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Research Application Summary

Effect of incubation water flow rate and egg population density on hatching success of *Oreochromis karongae*

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Abstract

Research was conducted to assess the combined effect of water flow rate and incubation egg population density on egg hatching period, hatchability, total mortality and fry survival of *Oreochromis karongae*. The treatments were (1) 0.09 l/sec and 125 eggs/l, (2) 0.09 l/sec and 417 eggs/l, (3) 0.17 l/sec and 125 eggs/l, and (4) 0.17 1/sec and417 eggs/l. There was a significant interaction effect whereby high flow rate (0.17L/sec) and low egg population density (125 eggs/l) resulted in the shortest hatching period of 4.61±0.07 days ($p<0.05$). There was no significant interaction effect between flow rate and egg population density on hatchability, total mortality, and fry survival ($p>0.05$). However, fry survival (74.56±9.48%) was higher at high incubation egg population density than at low incubation egg population density (24.37±37.79%) ($p<0.05$). The hatchiéng success is higher than reported in the past for Tilapias in Malawi. These results indicate that there is potential to increase *O. karongae* fry production by manipulating water flow rates and egg population density combinations during incubation.

Key words: Egg density, flow rate, hatchability, incubation, *Oreochromiskarongae,* survival

Résumé

La recherche a été menée pour évaluer l'effet combiné de taux d'écoulement de l'eau et de la densité d'incubation de la population des œufs sur la période d'éclosion des œufs, la capacité d'éclosion, la mortalité totale et la survie des gosses (menu fretin) d'*Oreochromis karongae*. Les traitements étaient: (1) 0,09 l/sec et 125 œufs /l, (2) 0,09 l/sec et 417 œufs/l, (3) 0,17 l/sec et 125 œufs/l, et (4) 0,17 1/sec and 417 œufs/l. Il y avait un effet significatif d'interaction dans lequel débit élevé (0.17L / s) et une faible densité de population d'œuf (125 œufs / l) ont donné lieu à la période d'incubation plus courte de 4,61 ± 0,07 jours (p <0,05). Il n'y avait aucun effet d'interaction significative entre le taux élevé d'écoulement et la densité de population d'œuf sur le fait d'éclosion, la mortalité totale et la survie des alevins (p> 0,05). Cependant, la survie des alevins (74,56 ± 9,48%) a été supérieure dans la haute densité de population d'incubation des œufs qu'à la faible densité de population incubation des œufs (24,37 ± 37,79%) (p <0,05). Le succès de l'éclosion est plus élevé plus que ce qui a été rapporté dans le passé pour les

Tilapias au Malawi. Ces résultats indiquent qu'il existe un potentiel pour la croissance de la production d'alevins de O. karongae en manipulant les écoulements d'eau et des combinaisons de densité de population d'œufs pendant l'incubation.

Mots clés: Taux d'écoulement, incubation, la densité des œufs, Oreochromi skarongae, faire éclore, la survie

Background

Tank based fish hatchery and production systems using water recirculation continue to grow worldwide. Among other reasons, the quest for controlled environment, limitations to water availability, and the need for increased system carrying capacity and efficiencies contribute to the growing interest in such systems (Losordo and Hobbs, 2000). For example, due to presence of water treatment, the make-up water needs of recirculating systems, about $1m^3$ kg^{-1} of feed, are 100 times lower than in traditional flow through systems (MacMillan, 1992; Blancheton *et al.,* 2007). For endemic fish species with limited fecundity and dwindling wild catches, such as *Oreochromis karongae* (Banda *et al,* 2003), there is need for massive production of seed for both aquaculture and restocking purposes. However, current hatchery systems especially in Malawi have registered little success in the production of *O. karongae* seed (Valeta *et al.,* 2013). Long hatching period (9-14 days), high egg and larvae mortalities and low hatchability (around 50%) of *O. karongae* has been reported by hatcheries and individual farmers.

Literature summary

Incubation is a critical point in the hatching process. Different egg characteristics are known to affect hatchability success in fish. During incubation, fish eggs need to be adequately suspended in the incubator water column to avoid sticking together and allow access to dissolved oxygen. To achieve this, flow of water into the incubation jar must be properly designed. A study by Shiotani *et al.* (2005) showed that mass mortality of *Epinephelus septemfasciatus* (grouper) larvae depended on hydrodynamics factors such as flow rate in larval rearing tank. Optimum hydrodynamic conditions in aquaculture facilities are determined by species requirements and waste elimination. The main design parameters that influence tank hydrodynamics including flow pattern and average velocities are the geometry and water inlet and outlet characteristics (Timmons *et al.,* 1998; Oca *et al.,* 2004; Masaló, 2008). Therefore, rate of water flow into the incubation system would be of paramount importance as it would influence the nature and extent of water and egg circulation. Furthermore, it is not known whether or not and to what extent egg population density would affect hatching of *O. karongae* eggs. Watanabe *et al.* (1992) suggests that egg population density does not affect hatching rate in Florida red tilapia. However, eggs of *O. karongae* are slightly larger (Average 3.5 mm) as observed by Msiska and Costa-Pierce (1999), than those of Florida red tilapia (average 3mm) (Chapman, 2012). Thus, the main objective of this study was to assess the effects of combinations of water flow and egg population density on hatching period, hatchability, total mortality and fry survival in *O.karongae* eggs.

Study description

The research was conducted at the National Aquaculture Center, Zomba, Malawi. One hundred (100) female, and fifty (50) male broodstock were conditioned separately for three months in earthen ponds, after which they were mated in hapas. They were fed with soybased meal of 35% CP at 5% body weight. Female broodstock were closely observed for eggs after 1-2 weeks of mating. Eggs were collected from the mouth of the female brooders, and were cleaned and sorted by stage of development (Geffen *et al.,* 2006). Stage I eggs were separated, counted and assigned to the treatments in 1.2 litre incubation jars in a recirculating system. The hatching experiment took up to two weeks. Two factors were tested, namely, water flow rate (Q) and egg population density (D) in 1.2 litre incubation jars. Each factor was tested at two levels and replicated three times in a 2×2 factorial experiment. Thus a total of four treatments were randomly allotted to the incubation jars. The treatments were: 0.09 L/sec×125 Eggs/L; 0.09 L/sec×417 Eggs/L; 0.17 L/sec×125 Eggs/L; and 0.17 L/sec×417 Eggs/L. Flow rate was controlled using pipe valves and timers. Water was exchanged daily at 20% replacement rate using ground water. The experiment was carried out over a period of 14 days in September, 2012. Temperature, pH and dissolved oxygen were measured three times a day at eight hour intervals, using water checker. Water samples were collected in 500ml plastic bottles twice a week for ammonia analysis (Titration method). Egg development was monitored four times daily, and stage advancement was confirmed through observations under the light microscope (×40) and time was recorded. Time taken for eggs to hatch was computed in Excel (2010). All data were entered into Excel (2010) and exported to SPSS 16.0 for analysis. All percent data were arcsine transformed for analysis, and means were back-transformed for reporting.

Results

There was a significant interaction effect between flow rate and incubation egg population density ($P<0.05$). The lowest incubation egg population density and highest flow rate combination (125 eggs/L) at 0.17 L/sec) resulted in the shortest hatching period (4.61±0.07days), while the rest of the treatments took longer (7.46±0.07days) to hatch ($p<0.05$).There was no significant interaction effect between flow rate and incubation egg population density on hatchability, total mortality (defined as percent of dead embryos and larvae over the initial egg stock) and fry survival ($p>0.05$). Therefore, main effects were tested for mean differences using LSD. At high incubation egg population density, fry survival (74.56±9.48%)) was higher than at low incubation egg population density (24.37±37.79%) ($p<0.05$). However, there were no significant differences in mean hatchability, total mortality, and fry survival ($p>0.05$) between 0.09 and 0.17 l/s incubation water flow rates.

There were no significant differences in temperature ($p>0.05$) across the treatments.

However, there was a significant interaction effect of flow rate and egg population density on dissolved oxygen, pH and ammonia concentration ($p<0.05$). Dissolved oxygen concentration was higher in treatment combinations with high flow rate (6.80±0.30-6.83±0.32mg/l) than low flow rate (5.80±0.00-6.00±0.17mg/l) ($p<0.05$). Significant differences were observed in pH ($p<0.05$) but all values were between 7 and 8 (7.6±0.05 and 7.9±0.20). Ammonia level was owest (0.10±0.01mg/l) in the treatments with highest flow rate and highest egg population density and was highest (0.27±0.01mg/l) in the treatment with the lowest flow rate and highest egg population density, respectively.

Discussion

The combination of high water flow rate and low egg population density resulted in a shorter hatching period which likely enhanced physiological activity of the *O. karongae* eggs during incubation. Adequate flow of water is needed to provide constant motion of eggs, uniform distribution of dissolved oxygen and prevent eggs from sticking and stagnating, which would promote fungal infections. Although microbial assays were not done in this study, elsewhere Rach *et al.* (1995) observed that operating incubators at high flow rates reduced instances of fungal infections of trout eggs. Egg population density also has an effect on hatching. In a previous study with incubating eggs at similar temperature and flow rate, and a higher egg population density of 265 eggs, Valeta *et al.* (2013) recorded longer hatching period (7.3 days) compared to in the present study (4.6 days). The present study did not observe significant differences in hatchability values at different egg population densities. Similar observations were also made by Watanabe *et al.* (1992) in which hatchability of Florida red Tilapia in brackish water was 63.9±1.1 -65.3±4.7% for incubation densities 462-1846 eggs/l. While incubation conditions may vary widely among studies, the survival rates obtained in this study were higher than those obtained in Florida red Tilapia. It is unlikely that high hatchability and reduced hatching period at this high temperature (29°C) may be as a result of precocious response to hypoxia because dissolved oxygen was adequately high across the treatments (around 6.0mg/l). Therefore, the improved availability of oxygen to developing eggs achieved by both flow rates likely enhanced egg development. High fry survival rates observed at the high flow rate further testify to the fact that the larvae did not hatch prematurely as oxygen was adequate. Delivery of oxygen to developing embryos is achieved both by diffusion and by entry of oxygen dissolved in the water into the egg as a result of the osmotic gradient (Alderdice *et al.,* 1984). The osmotic gradient would be steepened by high partial oxygen pressures created by high flow-rates (Czerkies *et al.,* 2001).

In both low and high flow rates, the small size of the incubation jars (1.2 l) and water inlet pipe (0.5″) ensured water pressure high enough to keep the eggs churned and in continuous motion, which is desired for aeration of eggs during incubation. These findings suggest that there is a potential to reduce the hatching period further by establishing the optimum combination of flow rate and egg population density. Furthermore, considering that increase in egg population density during incubation did not reduce survival of larvae and fry, there is room for increasing both egg population density and flow rate during incubation. The other positive observation is that increased flow rate maintained ammonia below 0.20mg/l, and the dissolved oxygen and pH levels were also within acceptable limits for Tilapia eggs. This is evidence that the flow rate, assisted by the 20% water replacement, was good enough to flush out metabolites to ensure good water quality, without external aeration. The challenge is to assess the levels of flow rate and density that give the best technical and economic advantage arising from the incubator's fry productivity that accrues to reduced hatching period, i.e., increased production cycles, and increased fry survival. Kamler (2002) found that while temperature is the major extrinsic factor, egg size (diameter) is a major intrinsic factor affecting ontogenetic rate of development in fish larvae.

It is not yet well established whether the size of the egg also influences the optimum density for a particular species, i.e., if the robustness of bigger eggs is depressed under high incubation egg density. For example, Regnier *et al.* (2012) observed that smaller eggs of Rainbow trout (*Salmo trutta)* had higher survival rates than bigger eggs. Generally *O. karongae* eggs are bigger in size than for other Tilapias such as the commonly cultured *O. shiranus,* which have shown higher survival rates in hatcheries at room temperatures. However, the survival rates obtained in this study are higher than those reported for *O. shiranus* by most hatcheries in Malawi. It would be expected that more conclusive results would come from incubating eggs of *O. karongae* and *O.shiranus* under similar conditions such as used in the study. In other species such as Chinook salmon (*Oncorhynchus tshawytscha*), larvae and fry from bigger eggs exhibited enhanced growth and better survival than those from smaller eggs (Einum and Fleming, 2000; Heath *et al.,* 2003).

These findings suggest that, in general, size alone may not have bigger influence on hatching success and fry survival. Instead, other factors such as species, technical and bio-physical conditions during incubation, including flow rate, may also have significant influence on the egg evolution (Regnier *et al.,* 2012). It is surprising to note that fry survival rate was lower at lower incubation densities. It is possible that the fry were heavily tossed within the bottle which resulted in physical damage, stress and mortality.

In wild fisheries, mechanical action of high velocities has been held responsible for high mortalities in natural redds for Atlantic salmon (Chapman, 1988; Lisle and Lewis, 1992; Crisp, 1993, 1996; Guerrin and Dumas, 2001a, b). Egg-to-fry stages are known to be very fragile and while they require adequate oxygen circulation (Dumas *et al.,* 2007), they may not survive mechanical stress imposed by rapid churning likely taking place at low incubation egg densities.

Conclusion

There was an interaction effect between water flow rate and incubation egg population density on hatching of *O. karongae.* High flow rate and low egg densities enhanced hatching resulting in reduced hatching period and increased fry survival, while maintaining good water quality. However, hatchability was not significantly affected by both flow rate and egg population density. Therefore, there is need to establish the optimum combination of flow rate and egg population density that will result in short hatching period and high survival of large numbers of fry. Future research should aim at assessing more hydrodynamic patterns of flow in the incubation jars to optimize hatching success at higher egg population densities in *O. karongae*.

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