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Qualitative and quantitative changes in testes of *Cirrhinus mrigala* (Ham.) through multiple induced stripping

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ABSTRACT

Quantitative evaluation of the male gonad and gametes in *Cirrhinus mrigala* was studied in seasonal cycle as well as in the multiple stripping sequences during breeding season to assess the efficacy of males for multiple uses. The peak GSI was observed during July and least in January. The matured testes during June showed a GSI 2.175±0.01 that reduced to 0.88±0.01 soon after stripping. The same again increased up to 1.81±0.08 within 4 weeks in brood rearing ponds. The milt yield, spermatocrit value and sperm count reduced drastically in first week after first stripping (milting) and slowly increased on fourth week. The histo-anatomical study of testes through seasonal cycle and during inter-milting (stripping) period implies that the testes do not enter to any resting phase in later. The sequential growth of germ cells in spermiogenesis completed within few weeks during inter-milting period in a multiple sequence of milting. The spent male broods were found to be ready for subsequent use within 30 days after first stripping.

Key words: *Cirrhinus mrigala,* GSI, Milt, Spermatocrit, Stripping, Testes

Indian major carps could be bred 2 to 4 times in a year (Bhowmik *et al*. 1977, Chondar 1986, Gupta *et al.* 1995). Studies on the multiple spawning in female individuals are known to some extent (Routray 2003) but limited information is available about male carps (Chondar 1986). Increased rate of fertilization in induced breeding depends upon quantity and quality of milt produced during this period (Routray 2003, Thomas *et al*. 2003, Bozkurt *et al.* 2009, Verma *et al.* 2009). Similarly, studies on seasonal changes of testes of cultivable carps in Indian waters especially their maturity status and annual cycles are meager (Kurokura *et al.* 1984, Das 1988, Kumar 1998, Gupta and Rath 2006). Milt collection by stripping from carp males during artificial fertilization is a regular practice in hatcheries. However, there is no systematic study on the milt quality and quantity by multiple induced stripping of these species. The present study aimed to analyze the qualitative and quantitative changes in testes of *C. mrigala* through induced stripping in multiple spawning programmes.

MATERIALS AND METHODS

Spent mrigal male brood (milters) of preceding years (2+ years age groups) were tagged by M. Procian blue following the tagging technique as described by Khan *et al*. (1988) in brood ponds at Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneshwar. Milters were sacrificed and sampled monthly for a complete calendar year for the study of GSI and histo-anatomy. Mature milters (20) were induced milted with ovaprim @ 0.2 ml /kg body weight in June, and spent were again reared with 2nd dye mark for subsequent milting. Milt sample were analyzed for volume, spermatocrit value, sperm motility and sperm count. Spermatocirt values (packed sperm cells) of milts were determined by micro-haematocrit apparatus with RPM 10000×g for 10 min. Motility of milt were assessed by activating the sample with a drop of sterile water on a glass slide and observed under an inverted microscope (×200) attached with a CCD camera.

Table 1. Quantitative evaluation of milt of *C. mrigala* at weekly intervals

Parameters	Weight of fish (Kg)	Motility (%)	Spermatocrit value (%)
Initial	1.25± 0.02^{a}	92.75±2.87^{a}	91.75±4.71^{a}
1st week	1.23±0.22^{a}	13.5±3.87^{d}	15.75±3.30^{e}
2nd week	1.16±0.18^{a}	22.5±5.50^{c}	31.75±0.18^{d}
3rd week	1.21±0.19^{a}	44.5±7.59^{b}	60.5±6.55^{c}
4th week	1.26±0.20^{a}	87.25±2.21^{a}	78.75±6.39^{b}

Values are expressed as mean±SD (n=9). Values having different superscripts differ significantly in a column (P<0.05).

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The motility was recorded in computer by using the Biovis motility software. Sperm count was done by haematocytometer and expressed as number of spermatozoa per cubic mm. The milters were induced Ovaprim (Salmon GnRH + domperidone, Ovaprim) @ 0.2 ml/ kg body weight and milt collection was done with weekly intervals to assess the quality and quantity of milt. Gonadosomatic index was calculated using a formula, i.e. weight of testes/ total body weight of fish×100 and histo-anatomical study were done to ascertain the quantitative changes in testes during the study period. A piece of male and female gonad were fixed in Bouin's solution, dehydrated in alcohol, embedded in paraffin and sections, 5–7 µ thick, were prepared. Sections were stained with haematoxylin and eosin. Slides were examined under a phase contrast microscope.

RESULTS AND DISCUSSION

In the present study mean gonadosomatic index of mrigal during seasonal cycles showed lowest value (0.22±0.01) in January while the peak was found in July (2.26±0.114). Milting was observed since April with GSI of 0.80±0.03. The mean GSI (2.17±0.01) of non-induced milters during June has gone down to 0.88±0.02 soon after induced milting (stripping). The same further reduced after 1 week and slowly increased up to 1.81±0.08 within 4 weeks after first milting. The GSI in seasonal cycle reduced slowly up to the resting season. The GSI of mrigal dropped to 0.88 soon after stripping, certainly due to ejaculation of voluminous milt. The same was further reduced to 0.57 that implies that the ejaculation process also persisted for more hours after peak milting by stripping. Multiple usages of males in traditional induced breeding programmes have been recorded by practitioners. Badapanda *et al*. (1982) and Chondar *et al.* (1986) could utilize males for multiple times during breeding season. Gupta *et al*. (1995) reported three to four time uses of *C. catla* milters in multiple breeding experiments but did not quantify the efficacy of males. Similarly, it was reported that Indian major carps could be reused within a time lapse of 30 to 45 days between 2 successive spawning (Gupta and Rath 2006). Although these reports envisaged the possibilities of multiple spawning of milters, the gonadal readiness and milt production during subsequent breeding was not reported earlier. Multiple milt yield was also reported in *Labeo calbasu* and *L. rohita in* spawning season of carps (Routray 2003). Gupta and Rath (2006) further added that the time interval between successive milt yields is not the parameter that decides the use of milter for breeding operation, rather the milt quality with more than 60% spermatocrit value and 4+ sperm motility (scored in a index of 5–point scale) can be used subsequently. In the present study, the milt parameters such as milt yield, motility, sperm count and spermatocrit value were more suitable for use in breeding programmes in agreement with earlier workers. A seasonal change on male gonadosomatic index of carps is an indicator of gonadal status of brood fish. Verghese (1975) recorded a GSI range of 0.09 to 0.13% in male *Cirrhinus reba* during resting season and the same was as high as 0.89 to 3.50% during peak breeding season. Selvaraj *et al.* (1972) and Das (1988) reported the maximum mean GSI of *Labeo boggut* and *Labeo bata* as 3.5 and 5.96% respectively during breeding season. Testis of *Cyprinus carpio* exhibits highest GSI during pre-spawning period up to 3.36 whereas, grass carp and silver carp showed a GSI (testis) of 2.4 and 0.6 respectively (Chondar 1986).

Increased rate of fertilization in induced breeding depends upon quantity and quality of milt produced (Bozkurt *et al.* 2009, Verma *et al.* 2009). The milt yield, spermatocrit value and sperm count of mrigal on first stripping were 6.92±1.41 ml / kg, 91.75±4.77% and 2.94±0.43×10^7/ mm^3 respectively. These 3 parameters reduced drastically in the first week after first stripping (milting) and slowly increased up to 5.7±0.72 ml/kg, 78.75±6.39% and 2.62±0.32 10^7/ mm^3 respectively on fourth week. Spermatocrit value of milt and motility of spermatozoa of *Labeo rohita* in the range of 65 to 80% and a motility score of more than 4+ was found to be ideal for fertilization of eggs (Routray *et al*. 2006). Moreover, the present study showed that reduced spermatocrit value, sperm count and motility during first and third week were certainly due to the absence of adequate spermatozoa in the seminiferous tubules as observed in the histo-anatomical structures. The histo-anatomical study showed the presence of more number of spermatozoa in the testis during June and July whereas testes entered into the resting phase during November to January. The milted testes during multiple stripping experiments were different from the annual testes cycle. Initial stripped testes showed emptiness and shrunken seminiferous tubules with residual spermatozoa. Some tubules also retained large number of spermatocytes at different stages of development and spermatids. The size reduction was clearly evident in spermatogonia of third and fourth week after stripping. Thus, the sequential growth of germ cells in spermiogenesis is completed within few weeks during inter-milting period in a multiple sequence of breeding or milting. Post-stripped testes of 20 days showed large number of spermatids inside the tubular lumen with peripheral spermatogonia which could not be distinctly observed. Testes sections during the fourth week were filled with numerous spermatozoa comparable with the testes of peak phases. Thomas *et al*. (2003) described that during resting phase, the seminiferous tubules are filled with spermatogonial cells and called those structures as spermatic cords. In this study, similar structures were found from November to January and numerous spermatogonia in sequences are transformed to spermatozoa through spermatocytes and spermatids in subsequent months. The spermatogonia were observed distinctly as hyper-trophid cells in post-stripped testes up to second week after stripping. As the number of spermatocytes and spermatids increased in the lumen of the tubules, spermatogonial activity was

reduced. It was evident as in distinct spermatogonia of third and fourth week after initial stripping. Lin and Peter (1996) and Bhatacharya (1999) described that GtH-II hormones control final maturation of teleostean gametes and spawning. The reduced gonadal cycle during inter-spawning period may be due to inducing agent that ultimately triggers the formation of GtH-II. In the present study, the sequential growth of germ cells in spermiogenesis was completed within few weeks during inter-milting period in a multiple sequence of milting. This usually takes few months in a seasonal cycle of traditional breeding system. The spent male broods were found to be ready for subsequent use within 30 days after first stripping in multiple milting.

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REFERENCES

Badapanda H S, Das P K and Pathak G C. 1982. Observation of the frequency of inter-seasonal maturity and bundh breeding of *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) at Sonepur, Orissa. *Indian Journal of Fisheries* **29**: 1–2.

Bhatacharya S. 1999. Recent advances in the hormonal regulation of gonadal maturation and spawning in fish. *Current Science* **3**: 342–49.

Bhowmick R M, Kotwal G V, Jana R K and Gupta S D. 1997. Experiments on second spawning of major Indian carps in the same season by hypophysation. *Aquaculture* **12**: 149–55.

Bozkurt Yusuf, Ogretmen Fatih Secer, Faik Sertel and Ercin Ugur. 2009. Relationship between Seminal Plasma Composition and Spermatological Parameters in Scaly Carp (*Cyprinus carpio*). *Journal of Animal and Veterinary Advances* **8 (**12): 2745–49, DOI: 10.3923/javaa.2009.2745.2749.

Chondar S L. 1986. Repeated breeding of Indian and Chinese major carps during the same spawning season. *Proceedings Second National Fish Seed Congress*. Fisheries Department, West Bengal and Ministry of Agriculture. Government of India. pp 40–43.

Das R C. 1988. Seasonal variation and effect of certain hormones in testicular choloesterol and gonadosomatic indices of teleost, *Labeo bata* (Ham.). *Himalayan Journal of Environmental Zoo*logy **2**: 1004–11.

Gupta S D and Rath S C. 2006. *Handbook of Fisheries and Aquaculture.* Directorate of Information and Publication of Agriculture, Indian council of Agricultural Research, New Delhi pp. 227–45.

Gupta S D, Rath S C, Dagupta S and Tripathi S D. 1995. A first report on quadruple spawning of *Catla catla* (Ham.) *Verterinariski Archive* **5** (5): 143–48.

Hora S L. 1945. Analysis of the factor influencing the spawning of Indian major carps. *Proceedings of National Institute of Sciences of India*. (B), **11**: 303–12.

Khan H A, Gupta S D and Reddy P V G K. 1988. Suitable dye for marking Asiatic carp. *Indian Journal of Animal Sciences* **58**: 1003–06.

Kumar K. 1998. A comparative study of various extenders for cryopreservation of carp spermatozoa. *Journal of Animal Science*. **58**: 1355–60.

Kurokura H, Hirrano R, Ronia M and Iwahasi M. 1984. Cryopreservation of carp sperm. *Aquaculture* **37**: 267–73.

Lin H R and Peter R E. 1996. Hormones and spawning in fish. *Asian Fisheries Science* **9***:* 21–33.

Routray P. 2003. 'Evaluation of cryopreserved milt of selected carp *L.calbasu* for fertilization and seed production, Ph.D thesis. Sambalpur University, Sambalpur, Orissa, 144.

Routray P, Choudhary A K, Dash S N, Verma D K, Dash C, Swain P, Jena J K, Gupta S D and Sarangi N. 2006. Cryopreservation of dead fish spermatozoa several hours after death of Indian major carp, *Labeo rohita* and its utilization in fish production. *Aquaculture* **261**: 1204–11.

Selvaraj C, Radhakrishnan S and Parmeshwaran S. 1972. Notes on the breeding season, fecundity and life history of a minor carp, *Labeo boggut* (Skyes) *Journal of Inland Fisheries Societies of India* **3**: 130–31.

Terner C. 1986. Evaluation of sperm motility for Cryopreservation. *Progressive Fish Culturist* **48**: 230–32.

Thomas P C, Rath S C and Mohapatra K D. 2003. *Breeding and Seed Production of Finfish and Shell Fishes.* Daya Publishing House, Delhi, 402.

Varghese P V. 1975. Internal rhythm of sexual cycle in carp *Cirrhina reba* (Ham.) under artificial condition of darkness. *Journal of Inland Fisheries Society of India* **7**: 182–88.

Verma D K, Routray P, Dash C, Dasgupta S and Jena J K. 2009. Physical and Biochemical Characteristics of Semen and Ultrastructure of Spermatozoa in Six Carp Species. *Tourkish Journal of Fisheries and Aquatic Sciences* **9**: 67–76.

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