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Growth and survival of olive barb, *Puntius sarana* (Hamilton 1822) larvae produced with cryopreserved versus fresh sperm

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Abstract

Despite the success in fertilization and hatching of fish eggs with cryopreserved sperm, report on growth and survival of larvae produced from frozen-thawed sperm is inadequate. The study evaluates the applicability of cryopreserved sperm for mass seed production by comparing the growth and survival of a popular food-fish olive barb, *Puntius sarana* (Hamilton 1822) larvae produced from cryopreserved and fresh sperm. The eggs were artificially fertilized with cryopreserved and freshly collected sperm, and the growth and survival of produced larvae from both group recorded up to 12 weeks. The independent sample *t*-test statistic showed the difference in lengths, $t(718) = 0.241$; $P = 0.810$ and weights, $t(718) = 0.412$; $P = 0.680$ were insignificant between two groups. There was also no significant difference, $t(718) = -0.758$, $P = 0.448$ in survival of larvae produced from cryopreserved and freshly collected sperm. The study indicates that larvae of olive barb produced from cryopreserved sperm are equally compatible in growth and survival as the larvae produced from fresh sperm. Therefore, cryopreserved sperm can be applied for artificial fertilization of *P. sarana* to supply quality seed for aquaculture.

Keywords: cryopreserved sperm, growth, survival, *Puntius sarana*

Introduction

Lack of good quality fish seed is considered one of the major challenges for the development of aquaculture in Bangladesh, and many other Asian countries (Siriwardena 2007). Cryopreservation (storage of biological materials in liquid nitrogen at −196°C) technique has the potential to overcome the bottleneck by supplying sperm from superior male during artificial fertilization. Sperm cryopreservation is a proven technique to supply sperm for artificial fertilization in a large number of freshwater and marine fish. In addition, commercial application of cryopreserved sperm and feasibility of gene banking has been investigated for many fish species (Butts, Feindel, Neil, Kovács, Urbányi & Trippel 2011). In Bangladesh, research on fish sperm cryopreservation started in 2004, and the protocols have been developed for several commercial and threatened fish species (Hossain 2010). The studies mostly focused on selection of suitable extenders and cryoprotectants, optimal dilution ratios of milt, and optimal cryoprotectant concentrations, post-thawed motility of sperm, fertilization and hatching rate (Nahiduzzaman, Hassan, Roy, Hossain, Hossain & Tiersch 2012).

To date, only 10 fish species are studied for the growth and survival of larvae produced with cryopreserved sperm worldwide. The growth and survival of offspring produced from cryopreserved

sperm has been studied for pabda catfish, *Ompok pabda* (Sarder, Saha & Sarker 2013), tiete tetra, *Brycon insignis* (Viveiros, Isaú, Caneppele & Leal 2012), silver barb, *Barbonymus gonionotus* (Rahman, Sarder & Rouf 2009), common carp, *Cyprinus carpio* (Sarder, Salam & Hussain 2007), steelhead trout, *Oncorhynchus mykiss* (Hayes, Rubin, Hensleigh, Reisenbichler & Wetzel 2005), turbot, *Scophthalmus* (*Psetta*) *maxima* (Chereguini, La Banda, García, Rasines & Fernández 2002), channel catfish, *Ictalurus punctatus* (Tiersch, Goudie & Carmichael 1994), African catfish, *Clarias gariepinus* (Van der Walt, Van der Bank & Steyn 1993), tilapia, *Oreochromis hornorum* (Chao, Chao, Liu & Liao 1987) and striped bass, *Morone saxatilis* (Kerby, Bayless & Harrell 1985).

The fish under study, olive barb, *Puntius sarana* (Hamilton 1822) is the largest barb native to inland waters of South-East Asia (Talwar & Jhingran 1991). The olive barb is a popular food-fish having high market demand in Bangladesh and in other South Asian countries (Jena, Das, Das & Mondal 2007; Hossain, Nahiduzzaman & Saha 2010) enlisted as critically endangered (CR) in Bangladesh (IUCN-Bangladesh 2000). Along with its conservation in natural habitat through establishing fish sanctuary and stock enhancement programme, the inclusion of the fish in carp polyculture system would contribute to the diversification of aquaculture system and conservation of the species (Hossain *et al.* 2010). As the fish is rarely available in the wild, there has been limited scope of collecting fish for fry production in the hatcheries. If the stored sperm can be made available on demand for research or commercial fry production, this would certainly contribute to both conservation and aquaculture enhancement of the species. Sperm cryopreservation protocol has already been developed for *P. sarana* to assist basic research and time/location independent supply of sperm for artificial fertilization (Nahiduzzaman, Hassan, Khanam, Mamun, Hossain & Tiersch 2011). Although the frozen-thawed sperm of the species is proven useful to fertilize eggs, the lower hatching rate of the thawed sperm made them incompatible with fresh sperm. In most of the studies, fertilization and hatching is presumed to be ultimate criterion of assessment of a cryopreservation protocol. However, prior to use preserved sperm for seed production and restocking porgramme, the growth performance and survival of larvae need to be studied to know the compatibility with fresh sperm.

In many fish species, cryopreservation reduces post-thawed sperm motility without affecting embryogenesis and larval growth (Viveiros *et al.* 2012). Freezing and thawing damage DNA of sperm and reduces fertility (Watson 2000; Zilli, Schiavone, Zonno, Storelli & Vilella 2003; Cabrita, Robles, Rebordinos, Sarasquete & Herráez 2005). Prior to start of mass seed production for any fish species with cryopreserved sperm, growth and survival of larvae need to be tested. Whether cryopreserved sperm exerts any deleterious effect on growth and survival of resulting *P. sarana* larvae is not known. Therefore, the objective of the study was to compare the growth and survival of *P. sarana* larvae produced with cryopreserved and fresh sperm.

Materials and methods

Collection and rearing of larvae

The experiment was carried out during July to November, 2012 in backyard hatchery of the Faculty of Fisheries, Bangladesh Agricultural University. Two groups of larvae were produced by fertilizing olive barb eggs with freshly ejaculated sperm and frozen thawed sperm following the protocol of Nahiduzzaman *et al.* (2011). After absorption of yolk sac, the larvae were transferred to three rectangular glass aquaria (120 × 50 × 35 cm) for each group. The initial density (number/L water) of the larvae was twenty five. Continuous aeration was supplied in the rearing tank, and water exchange took place once a week during the rearing period. The temperature throughout the study period was 24–31°C. The larvae were fed with finely minced hard-boiled chicken egg-yolk six times a day for first 3 days. During 3 days post-hatching period, larvae were fed to apparent satiation four times a day with live food (microalgae, zooplankton and crushed tubificid worm). After 4 weeks, live food was partially replaced with the pelleted starter feed (Paragon Feeds Limited, Bangladesh) until the end of the study period.

The growth and survival of larvae was studied out up to 12 weeks rearing period. Ten larvae were randomly sampled once in a week with three replicates. The total length (mm) and wet weight (g) were taken for growth study according to

Memiş, Ercan, Çelikkale, Timur and Zarkua (2009). The lengths of the fish were measured using Vernier calipers (Mitutoyo 500-196-20, Foshan, China) to the nearest 0.05 mm, and body weights were measured to an accuracy of 1 mg using digital scale (UW1020H, Kyoto, Japan). The dead larvae were counted and removed from the tank every day, and the survival rate were calculated by counting all dead larvae over the rearing period.

Calculation of growth and survival

Daily instantaneous length specific growth rates were calculated by fitting the exponential model: $L_t = L_0 e^{gt}$, Where, L_t = total length (mm) at time t, L_0 = estimated length at hatching following the absorption of yolk sac, g = instantaneous growth coefficient, and t = estimated age (days after hatching). The weight specific instantaneous growth rates were calculated by fitting the exponential model: $W_t = W_0 e^{gt}$, Where W_t = wet weight (mg) at time t, W_0 = estimated weight at hatching following the absorption of yolk sac, g = instantaneous growth coefficient, and t = estimated age (days after hatching).

Length-weight relationship of larvae was calculated by fitting the exponential model: $W = aL^b$, where W is the body weight (mg) and L is the total length (mm). Parameters a is the intercept and b is the slope of equation based on natural logarithms: $\ln W = \ln a + b\ln L$. Survival of larvae were calculated by fitting the exponential model $N_t = N_0 e^{-Zt}$ (Powell, Cheshire, Laban, Colvocoresses, O'Donnell & Davidian 2004), Where N_0 = estimated number of larvae at hatching, N_t = number of larvae at time t, Z = daily instantaneous mortality rate and t = estimated age (days after hatching).

Data analysis

All the mentioned equations were log-transformed to calculate the slope and intercept by linear regression. The instantaneous growth coefficients of larvae produced with cryopreserved sperm were compared with fresh sperm using independent sample t-test. The survival was arcsine-transformed to induce homogeneity and analysed using independent sample t-test. The differences were considered statistically significant at $\alpha = 0.05$. All data analyses were performed with SPSS version 20.0 (IBM Corporation, Armonk, NY, USA).

Results

Growth of larvae

The gain in length (mm) and weight (mg) of larvae were considered to measure the growth, and independent sample t-test performed to compare between larvae of two groups. The test statistic showed that the differences for both the lengths, $t(718) = 0.241$; $P = 0.810$ and weights, $t(718) = 0.412$; $P = 0.680$ were insignificant between the larvae produced with cryopreserved sperm and fresh sperm. The exponential equation fitting the daily instantaneous length based growth coefficient of larvae produced by cryopreserved sperm was $L_t = 0.999e^{0.038t}$ and the larvae produced by fresh sperm was $L_t = 1.029e^{0.037t}$. The weight specific instantaneous growth coefficient of larvae produced by cryopreserved sperm was $W_t = 3.071e^{0.81t}$ and the larvae produced with fresh sperm was $W_t = 3.135e^{0.80t}$. The gain in length (mm) and weight (mg) of larvae produced with cryopreserved sperm and fresh sperm are shown in Figure 1.

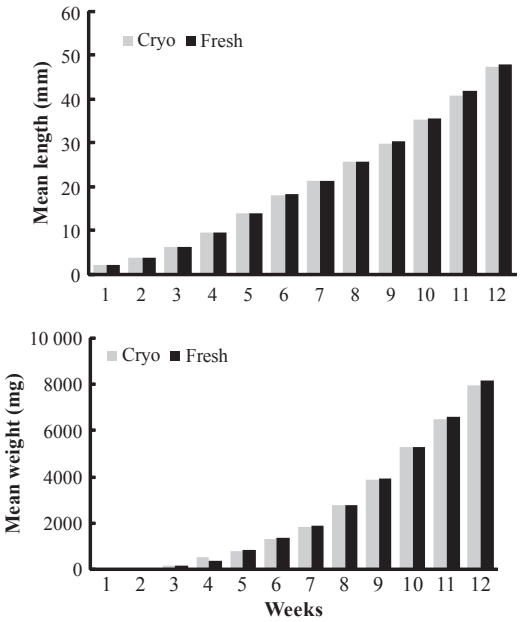

Figure 1 The mean length (mm) (top) and mean weight (mg) (bottom) of *Puntius sarana* (Hamilton 1822) larvae produced with cryopreserved sperm and fresh sperm.

Length-weight relations

The Pearson correlation (r) among length and weight of cryopreserved sperm was 0.947, and fresh sperm was 0.978 (Fig. 2). Coefficient of determination (R^2) of larvae produced with cryopreserved and fresh sperm was 0.897 and 0.956 respectively.

Survival of larvae

While compared the survival rate, t-test statistic showed that there was no difference, t (718) = −0.758, P = 0.448 between the larvae produced with cryopreserved sperm (M = 57.53, SD = 5.87) and fresh sperm (M = 57.20, SD = 5.78) (Fig. 3). The exponential equation fitting the instantaneous daily mortality of larvae produced by cryopreserved sperm was $N_t = 67.852^{-0.227t}$ and fresh sperm was $N_t = 67.803^{-0.233t}$.

Discussion

Fish sperm cryopreservation is proven successful to produce viable offspring in a number of studies, however, only a few of the studies reported the growth and survival of larvae. In our study, no significant differences were observed in the instantaneous growth coefficient in length and weight of *P. sarana* larvae produced with cryopreserved and fresh sperm. Similar growth rates were observed among the larvae produced with frozen thawed sperm and fresh sperm in butter catfish, *Ompok pabda* (1–6 weeks) (Sarder *et al.* 2013), tiete tetra, *Brycon insignis* (0–112 days) (Viveiros *et al.* 2012) and turbot, *Scophthalmus* (*Psetta*) *maxima* larvae

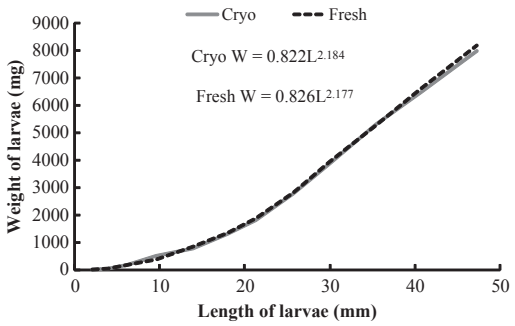

Figure 2 Length weight relationship of *Puntius sarana* (Hamilton 1822) larvae produced with cryopreserved sperm and fresh sperm.

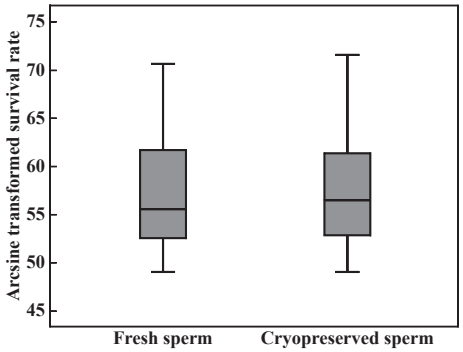

Figure 3 Survival rate of *Puntius sarana* (Hamilton 1822) larvae produced with cryopreserved sperm and fresh sperm.

(4–12 months) (Chereguini *et al.* 2002). In addition, tilapia, *Oreochromis hornorum* larvae produced with frozen thawed sperm showed similar growth compared with the fresh sperm up to 18 months trial (Chao *et al.* 1987). No significant differences observed ($P > 0.05$) in growth of channel catfish, *Ictalurus punctatus* (2–12 months) (Tiersch *et al.* 1994) and striped bass, *Morone saxatilis* (0–1.5 months) (Kerby *et al.* 1985) fry produced with cryopreserved sperm or untreated sperm. In contrast, significant differences in growth were observed in African catfish, *Clarias gariepinus* larvae produced with cryopreserved sperm, and the differences were attributed to the variability among the rearing tanks (Van der Walt *et al.* 1993). The results of different studies including the present one suggest that sperm cryopreservation has no deleterious effect on the growth of offspring.

There were strong relation between length and weight of larvae from both group (cryopreserved, r = 0.947; fresh r = 0.978). In addition, coefficient of determination (cryopreserved, R^2 = 0.897; fresh, R^2 = 0.956) suggests that the data fitted well in the regression line in both groups. The ideal b value indicates the rate of increase in length is proportional to the rate of increase in weight. The slope of the regression (b) of larvae produced with cryopreserved and fresh sperm was 2.184 and 2.177 respectively. Under natural condition, b value remains constant at 3.0 in adult fish (Martin 1949), and higher deviation is uncommon from this ideal value (Beverton & Holt 1957). The R^2 = 0.98 and b = 3.18 is found for *P. sarana* from Indian rivers (Sani, Gupta, Sarkar, Pandey, Dubey & Singh Lakra 2010). The devia-

tions from ideal b value is caused by a number of factors (Bagenal & Tesch 1978), and the lower b value of *P. sarana* in our study is assumed to be attributed to the rearing of larvae in tanks rather than in the natural condition.

Freezing and thawing lead to structural damage of sperm that cause lower fertilization and hatching of eggs (Billard 1983; Lahnsteiner, Weismann & Patzner 1992). Lower hatching rates were observed during eggs fertilized with frozen-thawed sperm compared with fresh sperm (Zilli *et al.* 2003; Nahiduzzaman *et al.* 2011). Cryopreserved sperm affected embryonic development and reduced survival of the eyed-eggs and larvae of trout (Pérez-Cerezales, Gutiérrez-Adán, Martínez-Páramo, Beirão & Herráez 2011). In this study, survival rate was similar between the two groups of larvae of *P. sarana*. No significant differences were also observed ($P > 0.05$) in the survival of fry produced with cryopreserved and fresh sperm in butter catfish (Sarder *et al.* 2013), turbot (Chereguini *et al.* 2002) and common carp (Moczarski 1977). Therefore, the study suggests that once the larvae are hatched out, there is no detrimental effect of cryopreservation on the survival of larvae.

Cryopreservation has deleterious effect on sperm quality, however, the development of larvae fertilized with thawed sperm was similar to that of fresh sperm. The present study suggests that cryopreservation technique can be applied to produce viable offspring of *P. sarana* without any difference in growth and survival of larvae. This would definitely contribute to the commercialization of cryopreserved fish sperm for hatchery operation and *ex-situ* conservation of the critically endangered olive barb.

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