STUDY OF GONADAL DEVELOPMENT IN STRIPED CATFISH
PANGASIANODON HYPOPHTHALMUS (SAUVAGE, 1878) DURING BREEDING SEASON IN PUNJAB, INDIA

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ABSTRACT: The present study was conducted to understand the reproductive biology and gonadal development of Freshwater striped catfish Pangas (Pangasianodon hypophthalmus) reared under the agro-climatic conditions of Punjab. Condition factor (K) of female fish ranged between 0.81 – 1.48, while in male fish 0.92 – 1.12. In both sexes K value recorded significantly (P<0.05) high in the month of July. Gonado somatic index (GSI) in female fish ranged 4.28 – 11.37, while in male fish 3.25 – 8.65. In both sexes K value and GSI recorded significantly (P<0.05) high in the month of July. In liver of female fish active hepatocytes along with large nucleoli showing biosynthesis activity observed in May, while in August, hepatocytes were completely exhausted. In hepatocytes of male fish no major change was observed. Ovarian development in the month of May showed developmental stages of ova, while germ cells attained the mature stage in July and continued upto August. Testicular development was arrested at spermatogenic stage in May, while in July and August testis were dominated by spermatozoa stage showing peak developmental stage.

Key words: Pangasianodon hypophthalmus, gonadal development, histology, liver, gonads, hepatocytes.

INTRODUCTION

Freshwater striped catfish Pangasianodon hypophthalmus belongs to the family Pangasiidae is an important food fish and serves as major source of protein for local consumers in Southeast Asian countries and also have commercial potential (Srirphairoj, 2010). In India, P. hypophthalmus was first introduced in 1997 in the state of West Bengal from Bangladesh. At present this fish is being widely cultured in many states particularly Andhra Pradesh, West Bengal, Kerala, Odisha and being farmed in more than 40000 ha and its production accounts for 0.7 million tonnes (Singh and Lakra, 2012).

In Punjab, Pangas is in good demand due to less intramuscular spines, soft texture, low in price etc., but at present; it’s demand is being fulfilled mainly through the import from other states; particularly from Andhra Pradesh. Standardization of induced breeding of different species of Pangas catfish reported by Potaros and Potaros (1974) for Pangasius sutchi in Bangkok, Meenakaran (1986) for Pangasius panga in South Sumatra, Indonesia, Thailand; Rahman et al (1993) for Pangasius sutchi in Bangladesh. In India induced spawning of Pangasius sutchi reported by Chattopadhyay et al (2002) and Chand et al (2011) still it’s breeding and seed production is a challenge under the agro-climatic conditions of the state due to lack of the information related to reproductive physiology, gonadal maturity and spawning season.

Success of the fish culture chiefly depends on timely availability of quality seed. To establish breeding programme of fish species, it is important to understand the dynamics of reproductive events, which occur during gonadal development. Therefore the present study was conducted to understand the reproductive biology and gonadal development of Pangas reared under the agro-climatic conditions of Punjab.

MATERIALS AND METHODS

The study was conducted at the Fish Farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India (30.54° N latitude, 75.48 °E longitude and an altitude of 247 m above mean sea level). The juveniles of P. hypophthalmus were procured from Kolkata, West Bengal and raised in earthen pond during summer (April to November) and in polyhouse (equipped with electrical heating system) during winter (December to March). Fishes were fed with formulated floating feed @ 2% of body weight; in two installments. Nutritionally, feed contains 28.0% crude protein.
formulated through balanced rationing of agro-industrial by-products i.e. rice bran (30%), de-oiled ground nut (30%), de-oiled soybean (25%), fish meal (13%) and vitamin – mineral mixture (2%). In its third year of rearing, gonadal development was studied for Condition Factor (K), Gonadosomatic Index (GSI) and histological changes in liver, ovaries and testis along with water quality of pond water from May to August, 2016.

During the present study, water quality with respect to temperature, pH, dissolved oxygen, alkalinity and hardness were assessed as per standard methods given in APHA (2005).

Every month three female and male; each were taken out and dissected after taking length and weight, and the intact ovary/ testis were taken out to measure gonad weight of respective sexes and following parameters were calculated:

Fulton’s condition factor (K): Fulton’s condition factor (K) was calculated according to Htun-Han (1978) equation as per formula given below: $K = \frac{W}{L^3} \times 100$

Where, $W = \text{weight of fish (g)}$, $L = \text{Length of fish (cm)}$

The Gonadosomatic Index (GSI) was recorded as per the formula given by Vladykov (1956) as

$$\text{GSI(\%)} = \frac{\text{Weight of gonad (g)}}{\text{Weight of fish (g)}} \times 100$$

Liver and gonads were further cut into pieces and fixed in Bouin’s solution and further processed for histological studies as per the method suggested by Humason (1979). Paraffin sections were cut at 5 micrometer thickness and stained with Hematoxylin-Eosin (HE) for histological observations prior to microphotography. Leica model no. DM 3000 has been used for microscopy studies of histology. Statistical program SPSSv16 (SPSS Inc.2008) was used for data analysis.

**RESULTS AND DISCUSSION**

Fish, being an aquatic vertebrate is directly influenced by water quality parameters. During the experimental period physico-chemical parameters varied within the desirable range for fish culture as suggested by Boyd (1990) and Bhatnagar and Devi (2013) for warm water fish species. No abrupt change in water quality recorded during the study period. Details of the water quality recorded during the study period are given in Table 1.

Different biological indicators like K value and GSI is more often used to observe the progression of gonadal development w.r.t. health status. Condition factor of a fish reflects physiological and health status of fish Ndiaye et al (2015). In present study, K value of female fish ranged 0.81 – 1.48. Significantly ($P<0.05$) higher K value recorded in the month of July, while it was low in May. In male; K value ranged 0.92 – 1.12. Male also showed significantly ($P<0.05$) higher K value recorded in the month of July, while it was low in May. Gonadosomatic Index exerts the performance of gonadal development in fish. In female, GSI ranged 4.28 – 11.37 and was significantly ($P<0.05$) high in July, while low in August. In male; GSI ranged 3.25 – 8.65, it was also significantly ($P<0.05$) high in July and low in August. Change in K value and GSI may be mainly due to change in volume and weight of gonad. Condition factor reflects the well-being of fish, while GSI is more often used as reliable indicator of its maturity (Rae and Calvo, 1995).

In the month of May, liver of female *P. hypophthalmus* exerted with active hepatocytes along with large nucleoli (Fig. 1) showing biosynthesis activity in progress, while in July reduced biosynthesis activity exhibit less biosynthesized content (Fig. 2). In August, hepatocytes were completely exhausted (Fig. 3) may be

### Table 1: Physico-chemical parameters of water in pond.

<table>
<thead>
<tr>
<th>Water parameters</th>
<th>Mean ± SE</th>
<th>Range</th>
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<tbody>
<tr>
<td>Temperature (°C)</td>
<td>28.32 ± 3.58</td>
<td>24.74-31.90</td>
</tr>
<tr>
<td>pH</td>
<td>7.20 ± 0.05</td>
<td>7.15-7.25</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg l⁻¹)</td>
<td>7.35 ±0.25</td>
<td>7.10-7.60</td>
</tr>
<tr>
<td>Total Alkalinity (mg l⁻¹)</td>
<td>230.00 ±12.30</td>
<td>217.70-242.30</td>
</tr>
<tr>
<td>Hardness (mg l⁻¹)</td>
<td>214.00±6.80</td>
<td>207.20-220.80</td>
</tr>
<tr>
<td>Ammonical - nitrogen NH₃-N (mg l⁻¹)</td>
<td>0.082±0.006</td>
<td>0.076-0.088</td>
</tr>
<tr>
<td>Phosphate- phosphorus PO₄-P (mg l⁻¹)</td>
<td>0.521±0.04</td>
<td>0.023-0.92</td>
</tr>
<tr>
<td>Nitrate - nitrogen NO₃-N (mg l⁻¹)</td>
<td>0.558±0.08</td>
<td>0.163-0.850</td>
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### Table 2: Mean ±SE ($P<0.05$) of condition factor (K) and Gonadosomatic Index (GSI) of female and male *P. hypophthalmus*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female</th>
<th>Male</th>
</tr>
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<tr>
<td></td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td>Condition Factor (K)</td>
<td>0.81± 0.03</td>
<td>1.04± 0.05</td>
</tr>
<tr>
<td>Gonadosomatic Index (GSI)</td>
<td>8.80± 0.12</td>
<td>10.08± 0.68</td>
</tr>
</tbody>
</table>
Fig. 1: Active hepatocytes with large nucleoli (→) and biosynthesized material in the liver of female *P. hypophthalmus* in May. HE × 200

Fig. 2: Hepatocytes having large nucleolar size (→) and reduced biosynthesized material in the liver of female *P. hypophthalmus* in July. HE ×200

Fig. 3: Hepatocytes having large nucleolar size (→) and exhausted biosynthesized (vacuolization) material in the liver of female *P. hypophthalmus* in August. HE ×200

Fig. 4: Active but small hepatocytes having small nucleolar size (→) containing moderate biosynthesized material in the liver of male *P. hypophthalmus* in August. HE ×200

Fig. 5: Ovary of *P. hypophthalmus* showing primordial germ cell (PGCs, →), oogonials (●), immature oocytes with numerous nucleoli (⇒) in the month of May. HE ×50

Fig. 6: Ovary of *P. hypophthalmus* showing mature stage of ova (⇐), full with yolk granules (●) in the month of July. HE ×100
Fig. 7: Ovary of *P. hypophthalmus* showing separation of follicular layer (■), full with yolk granules (▲) in the month of July. HE ×200

Fig. 8: Ovary of *P. hypophthalmus* continuing dominance of mature ova (■), in the month of August. HE ×50

Fig. 9: Ovary of *P. hypophthalmus* showing separation of follicular layer (■), full with yolk granules (▲) in the month of August. HE ×200

Fig. 10: Testis of *P. hypophthalmus* showing spermatogonium (→), spermatocytes (□) and spermatids (▲) in the month of May. HE ×200

Fig. 11: Testis of *P. hypophthalmus* showing spermatocytes (□), spermatids (▲) and spermatozoa in the month of July. HE ×200

Fig. 12: Testis of *P. hypophthalmus* showing spermatozoa (►) in the month of August. HE ×200
due to mobilization of biosynthesized content due to which vacuolization condition developed. In liver of male fish, active but small hepatocytes having small nucleolar size showing moderate biosynthesized content (Fig. 4). Rae and Calvo (1995) suggested that reduction in biosynthesized content occurs due to mobilization of energy stored from liver to ovary for egg production and maturation, same trend recorded in the present study.

Ovarian development in the month of May showed developmental stages of ova including primordial germ cell (PGCs), oogonial cells and immature oocytes (Fig. 5), while it attained the mature stage in July (Figs. 6 & 7) and continued up to August (Figs. 8 & 9). Oogenesis is the process of transformation of primordial germ cells (PGCs) into ova, ready to be fertilized followed by embryonic development. Normally six oogenesis stages i.e. formation of PGCs, transformation of PGCs into oogonia and transformation of oogonia into oocytes (onset of meiosis), vitellogenic growth of oocytes while under meiotic arrest, resumption of meiosis (maturation) and expulsion of the ovum from its follicle (ovulation) are associated with gonadal development and maturation in several teleosts (Patino and Sullivan, 2002). Phenomenon of spawning largely depends on vitellogenesis and oocyte maturation (Micale and Perdichizzi, 1994; Micale et al., 1999).

Testicular development in the month of May showing progression of spermatogenic stage evident by cellular stages as spermatogonium, spermatocytes and spermatids, while in July and August testis were dominated by spermatooza stage (Figs. 11 & 12) showing peak developmental stage. Spermatogenesis start with spermatogonium or stem cell increases geometrically in species-specific number of mitotic cell division. In present study, development of cellular stages clearly evident that pangas attains its peak maturity during the month of July and August under the agro-climatic conditions on Punjab.

CONCLUSION

Knowledge of the gonadal developmental stages of fish is important to understand the reproductive performance for the purpose of developing a breeding programme. In present study, it was observed that Pangas (P. hypophthalmus) attend peak maturity in the month of July and maintains mature stages of germ cell in August also under the agro-climatic conditions on Punjab.

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