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Alternative artificial incubation system for intensive fry production of Nile tilapia (*Oreochromis niloticus*)

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Abstract
Jar incubation system is a well established artificial incubation system for intensive fry production of Nile tilapia. However, this system needs special hatchery structure and huge amount of water for circulation of eggs. The present study aimed to explore a simple, economic and water efficient alternative incubation system appropriate for small-scale hatchery operators. Two incubation systems, i.e. atkin incubation system and aquarium incubation system were compared with jar incubation systems in terms of water use, hatching rate and subsequent survival of larvae. Results showed that the amount of water used was significantly higher in atkin (127.0±3.1 m^3) and jar (36.8±4.9 m^3) incubation systems compared to aquarium (0.05±0.0m^3) incubation system. The hatching percentage was significantly higher in jar incubation system (95.5±0.6%) compared to aquarium (65.2±7.7%) and atkin (57.8±2.2%) incubation systems. Hatching tended to occur slightly earlier in the jar incubator than other systems. After 7 days of rearing, the mean larval survival rate was highest in jar incubation (96.9±0.5%), intermediate in aquarium incubation (90.9±3.4%) and lowest in atkin incubation (81.0±3.1) system (P<0.05). The dissolved oxygen was significantly higher in aquarium (6.1±0.0 mg/L) than jar (3.0±0.0 mg/L) and atkin (3.0±0.1 mg/L) incubation systems. Further experiments indicated that about 5000 eggs can be successfully hatched with a hatching rate of 95% and reared to swim-up fry in in 50 L size glass aquarium with water exchange twice daily. This system is best suited for incubation of late stage eggs and rearing of newly hatched larvae up to free swimming stage. The results indicate that aquarium incubation can be used as an alternative of jar incubation system for Nile tilapia eggs, especially in water scarce areas.

Keywords: Nile tilapia, jar incubation, aquarium incubation, atkin incubation, hatching rate

Introduction
Nile tilapia (*Oreochromis niloticus*) is the most commonly cultured freshwater fish in the world with over 3.4 million tonnes production [1]. An adequate supply of high quality seed is one of the major challenges facing the tilapia aquaculture industry [2]. Tilapia seeds can be produced in hapas, concrete tanks, fiberglass tanks and earthen ponds [3]. Natural breeding is considered the main advantage of tilapia over other species, but as the farming become more commercial, demand for large and uniform fish increased [4]. This created a large demand for good quality monosex fry. It is almost impossible for the traditional hatcheries to produce and supply a large quantity of fry using traditional methods. Artificial incubation of eggs will increase the fry production and economic efficiency of a commercial hatchery operation [5]. Moreover, fry produced from artificial incubation system are of known age and therefore suitable for application of hormone treatment for the production of monosex male fingerlings.

In artificial fry production system, the fertilized eggs are collected from the female's mouth and transferred to a hatchery unit for artificial incubation. A wide variety of devices are used for incubating tilapia eggs in different countries [6, 7, 8, 4]. The recent and most commercial method is the incubation of eggs in plastic jars of about 4 litre size [4]. Jar incubation method is cheap, easily available and more transparent so that the hatchery operators can see the egg movement easily and they are also lighter and easier to handle. This method is commercially used by most hatchery operators in China, Thailand, Bangladesh and many other countries. However, this method consumes large volume of water and running such system is not possible in places where water scarcity occurs. Thus, there is a need to find the solutions which is easy, cheap, water efficient and more practical for small-scale farmers.

Among the known and used techniques of egg incubation, use of atkin (specially designed unit of fiber glass for egg incubation) is well established for species having heavy eggs that settle

down easily [9, 10]. In atkin, a number of screened, flat bottomed trays can be placed inside each compartment in a horizontal series. The water flow in each compartment is adjusted so that each tray receives water through eggs from below. However, this system also needs a continuous supply of water. But compared to incubation jar, with respect to eggs loaded, supply of water is relatively lower. Use of glass aquariam with well aeration system might be another alternative method for incubation of tilapia eggs. In the present study, we tested the possibility of using atkin and glass aquarium for incubation of Nile tilapia eggs. The objective of this study was to compare the amount of water needed, hatching rate and larval survival rate of Nile tilapia in various incubation systems.

Materials and Methods

This experiment was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal during April to June 2017. Rearing of Nile tilapia broods was done in hapa placed in a cemented pond and were allowed to breed naturally in hapas. Fertilized eggs were collected from the mouth of five females, randomly mixed, counted and placed in their respective incubation systems. The water source used in the incubation systems was from underground tap water. Two sets of experiment were conducted.

In the first experiment, three incubation treatments were tested: (1) Jar incubation, (2) Atkin incubation and (3) Aquarium incubation. All treatments were replicated thrice. 200 eggs of eyed-stage (yellow brown color) were stocked in each incubation unit. Brief description of each incubation unit is as follows:

Jar incubation system

In this system, 1.5 L plastic jars with 20 cm x 20 cm perforated aluminium incubation tray were used. The eggs were held in upwelling condition until hatch and swim up. Continuous flow of water was maintained using a pipeline of CPVC fittings in a way that eggs remains in continue moving condition. Water flow rate and total water used were measured.

Atkin incubation system

In this system, fertilized eggs were incubated in atkin's incubator trays by allowing one layer of eggs on single mess screen trays in flow through system. The size of the atkin was 2.0 m x 0.4 m and the size of tray was 30 cm x 30 cm. Water flow in the incubation trays was maintained at the discharge rate of 7-9 L/min. Dead eggs were removed using droppers to protect from the fungal infection.Water flow rate and total water used were measured.

Aquarium incubation system

In this system, fertilized eggs were incubated in glass aquaria (60 cm x 30 cm x 45 cm) containing 50 L water volume. The aquarium was well aerated using two airstones. Water replacement was not done.

The water quality in the different incubation systems such as dissolved oxygen (Lutran oxygen Meter DO-5510), pH (Lutran pH Meter YK-21 pH) and temperature (Lutran oxygen Meter DO-5510) were recorded twice daily during the experiment.

The fertilized eggs that were incubated for hatching were observed regularly to remove spoiled eggs so that they don't contaminate the other viable eggs. After hatching has been completed in each group of eggs, all hatched larvae were counted and hatching percentages were determined from the initial number of ova used. Survival percentages of 7-days-old larvae were determined from the initial number of hatched larvae. Following formulae were used to calculate hatching rate and larval survival:

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish survived}}{\text{Number of fish hatched}} \times 100$$

A second experiment was conducted to improve the hatching and larval survival rate in the aquarium incubation system, with different levels of water exchange. Three levels of water exchange were tested: (1) No water exchange, (2) Water exchange - one time a day, and (3) Water exchange - two time a day. About 90% water was exchanged in each time. The stocking density was 1000 eggs per aquarium. The aquarium was well aerated using two air stones. Water temperature, dissolved oxygen and pH was monitored twice daily during the experiment.

Based on the results of second experiment, a complementary experiment was carried out to assess the usefulness of aquarium for incubating a large number of eggs. In this experiment, 5000 eggs of late-stage (dark brown color with hair like tail) were placed in 50 L aquarium with well aeration. The water was exchanged twice daily. Other procedures were equivalent to those described for the first and second experiments.

The hatching percentages and the survival rates of 7-days-old larvae obtained as a function of the method used for egg incubation were compared using one-way analysis of variance (ANOVA) in SPSS (version 16.0) statistical software package (SPSS Inc., Chicago). When necessary, arcsine transformation of data was carried out in order to stabilize the residual variance. Differences were considered significant at the 95% confidence level ($p<0.05$). All means were given with ± standard error (S.E.).

Results

Experiment-I

Hatching rate

The hatching rates obtained as a function of the different systems of egg incubation tested are presented in Table 1. Mean hatching rate was 95.5±0.6, 57.8±2.2 and 65.2±7.7% in Jar incubation, Atkin incubation and Aquarium incubation systems, respectively. The hatching percentage was significantly higher in jar incubation system compared to atkin and aquarium incubation systems ($P<0.05$). There was no significant difference in hatching percentage between atkin and aquarium incubation systems ($p>0.05$).

Table 1: Mean (±SE) incubation period, hatching rate and larval survival rate of Nile tilapia in different incubation systems. Mean values with different superscript in the same row are significantly different (P<0.05).

Parameters	Jar incubation	Atkin incubation	Aquarium incubation
Number of eggs incubated	200.0±0.0 [a]	200.0±0.0 [a]	200.0±0.0 [a]
Incubation period (hour)	62.0±1.0 [a]	63.0±1.0 [a]	63.0±1.0 [a]
Hatching rate (%)	95.5±0.6 [a]	57.8±2.2 [b]	65.2±7.7 [b]
Larval survival rate at 7-days	96.9±0.5 [a]	81.0±3.1[b]	92.9±3.4[a]
Water requirement (m^3)	36.8±4.9[b]	127.0±3.1[a]	0.05±0.0[c]

Hatching time

Hatching of eggs started from 60 hours of loading and yolk sac was completely absorbed in about 140 hours after hatching. The hatching time of eggs in different incubation systems are presented in Table 1. Mean incubation period was 62.0±1.0, 63.0±1.0 and 63.0±1.0 hours in Jar incubation, Atkin incubation and Aquarium incubation systems, respectively. The only detected difference was an hour earlier hatching of eggs incubated in jar incubator compared to other incubation systems. However, there was no significant difference in incubation period among different incubation systems (p>0.05).

Larval survival

After 7 days of rearing, the mean survival rate of Nile tilapia larvae issued from the different incubation systems tested in the first experiment varied between 81.0 and 96.9% (Table 1). Mean larval survival rate was highest in jar incubation (96.9±0.5%), intermediate in aquarium incubation (90.9±3.4%) and lowest in atkin incubation (81.0±3.1) systems (P<0.05).

Water quality

Daily mean and range of water temperature, dissolved oxygen and pH in each treatment during the experimental period are given in Table 2. The temperature, dissolved oxygen and pH varied between 24.6 to 26.5°C, 3.0 to 6.1 mg/L and 7.2 to 7.5, respectively in different incubation systems.

The temperature and dissolved oxygen were significantly higher in aquarium incubation system than other systems (P<0.05). However, there was no significant difference in temperature and dissolved oxygen between jar and atkin's incubation systems (p>0.05).

Table 2: Mean (±SE) and range of water quality parameters in different incubation systems. Mean values with different superscript in the same row are significantly different.

Parameters	Jar incubation	Atkin incubation	Aquarium incubation
Water temperature (°C)	24.8±0.3 [b] (23.5-25.9)	25.0±0.0 [b] (24.0-26.3)	26.5±0.2 [a] (24.0-27.4)
Dissilved oxygen (mg/L)	3.0±0.0 [b] (2.8-3.5)	3.0±0.1 [b] (2.7-3.4)	6.1±0.0 [a] (5.5-6.4)
pH	7.2 (7.1-7.4)	7.5 (7.3-7.7)	7.5 (7.4-7.7)

Experiment-II

In the second experiment, the effect of water exchange on hatching and larval survival rate was tested in aquarium incubation system. Results showed that the mean hatching rate was highest in 2 times/day water exchange system (95.1±0.6%), intermediate in 1 times/day water exchange system (93.1±0.6%) and lowest in no water exchange system (70.2±0.4%) (P<0.05) (Table 3). Similarly, mean larval survival rate at 7-days was highest in 2 times/day water exchange system (96.3±3.4%), intermediate in 1 times/day water exchange system (87.6±2.1%) and lowest in no water exchange system (78.8±3.1%) (P<0.05) (Table 3).

Table 3: Mean (±SE) number of eggs incubated and larval survival rate of Nile tilapia in aquarium incubation system. Mean values with different superscript in the same row are significantly different (P<0.05).

Parameters	No water exchange	Water exchange (1 time/day)	Water exchange (2 time/day)
Number of eggs incubated	1000.0±0.0[a]	1000.0±0.0 [a]	1000.0±0.0 [a]
Hatching rate (%)	70.2±0.4[c]	93.1±0.6 [b]	95.1±0.6[a]
Larval survival rate at 7-days	78.8±3.1 [c]	87.6±2.1 [b]	96.3±3.4 [a]

Daily mean and range of water temperature, dissolved oxygen and pH in each treatment during the experimental period are given in Table 4. There were no significant difference in water temperature among different water exchange systems (p>0.05). However, the dissolved oxygen was significantly higher in 2 times/day water exchange system than other systems (P<0.05) (Table 4).

Table 4: Mean (±SE) and range of daily water quality parameters in different incubation systems. Mean values with different superscript in the same row are significantly different.

Parameters	No water exchange	Water exchange (1 time/day)	Water exchange (2 time/day)
Water temperature (°C)	27.3±0.2[a] (26.5-28.0)	26.9±0.2[a] (25.5-27.5)	26.8±0.3 [a] (25.5-27.4)
Dissolved oxygen (mg/L)	5.6±0.1 [b] (5.2-5.8)	5.8±0.1 [b] (5.5-6.0)	6.3±0.0 [a] (5.8-6.5)
pH	7.3 (7.2-7.5)	7.4 (7.1-7.6)	7.5 (7.2-7.7)

In the complementary experiment, in which larger quantities of last-stage eggs and newly hatched larvae (1-day) were used in high density, the larval survival rate at 7-days was significantly higher in 2 times/day water exchange system (92.4±2.2%) compared to no water exchange system (54.4±3.4%) (P<0.05) (Table 5).

Table 5: Mean (±SE) number of eggs incubated and larval survival rate of Nile tilapia in aquarium incubation system. Mean values with different superscript in the same row are significantly different (P<0.05).

Parameters	No water exchange	Water exchange (2 times/day)
Number of eggs incubated	5000.0±0.0[a]	5000.0±0.0[a]
Larval survival rate at 7-days	54.4±3.4[b]	92.4±2.2[a]

Discussion

Economic seed production is one of the major factors which directly affect on sustainability and productivity of any aquaculture system. The practical and economic benefits of artificial incubation systems depends upon efficient water use, high rates of survival during incubation and this should be optimized and standardized prior to establishment of commercial systems [11]. Jar incubation system is a well-established and widely used artificial incubation system for intensive fry production of Nile tilapia [4]. Although thejar hatching system is cheap, easy to operate and more transparent, this system needs special hatchery structure and huge amount of water for circulationof eggs. The results of the present study showed that about 36.8 m^3 water is needed for a single jar of incubation capacity of 10000 eggs. Running such system is not economic in the places of water scarcity.

In the present experiment, we tested two incubation systems, i.e. atkin incubation system and aquarium incubation system as an alternative for the jar incubation system. Atkin incubation system is commonly used for incubation of rainbow trout (*Oncorhynchus mykiss*) and sahar (*Tor putitora*) eggs in Nepal [9, 10]. This system is best suited for sinking and non-sticky type of eggs. Our result showed that atkin incubation requires significantly huge amount of water (127.0±3.1 m^3) compared to jar and aquarium incubations. The hatching and larval survival rate in atkin was also very poor. In atkin trays, eggs and yolk-sac larvae clogged followed by fungal attacks (*Saprolegnia* sp.). This result suggests that there is no additional advantage of using atkin incubation over jar incubation system for incubating Nile tilapia eggs.

The aquarium incubation system needed only 0.05 to 0.9 m^3 of water depending on water exchange or non-exchange system. About 65-70% eggs were hatched and most of them survived to fry stage in aquarium incubation without exchange of water. The poor hatching rate might be associated with accumulation of high CO_2 and NH_3. It has been suggested that it is important to remove harmful metabolites such as CO_2 and NH_3 excreted by the developing eggs during incubation [12]. Moreover, mechanical trauma of eggs in artificial incubators associated with poor system design has been identified as the major problem causing poor hatchability [13]. Although the hatching rate is significantly lower in aquarium incubation than the jar incubation, there is no significant difference in larval survival rate between these two systems. Further, if we compare the water use and economics of these two systems, the cost for per unit fry production is far below in aquarium incubation compared to jar incubation system. Our second experiment showed that the hatching rate can be improved to 95% with exchange of water twice daily.

The dissolved oxygen was significantly higher in aquarium incubation system than jar and atkin systems (P<0.05). The higher concentration of dissolved oxygen was due to well aeration system as two air stones were provided in each aquarium. High dissolved oxygen is very important to achieve high hatchability rates and survival of tilapia fry [14]. In aquarium, the dissolved oxygen concentration was more than double than those of jar and atkin. Thus, the dissolved oxygen is not a cause of poor hatching in aquarium incubation system.

Although the water temperature in jar incubation system is lowest than atkin and aquarium incubation systems, the incubation period was slightly shorter. This earlier hatching in jars was assumed to be a consequence of the slight mechanical agitation of eggs. By contrast, no difference was found between the atkin and the aquarium incubation system. This result indicates that mechanical agitation induce early hatching of eggs. It has been reported that large variations in the embryos development time of *Oreochromis niloticus* and *O. mossambicus* eggs as a function of the water exchange and shape of the incubator used [13]. However, there is a need for more studies on this aspect because it was ascertained in other species that small temperature changes can cause considerable variation in the embryonic development of fish [15].

The present experiment showed that the aquarium incubation system is a simple, cheap and water efficient. Another advantage of this system is that temperature can be controlled with aquarium heaters. Using this system, single batches of at least 5000 eggs can be successfully hatched and reared to swim-up fry in 50 L size glass aquarium with minimal losses. Further, the present study gives evidence that aquarium system is best suited for incubation of late-stage eggs and rearing of newly hatched larvae upto free swimming stage. Jar incubation until hatching followed by aquarium rearing to 7-days increases fry survival and saves a huge amount of water.

Conclusion

The present experiment concluded that aquarium incubation system can be used as an alternative of jar incubation system for Nile tilapia eggs, especially in those areas where water is scarce. Since no advanced techniques are involved, inexpensive material, ability to operate by a single fish farmer in a small area, this mini hatchery could be used as an effective facility to produce tilapia fry to fulfill seed requirements of small scale fish farming systems. Further experiment will verify if it is possible to increase egg numbers in aquarium by increasing aeration and/or water exchange.

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