A RECIRCULATING INCUBATION SYSTEM FOR HATCHING SMALL BATCHES OF FISH EGGS

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ABSTRACT
This paper describes the design, construction and evaluation of the capacity of a re-circulating incubation system for hatching small batches of fish eggs. The system consisted of forty eight 50-mL plastic centrifuge tubes as incubation units connected to UV-sterilized water supply. A 1.0-mL serological pipette was extended through a rubber stopper to the bottom of each centrifuge tube to provide gentle rolling of eggs. Water flowed out of the incubation unit through a small section of glass and then plastic tubing inserted through a second hole in the rubber stopper to a PVC drain manifold. The system was used to hatch successfully small batches of artificially fertilized, naturally spawned eggs (50-150) of isolated blue tilapia Oreochromis aureus females. The best hatching success (36%) was achieved at the stocking density of 50 eggs/tube. The poor hatchability was attributed to low egg viability.

INTRODUCTION
In some research studies related to controlled artificial reproduction, genetic manipulation and division of a clutch of eggs into small batches to which treatments are applied can control for maternal or paternal effects and maximize utilization of the spawn. Available conventional incubation systems designed and constructed from commercial containers for mass fry production are not suitable for experimental studies, because they are usually too big and do not allow direct observation of developing eggs and embryos. Smaller incubation jars that are being used currently for experimental studies have been designed and constructed from clear, disposable, plastic beverage bottles to permit direct observation of developing eggs and embryos and they generally justify cost and construction time (Rottman and Shireman, 1988; Goodfellow et al., 1985; Macintosh and Little, 1995; Bates and Tiersch, 1995; Gleen and Tiersch, 1997). Although, some of these jars have volumes as small as 2.3-L to hold a minimum of 2,000 eggs (Gleen and Tiersch, 1997), they are still not suitable for experiments involving several treatments and replications in fish species with low egg production of only a few hundred eggs like mouth-brooding (Oreochromis) tilapia.

This study was to design and evaluate an incubation system specifically for hatching several batches of small numbers of eggs involving fish with low egg production.
MATERIALS AND METHODS
System design and construction
An incubation system was designed and assembled taking into consideration water quality requirements for hatching tilapia eggs. It was built from 50-mL capacity plastic centrifuge tubes, a supporting wooden rack, a sump (100-L aquarium), a small submersible pump (25 W), an ultraviolet sterilizer (8 W), immersion heaters and a water distribution manifold of polyvinyl chloride (PVC) pipe (Fig. 1). The heaters were installed inside the sump to maintain water temperature around 28±2°C. The submersible pump was also placed in the sump and was connected with flexible plastic tubing through the UV-sterilizer to a water distribution manifold of 1.27 cm diameter PVC pipe. The incorporation of the UV device was to control fungal and bacterial invasion of the eggs, which is recognized as the primary cause of poor hatchability and low survival of artificially hatched eggs (Rana, 1988). The manifold was tapped at 15-cm intervals with aquarium valves, which controlled water-flow at 1-L min⁻¹ into the plastic centrifuge tubes serving as incubation units. A down-welling water distribution assembly was inserted into each round-bottomed centrifuge tube. To simulate the churning action of oral incubation in mouth-brooding tilapias (Little et al., 1993), the assembly consisted of a 1.0-mL serological pipette that extended to the bottom of the incubation unit to provide gentle rolling of eggs. Water flowed out of the incubation unit through a small section of glass and then plastic tubing inserted through a second hole in the rubber stopper to a drain manifold of PVC pipe that returned water to the sump. The recirculation system had a capacity of 48 incubation units arranged on a two-tier wooden shelf with two rows of 12 units per tier. The incubation units could be removed easily to facilitate closer examination of embryonic development under the dissecting microscope, removal and counting of dead eggs and embryos or cleaning in a disinfectant bath and rinsing in a clean water bath.

Evaluating the capacity of the incubators
Eggs of blue tilapia *Oreochromis aureus* were used to evaluate the capacity of the incubation units. Naturally spawned eggs were removed from the mouths of five isolated females, 5-10 minutes after ovoposition, and placed in calcium-free Hank’s balanced salt solution (C-F HBSS) to extend viability. Eggs were fertilized with pooled sperm (motility>70%) stored overnight in a refrigerator. The 50-mL capacity incubation units were stocked with 50, 100 or 150 fertilized eggs in triplicates and incubated at water temperature of 28±2°C, pH of 8.0 and alkalinity of 80 mg L⁻¹ CaCO₃. Water flow rate was regulated at 1.0-1.2 mL min⁻¹ (Rach et al., 1995) to roll the egg batches gently in the round-bottom centrifuge (incubation) tubes to simulate the churning action of oral incubation of mouth-brooding (*Oreochromis*) tilapia. Dead eggs or embryos were removed and counted daily. The number of developing eggs surviving to the non-pigmented eyed, pigmented eyed and freshly hatched yolk-sac fry stages was determined after 48, 72 and 96 h post-fertilization.

Data analysis
Data on egg survival to hatch were subjected to analysis of variance (ANOVA) using the general linear models (GLM) of SAS (SAS Int., 1989). Means were separated using Fisher’s protected least significant difference (LSD) test at P<0.05.

RESULTS
The proportion of eggs surviving to hatch at incubation temperature of 28±2°C and water flow rate of 1.0- to 1.2-L min⁻¹ was generally low (23-36%) and survival decreased as development advanced (Table 1). Egg survival to hatch was density-dependent. At all the developmental stages investigated, the survival attained for the stocking density of 50 eggs/tube was significantly (P<0.05) higher than what was attained for either the 100- or 150 eggs/tube. No maternal effect on hatchability among the experimental females was observed. Fungal infection was not detected during the experiment to evaluate the capacity of the incubation units, not even on dead eggs or embryos, indi-
cating that the water quality was good. Apparently, the UV-sterilization effectively prevented fungal growth in the re-circulating water.

**DISCUSSION**

The need to provide a suitable incubation environment for fertilized fish eggs, while reducing fungal growth which is usually responsible for poor hatching and survival, has generated considerable research interest in the design and construction of cost-effective hatching jars in recent years (Goodfellow *et al.*, 1985; Rottmann and Shireman, 1988; MacIntosh and Little, 1995). Various containers, which allow upwelling of water, have been used to incubate fish eggs. Gleen and Tiersch (1997) constructed incubation jars from 2.3-L capacity plastic bottles and hatched between 2,000 and 80,000 eggs of common carp *Cyprinus carpio* (koi strain). Bates and Tiersch (1995) used inverted plastic bottles as culture units in recirculation systems for raising channel catfish (*Ictalurus punctatus*) fry. Macintosh and Little (1995) built hatching jars from disposable polycarbonate plastic bottles to hatch *Oreochromis* tilapia eggs. However, under identical water quality conditions, round-bottom, down-welling containers in which eggs are rolled by UV-sterilized water generally produced higher hatchability than conical, upwelling containers (Rana, 1986a, b). This shows that mechanical stress caused by friction between developing embryos and the surface of the container, which is often the main cause of mortality, is greater in conical containers than round-bottom containers. Rana (1988) hatched *Oreochromis* tilapia eggs incubated in conical containers and round-bottom containers at water temperature of 28°C within 72-84 h and 90-102 h respectively compared with natural hatching time of 96-102 h after spawning. Apparently, mechanical stress in the conical containers induced premature hatching.

The present incubation system which combines essential features of previous systems was successfully tested with mouth-brooding blue tilapia *O. aureus* eggs at stocking densities of 50, 100 and 150 eggs/50 mL. The eggs hatched within the natural hatching time of 96 h after artificial fertilization (Rana, 1988), indicating that the system did not induce premature hatching conditions. The best hatching success (36%) was attained at the lowest stocking density. Since the physico-chemical quality of the incubation water was optimal (Wohlfarth and Hulata, 1983; Rana, 1988; Boyd, 1990) and fungal infestation was not detected the generally poor hatchability probably originated from the egg source. Natural spawns from isolated females were used. It has been demonstrated in a previous study that the viability of eggs from this source is inferior to that of stripped eggs (Owusu-Frimpong, 2008).

<table>
<thead>
<tr>
<th>Egg density (No./50mL)</th>
<th>Egg batches</th>
<th>Non-pigmented eyed embryos at 48 h</th>
<th>Pigmented eyed embryos at 72h</th>
<th>Yolk-sac fry at 96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>5</td>
<td>67 a</td>
<td>62 a</td>
<td>36 a</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>50 b</td>
<td>47 b</td>
<td>32 b</td>
</tr>
<tr>
<td>150</td>
<td>5</td>
<td>34 c</td>
<td>35 c</td>
<td>23 c</td>
</tr>
</tbody>
</table>

Means sharing a common letter in the columns were not significantly (P>0.05) different.
The unique feature of the present system is the size of the incubation unit that allows egg batches as small as 50 eggs to hatch successfully. This is an obvious advantage of this system over all other existing systems similar to it. The system is suitable for application in research studies including genetic manipulations allowing for separation of treatments in fish species such as mouth-brooding (*Oreochromis*) tilapia, which have low fecundity and non-adhesive eggs. Thus the system has the potential to stimulate research investigations into tilapia breed improvement for aquaculture. It may also be used to hatch adhesive eggs of other low fecund fish species if the adhesive properties of the eggs can be removed, for example, by immersion in a bath of 0.4% NaCl and 0.3% urea to ensure adequate oxygenation (Woynarovich, 1962) prior to incubation.

REFERENCES


Fig. 1: Schematic Diagram of the Recirculation Incubation system