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SHORT PAPER

Histological Study of Sex Differentiation in Bighead Carp (*Hypophthalmichthys nobilis*), Grass Carp (*Ctenopharyngodon idella*), Silver Carp (*Hypophthalmichthys molitrix*) and Catla (*Catla catla*)

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Abstract

Time of sex differentiation has been identified in four major commercial carps; bighead carp (*Hypophthalmichthys nobilis*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) and catla (*Catla catla*). Histological differentiation of germ cells has not been previously studied in these species except grass carp. Developmental process of gonads in these species was studied from fertilized egg stage till completion of sex differentiation. Identification of this time is important to find out an appropriate time for sex reversal treatment to produce monosex culture at commercial level thus eliminating the growth rate differences between both sexes. Sex differentiation was observed at 784^{o}dph (degree days post-hatch) (28 days post-hatch), 1215 ^{o}dph (45 days post-hatch), 786^{o}dph (28 days post-hatch) and 840 ^{o}dph (28 days post-hatch) in bighead carp, grass carp, silver carp and catla, respectively.

Keywords: Sex differentiation; bighead carp; grass carp; silver carp; catla.

Introduction

Bighead carp, grass carp, silver carp and catla are major commercial carps in Asia (FAO, 2016). These species have been introduced in Pakistan almost four decades ago to meet the increasing demand of protein except catla which is an indigenous species. Problems of differential growth rate between both sexes and early maturation in males has been an issue for commercial farmers in these species (Kuronuma, 1968; Schrank and Guy, 2002; Williamson and Garvey, 2005). This problem can be addressed by production of monosex population (Devin and Nagahama, 2002). Monosex production by the application of hormone treatment has been successfully performed in several species such as tilapia (*Oreochromis niloticus*; Ridha, 2011), common carp (*Cyprinus carpio*; Gomelsky, 1985), eel (*Anguilla japonica*; Andersen *et al*., 1996), rainbow trout (*Onchorhynchus mykiss*; Amini and Tala, 2003), chinook salmon (*Oncorynchus tshawytscha*; Baker *et al*., 1988), coho salmon (*Oncorhynchus kisutch*; Piferrer and Donaldson. 1989) and brook trout (*Salvelinus fontinalis*; Fatima *et al*., 2016). However, identification of time of sex differentiation is crucially important for successful hormonal treatment.

Sex differentiation is the process by which the various molecular, genetic and physiological mechanisms produce a male or female from a zygote of a given genotype and parents in a given environment (Bull, 1983). At this developmental stage, gonads can be clearly differentiated as ovaries and testes histologically if not morphologically identified. Sex differentiation in gonochorist fish species can be indirect or direct. In indirect sex differentiation, undifferentiated gonads firstly develop into ovaries and subsequently into testes or ovaries. On the other hand, in direct sex differentiation, undifferentiated gonads develop directly into ovaries or testes (Yamamoto, 1969). Nakamura *et al*. (1998), Devlin and Nagahama (2002), Strüssmann and Nakamura (2002), Penman and Piferrer (2008) and Sandra and Norma (2010) have extensively reviewed the factors affecting sex differentiation, with genetic, endocrine, environmental and social factors all playing an important role.

Gonadal differentiation has not been previously studied in above mentioned major carps except grass carp (Jensen and Shelton, 1983).On the other hand, monosex culture production has not been studied in any of these species. For this purpose, a comprehensive histological study will help to identify the time of sex differentiation during the post-hatch period that could be the most responsive stage for future endocrine sex-reversal treatment. Therefore this study was conducted to determine the time of

hatch and characterize gonad differentiation in these species during the post-hatch period. Data given in present study could be very useful to design an appropriate protocol for hormone treatment in future.

Materials and Methods

Present study was performed after approval of animal ethics committee, Lahore College For Women University, Lahore. Study was performed at hatchery and ponds facility of Fisheries Research and Training Institute, Lahore and Gujranwala. Study period was from April – May, 2016. Brood stock of varying age was used for artificial breeding. Standard protocol of artificial breeding for carps was followed. Males were given 0.21cc/kg and females were given 0.5 cc/kg of ovaprim. Fertilized eggs were kept in recirculation tanks at 26±0.50 ºC in silver carp and grass carp while water temperature at this stage was 29±0.50 ºC in case of catla and bighead carp. At yolk sac absorption stage, water temperature was 26±0.50 ºC in case of silver carp, grass carp and catla while 29±0.50 ºC in case of bighead carp. After yolk sac stage, fry were transferred to earthen ponds. Average temperature of pond water till end of study was recorded at 28±0.50 ºC, 30±0.50 ºC, 27±0.50 ºC and 26±0.50 ºC in case of bighead carp, catla, grass carp and silver carp, respectively. Photoperiod was recorded 14 hours Light: 10 hours dark in April while 15 hours light: 9 hours dark in May.

Samples were collected for all developmental stages (Fertilized eggs, yolk sac stage and juvenile) and temperature of pond for each stage was recorded at daily basis. Sampling was carried out on alternate days at pre-hatch, hatch and one week post-hatch stages followed by once a week over the remaining study period. A total of ten samples were collected at each sample point over the period of two months (April – May, 2016). Samples were preserved in Bouin's solution (75ml saturated aqueous solution of picric acid, 25ml formalin (40% aqueous solution of formaldehyde, 5ml glacial acetic acid) until processed for histology.

Histological study of collected samples (middle section of whole fish) was performed using the routine procedure. Dehydration of the tissues was achieved by passing them through different grades of alcohol (70%, 90% and 100%). After dehydration in 100% alcohol tissues were passed through two exchanges of xylene. After dehydration, tissues were processed through two exchanges of paraffin wax. Tissues were embedded to prepare blocks and sectioned at 5µm thickness using the microtome (Shandon, UK). Routine haematoxyline and eosin staining was performed to attain the tissues. Tissues were dewaxed at 60ºC and then passed through two exchanges of xylene, 100%, 90%, 70% and 50% alcohol. After hydration, tissues were stained in haematoxyline and eosin solution. After staining, tissues were passed through grades of 50%, 70%, 90%, 100% alcohol and xylene. Sections were mounted with DPX (Merck, Germany). Microphotographs of tissues were taken at different magnifications by a high resolution microscope (Meiji Techno, Japan).

Results

Hatching was observed at 24 hours post-fertilization at 26ºC (26 degree days post-fertilization [26 ºdpf]) in silver carp and grass carp. In catla and bighead carp, it was observed at 24 hours post-fertilization at 29ºC (29 ºdpf) and 30 hours post-fertilization at 28ºC (28 ºdpf), respectively. Yolk sac absorption took three days post-hatch at 26.00 ± 0.50 ºC (78ºdph) in silver carp, grass carp and catla. In bighead carp, it took four days at 29.00 ± 0.50 ºC (116 ºdph). Histologically, shape of gonads was observed to be oval or peer shaped. Position of gonads was below the swim bladder near the gut. Undifferentiated gonads were observed containing a few primordial germ cells. Sex differentiation was observed at 784ºdph, 1215 ºdph, 786ºdph and 840 ºdph in bighead carp, grass carp, silver carp and catla, respectively. Histologically, differentiated testes and ovaries contained spermatogonia and oogonia, respectively (Figure. 1).

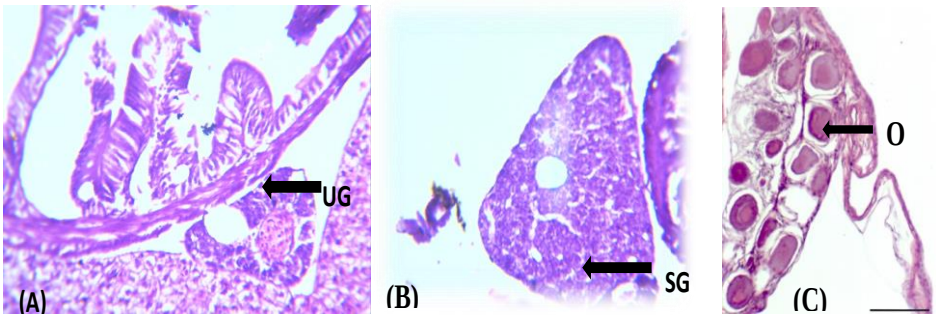

Figure. 1. Histological development of gonads. (A) Undifferentiated Gonad, 400X (B) Testis. 40X (C) Ovary, 40X. SG: spermatogonium, O: oogonium, UG: Undifferentiated Gonad. Histologically, differentiated gonads showed similar structure in all four species

Discussion

Present study identified the histological development of gonads and time of sex differentiation in four major carps in Asia which has not been reported previously except grass carp (Jensen and Shelton, 1983). The hatching period is critically important in gonadal differentiation of teleosts, as sex differentiation as defined by histology may occur on hatching or shortly thereafter in warm water species (Jensen and Shelton. 1983; Nakamura *et al*. 1998; Selim *et al*., 2009). Hatching period in all four species was 24 hours post-fertilization which indicates effective control of water temperature over hatching period (Devlin and Nagahama, 2002). It further eliminates the probability of gonadal development at the time of hatch due to its extreme short duration. Indifferent gonads were observed over the period of about three weeks post-hatch in all four species. Undifferentiated gonads contained a few primordial germ cells in all species as reported previously in teleosts (Jensen and Shelton, 1983; Parmentier and Timmermans, 1985; Uguz, 2008; Sacobie and Benfey, 2005; Fatima *et al*., 2011). Based on present results, it is apparent that sex differentiation occurs after one month post-hatch in these species under regional climatic conditions. Jensen and Shelton (1983) reported differential patterns of development in presumptive ovaries and testes in grass carp. They observed that presumptive testes had thin stalk of stromal tissue attached to the peritoneum while presumptive ovaries were broader in cross section with an expanded area of peritoneal attachment. This change appeared to be a characteristic of anatomical differentiation of gonads. However, no such distinct anatomical differences were observed in present study. At the time of sex differentiation, both ovaries and testes could be identified by presence of oogonia and spermatogonia although ovaries were found to be slightly larger than testes in cross section. Jensen and Shelton (1983) reported gonadal differentiation at 45 days post-hatch which coincides with the finding of present study.

Present study shows that sex differentiation in all four species occurs after 3 weeks post-hatch which indicates that the most labile period for hormonal treatment may be about 1 – 2 weeks post-hatch under ambient conditions when indifferent gonads were being developed. This information will be useful for designing an appropriate protocol to skew the sex ratio towards monosex (either all-male or all-female) production of these species in the regional Aquaculture industry. Application of this technique will help to eliminate the growth rate differences between both sexes thus improving early availability of marketable sized fish.

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