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The effects of stocking density, Tubifex feeding and monosex culture on growth performance of guppy (*Poecilia reticulata*) in a closed indoor recirculation system

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ABSTRACT

The purpose of this study was to determine the optimal stocking density, effects of tubifex (*Tubifex tubifex*) feeding and monosex culture on growth performance of guppy (*Poecilia reticulata*) in a recirculation system. Three separate experiments were conducted in 200-L cylindrical fiberglass tanks, at 1.5/h water turnover rate at 26 °C water temperature. In Experiment 1, the fish (2.71 cm and 0.21 g) reached 4.56–4.99 cm length and 1.28–1.83 g weight at the at end of the culture period of 100 days in four stocking densities (1, 2, 3 and 4 fish/L), and 3 fish/L was found to be the optimal density under the present experimental conditions. In Experiment 2, the fish (2.68 cm and 0.20 g) were fed four different tubifex feeding frequencies (0, 1, 2 and 3 days/week) during a rearing period of 80 days. The growth of fish was increased (4.25–4.47 cm and 0.96–1.26 g) depending on the feeding frequency of tubifex, and the best growth was obtained in 3 days/week tubifex feeding ($P < 0.05$). In Experiment 3, the fish (3.20 cm and 0.27 g) were found to grow significantly better in monosex culture (4.47 cm, 1.09 g) than in the mixed culture (4.32 cm, 1.01 g) during the rearing period of 80 days. Also, in both culture types, the growth performance of females was about twice that of males ($P < 0.05$). In all the three trials, the fish reached to marketable size in as short as 80–100 days with very high survival rates (97–99%) even under the high stocking density of 3 fish/L. The present results are very promising in terms of providing higher yields per unit area as well as raising guppies in subtropical and even temperate climatic regions using closed RAS culture systems that can be applied worldwide.

1. Introduction

With a turnover of US $ 15 billion per year and an annual growth of 14%, ornamental fishery is an important commercial activity and one of the world's most popular hobbies (Tlusty et al. 2013; FAO 2014). This sector continues to contribute to the economic development of under-developed countries, especially in the tropics (Lee and Newman 1997; Paripatananont et al. 1999; Lovell 2000; Gouveia et al. 2003). Among the ornamental fish species, guppy is one of the best known and most commonly marketed fish worldwide after the goldfish (Ghosh et al. 2003; FAO 2014).

Ornamental fish farming is mainly practiced in outdoor ponds in the tropical south-east Asian countries due to climatic advantages. In such traditional farming systems, the yield obtained from guppy as well as some other species is generally low, not > 0.3 fish/L (Fernando and Phang 1985). It is though that recirculation aquaculture systems (RAS) might be more advantages in the production of ornamental fish, as such systems may support higher survivals and growth performance under high fish stocking densities due to easier and better management of water quality. Additionally, RAS technology can enable farmers to produce more uniform size crops, higher yield in less land area and water volume. Although the investment cost of this system is relatively high, this disadvantage can easily be compensated by the fact that the price of ornamental fish is much higher than the fish intended for human consumption. Nonetheless, except for a few countries like Israel, neither guppy nor other aquarium fish are commonly farmed in RAS. Furthermore, no enough data is available so far in the literature concerning RAS aquaculture growth performance of the guppies until marketable size, though Vine and Kaiser (1996) and Kaiser et al. (1998) studied stocking densities of guppies in relation to water turnover rate in closed recirculation systems. Another study on swordtail (*Xiphophorus helleri*), a member of the same family (Poeciliidae) with guppies, dealt with only stocking density and water cycle (Vine and Kaiser 1996; Oliver and Kaiser 1997). Ako et al. (2005) further studied behavioral limitations during the growout of swordtail fish under high stocking densities (1, 3 and 6 fish/L) in recirculation systems.

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Tubifex (*Tubifex tubifex*, Annelida) is a living bait which is widely used in the production of ornamental fish due to its suitable size and availability. It is stable in nutrient components and rich in n-3 (C18: 3n-3 and C20: 5n-3) and n-6 (C18:2n-6 and C20:4n-6) fatty acids (Yanar et al. 2003). This live feed has been proven to enhance growth performance of ornamental fish such as *Chitala chitala* (Sarkar et al. 2006) and *Betta splendens* (Mandal et al. 2010). The red earthworm (*Eisenia fetida*), another annelid species, has been shown to increase growth (Kostecka and Paczka 2006) in guppies, but potential of tubifex has not yet been tested.

Monosex culture is a preferred aquaculture model in some fish species due to differential growth rates between sexes. However, since the guppies are sold in pairs on the market, the essence of our work was not a monogamous aquaculture model in which advantageous sex is preferred, but rather a comparison of mixed cultures with male and female grown separately. In the mixed production, the presence of male and female in the same place accelerate the formation of gametes by the secretion of reproductive hormones, and this inturn slows down the somatic growth (Nishibori and Kawata 1993). On the other hand, rivalry for partnership (sexual selection) also negatively affects growth. Thus, monosex culture saves energy spent on reproduction and competition (Rodgers et al. 2006; Stejskal et al. 2009; Githukia et al. 2015). It is predicted that guppies will exhibit better growth performance with monosex culture by avoiding the above-mentioned negativities in the mixed cultivation.

In this study, three experiments were designed to determine the optimal stocking density, the effects of tubifex feeding, and monosex vs mixed culture model on the rearing performance of guppy under indoor RAS conditions.

2. Materıals and methods

2.1. General culture procedures

In this research, three experiments were carried out to determine optimal stocking density, the effects of tubifex feeding as a supplementary fresh food, and monosex vs mixed culture on the growth performance of the guppy, *Poecilia reticulata*, under indoor RAS culture conditions. The experiments were conducted in Cukurova University, Faculty of Fisheries, Adana - Turkey. A red tail variety of guppies was produced in Freshwater Research and Production Station of the above mentioned faculty for the experiments.

In the experiments, granulated juvenile fish feeds (containing 60% protein and 10% fat) produced by Camlı Feeds Company (İzmir -Turkey) in size ranging from 150 and 1200 μ were used. The fish fed four meals a day to satiation. In order to calculate the FCR separately for each experimental group, at the beginning and end of each sampling period (20 days), a certain amount of feed was weighed into a container allocated to each tank and then the remaining unconsumed feed was measured at the end of the period. All the experiments were performed in a RAS containing a sump tank, a sand filter, a biological filter, all connected with a pump and pipes to 12 round fiberglass tanks (200 L, 70 cm diameter and 65 cm height). Depending on density and size of fish, the tanks were provided with a water cycle of 1.5 turnover rate per h and oxygenation was supported by supplying air. Any remaining feed and feces were siphoned off the bottom of the tanks and 5% freshwater was replaced every day.

The contents of ammonium (NH_4^+-N), nitrit (NO_2^--N) and nitrat (NO_3^--N) in RAS were measured spectrophotometrically (Spectroguant ® NOVA 60 Merck), while water hardness by titrimetric method (APHA 1998) once a week. Water temperature, dissolved oxygen level (Oxyguard Handy Polaris) and pH (with Testo 206) were measured daily. Water quality parameters were; temperature 26.2–26.7 °C, oxygen 6.26–6.62 mg/L, pH 7.61–7.67, ammonium 0.10–0.20 mg/L, nitrite 0.02–0.03 mg/L, nitrate 30.13–56.96 mg/L, and hardness 305 mg $CaCO_3$/L. These water quality parameters are within the optimal range for the fish and did not show fluctuations during the experiments.

Total length and live weights of fish were measured at intervals of 20 days and 30–40 fish samples were used from each tank at each sampling period. T he fish were anesthetised with phenoxyethanol (300 ppm) to facilitate measurements. A 12 h light and 12 h dark photoperiod was applied during the whole experimental periods, ranged between 80 and 100 days, considering the market size of fish (4–6 cm total length and 1–2 g).

Experiments were conducted according to the European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

2.2. Experiment 1 (stocking density)

In order to determine optimal stocking density in RAS system, four experimental groups were formed, namely S1 (1 fish/L), S2 (2 fish/L), S3 (3 fish/L) and S4 (4 fish/L) and the cultivation period lasted for 100 days. The stocking densities of the second and third experiments were adjusted to the optimal stocking rate to be determined in this trial.

2.3. Experiment 2 (tubifex feeding)

To determine the effects of tubifex feeding frequency on growth performance of guppies, the fish were fed continuously with granulated feeds (T0), 1 day tubifex + 6 days granulated feeds (T1), 2 days tubifex + 5 days granulated feeds (T2), 3 days tubifex + 4 days granulated feeds (T3). The fish were stocked as 3 pieces/L and fed four times daily during the experimental period, which lasted for 80 days. Tubifex (0.2–0.3 mm in diameter and 3–4 cm in length) were stored in refrigator at − 20 °C and were thawed before they were given to the fish at each feeding time.

2.4. Experiment 3 (monosex vs mixed culture)

In order to compare culture performance of guppy in monosex and mixed culture, three groups were formed, consisting of a group of totally male (Monosex Male Group), second group of totally female (Monosex Female Group), and the third group 50% male and 50% female (Mixed Culture Group). Fish are stocked in tanks in 3 pieces/L and the culture period lasted for 80 days. Three tanks were allocated for each group (total 9 tanks) in RAS. The fish were fed with granulated feeds to satiation (as described above) four times a day. At 20 days intervals, 40 inviduals from male and females from the monosex culture groups, as well as 40 males and 40 females from the mixed culture group (a total of 80 fish) were separately measured throughout the experiment. The data of monosex groups were pooled together while comparing the results with those of the mixed group. Five different comparisons were made namely as; Monosex (Male + female) vs mixed culture, males vs females in monosex culture, males vs females in mixed culture, males in monosex culture vs males in mixed culture, and females in monosex vs females in the mixed culture. Equal number of indivuals was pooled together to make the above comparisos.

2.5. Statistical analyses

All the data were reported as means ± standard error (s.e.) throughout the text. The data were analyzed by one-way ANOVA at a significance level of $P < 0.05$ following confirmation of normality by Levene's test and homogeneity of variance by Shapiro and Wilk's test. Where significant differences were detected, data were subjected to Duncan post hoc test for identifying any significant difference among the treatment groups. All computations were performed using SPSS20.0 (SPSS Inc., Chicago, IL, USA).