomatic index are the sign of sexual maturity and growth in fishes. Gonad weight provides valuable information on different reproductive stages of the fish¹². The study of seasonal variation in the histomorphology of gonads of *Clupisoma naziri* revealed the preparatory stage and spawning stage in spring and summer season respectively. Both male and female gonads reach to their maximum weight in summer which decreases after spawning and fish becomes in spent stage. These findings were supported by the studies of Gaber, in *Bagrus* species⁴. In similar to the findings of Lawson, 2011; Cek and Yilmaz, 2007; Suwanjarat *et al.*, 2005; Shrestha and Khanna, 1978 in different teleost's; the results of present study show no spermatozoa in testicular lobules during spring season (preparatory phase) while in summer (spawning phase) the testicular lobules contained the spermatozoa with some spermatogenic cells^{5,3,9,8}.

The thickness of the tunica albuginea in ovaries varies throughout the year depending on the season. In spring it was observed thick (preparatory phase) and in summer it became thin (spawning phase), these findings were in line with the study on *B. domac* and in the ovary of *O. niloticus*^{4,5}. During summer season the ovarian follicles or vitellogenic follicles were determined by the presence of large yolk vesicles in *C. gariepinus* and in *Bagrus*^{10,4}. In addition, the results of gonadosomatic index (GSI) coincided with the histology of the gonads where they were observed higher in summer season and lowest during spring season when gonads were in preparatory and spawning phase respectively. The findings of the present research works will be helpful for the better understanding of reproductive biology of this economically valuable catfish to bring it under captive breeding and production in Pakistan.

Table I. Seasonal variations in the mean body weight (gm), Gonads weight (gm) and Gonadosomatic index of *C.naziri*.

Group	Sex	Season	Mean± SE	P-Value
BW	Male	Spring	119.67 ± 14.6	0.108 ^{NS}
		Summer	89.33 ± 1.76	
	Female	Spring	62.25 ± 3.183	0.006 ^S
		Summer	110.75 ± 14.5	
GW	Male	Spring	0.14 ± 0.02	0.19 ^{NS}
		Summer	3.33 ± 0.57	
	Female	Spring	2.79 ± 1.81	0.141 ^{NS}
		Summer	5.61 ± 4.89	
GSI	Male	Spring	0.11 ± 0.03	0.025 ^S
		Summer	3.71 ± 0.54	
	Female	Spring	2.43 ± 0.99	0.129 ^{NS}
		Summer	3.71 ± 2.97	

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

Figure. 1: Male gonad of *Clupisoma naziri* during spring season (Preparatory Phase).

S = Spermatozoa

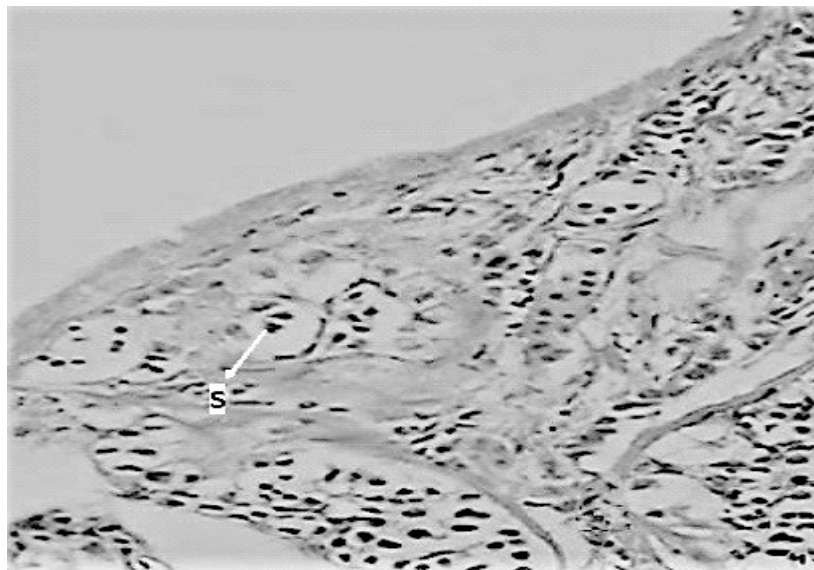


Figure. 2: Male gonad of *Clupisoma naziri* during summer season (Spawning Phase).

S = Spermatozoa, TA = Tunica albuginea.

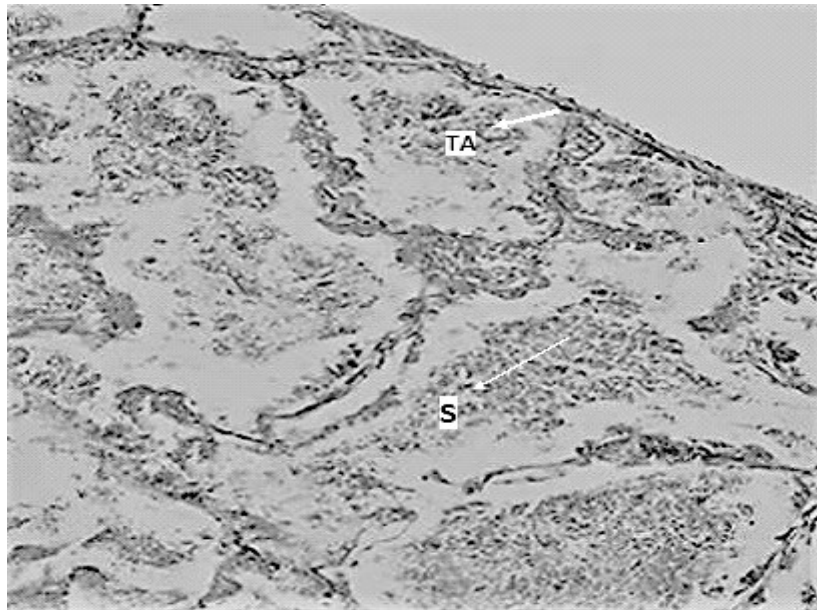


Figure. 3: Female gonads of *Clupisoma naziri* during spring season (preparatory phase). N= Nucleus, E= Evagination in nucleus, YV= Yolk vesicles



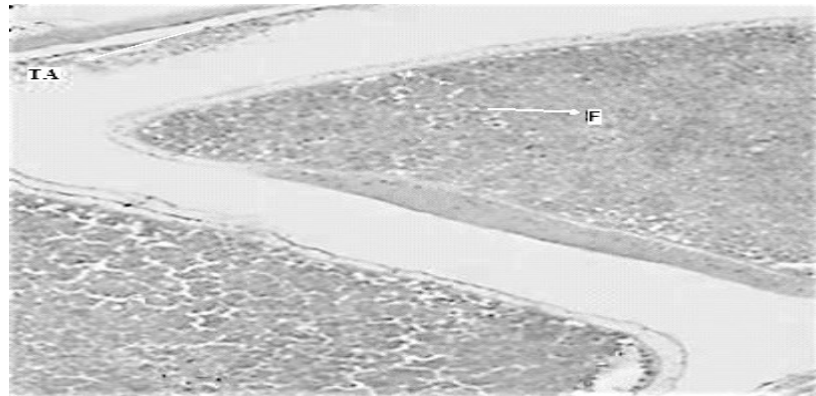


Figure. 4: Female gonads of *Clupisoma naziri* during summer season (Spawning phase) F= Follicle, TA= Tunica Albuginea

4. CONCLUSIONS

It was concluded that the gonadosomatic index (GSI) values rise during breeding season and reduce during non-breeding season, which is compatible with histomorphological gonad results. According to the current study, *C. naziri* only breeds once a year during the summer season, and this information will benefit aqua breeders in the rearing of this commercially important catfish in Pakistan.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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