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Short communication

Evaluating quality of Nile tilapia (*Oreochromis niloticus*) eggs and juveniles from different commercial hatcheries

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ABSTRACT. Our objective was to assess the quality of eggs and juvenile of Nile tilapia (*Oreochromis niloticus*) from broodstock of three commercial hatcheries (H1, H2, and H3). Larval weight was significantly higher for H2 and H3 hatcheries ($P < 0.05$). After 45-day growth, weight of juveniles of H2 was higher, while specific growth rate was significantly higher for H1 and H2 ($P < 0.05$). After the ammonia stress test, no significant differences in mortality were found among larvae from the three hatcheries, although a positive relationship between carbohydrate content in eggs and juvenile mortality was observed ($P < 0.05$). After the salinity stress test, the lowest and highest mortality ($P < 0.05$) occurred respectively in tilapia larvae from the H1 (24.2%) and H3 (57.8%) hatcheries. We concluded that egg quality was not affected by tilapia broodstock from the three hatcheries studied, but differences were obtained for growth performance, carbohydrate content, and survival of juveniles when exposed to the salinity stress test. These three indexes should be considered as potential juvenile quality criteria for tilapia.

Keywords: *Oreochromis niloticus*, quality criteria, eggs, juveniles, tilapia, broodstock.

An adequate supply of high quality eggs and larvae is one of the major challenges facing the tilapia aquaculture industry (Lupatsh *et al*., 2010). Most studies dealing with tilapia egg and larvae quality focus on broodstock nutrition, since an adequate diet of brooding females promotes gonadal development and performance of eggs and larvae (Bhujel *et al.*, 2001). Yet, some studies indicate that egg quality and larvae development are affected by genetic variability of broodstock. For instance, Phu *et al.* (2015) observed variability in quality of tilapia progeny when females from different sources were fed the same diet, suggesting that variability is partly attributed to differences in genetic characteristics of females.

Oreochromis niloticus was introduced in Mexico in the 70's (Arredondo & Lozano, 1996), but the lack of renewable broodstock and genetic variabilty led to unfavourable traits, including smaller sizes, early sexual maturation, and deformities (Barriga-Sosa *et al.*, 2004). Endogamic processes resulting from the introduction of small broodstock from the same source can result in lower quality eggs and larvae (Osure & Phelps, 2006). In this study, we assess the quality of eggs and juveniles Nile tilapia from broodstock of three commercial hatcheries in Mexico. There are no antecedents in the litearature of such studies.

Groups of adult Nile tilapia (250 females and 50 males) used in Mexico by tilapia growers, were obtained from three commercial hatcheries (H1, H2 and H3, hereafter), transported and maintained at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa (CIIDIR, Guasave, Sinaloa, México). After acclimation, 15 fish (297.03 ± 36.28 g; 5 males and 10 females from each hatchery) were selected, weighed, ventrally labelled using microchips (Avid ® México SA de CV), and distributed in a circular tank (7.3 m^3 capacity). Aereation in each tank was provided by perforated PVC piping connected to a 2 hp blower. Photoperiod was natural throughout all study. Water temperature was maintained at 28 ± 1°C. Every week, 30% of the water volume of all tanks was renewed. Fish were fed to appa-

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rent satiation twice (09:00 and 17:00 h) with a commercial diet (Purina©, 35% crude protein). The number of gravid females in each tank was monitored every week.

A total of twenty eggs (36-48 h after fertilization) from each broodstock group were collected from the mouth of gravid females to determine: 1) Major and minor length (using a microscope and an micrometer ruler), 2) egg volume = ¾π (2 × minor radius × major radius) (Van der Merwe, 1970), 3) egg area = π (minor radius × major radius) (Van der Merwe, 1970), 4) wet weight using an analytic balance (±0.0001 g), 5) total number of eggs in the total mass of eggs, 6) protein, lipid, and carbohydrate content (mg g^{-1}), and 7) hatching rate = $L \times (L + H)^{-1} \times 100$ (were L is the number of larvae; and H is the number of eggs). Fertilized eggs from each group were incubated (28 ± 1°C) using 18 McDonald type incubators (6 L capacity) maintining constant aeration and setting water temperature at 28 ± 1°C with a 100 watts heater. Once larvae consumed the yolk sack, zootechnical parameters and tolerance to stress were determined. Samples of tilapia fertilized eggs (n = 20) and juveniles (n = 10) from each broodstock tank were individually homogenized in 3 mL cold saline solution (1.2% NaCl) to obtain a crude extract. Lipid content was measured by the sul-phovanillin method (Barnes & Blackstock, 1973). Absorbance was recorded at 560 nm in a microplate reader (Thermo/LabSystems Multiskan Multisoft, Basingstoke, UK), using a mixture of triglycerides (12 mg mL^{-1}) and cholesterol (8 mg mL^{-1}) as standard (Palacios *et al.*, 2000). Protein concentration was determined (Bradford, 1976) using bovine serum albumin as standard, after digestion of the homogenate for 30 min at room temperature, using 0.5 N NaOH. For carbohydrates analysis, proteins were precipitated with 20% TCA and centrifuged at 2,600 g for 10 min at 4°C. Then, free glucose in the supernatant, resulting from acid hydrolysis, was quantified by the Anthrone method (Van Handel, 1965), using glucose as the standard.

A 45-days growth trial was conducted in 18 square-shaped 270 L plastic tanks (6 tanks per broodstock group). Stocking density was adjusted at 50 larvae per tank, with an individual mean initial weight of 0.13 ± 0.001 g. Water temperature was set at 28 ± 1°C with a 100 watt heater, and aeration was continuously provided with air diffusers connected to a 2 hp blower. Water temperature and dissolved oxygen were monitored twice daily (8:00 h; 16:00 h). Fish were daily fed with a commercial diet (Purina, 45% crude protein, 15% lipids) at 09:00 h, 14:00 h, and 17:00 h, adjusting feeding ratio at 10% biomass. About 30% of the water was replaced each week. The following parameters were calculated: 1) Specific growth rate (SGR) = $[\ln(fw) - \ln(iw)] \times 100 \times t^{-1}$; where fw is the final weight, iw is the initial weight, and t is the days of cultivation (Hopkins, 1992), 2) feed conversion ratio (FCR) = feed consumed × weight $gain^{-1}$, 3) survival (%) = final number of fish ×100 × initial number of $fish^{-1}$, 4) protein, lipid, and carbohydrate content (mg g^{-1}).

Salinity and ammonia stress tests were performed in 1000 mL beakers immersed in a bath set at 28 ± 1°C. In both tests, three beakers (10 juveniles per beaker) were used for juveniles from each broodstock group. Time of exposure and levels of salinity and ammonia were used on the basis of LC_{50} (Probit's test) determined in a previous test. The resulting LC_{50} for the 24 h trial was 19.3 of salinity and 35.9 mg L^{-1} ammonia. Mortality was defined as no movement after physical stimulation. Data were analysed for normality and homoscedasticity (Zar, 2010). One-way ANOVA and *post-hoc* Tukey's test were used to detect differences among eggs and juveniles quality criteria from the broodstock groups. Survival was transformed to natural logarithm before analysis. Regression analyses were conducted to detect possible relationships between egg and juvenile quality criteria. Statistica 7.0 (StatSoft, Tulsa, OK) was used to for all analyses, with significance set at $P < 0.05$.

There were no significant differences in wet weight, size, number, and hatching rate of eggs among the brooders ($P > 0.05$, Table 1).

No significant differences were detected in the biochemical components of eggs and juveniles ($P > 0.05$, Table 2), although the carbohydrate content in juveniles from the H2 group was higher (Table 2, $P = 0.0074$).

After hatching, a significantly higher larval weight was measured for the H2 and H3 groups ($P < 0.001$). At the end of the trial, juveniles from the H2 group were heavier (1.2 g, $P < 0.001$) (Table 3). SGR was significantly higher for the H1 and H2 groups ($P < 0.001$) (Table 3). No significant differences were detected in survival and feed conversion ratio ($P > 0.05$; Table 3).

For the ammonia test, there was no significant difference in mortality among the juveniles from the three groups (Table 4). A significant positive relationship between carbohydrate content in eggs and juvenile mortality was found ($R^2 = 0.40$; $P = 0.0035$; Fig. 1).

When small fish were challenged with salinity test, significant differences among the tilapia juveniles from the three groups were found (Table 4). Similar mortality trend was observed between the H2 group (40.6%) and the other groups (Table 4).

Table 1. Mean values (±SE) of wet weight, number, hatching rate, major length, minor length, area and volume of eggs of Nile tilapia from different hatcheries.

	Broodstock group		
	H1	H2	H3
Egg weight (g)	0.019 ± 0.10	0.017 ± 0.1	0.01 ± 0. 05
Eggs per spawn	860 ± 68.1	992 ± 60.3	763 ± 66.2
Hatching rate (%)	48.55 ± 1.20	47.65 ± 1.10	48.25 ± 1.00
Major length (mm)	2.73 ± 0.05	2.72 ± 0.08	2.81 ± 0.05
Minor length (mm)	2.21 ± 0.09	2.19 ± 0.09	2.36 ± 0.04
Area (mm^2)	4.71 ± 0.40	4.65 ± 0.24	5.16 ± 0.32
Volume (mm^3)	68.56 ± 7.78	67.77 ± 7.87	77.88 ± 10.28

Table 2. Mean values (± SE) of protein, lipid, and carbohydrate content in eggs and juvenile of Nile tilapia from three (H1, H2 and H3) different hatcheries.

		Broodstock group		
		H1	H2	H3
Protein (mg g^{-1})	Eggs	721.6 ± 20.4	640.1 ± 61.6	739.9 ± 22.1
	Juveniles	417.5 ± 22.7	341.1 ± 61.4	297.9 ± 30.3
Lipid (mg g^{-1})	Eggs	165.3 ± 7.7	129.2 ± 15.4	156.1 ± 30.8
	Juveniles	240.1 ± 10.6	221.3 ± 17.0	235.8 ± 14.6
Carbohydrate (mg g^{-1})	Eggs	7.9 ± 10.50	9.6 ± 1.74	8.3 ± 0.80
	Juveniles	22.1 ± 3.37^b	54.1 ± 9.65^a	25.0 ± 8.62^b

Comparison of different brooders of Nile tilapia *O. niloticus* is not straightforward because many factors affect performance (Osure & Phelps, 2006). It is well accepted that the biochemical composition of eggs depends on diet, age of broodstock, genetic differences and spawning season (Faulk & Holt, 2008). In our study, tilapia broodstock from different hatcheries were fed with a commercial diet adequate for reproduction, which explains the similarity in biochemical composition of eggs from the three broodstock groups, despite the different origin of brooders. Gunasekera *et al.* (1996) found a direct correlation between the dietary protein level of *O. niloticus* broodstock and content of egg protein, with a protein content in eggs of 57.7-60.3%, which is similar to 62.6% reported by El-Sayed & Kawanna (2008), and 63% in our results. In this study, there was no evidence that broodstock groups influenced productive performance of females (number of eggs and hatching rate) and egg quality (weight, size and biochemical content).

Bray & Lawrence (1992) stated that postlarval weight is a reliable parameter to measure quality of juvenile shrimp because physiological differences among populations can be distinguished even at early life stages, which coincides with our results considering that larval weight, final weight of juvenile and SGR varied significantly with the origin of broodstock, just after hatching and at the end of the experiment. Biochemical composition of juveniles can be influenced by age, sex, season and environmental conditions, such as diet (Penney *et al.*, 2006). In our study, better growth occurred in juveniles having the highest content of carbohydrates (H2 group).

The obtained results for the ammonia test (LC_{50} = 35.9 mg L^{-1}) suggest that juvenile Nile tilapia are ammonia tolerant fish (Hargreaves, 1998) compared with sensitive fish, such as the carp *Cirrhinus mrigala* (LC_{50} = 26.4 mg L^{-1}; Das *et al.*, 2004) for the same exposure period (24 h). All challenged fish displayed similar responses to the ammonia test, suggesting that origin of brooders to measure the ammonia resistance is not determinant. The LC_{50} for salinity obtained in our study is difficult to compare with other reports, mainly because most studies tested long term effects of high salinity using gradual acclimatation protocols (Lemarié *et al.*, 2004). The LC_{50} in our study (salinity of 19.5) is close to the 18 of found by Suresh & Lin (1992) for the same species, when directly tested at different salinities. Our results from the salinity stress test showed that the highest mortality occurred in larvae from the H3 group, which is a genetically manipulated broodstock that yields 98-100% males after crossing YY "supermales" with XX normal females (Mair *et al.*, 1997). The osmoregulatory capacity of the H3 group could be affected by sex-linked hormones or other characteristics linked to genetic manipulation (Madsen

Table 3. Zootechnical parameters (Mean ± SE) larvae and juvenile of Nile tilapia from three commercial hatcheries. SGR: specific growth rate, FCR: feed conversion ratio.

	Broodstock group		
	H1	H2	H3
Larvae weight (g)	0.09 ± 0.05^{b}	0.15 ± 0.11^{ab}	0.15 ± 0.05^{a}
Juvenile final weight (g)	0.81 ± 0.34^{b}	1.20 ± 0.06^{a}	0.89 ± 0.40^{b}
SGR	10.03 ± 0.14^{a}	9.40 ± 0.18^{ab}	9.36 ± 0.2^{b}
Survival (%)	62.00 ± 26.00	64.00 ± 9.66	82.67 ± 10.47
FCR	1.57 ± 0.5	1.59 ± 0.6	1.49 ± 0.1

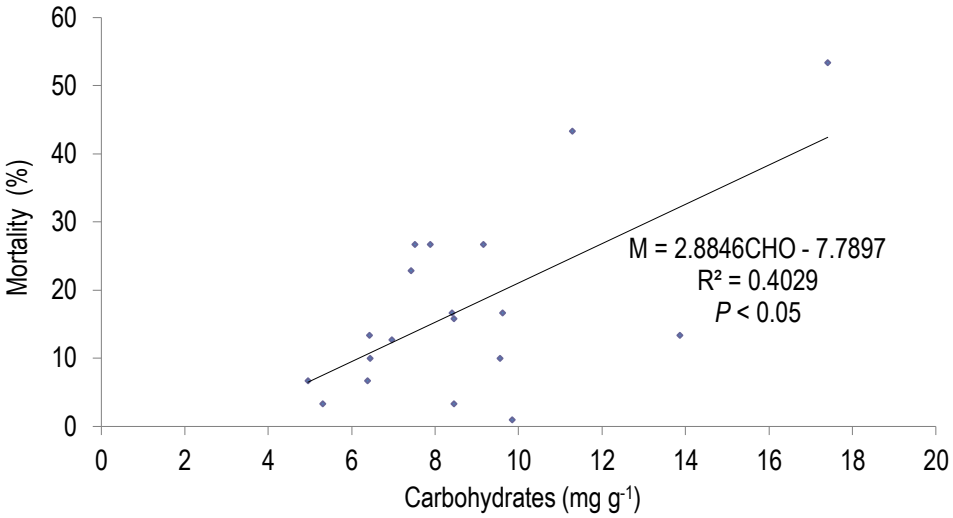

Figure 1. Relationship between juvenile mortality (M) and carbohydrate (CHO) content in eggs of Nile tilapia from three different hatcheries after ammonium stress tests.

Table 4. Mortality mean values (% ± SE) of tilapia juveniles from three different hatcheries after challenge tests (ammonia and salinity).

	Broodstock group		
	H1	H2	H3
Ammonia	17.6 ± 4.3	22.8 ± 8.75	12.6 ± 1.58
Salinity	24.2 ± 12.5^{b}	40.6 ± 7.7^{ab}	57.8 ± 12.5^{a}

& Korsgan, 1991). It is accepted that salinity resistance is related to the interaction of factors such as temperature, pH (Hui *et al.*, 2014) and hardness (Bart *et al.*, 2013), which could affect hatching rate, length, yolk sac size, embryogenesis, survival, growth and oxygen consumption of tilapia (Rodríguez-Montes de Oca *et al.*, 2015; Fridman *et al.*, 2013).

We concluded that juvenile growth, carbohydrate content in eggs and juveniles, and resistance to salinity stress are adequate criteria to assess the quality of eggs and juvenile of Nile tilapia from broodstock of different origin.

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