GROWTH ENHANCEMENT, SURVIVAL AND DECREASE OF ECTOPARASITIC INFECTIONS IN MASCULINIZED NILE TILAPIA FRY IN A RECIRCULATING AQUACULTURE SYSTEM

[MEJORAMIENTO DEL CRECIMIENTO, SUPERVIVENCIA Y DISMINUCIÓN DE INFECCIONES ECTOPARASITARIAS EN CRÍAS DE TILAPIA MASCULINIZADAS EN UN SISTEMA DE RECIRCULACIÓN ACUÍCOLA]

Isabel Jiménez-García[1*], Carlos R. Rojas-García[2], Carlos N. Castro-José[1], Salim Pavón-Suriano[1], Fabiola Lango-Reynoso[1] and Ma. del Refugio Castañeda-Chávez[1]**

[1]Instituto Tecnológico de Boca del Río. Km 12 Carretera Veracruz-Córdoba, CP. 94290, Boca del Río, Veracruz, Mexico. cncj_2448@hotmail.com, salimset@hotmail.com, fabiolalango@yahoo.com.mx, reyda64@yahoo.com.mx
[2]Universidad Católica de Temuco, Casilla 15D, Temuco, Chile. carlosrojas@uct.cl
***Present address: CCMAR Universidade do Algarve, Campus de Gambelas, 8005-139 Faro – Portugal*
**Corresponding Author: isabel_jimenez@fastmail.fm*

SUMMARY

Under lab conditions, tilapia fry at culture densities of 8 fish/L^{-1} can reach a body weight of 0.5 to 1.0 g after the masculinization phase. In commercial hatcheries, the stocking density is four to six times higher, and consequently the occurrence of ectoparasitic infections also rises. The aim of this study was to examine the growth and survival of masculinized Nile tilapia (*Oreochromis niloticus*) fry in a recirculating aquaculture system (RAS). The fry, which were naturally parasitized by protozoan of the genera *Trichodina*, *Ambiphrya* and *Chilodonella*, weighed 0.013 ± 0.003 g and were reared in replicated tanks (N = 3) during 32 days at density of 18 fish/L^{-1} in the RAS to maintain good water quality, which was achieved especially during the first 22 days of fish rearing. The infection parameters and growth were monitored twice a week. The final fish weight was 1.17 ± 0. 6 g and survival 99.5%. The most frequent parasites were *Trichodina* and *Gyrodactylus cichlidarum* (Monogenea). Although nitrogen compounds increased significantly over the last 10 days of fry rearing, final growth and survival were higher than those reported, additionally, the ectoparasitic infections were relatively low.

Key words: *Oreochromis niloticus*; *Gyrodactylus*; *Trichodina*; parasites; tilapia rearing.

RESUMEN

En laboratorio, crías de tilapia a densidad de cultivo de 8 peces/L^{-1} pueden alcanzar peso corporal de 0.5 a 1.0 g después de la fase de masculinización. En criaderos comerciales, la densidad de siembra se eleva cuatro a seis veces, y con ello la ocurrencia de ectoparasitosis. El objetivo del estudio fue examinar el crecimiento y supervivencia de crías de tilapia nilótica (*Oreochromis niloticus*) masculinizadas en un sistema de recirculación acuícola (SRA). Los peces, parasitados originalmente y de forma natural por protozoarios de los géneros *Trichodina*, *Ambiphrya* y *Chilodonella*, pesaron 0.013 ± 0.003 g y fueron criados en tinas replicadas (N = 3) durante 32 días, a densidad de 18 peces/L^{-1} en el SRA para mantener buena calidad del agua, lo que se logró especialmente los primeros 22 días. Los parámetros de infección y el crecimiento fueron monitoreados dos veces por semana. El peso y la supervivencia final de los peces fueron 1.17 ± 0.36 g y 99.5%. Los parásitos más frecuentes fueron *Trichodina* y *Gyrodactylus cichlidarum* (Monogenea). Aunque los compuestos nitrogenados aumentaron significativamente los últimos 10 días de cultivo, el crecimiento y supervivencia final de las crías de tilapia fueron mayores a los reportados, y las parasitosis fueron relativamente bajas.

Palabras clave: *Oreochromis niloticus*; *Gyrodactylus*; *Trichodina*; parásitos; crianza de tilapia.

INTRODUCTION

Stocking density, food, feeding regimes, water quality and photoperiod are all factors that affect the growth of tilapia fry, yet studies of these are not well documented or are controversial (El-Sayed, 2006). In general, it has been said that masculinized tilapia fry should have a mean weight of 0.4 g after 4 weeks of

growth (Rakocy, 2005), or from 0.5 to 1 g (with 20% mortality) after 30 days (Rakocy, 1989).

Despite the potential risk of parasitic infections in pisciculture, information about fluctuations in infection parameters and their effect on the first phases of tilapia fry rearing is scarce, thus the level of risk that cultures are exposed to is unknown. Parasitic loads are assumed to be high during rearing due to the use of management practices that favor them, such as high stocking densities (> 40 fry L^{-1}), which not only increase social stress in fish (El-Sayed, 2006), but also inevitably reduce water quality (Ellis *et al*., 2002) and increase transmission rates of parasites with direct life cycles (Lafferty and Kuris, 1999), such as some ectoparasites. Within this group, protozoans and monogeneans can affect fish growth (Barker *et al*., 2001), and they frequently occur in cultures of tilapia fry (El-Sayed, 2006). In addition, such ectoparasites can be vectors of bacterial diseases that affect the health not only of the fish, but also of consumers (Xu *et al*., 2007), and they might reduce the efficiency of vaccines against *Streptococcus iniae*, a bacteria that causes heavy losses in tilapia cultures (Martins *et al*., 2011). The aim of this research was therefore to examine the growth, mortality and ectoparasitic infections of Nile tilapia (*Oreochromis niloticus*) fry during masculinization.

MATERIALS AND METHODS

The recirculating aquaculture system (RAS) was made of three 18.3 L plastic rectangular tanks, connected to a 60 L up-flow mechanical filter with 11.8 L of gravel (5 to 10 mm ϕ) as the filter medium. Effluent was treated in a trickling biofilter with 20 L of plastic 3-cm diameter biospheres as the attachment substrate for nitrifying bacteria. The water was recirculated through the RAS with a 1000 L/h^{-1} pump, and the culture tanks were continuously aerated. Between 5 and 10 L of water were replaced daily as a result of losses due to evaporation and siphoning of solid waste.

The experimental fish were fry at seven days post-hatching, with an initial weight of 0.013 ± 0.003 g and total length of 0.87 ± 0.07 cm, cultured in a commercial hatchery at high density in a flow-through system. The original batch of fish had been naturally parasitized by ciliated protozoa of the genera *Trichodina*, *Ambiphrya* and *Chilodonella*, and on day 4 of the study an additional species was recorded, the monogenean *Gyrodactylus cichlidarum*.

Nine hundred and ninety fry were placed in the three RAS culture tanks at density of 18 fish/L^{-1} and masculinization and the monitoring of parasitic infections began. For the first 28 days, the fish were fed at 20% of their biomass with meal containing 52% protein and the 17α-methyltestosterone hormone from the manufacturer, and the remaining four days they were fed at 15% of their biomass with tilapia feed containing 32% protein. Food was given from 9:00 a.m. to 5:00 p.m. in nine rations throughout the day. The number of dead fish was recorded daily during feeding times.

A sample of 20 fry from the original batch were examined prior to placing them in the recirculation system to determine the initial parameters of parasitic infection (sampling 1, day 1): prevalence (percentage of fish from a sample positive for a particular parasite species) and mean intensity (mean number of organisms of a particular parasite species per host parasitized with that species in the sample) (Bush *et al*., 1997).

Parasitological evaluations and biometric measurements were performed twice a week. Eight fry were taken from each tank and euthanized with a transverse cut to the head. An optical microscope (4X and 10X) was then used to observe the skin, the fins and smears prepared with mucus from both sides of the body. The type and number of parasites were recorded. The weight was measured using an analytical balance (0.0001 g readability) and total length was measured with a millimeter ruler with the help of a stereo microscope.

Throughout the study, water temperature and oxygen levels were measured daily using a mercury thermometer and a Hanna HI 83203 photometer, respectively. Ammonia, nitrite and nitrate levels in the water were measured every three days with the same photometer. The pH level was recorded weekly with a Hanna pH211 pH meter.

To compare prevalence, X^2 tests were carried out using the Statistica 7 package ($p < 0.05$). An analysis of variance was conducted to evaluate the differences between the three tanks with respect to the weight and size of the fish and the number of parasites. As no significant differences were observed for these variables, the data from the tanks were pooled.

RESULTS AND DISCUSSION

Mean values for the physical and chemical parameters of the culture water are shown in Table 1. Oxygen, temperature and pH were within the optimum levels for rearing Nile tilapia (El-Sayed, 2006; Drummond *et al*., 2009), which stimulates the feeding response and reduces the susceptibility of the fish to disease (Plumb, 1999; Phelps and Popma, 2000). Total ammonia concentration was close to the recommended limit (0.1 mg/L^{-1}) for the first 22 of the 32 days of the culture (0.055 ± 0.058 mg/L^{-1}); however, it increased notably over the final 10 days, and as a result, nitrite and

nitrate levels also increased (Table 1). This situation was apparently due to unexpected changes in the water volume or flow of the RAS associated with greater-than-expected water evaporation during the afternoon-night, such that by the next morning, the volume of water was considerably less. Once this situation was corrected by increasing the monitoring and by replenishing the water in the system, the ammonia level started to decrease. With respect to nitrite, it is a compound that can be highly toxic to fish, especially with increased alkalinity and water temperatures above 30 °C (Emerson *et al*., 1975), conditions that were not present in this study. The tolerance of Nile tilapia to nitrite is also influenced by the size of the fish; fry of 4.4 g have been observed to be more tolerant to nitrite than large fish (90.7 g), and the LC_{50} for 96 h of exposure to nitrites is 81 and 8 mg/L^{-1} for small and large tilapia, respectively (Atwood *et al*., 2001). Although the increase in ammonia and nitrites was significant over the last 10 days of this study, the levels were relatively low and short-lived, such that the fry never stopped eating and ultimately surpassed the weight and survival recorded in the literature for the masculinization period.

Table 1. Values (mean ± SD) of the physical and chemical parameters of the water during the masculinization of Nile tilapia (*Oreochromis niloticus*) fry (32 days). Values are also provided for two periods: days 1 to 22 and days 23 to 32. Brackets indicate minimum and maximum values observed.

	Days 1 to 32	Days 1 to 22	Days 23 to 32
Oxygen (mg/L)	6.20 ± 0.90	6.50 ± 0.62 (4.8-7.6)	5.53 ± 1.10 (4.0-7.20)
Temperature (ºC)	28.7 ± 0.70	28.7 ± 1.0 (26-29.5)	28.8 ± 0.8 (28-30)
pH	7.25 ± 0.50	7.51 ± 0.20 (7.2-7.75)	6.80 ± 0.60 (6.3-7.40)
Ammonia (mg/L^{-1})	0.25 ± 0.33	0.055 ± 0.058 (0-0.15)	0.61 ± 0.34 (0.30-0.99)
Nitrite (mg/L^{-1})	2.54 ± 3.40	0.43 ± 0.41 (0.07-0.90)	5.10 ± 3.91 (1.6-3.90)
Nitrate (mg/L^{-1})	35.06 ± 38.0	9.85 ± 6.73 (0-19.50)	72.90 ± 36.10 (25.3-95.0)

The original batch of fish had three genera of ciliated protozoans from the outset, two of which were only found in the first week of the study, which were *Chilodonella* sp. at sampling 1 (day 1) (prevalence 27%, intensity 3 ± 2 organisms per parasitized fish) and *Ambiphrya* sp. at samplings 1 (day 1) and 2 (day 4) (prevalence 85 and 45%, respectively, intensity 2 ± 1 at both samplings). The most frequent parasites were *Trichodina* sp. and the monogenean *Gyrodactylus cichlidarum*, both of which showed a significant rise ($p < 0.05$) in prevalence starting on day 25 (Figures 1 and 2).

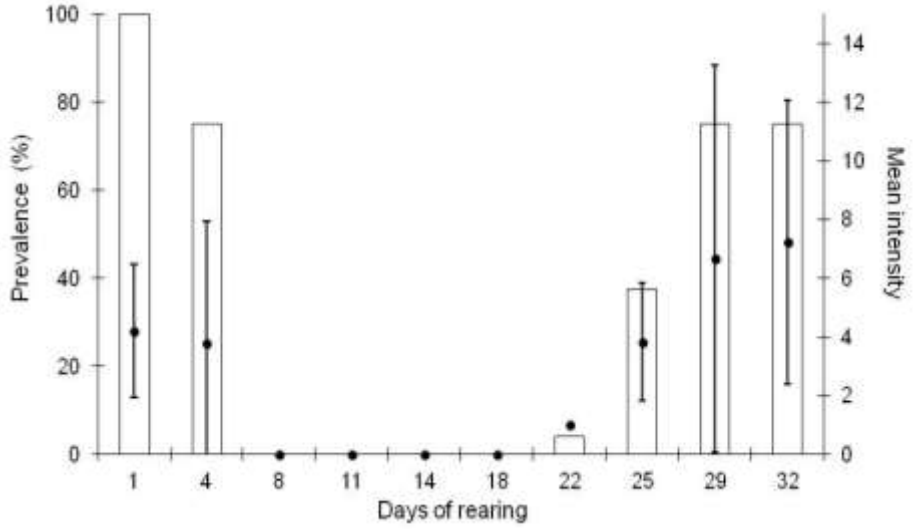

Figure 1. Infection parameters, prevalence (bars) and mean intensity ± SD (points) of *Trichodina* sp. during the masculinization of Nile tilapia (*Oreochromis niloticus*) fry.

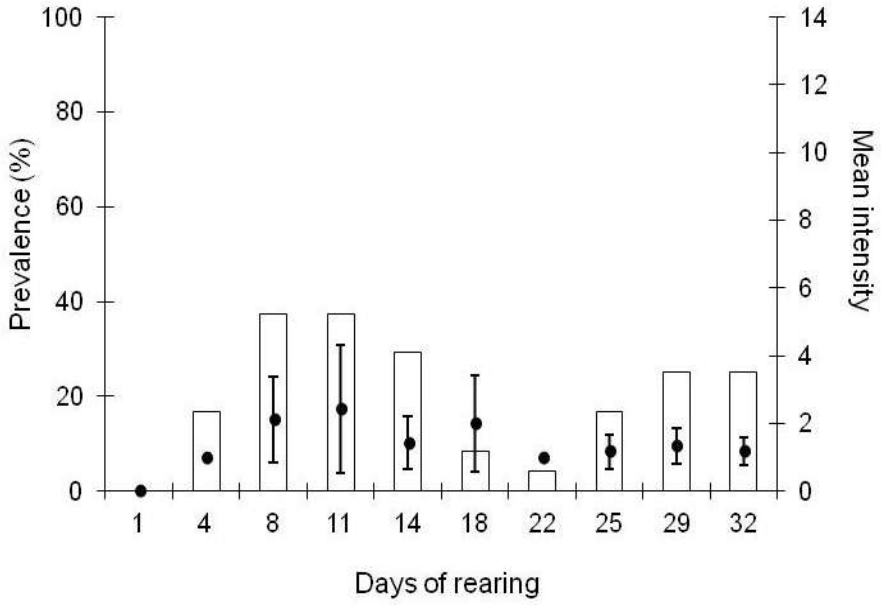

Figure 2. Infection parameters, prevalence (bars) and mean intensity ± SD (points), of *Gyrodactylus cichlidarum* during the masculinization of Nile tilapia (*Oreochromis niloticus*) fry.

The increase in the total number of parasites (*Trichodina* + monogeneans) since day 25 was associated with the increased concentration of ammonia recorded beginning on day 25 of the culture (Figure 3). Infestations of *Trichodina* and monogeneans have been observed to be indicators of deteriorating water quality (e.g., over-population of fish, high ammonia or nitrites, organic pollution or low oxygen) (El-Azez, 1999; Noga, 2000).

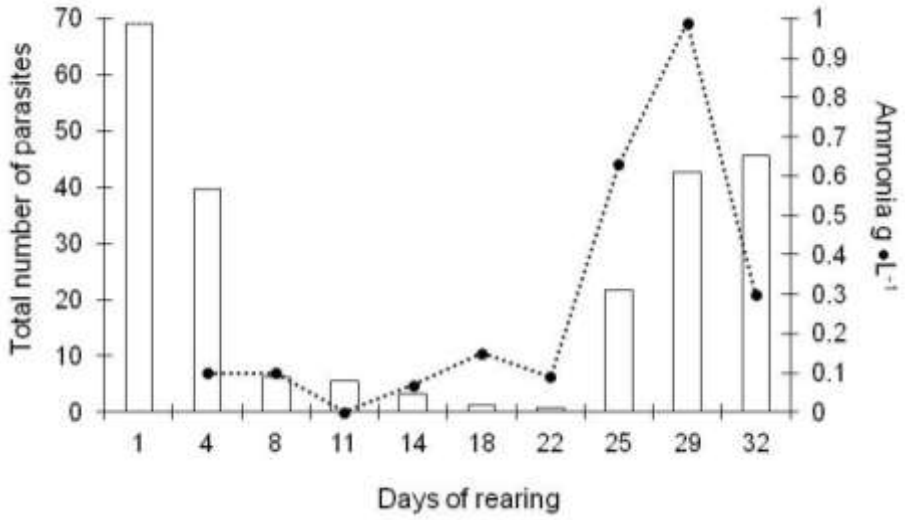

Figure 3. Total number of parasites (protozoans + monogeneans) (bars) and total ammonia concentration (dotted line) in the culture water during the masculinization of Nile tilapia (*Oreochromis niloticus*) fry.

At the end of the study, the fish reached a mean weight of 1.17 ± 0.36 g and a total length of 4.0 ± 0.4 cm (Figure 4), values which are higher than those mentioned by Rakocy (1989) of 0.5 to 1.0 g for a culture density of 8 fry/L^{-1} and than those reported by Popma and Lovshin (1995) of 0.15 to 0.80 g for sex-reversed fry over a 30-day period. The stocking density (18 fry/L^{-1}) probably played an important role in the growth rates, as at a density of 40 fry/L^{-1} the weight, length and survival recorded for sex-reversed

fry cultivated at 28 °C were 0.37 to 0.39 g, 2.67 to 2.78 cm, and 64.7 to 70.6%, respectively (Drummond *et al*., 2009). Lastly, survival (99.5%) was above the 80% reported by Rakocy (1989).

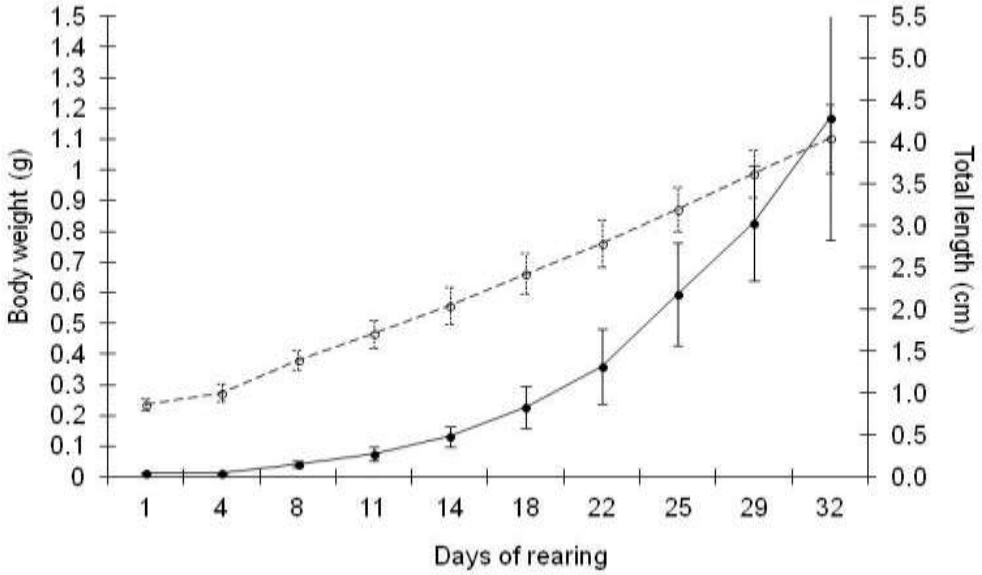

Figure 4. Growth in body weight (dotted line) and total length (solid line) of Nile tilapia (*Oreochromis niloticus*) fry during masculinization. Bars: ± 1 SD.

CONCLUSION

When Nile tilapia fry are cultivated at a density of 18 fish/L^{-1} and with good water quality, they can exceed their expected final weight (1 g), present virtually zero mortality, and naturally control or eliminate their ectoparasites. The rise in parasitic infections associated with a significant increase in nitrogen compounds from days 25 to 32 of the culture reinforces the importance of maintaining these parameters under control.

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