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Original Article

Oogenesis, spermatogenesis and hatching rate of seawater-acclimated Nile Tilapia, *Oreochromis niloticus*♣

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Abstract

Seawater (SW)-acclimated Nile tilapia, *Oreochromis niloticus*, can grow and survive but rarely have offspring under an elevated salinity environment. To find out the cause(s) of this problem, the size and histology of the gonads of the SW-acclimated Nile tilapia were determined, which revealed that the gonadosomatic index and histology of the gonads of the SW-acclimated fish did not differ from those of the freshwater (FW)-acclimated ones. However, the embryos of the SW-acclimated *O. niloticus* could tolerate salinity from 0 to 15 ppt, with a survival rate of embryos more than 80%, but 0% under 20-ppt. However, when the embryos were incubated under 10 ppt up to the stage of eye formation, and thereafter the salinity was abruptly raised to 20 ppt, >90% of them survived. The results suggested that SW-acclimated *O. niloticus* have normal oogenesis and spermatogenesis, and their embryos can survive salinity above 15 ppt if the salinity is raised stepwise.

Keywords: reproduction, seawater-acclimated Nile tilapia, *Oreochromis niloticus*, embryonic tolerance, gonadal development, hatching

1. Introduction

Nile tilapia *Oreochromis niloticus*, generally considered as a freshwater (FW) fish, has been cultured worldwide and its commercial production has been second only to the Chinese carp (Food and Agriculture Organization of the United Nations [FAO], 2020). The fish, however, can be acclimated to elevated salinity, comfortably in brackish water and even in full-strength seawater (SW, 30-35 ppt) if the salinity is gradually increased (Luan, Olesen, Ødegard, Kolstad, & Dan 2008; Schofield, Peterson, Lowe, Brown-Peterson, & Slack, 2011; Withyachumnarnkul *et al*., 2017). In Thailand, the fish has been produced commercially either by direct stocking in earthen ponds or net cages floating in freshwater canals, rivers, and lakes (Lebel *et al*., 2013). The cage culture produces environmental pollutions and obstructs free water flow and transportation in public waterways (Ingthamjitr, Paankhao, Lueangtongkham, & Ooparikatipong, 2017; Mwebaza-Ndawula, *et al*., 2013). For these reasons, the Thai government has issued regulations to limit the area of cage culture of Nile tilapia in public waterways; the regulations, if fully reinforced, would force the fish growers to find other venues to rear the fish. Besides inland freshwater ponds, another possible rearing place would be the reservoir in the existing shrimp farms, which could conveniently

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accommodate a large volume of fish. For many shrimp growers, stocking tilapia in the reservoir, or the co-culture shrimp and fish in the same pond, has been a common practice and the co-culture could strengthen the shrimp health (Tendencia, de la Pena, & Choresca, 2006; Troell, 2009; Yi & Fitzsimmons, 2004). However, the only problem is that the tilapia for the co-culture must be able to thrive well under elevated salinity, either brackish or full-strength seawater. For brackish water below 20 ppt, ordinary Nile tilapia may survive reasonably well; however, with salinity above 20 ppt, the fish requires a certain period of adaptation. To serve this purpose, *O. niloticus* were acclimated to full-strength SW but the growth and survival rates of the fish were significantly lower than those of the FW-acclimated ones (Withyachumnarnkul *et al*., 2017).

It was observed that SW-acclimated *O. niloticus* rarely generate offspring under full-strength SW. Whether the problem is due to an inability of the fish to have normal oogenesis/spermatogenesis, or an inability of the embryos to survive under high salinity, is not known. As tilapia are mouth-brooders; the females keep fertilized eggs in their mouth and the embryos develop in the mouth chamber until hatching. Therefore, the survival of the embryos would depend on their tolerance to environmental salinity. Therefore, this study aimed to, firstly, determine oogenesis and spermatogenesis through the histological features of the gonads of the SW-acclimated *O. niloticus*, and secondly, determine the survival rate of the fish embryos under elevated salinity.

2. Materials and Methods

2.1 The gonadosomatic index and the histology of gonads

Both the FW- and SW-acclimated *O. niloticus* were kept in a 5m × 5m rectangular net cage, with 1.5m deep floating in a 0.5ha pond; the salinity of the water rearing the FW-acclimated fish was 0-3 ppt, and that for the SW-acclimated fish was 27-30 ppt. Both groups of fish were stocked at the density of 10 individuals/m^3 of water and provided with commercial pellets (30% protein) three times daily (08.00, 13.00, and 18.00 h), initially at 4% biomass per day and were adjusted from meal to meal to assure *ad lib* consumption. The water was aerated by paddlewheel, and the water quality: total ammonia nitrogen (TAN), nitrite, pH, alkalinity, and dissolved oxygen (DO); was daily monitored to assure optimum levels for the fish health. Water exchange in the pond was performed whenever either TAN or nitrite level was above 0.5 ppm and DO was kept between 4-6 ppm at all times.

Females (200-300g BW) and males (300-500g BW) of FW- and SW-acclimated *O. niloticus* were randomly sampled from the net cages, 9-11 individuals per group. They were individually euthanized with 100ppm Eugenol, BW was determined, and gonads were isolated and weighed. The gonadosomatic index (GSI) was calculated as: gonadal weight × 100/BW.

Small pieces (10-20 mg) of the ovaries and testes were fixed in 10% neutral formalin for 24 h, after which the formalin was changed to 70% ethanol and the fixed tissues were kept in 4 ºC until being processed through standard paraffin sectioning. Briefly, the fixed tissues were dehydrated through a series of rising concentrations of ethanol, embedded in paraffin, sectioned with a microtome at 5-6 µm thickness, and stained with hematoxylin and eosin (H&E).

2.2 The survival of fish embryos under different water salinity

Sexually mature SW-acclimated *O. niloticus* that had been reared under 27-30 ppt were re-acclimated to 0 ppt, by 3 ppt reduction per day. This was carried out in a 2.5m^3 round canvas pond, with 2m diameter and 1m high, containing 0.8m water deep. The pond was stocked with 9 females (200-300g BW) and 3 males (300-500g BW), which is considered the optimum ratio and density for tilapia breeding (Hughes & Behrends, 1983; Obi & Shelton, 1988; Tahoun, *et al*., 2008). The fish were provided commercial pellets (35% crude protein) at 4% biomass per day. Water quality was monitored as described, except that 70% water exchange was performed daily.

Three weeks after acclimation to 0 ppt, mouth-brooded females were identified and clutches of eggs were taken out for incubation in 5L round-bottom flasks, with mild aeration to keep the water gently move in the flask. Each flask contained 500-600 eggs, which were accurately counted. During the incubation, dead embryos (opaque appearance) were recorded twice daily (at 07.00 and 19.00 h) and eliminated. Water exchange was performed daily at 70%. The percentage of live embryos was determined daily until all the embryos hatched out as "swim-ups" carrying yolk sacs in their abdomen, which usually fell on days 4-5 of incubation.

The embryos were tested for salinity tolerance at four levels: 0, 10, 15, and 20 ppt. Each level of the salinity test comprised 5 replicates. The total hatching rate (HR) was determined when all the embryos turned into swim-ups.

As it turned out that the embryos could tolerate 0, 10, and 15 ppt water, but not 20 ppt water, a further test was performed to find out the survival rate of the embryos if they were incubated at 10 ppt to day 3, followed by an increase to 20 ppt. It was hypothesized that the embryos may be able to hatch at a salinity higher than they normally do if they have time to develop sufficient osmoregulation mechanisms.

2.3 Statistical analysis

The numerical data were expressed as means ± SD (N) and statistical analysis was performed by ANOVA followed by Tukey's test, and also by Student's t test. Statistical significance was set at $p<0.05$.

3. Results and Discussion

3.1 The GSI and histology of the gonads

The GSI of the FW- and SW-acclimated *O. niloticus* were not significantly different within the same sexes (Figure 1). A wide variation of the GSI suggests that the gonads of the fish were at different stages of development; at more advanced stages, the size of the gonads would be larger than that of the earlier stages. It is likely that the gonads of the SW-acclimated fish, similar to the FW-acclimated ones, were capable of generating gametes, which could be revealed by

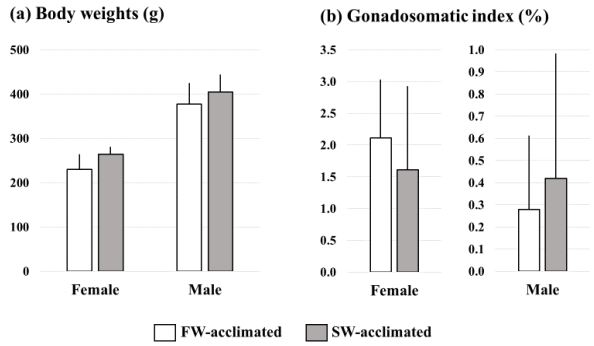

Figure 1. The bodyweights (a) and gonadosomatic indices (b) of the female and male freshwater (FW)- and seawater (SW)-acclimated Nile tilapia, *O. niloticus*. N = 9-11

their histological features.

Histology of the gonads of the FW- and SW-acclimated fish of both sexes showed no distinctive difference (Figures 2 and 3). Among other features, the ovaries of both groups of fish were composed of normal immature and mature oocytes. Likewise, the testes of both groups of fish revealed normal features of spermatocytes and spermatozoa. These results suggest that SW-acclimated *O. niloticus* have normal oogenesis and spermatogenesis.

3.2 The survival of fish embryos under different water salinity

The percentage of viable embryos incubated under all the salinity levels (0 to 20 ppt) was higher than 85% on days 1 and 2 of the incubation (Figure 4). On day 3, the percentage of the 0-, 10-, and 15-ppt groups remained the same as on day 2, but that of the 20ppt group was decreased markedly to approximately 15%. On day 4, the percentage of the 0-, 10-, and 15-ppt groups remained the same, with a large number of embryos hatched out as swim-ups carried yolk sacs in their abdomen. On the contrary, the percentage of the 20ppt group dropped to 6% with no appearance of swim-ups. On day 5, the percentage of the 0-, 10-, and 15-ppt groups remained above 80%, with all embryos hatching out, while all the embryos of the 20ppt group died.

Among the 0-, 10-, and 15-ppt groups, the total hatching rate of the 10ppt group was the highest, although the value did not differ statistically from that of the other two groups. The internal osmolality of the fish embryo is approximately 300 mOsm (Machado & Podrabsky, 2007), which is equivalent to 9ppt salinity. This may explain why the embryos survived better under 10 ppt, the condition being close to isotonic salinity. Under that condition, the embryos may not have to spend much of their energy to defend their internal osmolality, compared to being under hypotonic (e.g., 0 ppt) or hypertonic (e.g., 15 ppt) environment. Based on this finding, it is advisable that hatching of fertilized eggs of Nile tilapia should be under 10 ppt water, instead of freshwater.

This finding thus revealed that embryos of the SW-acclimated *O. niloticus* could survive within the salinity range from 0 to 15 ppt, but not at 20 ppt, which is similar to the previous report (Watanabe, Kuo, & Huang, 1985). All these results suggest that the SW-acclimated Nile tilapia have a normal reproductive function, but their eggs cannot develop to

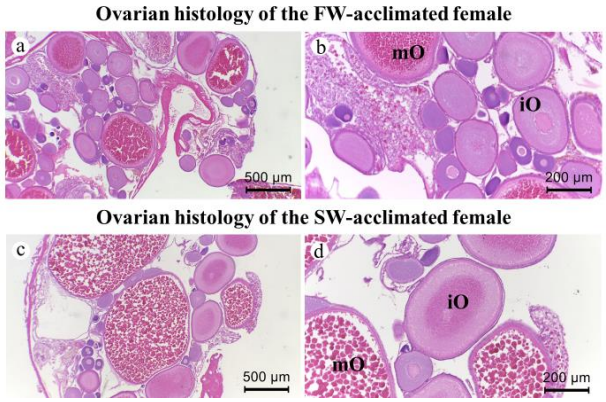

Figure 2. The ovarian histology of the female freshwater (FW)- (a and b) and seawater (SW)- (c and d) acclimated Nile tilapia, *O. niloticus*. H&E staining. iO, immature oocyte; mO, mature oocyte

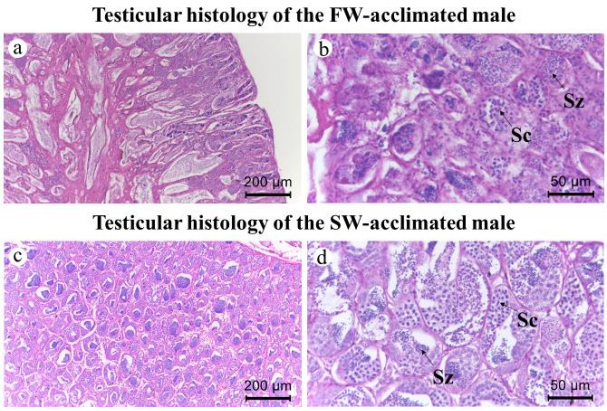

Figure 3. The testicular histology of the male freshwater (FW)- (a and b) and seawater (SW)- (c and d) acclimated Nile tilapia, *O. niloticus*. H&E staining. Sc, spermatocyte; Sz, spermatozoa

full-term embryos under salinity of 20 ppt and above.

In the last experiment, as the embryos had developed under 10 ppt salinity to day 3, when eye formation was observed, thereafter the salinity was raised abruptly to 20 ppt. In that situation, it was found that the embryos survived well and hatched out as normal swim-ups, and the total hatching rate was not statistically different from that of the 10ppt group (Figure 5). This result suggested that the embryos can tolerate 20ppt salinity if they had not to face that salinity from the beginning but only after a certain period of embryonic development. The embryos may need time to develop mechanisms to enable them to defend their internal osmolality, and once that defense exists they could tolerate 20ppt or higher salinity. One of the mechanisms of fish embryos to accomplish this task is to develop mitochondrial-rich (MR), or chloride cells, on the yolk sac epithelium (Hiroi, Kaneko, & Tanaka, 1999; Hiroi, Kaneko, Uchida, Hasegawa, & Tanaka, 1998; Katoh, Shimizu, Uchida, & Kaneko, 2000). The MR cells contain a high concentration of Na^+/K^+ ATPase, which regulates levels of Na^+, K^+, and H^+ of the embryo. The result of this experiment may lead to a practical application that SW-acclimation of *O. niloticus* might be carried out as early as during embryonic development, instead of at the

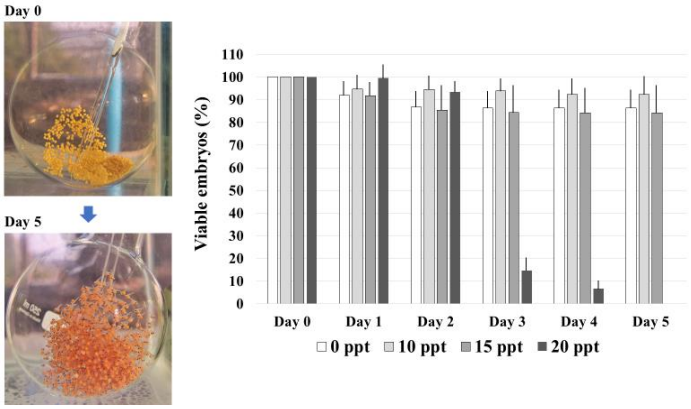

Figure 4. The percentage of viable embryos of seawater (SW)-acclimated Nile tilapia, *O. niloticus*, in the 5L incubation round-bottom flask, from day 0 to day 5, at which all the embryos hatched out as swim-ups with attached yolk sacs. The test was performed in four groups under different salinities, each with 5 replicates.

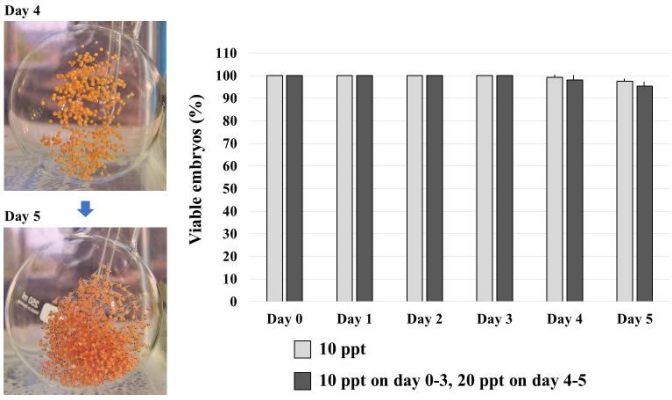

Figure 5. The percentage of viable embryos of seawater (SW)-acclimated Nile tilapia, *O. niloticus*, in the 5L incubation round-bottom flask, from day 0 to day 5, at which all the embryos hatched out as swim-ups with attached yolk sacs. The test was performed in two groups, each with 5 replicates: the first group was incubated under 10 ppt throughout and the second one under 10 ppt until day 3 and changed to 20 ppt on days 4 and 5.

fingerling stage, which would shorten the period of acclimation to the elevated salinity, and probably enhance the survival rate of the fish.

4. Conclusions

Nile tilapia, *O. niloticus*, acclimated to full-strength seawater (SW), have normal oogenesis and spermatogenesis, but their generation of offspring is limited by an inability of the embryo to survive salinity higher than 15 ppt, but a stepwise increase in salinity enables the embryo to survive up to at least 20 ppt. These findings could change the future of the hatchery generation of SW-acclimated Nile tilapia.

Acknowledgements

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