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RESEARCH PAPER

Status and problems of monosex tilapia (*Oreochromis niloticus*) seed production using androgen hormone in Bangladesh

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Abstract

Nile tilapia (*Oreochromis niloticus*) has become a fast-growing aquaculture species in Bangladesh. This study investigated the status of existing practices for monosex tilapia production in aquaculture farms of Bangladesh. The data were collected using a structured questionnaire. The survey revealed that own hatchery origin brood fishes were used by 50%, 44% and 33% hatcheries in Mymensingh, Jessore and Cumilla, respectively. The weight of broods ranged from 200 to 350 g. The majority of hatcheries of Mymensingh (50%) and Jessore (83%) maintained male: female ratio of 1:2, but 50% hatcheries in Cumilla maintained 1:1 ratio for breeding. Average dose of hormone (17α-methyltestosterone) for producing monosex (all male) tilapia seed was 77.50 mg/kg feed, 57.50 mg/kg feed, 51.00 mg/kg feed in Mymensingh, Jessore and Cumilla, respectively. The average sex reversal rate was the highest (92.5%) in Mymensingh followed by 89% in Jessore and 83% in Cumilla. The highest average survival rate of fry was found in Mymensingh (77%) followed by Jessore (75%) and Cumilla (63%). Monitoring of monosex tilapia production process using 17α-methyltestosterone is needed to help enhance the quality and maximize the production of tilapia monosex seed.

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the fastest growing aquaculture species in the world due to its ability to grow and adapt in a wide range of environmetal parameters (Hasan et al., 2021; Hossain et al., 2022). According to FAO, 7 million mt tilapia has been produced globally in 2020, and its production will exceed 10 mt by 2030, contributing more than 10% to the world total fish production (Guenard, 2020). As tilapia is growing throughout the world like chicken, it is called aquatic chicken (Mair et al., 1995). The species was imported to Bangladesh in

1974, but its culture was not successful due to lack of knowledge about its biology and aquaculture techniques. In early 90s, tilapia culture become very popular throughout the country due to development of the GIFT strain (Maria et al., 2016; Rabby et al., 2015, 2019).

One of the World's largest river flows consisting of the Ganges, the Brahmaputra, and the Meghna rivers, offer one of the World's richest biodiversity harboring about 800 species of fishes (Das et al., 2017; Hossain & Rabby, 2020; Rahman et al., 2020). Among the large number of cultured species tilapia, especially monosex tilapia, has become an important culture species in Bangladesh in last 15 years due to its faster growth, higher response to supplementary feed and high demand as a tasty food fish. The contribution of aquaculture is gradually increasing to the national fish production in Bangladesh (Afrose et al., 2022; Alam et al., 2014). Bangladesh ranked 4th in tilapia production in the World and 3rd in Asia in 2018 (Guenard, 2020). Monosex (all male) tilapia are produced by almost all commercial tilapia hatcheries rather than producing mixed-sex tilapia fry (Belton et al., 2011). Farmers are interested in the culture of monosex tilapia due to its higher growth rate than the female and less chance of undesired reproduction in the culture system (Henson et al., 2018). Tilapia has been cultured in different culture systems following different technologies. The rapid intensification of tilapia farming increases the demand of large number of monosex tilapia fry, and it is crucial to fulfil the increased demand (Mehrim et al., 2019). Methyltestosterone hormone is used widely to produce monosex tilapia fry. Moreover, the information about the culture systems and hatchery operations in Bangladesh are very limited. Therefore, this study was carried out to investigate the existing practices of tilapia seed production in the hatcheries and to identify the problems associated with tilapia hatchery operations in Bangladesh.

Materials and Methods

Study area and duration

Tilapia hatcheries are available throughout Bangladesh. But most of the tilapia hatcheries are present in the Mymensingh, Jessore and Cumilla districts. Therefore, these three districts were selected for data collection on tilapia hatchery operations. The data were collected from Trishal, Tarakanda and Mymensingh Sadar Upzilla under Mymensingh district, Abhaynagar Upzilla under Jessore district and Nangalkot Upzilla under Cumilla district (Figure 1), from different hatcheries between September and October 2018 based on the previous year's production of the hatcheries.

Figure 1. Study areas shown with red circles on the map of Bangladesh.

Target group, preparation of questionnaire and data collection

The information related to the tilapia farming and seed production was collected from hatchery owners, hatchery technicians and tilapia grow-out farmers. Six hatcheries from each district i.e., a total of 18 hatcheries were selected randomly for collecting data from the three selected districts. A structured questionnaire was prepared carefully to collect data focusing on the objectives of the study. This interview questionnaire was further edited to make it easy for the target group of people so that they could answer all the questions. Before collecting information, the interviewees were introduced with the objectives of the survey. Then questions were asked with necessary explanations for the better understanding by the interviewees. Hatchery owners, hatchery technicians and farmers were interviewed as the primary source of data on tilapia seed production. Some important data and information were also collected from different websites, published articles, district fisheries offices and Bangladesh Fisheries Research Institute. Both qualitative and quantitative data were collected.

Data processing and analysis

After collecting data from hatchery some unnecessary data were removed to eliminate errors and to make it consistent and rational. The collected data were analyzed using Microsoft Excel.

Results and Discussion

Pond preparation for brood development

The survey noticed that 67% of hatcheries of Mymensingh completely dried their ponds and 33% of hatcheries followed partial drying and poisoning for removing unwanted fish from ponds, but in both Jessore and Cumilla 83% of hatcheries completely dried their ponds and 17% of hatcheries preferred partial drying and poisoning. In nursery management, pond preparation is essential. The production would not be satisfactory if the pond is not well-prepared. Various methods have been used to remove predatory fish including pond drying, poisoning, repeated netting etc. Pond drying is considered as the most effective one to remove predatory insects and fishes, and unwanted fishes. Fish poisoning can be harmful to the environment and hazardous to human health. In this survey, it was found that most of the hatcheries followed the drying method and some hatcheries followed partial drying and poisoning methods for removing unwanted fish from the pond (Figure 2).

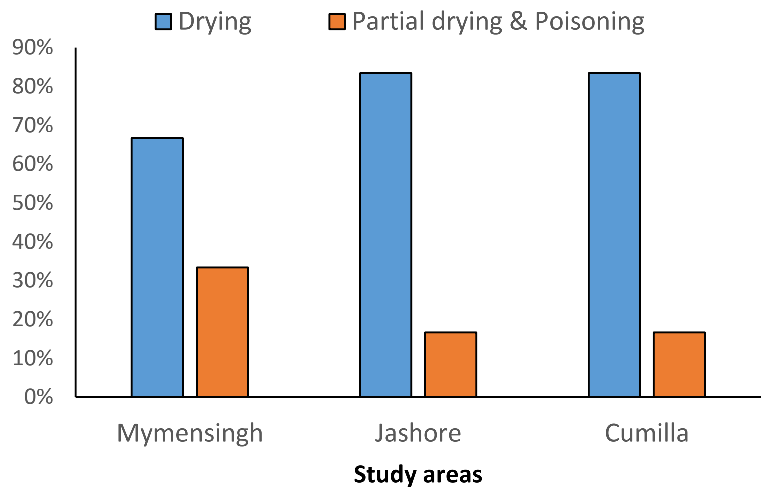

Figure 2. Processes followed during removal of predatory and unwanted fish.

Fifteen hatcheries out of 18 hatcheries used lime as disinfectant before introducing water into the pond. It was found that all the hatcheries of Mymensingh and Jessore district and two hatcheries of Cumilla district used lime as disinfectant. The average dose was 900 g/ 40.46 m^2 in Mymensingh which was higher than both Jessore and Cumilla. In Jessore and Cumilla the dose was 500 g/40.46 m^2 and 416 g/40.46 m^2, respectively (Figure 3). Uddin et al. (2017) reported that the dose of lime during pond preparation should be 1000-1500 g/40.46 m^2. The doses of lime varied from 600 to 1200 g/40.46 m^2 in India (Chakraborty & Banerjee, 2012). All the above results suggest that liming dose used in Mymensingh is satisfactory, but not satisfactory in Jessore and Cumilla. Liming is considered as important management activity for maintaining neutral pH, to prevent infectious diseases, to increase primary productivity, to remove turbidity and decompose organic substances from pond bottom.

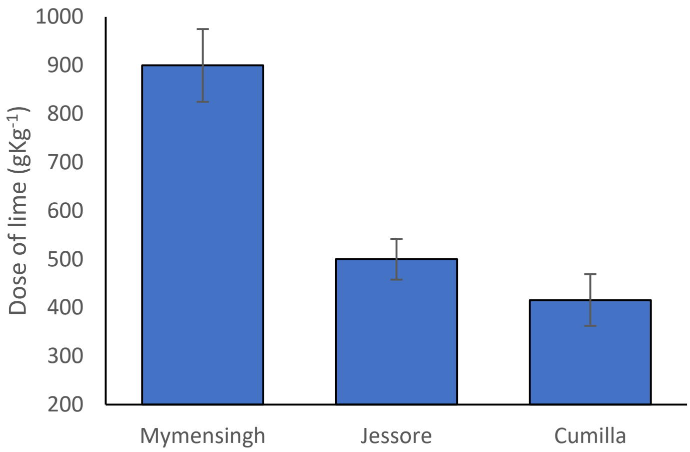

Figure 3. Average dose of lime used in different tilapia farms in the study area of Bangladesh.

Mainly underground water was used in the survey areas. Submersible pump, Diesel machine pump and electric motor pump was used for supplying water in the study areas. Electric motor pump was used in 50% of hatcheries of both Mymensingh and Jessore and 33% of hatcheries in Cumilla. Submersible pump was used by 17% of hatcheries in both Mymensingh and Jessore followed by 33% in Cumilla. Around 17% of hatcheries of both Mymensingh and Jessore used Diesel machine pump followed by 33% in Cumilla. All of three types of motors were used by 17% of hatcheries in both Mymensingh and Jessore (Figure 4).

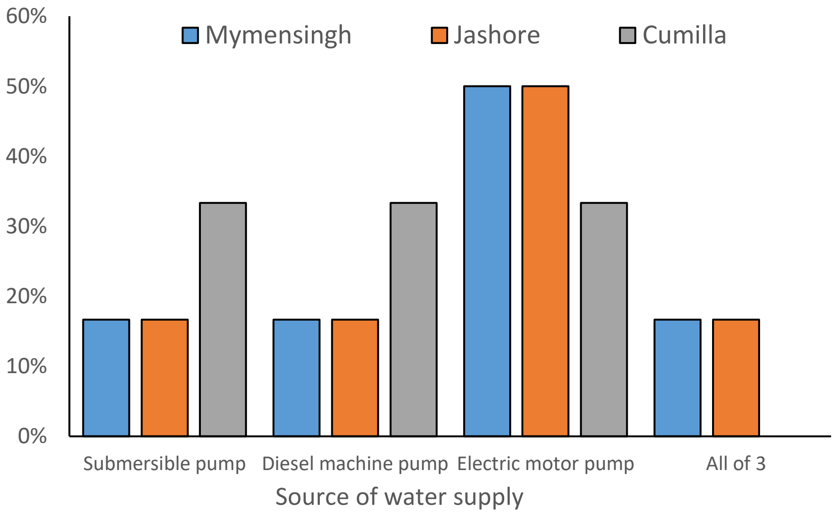

Figure 4. Source of water supply in ponds used for tilapia hatchery and farming.

Fertilizers were used during pond preparation as well as throughout the culture period depending on the primary productivity. The dose of fertilizers and application rate varied from hatchery to hatchery. Farmers used inorganic fertilizers like urea and triple super phosphate (TSP) to maintain water quality. In Mymensingh, average dosages of inorganic fertilizers such as urea and TSP were 162.5 g/40.46 m^2 and 175 g/40.46 m^2, respectively. Muriate of potash (MP) is also used by two hatcheries of Mymensingh, and its average dose was found 75 g/40.46 m^2. Cow dung was used by one hatchery in Mymensingh and one hatchery in Cumilla. The average dose of urea and TSP in Jessore was found 230 g/40.46 m^2 and 225 g/40.46 m^2, respectively. Average dose of urea was found 150 g/40.46 m^2 in Cumilla, but TSP was not used by any hatchery of Cumilla. The average dose of urea and TSP was found 190.9 g/40.46 m^2 and 200 g/40.46 m^2, respectively in the studied area. Average dose of various fertilizers was found higher in Jessore than Mymensingh and Cumilla. More fertilizers were used by the farmers in Jessore than that of Mymensingh region as the quality of soil and water in Jessore was poor. But the practice of fertilization in Cumilla region found in this survey was not satisfactory.

Source of Brood fish and tilapia strains used

Production of the hatchery depends on the availability of quality brood fish. In this survey study, it was found that majority of the hatcheries collected brood from their own hatchery and nearby hatcheries. In Mymensingh, 44% of the hatcheries used their own hatchery produced fish as brood fish and in Jessore and Cumilla the percentage was 33% and 50%, respectively. Some hatcheries also collected brood fish from nearby hatcheries to avoid inbreeding. In Mymensingh, 27.78% of hatcheries collected brood fish from nearby hatcheries followed by 33.3% in both Jessore and Cumilla. Some hatcheries also collected brood fish from BFRI (Bangladesh Fisheries Research Institute), WorldFish and other foreign countries. Lack of quality fish seeds is considered as one of the most important factors in fish production (Araf et al., 2021; Hossen et al., 2021; Khatun et al., 2017). Quality of brood fish plays a major role in the quality of seed production. Availability of good quality brood stock is very important to satisfy the future customers (Ali et al., 2021; Rahman et al., 2021). Currently, many growers are aware of the strain of tilapia fingerlings they want to grow (Agustin and Sur, 2004). In this survey, it was found that 50% of hatcheries of Cumilla used their own hatchery produced fish as brood fish followed by 44% in Mymensingh. Brood fish should be collected from different sources such as nearby hatcheries, BFRI and other foreign countries to avoid inbreeding which leads to the production substandard fish seeds and thus, restricts the rapid expansion of tilapia culture

All tilapia hatcheries in Cumilla used GIFT (Genetically improve firmed tilapia) strain of Nile tilapia. Some other strains of Nile tilapia were also used by some hatcheries in Mymensingh and Jessore. In Jessore, 17% of hatcheries used Nile tilapia (Genomar strain) collected from Philippines. Chitralada strain of Nile tilapia collected from Thailand was used by 17% hatcheries of Mymensingh. The hatcheries in Cumilla and 83% of hatcheries in both Mymensingh and Jessore used GIFT strain of tilapia which showed faster growth than other commonly used strain of tilapia, and 17% of hatcheries in Mymensingh used Chitralada strain and 17% of hatcheries in Jessore used Genomar strain. The GIFT strain was reported faster growth (60%) and better survival (50%) at harvest than the most commonly used strains in the Philippines and Israel (Barman & Little, 2011; Lind et al., 2015). Due to good reputation most hatcheries in Bangladesh used GIFT strain as brood stock and to produce mono-sex tilapia fry. GIFT strain was used as the only source of the brood stock by about 75% hatcheries in Bangladesh followed by GIFT strain along with Chitralada or Genomar (15%) and Chitralada or GenoMar (9%) (Mekkawy et al. 2017). GIFT was reported as 58% superior in terms of growth than locally available strains of Nile tilapia (Kamaruzzaman et al., 2009; Kohinoor et al., 2003)

Disinfection of fry before releasing in pond

While quality seeds and effective management measures are keys to maximize the harvest and profit of fish farming, it is equally important to ensure that only healthy fry are stocked. Before releasing fry into the pond, it is good to disinfect them to remove all harmful pathogens. In Mymensingh, all the hatcheries used various disinfectant to wash their fry before release in the pond as precaution measures against disease in their ponds. Salt and potassium permanganate are used by different hatcheries to disinfect their fry before releasing in the pond. But no hatchery in Cumilla followed this process and only 17% of hatcheries of Jessore disinfect their fry before stocking in their pond.

Fry stocking, male and female ration of brood

The fry of tilapia was generally stocked during April to September. The fries hatched during that time have been sold completely. Size of fries varied from 0.5-1 g. Average stocking density in Mymensingh was found as 200 fry/40.46 m^2 and in Cumilla 308 fry/40.46 m^2 but in Jessore it was found as 850 fry/40.46 m^2 which was higher than both Mymensingh and Cumilla (Table 1). The average stocking density was found as 453 fry/decimal in the studied area.

Table 1. Stocking density of tilapia fry in the grow out ponds of the study area.

Location (N=6 for each)	Average stocking density (Fry/ 40.46 m^2)	Minimum	Maximum
Mymensingh	200 ± 31.622	150	250
Jashore	850 ± 467.974	300	1500
Cumilla	308.33± 37.638	250	350
Total	452.77 ± 388.235	150	1500

Different hatcheries used different sex ratios for breeding purposes. The majority of the hatcheries in Mymensingh used 1:2 (male: female) followed by 1:3 (male: female) sex ratio for breeding. It was found that 50% of hatcheries follow 1:2 (male: female) sex ratio and 33% maintain 1:3 (male: female) sex ratio in Mymensingh. But in Jessore, 83% of hatcheries maintained a sex ratio of 1:2 (male: female) and the others (17%) used 1:3 (male: female). Again, in Cumilla, 1:1 (male: female) sex ratio was used in 50% hatcheries followed by 1:2 (male: female) in 33% hatcheries and 1:3 (male: female) in 17% hatcheries. The ratio of male to female brood fish is an important factor for the breeding success of tilapia. Sex ratios influence the number of fingerling production per female fish during breeding (Henson et al., 2018). In terms of maximizing genetic variability, a ratio of 1:1 is desirable but in practice, sub-ordinate fish may be excluded from spawning. Fewer than five females: one male appear to be within the optimal ranges in most situations. Especially when synchronized spawning is required, less than three females: one male is optimal (Naylor et al., 2021). Kohinoor et al. (2003) found that the male to female sex ratio of 1:4 produced higher breeding frequencies and lower intervals compared to 1:3 and 1:2, but Khalfalla et al. (2008) found that male to female sex ratio of 1:2 is the best for tilapia seed production. Alcántar-Vázquez et al., (2015) found the sex ratio of 1 male to 3 females is more economical for fry production. Most of the farmers in Kenya maintained male to female ratio of 1:1, but the sex ratio of the fish in the hatcheries resulted in low fingerling production per female fish during breeding, whereas the recommended male and female ratio is at least 1:2 (Ng & Wang, 2011).

Average weight of brood fish was found to be 200-250 g in 50% of hatcheries of Mymensingh and 33% of hatcheries of both

Jessore and Cumilla districts. Fifty percent of hatcheries in Jessore used brood fish weighing 250-300 g followed by 13% in Mymensingh and 33% in Cumilla for breeding. Brood fish weighing 300-350 g was also found to be used by 13% of hatcheries of three districts (Figure 5). The weight of brood fish ranged between 150-350 g (Figure 5).

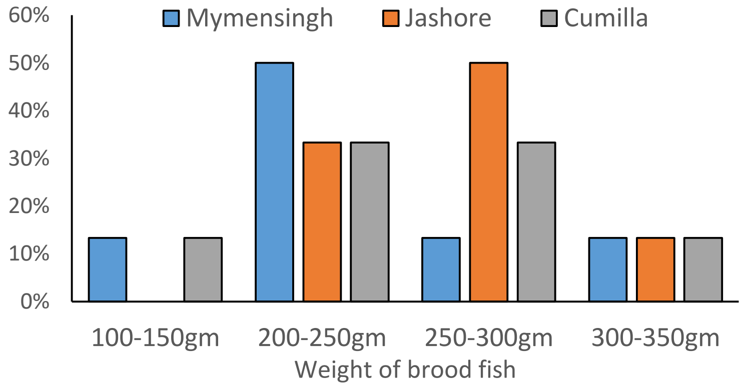

Figure 5. Weight of brood fish used in breeding of tilapia.

Different aspects of breeding in hatcheries

Breeding of tilapia in hatcheries is mainly done in hapa, tank and pond. This study indicates that 33% of hatcheries used pond, 50% of hatcheries used hapa and 16% used both pond and hapa in Mymensingh. All the hatcheries of Cumilla used hapa for breeding. Around 83% of hatcheries in Jessore used pond for breeding. All the hatcheries of Cumilla used hapa for spawning and 83% hatcheries in Jessore used pond for spawning. In a survey, Mekkawy et al., (2017) reported that in Bangladesh the majority of the hatcheries (75%) used only hapas for spawning and the remaining 25% used both hapas and ponds for spawning. In hatcheries, eggs may be infected by fungus and other pathogens. The primary reasons for disinfecting eggs are to remove fungus and other disease-causing agents. In this study, it was found that potash, salt, and formalin are mainly used by some hatcheries for egg disinfection but most of the hatcheries did not practice egg disinfection process. Fifty percent of hatcheries in both Mymensingh and Jessore followed this process but in Cumilla no hatchery followed it. Out of 18 hatcheries, only 6 hatcheries disinfected eggs before starting incubation. Incubation was mainly done in jars and trays; 72% of the hatcheries used tray and 28% used jar for incubation. Syphoning is required for removing eggshell from the jar and tray during incubation. Syphoning interval was found to be 2 hours in 33% of hatcheries, 1 hour in 28% of hatcheries and continuous syphoning was found in 16.7% of hatcheries.

Tilapia fries are fed with hormone treated feed to produce monosex tilapia. Duration of the treatment varies depending on the environment and the experience of the producer. In this survey, it was found that 50% of hatcheries in Mymensingh, Jessore and Cumilla did hormone treatment for 28 days, and 16%, 50% and 33% hatcheries did hormone treatment for 21 days in Mymensingh, Jessore and Cumilla, respectively. Thirty-three percent of hatcheries in Mymensingh did hormone treatment for one month. Surprisingly, 16.33% of hatcheries in Cumilla did hormone treatment for ten days only. About 95% of the fry can be effectively sex reversed into phenotypic male in 20 days. Sex reversing success is more consistent when the treatment duration is 25 to 28 days. Fast growing features of tilapia, *O.*

niloticus, can ensure maximum production per unit time (Beardmore et al., 2001). All the hatcheries of the studied area used 17alpha-methyltestosterone for sex reversal. Mair et al. (1995) stated that giving a powdered fish feed containing 17alpha-methyltestosterone to the first feeding (and still sexually undifferentiated) tilapia fry is the most common sex reversal treatment. Use of 17 alpha-methyltestosterone has been reported to be the most preferred sex reversal method in commercial uses (Celik et al. 2011). Megbowon & Mojekwu (2014) found that a variety of hormones are used for sex reversal of *O. niloticus* but 17 alpha-methyltestosterone is the most commonly used androgen.

Dose of hormone, feeding interval and survival rate of fry

Monosex (all male) tilapia is preferred due to their faster growth than females. The most efficient and least expensive method is sex reversal with the use of 17α-methyl testosterone (Megbowon and Mojekwu, 2013). Different hatcheries used different doses of this hormone. The dose of hormone ranged from 50 – 140 mg/kg feed in Mymensingh, 50 – 60 mg/kg feed in Jessore and 40 - 80 mg/kg feed in Cumilla. To prepare hormone treated feed hormone is diluted with alcohol for homogeneous mixing with the feed (Ferdous and Ali, 2011). In the survey area, all the hatcheries used ethyl alcohol, but the dose was different in different hatcheries.

Table 2. Spawn density of rearing hapa (a fixed net enclosed space).

Location	**Spawn density/ 0.092 m^2**
Mymensingh (N=6)	175 ± 45.93474
Jashore (N=6)	233.333± 32.04164
Cumilla (N=6)	145± 65.65059
Total (N=18)	184.444± 60.11971

*hapas are small confined rearing areas made by using bamboo and nets.

Feeding interval varied from hatchery to hatchery. In Mymensingh, mean feeding interval was found 3.7 hr followed by 3.5 hr in Jessore and 3.4 hr in Cumilla. After incubation, newly hatched spawn is released into the hapa for hormone treatment. Mean spawn density was found 1,884 spawn/m^2 in Mymensingh followed by 2,508 spawn/m^2 in Jessore and 1,561 spawn/m^2 in Cumilla (Table 2). The average weight of fry after hormone treatment was found 0.183 g in Mymensingh, 0.367 g in Jessore and 0.123 g in Cumilla. The mean weight in the studied area was found 0.2244 g and the minimum and maximum weight of fry was reported 0.10 g and 0.70 g, respectively. The survival rate of fry after hormone treatment was found 77% in Mymensingh which was higher than both Jessore and Cumilla. In Jessore, survival rate was 75% which was almost the same as Mymensingh but in Cumilla the rate was only 62.8% which was poorer than the other two districts (Figure 6).

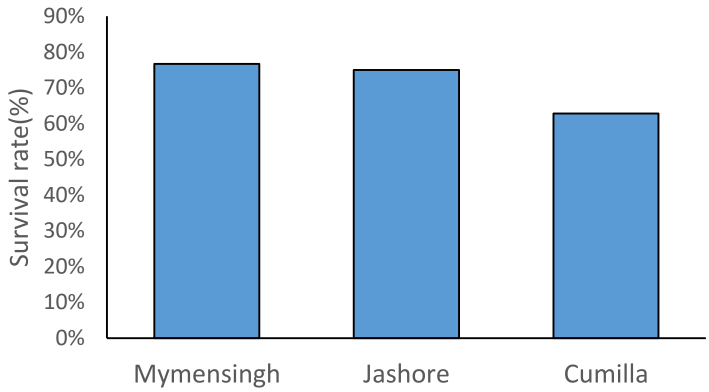

Figure 6. Survival rate of fry after 17α-methyltestosterone hormone treatment.

3.7 Reversal rate to monosex after hormone treatment

Androgen hormone (17-α methyl testosterone) is considered as the most effective and economically feasible method for producing all male tilapia populations (Mekkawy et al., 2017). Sex reversal rate after hormone treatment was different in the studied areas (Figure 7). In Mymensingh, it was found that 92.5% of fish became male after hormone treatment followed by 89.2% in Jessore and 83% in Cumilla. A key factor in the success of sex reversal treatments is the amount of hormone that is actually ingested by each individual fish during its labile period of sexual differentiation. In this survey, it was found that different hatcheries used different doses of hormone. Average dose of hormone was found 77.50 mg/kg feed in Mymensingh followed by 57.50 mg/kg feed in Jessore and 51 mg/kg feed in Cumilla. Fifty percent of hatcheries in Mymensingh, Jessore and Cumilla did hormone treatment for 28 days and 16.3%, 50%, 33.3% hatcheries in Mymensingh, Jessore and Cumilla, respectively did hormone treatment for 21 days. Thirty-three percent of hatcheries in Mymensingh did hormone treatment for 30 days.

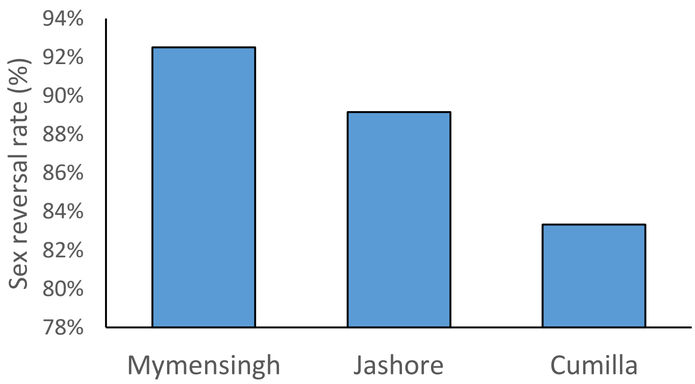

Figure 7. Sex reverse rate of 17α-methyltestosterone hormone treated tilapia fry.

The effectiveness of sex reversal treatment depends on the species, age at which hormone is administered, type and dosage of hormone, time and duration of treatment (Al-Hakim et al., 2013; Straus et al., 2013). El-Greisy and El-Gamal (2012) found in a study that the maximum sex ratios of male (95%) were recorded in the group treated with 60 mg of 17 alpha-methyltestosterone/kg of feed compared to the control group, 40 and 80 mg of 17 alpha-methyltestosterone/kg of feed treated groups. Vinarukwong et al. (2018) stated that tilapia aquaculture in Thailand commonly applies methyltestosterone at the dose of 60 mg/kg diet on the feeding of fish fry for 21 days to achieve male-monosex crop. This is similar to the dose used in

Mymensingh. Ferdous and Ali (2011) suggested that the optimum dose of methyltestosterone hormone was 60 mg /kg with a feeding period of 28 days after hatching, which is similar to the mean dose used in Jessore region. Dosage of 17α-methyl testosterone (MT) used to produce all male tilapia vary widely. Singh et al. (2018) stated that the dosage rates vary from 10-70 mg hormone/kg of diet for Nile tilapia, *O. niloticus* and the duration of administration of 17 alpha-methyltestosterone (MT) for masculinization of Nile tilapia varies from 14 to 60 days.

4. Conclusion

Bangladesh is one of the leading producers of Nile tilapia in the world. However, there are still some problems in the technical and management aspects. In the study area, i.e., Mymensingh, Jessore and Cumilla, many differences were found in brood stock management and hormone treatment processes between and within each district. More importantly, some of the techniques practiced have problems which have resulted in lower survival and percentage of males during sex-reversal. Own hatchery produced fish is used as brood fish in most of the hatcheries, and this is one of the major problems because repeated use of the same parent population will lead to poor brood stock management. Maintaining quality brood stock and proper hormone treatment with recommended standards would increase quality of seed, which is the pre-requisite for the expansion of tilapia industry in Bangladesh. Therefore, the necessary training on monosex tilapia hatchery management systems, nursery technology, brood stock replacement protocols and simple breeding techniques could be organized for the stakeholders related to tilapia farming and business, including hatchery and nursery operators, tilapia grow-out farmers and entrepreneurs. The training should include hands-on practice and field visits to a model hatchery which follows all protocols and maintains the quality of fry. A field trial should also be organized with the recommended doses and other doses of 17α-methyltestosterone practiced in Bangladesh for demonstration purpose with a view to promoting quality all-male (monosex) tilapia seeds for sustainable development and expansion of tilapia industry in Bangladesh.

Ethical approval

This research had been approved by animals' ethics committee of Sylhet Agricultural University Research System (SAURES), Bangladesh.

Data availability statement

The authors declare that data are available from authors upon a reasonable request.

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