Effects of stocking density on survival, food intake and growth of giant gourami (Osphronemus goramy) larvae reared in a recirculating aquaculture system

Otong Zenal Arifin, Vitas Atmadi Prakoso, Jojo Subagja, Anang Hari Kristanto, Simon Pouil, Jacques Slembrouck

Effects of stocking density on survival, food intake and growth of giant gourami (*Osphronemus goramy*) larvae reared in a recirculating aquaculture system

Otong Zenal Arifin[1], Vitas Atmadi Prakoso[1], Jojo Subagja[1], Anang Hari Kristanto[1], Simon Pouil[2], Jacques Slembrouck[2*]

[1] Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Bogor, Indonesia
[2] ISEM, IRD, Université de Montpellier, CNRS, EPHE, Montpellier, France

* Corresponding author: Jacques Slembrouck
Institut de Recherche pour le Développement (IRD)
Graha Kapital Lantai 4, Jl. Kemang Raya n°4
12730 Jakarta, Indonesia
E-mail: jacques.slembrouck@ird.fr
Phone: +62 (0) 21 71 79 46 51

Abstract

The influence of stocking density on survival, food intake, and larval growth was assessed in giant gourami (*Osphronemus goramy*) larvae reared in an indoor recirculating aquaculture system. Larvae aged eight days post-hatching were arbitrarily divided into six stocking density treatments (A: 0.6, B: 1.2, C: 2.4, D: 4.8, E: 9.6, F: 19.2 individuals L^{-1}; four replicates per treatment) and reared for three weeks. Tubifex worms, used as food, were kept continuously available for larvae. Samples of larvae were collected at days 0, 7, 14 and 21. Performance indicators - including survival rate (%), food intake (% and g ind^{-1}), total length (cm), body weight (g), specific growth rate (g day^{-1}), biomass gain (g L^{-1}), feed conversion ratio (FCR), condition factor (K) and coefficients of variation (%) - were measured. Water quality was checked throughout the experiment and parameters were maintained below critical thresholds for fish. The results showed no effect of stocking density on survival (> 98%) or size heterogeneity, although growth significantly decreased with increasing stocking density. At the end of the 21-day experiment, mean individual body weights were 563.2 ± 64.3, 461.0 ± 28.6, 288.8 ± 19.3, 170.2 ± 13.8, 113.6 ± 6.9 and 81.9 ± 2.3 mg, for groups A, B, C, D, E and F, respectively. Decreased growth may be due to reduction in food intake in larvae stocked at the highest densities. The consequences of intensification of larval rearing should be further investigated in the nursery and grow-out phases.

Keywords: Early life stages, Growth metrics, Rearing practices, Start-feeding, Tropical fish

1. Introduction

The giant gourami (*Osphronemus goramy*; Lacepède, 1801) is one of the main freshwater fish of economic importance in Indonesia. Pond aquaculture of giant gourami in Indonesia is a very old practice (Cuvier and Valenciennes, 1831; Pouil et al., 2019). Its annual production was over 119,000 t in 2014 and, had grown exponentially over the previous 15 years. Yet, for the first time in 2015, Indonesian production of this species, which is pursued by approximately 100,000 fish farmers mainly located on Java Island (79%; Badan Pusat Statistik, 2013), dropped slightly to 113,400 t (FAO, 2017). Nevertheless, knowledge on several aspects of giant gourami biology remains largely incomplete, particularly for the young life-stages.

The reliability of giant gourami aquaculture depends on fry availability, which is a limiting factor for fish farmers. Thus, as for many species, increasing survival during the larval phase should be one of the research priorities (Slater et al., 2018). Nevertheless, studies on rearing giant gourami larvae are scarce in the international literature (Ebrahimi et al., 2010; Amornsakun et al., 2014a, 2014b). Currently, larvae are typically produced under non-optimal conditions (i.e., outdoor ponds, stagnant water, etc.). For this reason, the quantity and the quality of giant gourami juveniles produced are generally low and highly variable (Etoh et al., 2011; Arifin et al., 2013; Nafiqoh and Nugroho, 2013; Budi and Supriyadi, 2015). Improvement of giant gourami juvenile production requires identifying and addressing the factors behind the variability observed in larvae production.

The success of larval production depends on environmental conditions, feeding strategies and rearing practices (Cowan et al., 2000; Kestemont et al., 2003). Among these factors, larval stocking density is known to affect larval performance. Effects of stocking density have been studied in young stages of several freshwater aquaculture species (e.g., El-Sayed, 2002; Sahoo et al., 2004; Keer et al., 2018). Nonetheless, the effects of stocking density on survival and growth may be variable or even contradictory (Niazie et al., 2013), depending on the species, rearing conditions and age of the fish (Saoud et al., 2008). For example, some studies have demonstrated that survival rate and growth are negatively affected by an increase in stocking density (El-Sayed, 2002; Keer et al., 2018; Sahoo et al., 2004) while other studies (Kaiser et al., 1995; Niazie et al., 2013) did not find any effects of stocking density on survival or growth rate. These results highlight the importance of better characterising the effects of stocking density on survival and growth of giant gourami larvae.

In this context, the objective of this study was to assess zootechnical performance through the assessment of survival, food intake, and growth of the larvae of giant gourami reared in a closed recirculating aquaculture system (RAS) at six stocking densities. The range of larval densities (from 0.6 to 19.2 larvae L^{-1} i.e., 150 to 4600 larvae m^{-2}) was selected in accordance with the recommendations of the "BPPSIGN" Centre (West Java Centre for the Development of Giant Gourami Culture) for the lower densities (0.6 to 1.2 larvae L^{-1}, i.e. 150-300 larvae m^{-2}) and extended according to a gradient of increasing production intensification.

2. Materials and methods

2.1. Origin of larvae

Giant gourami broodfish (3-4 years old), belonging to the local "Galunggung" strain (Arifin et al., 2017), were reared in a 200-m^2 outdoor pond at the Research and Development Installation of Germplasm for Freshwater Aquaculture (RIFAFE, Cijeruk, West Java, Indonesia). The broodfish were fed leaves of giant taro (*Alocasia macrorrhiza*) and commercial floating fish feed pellets (32% proteins, 5% lipids) distributed at a daily feeding rate of 1-2% of fish biomass, respectively. The broodfish pond was divided by net into 10 compartments of 16 m^2. Each compartment contained one male and three females. Bamboo nest supports and palm tree fibres were provided so that broodfish could build nests. The natural spawning event occurs once the male has chosen one ready female. After spawning, the male closes the nest and protects the eggs. Nests were monitored every two days, and eggs were removed one night after the spawning event. Thus, the larvae used in this experiment came from a single broodfish pair, allowing us to obtain a homogeneous response to the different stocking density conditions.

The buoyant giant gourami eggs were incubated in an experimental room in a 20-L plastic basin for ± 20 hours at a temperature of 29.0 ± 0.6°C (equivalent ~24 degrees-day). After hatching, larvae (BW: 5.6 ± 0.3 mg and TL: 4.9 ± 0.1 mm, n=30) were kept unfed in the incubation basin (following the typical fish farming practice), until the beginning of the experiment (i.e., 8 days post-hatching, dph).

2.2. Live prey maintenance

In this study, tubifex worms (*Tubifex tubifex*) were used as food according to local and traditional practices for giant gourami larval production described by the Standard National Indonesian (SNI, 2000). The benefits of tubifex worms for growth and survival rate of giant gourami were demonstrated by Lucas et al. (2015). Here, tubifex worms were used as the primary food for the larvae and throughout the fish nursery period between 8 and 30 days of age (i.e., first-feeding started before the yolk-sac depletion, Morioka et al., 2013). New batches of live tubifex worms were purchased weekly and stored in the experimental room (100-L aquarium; daily water change, temperature: 29.0 ± 0.6°C; light/dark cycle: 12:12 h) and kept unfed. To assess their nutritional quality, proximate analyses of tubifex worms were conducted after 3, 10 and 17 days of the experiment according to the procedures described in Cunniff (1999). Moisture was determined by weight loss upon drying at 105°C for 3 h. Crude protein was determined using the standard Kjeldahl procedure (Foss Tecator Kjeltec 8400 and Kjeltec Bucchi); lipid content after acid hydrolysis using the Weibull-Stoldt method (Slembrouck et al., 2018); crude ash by determining residue after heating at 550°C for 4-5 h in a muffle furnace, and crude fibre was determined as follows: macrophytes were extracted with 1.25% H_2SO_4 and

1.25% NaOH, then dried and samples were weighed, incinerated and reweighed. Results are summarised in Table 1.

[Table 1 is here]

2.3. Stocking density experiment

2.3.1. Experimental design

The experiment was conducted indoor under natural light (daylight intensity 60-4500 lux, night light intensity < 11 lux). Larvae were individually counted (n=4536) and measured (n=30, mean body weight: 14.4 ± 0.8 mg; mean total length: 10.1 ± 0.4 mm) and then arbitrarily assigned to 24 glass aquaria covered by transparent plastic sheets (30-L capacity; 40 x 30 x 30 cm, L x W x H) in a RAS. The 21-day experiment was started at 8 dph, a few days after mouth opening (Morioka et al., 2013) and when the ability of larvae to feed on tubifex worms was confirmed. To determine potential effects of stocking density on the zootechnical performance of *O. goramy* larvae, survival, food intake and growth, were evaluated at six different stocking densities as summarised in Table 2. The experiment was conducted as a completely randomized design with four replicates.

[Table 2 is here]

2.3.2. Feeding protocol and water quality monitoring

Larvae were fed every day except on the sampling days. The same quantity of live tubifex worms, carefully drained, was spread in the bottom of each aquarium twice a day at 8:00 and 16:00. Daily food quantities were in large excess for all treatments (Fig. 1) to promote non-limiting food conditions for larvae and to facilitate accurate estimation of ingestion. Through the entire experiment, the total amount of tubifex worms distributed in each aquarium was 465 g. Since tubifex worms are benthic and live on the bottom of the aquaria, they were continuously available for larvae without any degradation of water quality (see Table 3).

[Fig. 1 is here]

Prior to each larval feeding, tubifex worms were collected, rinsed and drained on a 50-µm mesh and weighed (to the nearest 0.1 g). To determine food intake, unconsumed tubifex worms were collected from each aquarium and weighed before the addition of the new ration of worms. The quantity distributed was kept constant in each aquarium. In the RAS, the filtration system consisted of filtration foams as the mechanical filtration medium and BioBall® carriers as bacterial support. Water flow into the rearing tanks was maintained at 33 L h^{-1} for the first four days of the experiment and then at 78 L h^{-1}. Water was added every day to compensate for evaporation and losses when aquaria were cleaned (approx. 5-7% of volume). Water quality was monitored in each aquarium once a week with direct measurements using a multi-parameter probe (Hanna HI 9829) for dissolved oxygen (DO), pH, total dissolved solids (TDS) and turbidity, and then by spectrophotometry analysis (Hanna HI 83399) for $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$. Temperature was monitored twice a day (at 08:00 and 16:00). Since no statistical differences were observed between the six experimental treatments for any of the parameters, data were pooled and are presented in Table 3. All the values indicate that water quality did not deteriorate and corresponded to appropriate rearing conditions for tropical freshwater fishes (Svobododá et al., 1993; Aryani et al., 2017).

[Table 3 is here]

2.3.3. Observations and measurements of larvae

Larvae from the experimental treatments (n=40-240 depending on the tested stocking density) were sampled at 15 dph (day 7), 22 dph (day 14) and 29 dph (day 21) during the experiment. The sample size and sampling frequency were selected in order to sample at least 10% of the total number of larvae at each stocking density while taking into account time needed and technical constraints of sampling procedure as well as stress for the larvae caused by handling. Sampled larvae were anaesthetised and their total body length (TL, mm) was measured under a stereomicroscope with a micrometre (accuracy ranging from 0.05 to 0.1 mm, depending on fish size and magnification). Body weight (BW, mg) was measured using a digital scale with an accuracy of 0.1 mg. After individual measurements, fish were returned to their respective aquarium. No mortality was observed following samplings. At 29 dph, all the aquaria were emptied and living larvae were counted to calculate survival rates.

2.4. Performance metrics

The effects of stocking density on zootechnical performance were determined by calculating the following parameters for each experimental treatment. Survival rates (*SR*), expressed as a percentage, were calculated by comparing the final number (N_f) with the initial number of larvae (N_i): SR (%) = [(N_f / N_i) x 100].

The specific growth in body weight (SGR_{BW}, %) was calculated according to the following equation: SGR_{BW} = [(ln BW_f - ln BW_i) / 21) x 100], where BW_i and BW_f are the initial and final body weights of fish, and 21 is the duration of the experiment in days.

The specific growth in total length (SGR_{TL}, %) was calculated using the same approach, as SGR_{TL} = [(ln LT_f - ln LT_i) / 21] x 100.

Heterogeneity of fish size (in body weight or total length) was assessed using the coefficient of variation (CV, %) calculated as: CV_{BW}= SD_{BW} / BW and CV_{TL}=SD / TL, where SD is the standard deviation for weight and BW is the average body weight (mg) and TL the average body length (mm).

Fish biomass gain per liter (BG, g L^{-1}) was calculated following the equation: $BG = [(N_f\, BW_f - N_i\, BW_i) / 30] / 1000$, where N_i and N_f are the initial number and the final number of larvae, BW_i and BW_f are the initial average body weight (mg) and the final average body weight respectively, and 30 is the volume of the aquarium in litres.

Total food ingestion per treatment (FI_{total}, %) was calculated as follows: FI_{total} = [(Food distributed - Food remaining) / Food distributed] x 100, where food distributed and food remaining are expressed in g.

Individual food intake (FI_{fish}, g ind^{-1}) was calculated according to the following equation: FI_{fish} = (Food distributed - Food remaining) / N_f where N_f is the final number of fish.

Feed conversion ratio (FCR) was calculated using the following equation: $FCR = F / (N_f\, BW_f - N_i\, BW_i$, where F is the total quantity of food intake in wet weight during the whole rearing period. F was determined as the total amount of uneaten food subtracted from the total amount of food provided.

The Fulton's condition factor (K) was calculated according to the relationship $K = BW_f / TL_f^3$ (Froese, 2006). The equation was multiplied by 100 to bring the value close to one. Fulton's condition factor predicts that the weight of a fish is proportional to its length cubed, allowing a direct comparison of nutritional conditions between individuals from the same species (Jin et al., 2015; Allen et al., 2018).

2.5. Statistical analysis

To account account the heterogeneity of the variances between the six experimental treatments due to the deliberately unbalanced sampling plan (see Section 2.3.3), Welch ANOVA (McDonald, 2009) was used to determine significant differences among treatments for growth (body weight and total length) and Fulton's condition factor. When significant differences were detected, the Games-Howell test (McDonald, 2009) with Bonferroni correction was performed to compare means.

For the other metrics used (see Section 2.4), data were first tested for normality (Shapiro's test) and homogeneity of variance (Levene's test) and, where necessary, data were arcsine or log transformed prior to analysis. One-way ANOVA was used to assess significance of differences among treatments. When significant differences were detected, Tukey's test was performed to compare means. The level of significance for statistical analyses was always set to $\alpha = 0.05$. All statistics were performed using R freeware version 3.3 (R Development Core Team, 2016).

3. Results

3.1. Survival rate

The survival rates (*SR*) measured at the end of the experiment were very high (98.6-100 %, Table 4) without any significant differences between the six stocking densities tested ($F = 1.31$, $p = 0.304$). Regardless of the experimental stocking density, larvae exhibited no aggressive behaviour throughout the experiment and no cannibalism was observed.

[Table 4 is here]

3.2. Growth and size heterogeneity

The growth of larvae reared at six different densities is indicated in Table 4. At the end of the experiment, the average total length (TL_f) and body weight (BW_f) of larvae ranged from 17.5 ± 1.0 mm and 81.9 ± 12.3 mg in treatment F and 31.7 ± 1.6 mm and 563.2 ± 90.3 mg in treatment A (Fig. 2). Growth was significantly less with increased stocking density ($F = 833.7$, $p < 0.0001$ and $F = 1382$, $p < 0.0001$ for BW_f and TL_f respectively), with the lowest growth observed for the larvae reared at the highest density (Fig. 2). This trend was quantified by the specific growth rate calculated for both body weight (SGR_{BW}) and total length (SGR_{TL}). Significant decreases in SGR_{BW} ($F = 425.3$, $p < 0.0001$) and SRG_{TL} ($F = 267.6$, $p < 0.0001$) were observed when stocking densities increased, with values varying from 17.5 ± 0.5 % for SGR_{BW} and 5.5 ± 0.2 % for SRG_{TL} in treatment A (0.6 larvae L^{-1}) to 8.3 ± 0.1 % for SGR_{BW} and 2.6 ± 0.0 % SRG_{TL} in treatment F (19.2 larvae L^{-1}). In this experiment, the commercial fry size ("Nguku", i.e., fish >2 cm in total length) was reached after 14 days of rearing (22 dph) for larvae reared at the lowest stocking densities (A: 24.0 ± 1.3 mm, B: 22.8 ± 1.24 mm and C: 21.5 ± 1.0 mm), whereas larvae from treatments D and E reached fry commercial size only seven days later (TL_D: 22.5 ± 1.6 mm and TL_E: 20.2 ± 1.1 mm). At the highest stocking density, the larvae had not reached the commercial size (TL_F: 17.5 ± 1.0 mm) even after 21 days of culture (Fig. 2). Although growth was reduced at high stocking densities, the biomass gain (*BG*), ranging from 0.33 ± 0.04 to 1.28 ± 0.04 g L^{-1}, increased significantly ($F = 155.3$, $p < 0.0001$) with increasing stocking density (Table 4).

Size heterogeneity as a function of stocking density was assessed at the end of the experiment (i.e., 29 dph) by the coefficients of variation for body weight (CV_{BW}) and total length (CV_{TL}). CV_{BW} ranged from 10.5 ± 3.0 to 19.9 ± 6.5% and CV_{TL} ranged from 3.9 ± 1.6 to 6.0 ± 2.0%. For CV_{BW} and CV_{TL}, no significant differences were found between any of the groups ($F = 1.974$, $p = 0.132$ and $F = 1.302$, $p = 0.307$) for CV_{BW} and CV_{TL} respectively (Table 4).

The Fulton's condition factors (*K*) estimated at the end of the experiment ranged from 1.38 to 1.77. Statistical analysis revealed significant decrease in *K* with increased stocking density ($F = 131.18$, $p < 0.0001$). Nevertheless, no statistical difference was found between treatments C, D and F (Table 4).

[Fig. 2 is here]

3.3. Food intake

The proportion of the total distributed tubifex worms (FI_{total}, %) effectively ingested in each aquarium was not affected by the stocking density ($F = 0.627$, $p = 0.681$) and remained similar in the six treatments (61-67%, Table 5). Furthermore, the minimum quantities of tubifex worms remaining at the end of each feeding period were never less than 6%. On the other hand, the total individual food intake (FI_{fish}, g ind^{-1}) during the 21 day the experiment was greatly affected by the stocking density (F=1857, $p < 0.0001$; Table 5 and Fig. 1). For the entire experiment, the highest individual food intake was 17.2 ± 0.82 g ind^{-1} for treatment A and only 0.60 ± 0.02 g ind^{-1} for treatment F. Similarly, the feed conversion ratio (*FCR*) was the highest at the lowest stocking density (i.e. treatment A, 31.6 ± 4.3) and decreased significantly ($F = 79.29$, $p < 0.0001$), with lowest values (8.3 ± 0.3) for the highest density treatment (F). The relationship between *FCR* and stocking densities was fitted using a single-component exponential model (R^2=0.94, $p < 0.0001$): $y = 24.14\ x^{-0.403}$ (Fig. 3).

[Fig. 3 is here]

[Table 5 is here]

4. Discussion

4.1. Effects of stocking density on survival rate and growth

There are few studies testing the influence of stocking density on survival and growth of giant gourami larvae. Moreover, the density ranges tested were often narrow (e.g. 0.3 to 0.7 fish L^{-1}, Ebrahimi et al., 2010; and 2.5 to 10 fish L^{-1}, Sarah et al., 2009). The present study provides quantification of the effects of stocking density on larvae considering a wider range of density (0.6 to 19.2 fish L^{-1}). The lowest stocking density treatments (i.e., 150-300 larvae m^{-2} or 0.6-1.2 fish L^{-1}) were based on the current recommendations from the "BPPSIGN" Centre.

Increasing intensification was applied until reaching densities 6-fold higher than what is observed among the fish farmers.

First, we compare our results to larval production reported in an on-farm survey of 39 small-scale farms and two training centers that produced “Nguku” in West Java province (mainly located in Bogor and Tasikmalaya districts) carried out in November 2016. Overall, the growth of the larvae reared in our recirculating aquaculture system (RAS) was higher than those reported from small-scale farms, where 22 to 90 days were needed to reach the 2-cm commercial size at stocking densities ranging from 111 to 714 larvae m^{-2} in small, stagnant outdoor ponds based on the farmer’s responses (n = 20, unpublished data). In addition, we observed very high survival rates for all the stocking densities (>98%) much higher than those reported by Javanese fish farmers (0-98% and 50% on average, n = 23 farmers) or in previous experiments (e.g., Verawati et al., 2015). Regardless of the experimental stocking density, larvae exhibited no aggressive behaviour throughout the experiment and no cannibalism was observed.

Overall, we observed high growth rates for giant gourami. Indeed, although we found similar results for higher stocking densities, Sarah et al. (2009) observed about 50% lower growth when larvae were maintained at 5 fish L^{-1} compared to our findings. Altogether, our results suggest that (1) larvae were reared under appropriate environmental conditions, and (2) RAS is a suitable method for improving juvenile production in giant gourami aquaculture.

We found that increasing stocking density had negative effects on the growth of giant gourami larvae. These results are in accordance with those of Sarah et al. (2009). Conversely, Ebrahimi et al. (2010) reported that very low stocking densities (< 0.7 fish L^{-1}) had no effect on the growth of young-stage giant gourami. These findings suggest that the lowest density tested in our study (0.6 fish L^{-1}) was the minimum value to detect effects of stocking density.

Several interpretations have been offered to explain the effects of stocking density on growth and survival in fish. In Reba carp *Cirrhinus reba* fry, lower survival rates observed at high stocking densities were attributed to stronger competition for food and space as well as increased stress (Keer et al., 2018). On the other hand, stocking density showed no negative effect on survival and growth in marbled spinefoot *Siganus rivulatus* juveniles, a result credited to the maintenance of water quality within the tolerance range for this species (Saoud et al., 2008). European perch *Perca fluviatilis* and European seabass *Dicentrarchus labrax* showed contrasting results for growth and survival depending on the life-stage considered (larvae and post-larvae) with regard to the occurrence of cannibalism (Kestemont et al., 2003). In the present study, water quality remained constant throughout the experiment and was not affected by stocking density. Survival was very high and did not vary significantly between stocking densities tested. Not surprisingly for a non-aggressive fish such as the giant gourami, no cannibalism was observed, likely contributing to the homogeneity of larval size at each stocking density (i.e. CV_{TL} = 4-6%; CV_{BW} = 13-20%). These results accord with those of a previous study on European perch *P. fluviatilis* (Król and Zieliński, 2015) larvae. However, the effects of stocking density on survival and growth are species-dependent (Huang and Chiu,1997; Szkudlarek and Zakęś, 2007), and results can vary for a given species (e.g., Baras et al., 2003; Król and Zieliński, 2015). At the end of the experiment, the Fulton's condition factors (*K*) decreased significantly for the four highest densities tested, suggesting that the larvae stocked in these experimental treatments were under poorer nutritional conditions. All together, these results indicate that, in the giant gourami, the decrease in larval growth due to stocking density is very likely related to lower food intake.

4.2. Effects of density on food intake and FCR

The effects of stocking density on food intake were assessed using tubifex worms as living prey. This live food source is commonly used to feed various fish species (Ravichandra Reddy et al., 1977; Le Thanh et al., 1999; Malla and Banik, 2015). Due to their high levels of protein and lipids (Bardach et al., 1972; Table 1) and their aquatic lifestyle, tubifex worms ensure an adequate nutritional intake for fish larvae without causing significant effects on water quality.

In the present study, we showed that the proportion of the total distributed tubifex worms effectively ingested in each aquarium did not vary with stocking density, indicating a large decrease in the quantity ingested by individual larvae with increasing stocking density (Table 5). Nevertheless, despite a reduction in individual food intake and slower growth at the highest stocking densities, we found a significant negative relationship between *FCR* and stocking density, indicating that the higher the larval density, the lower the *FCR*. Using a wider range of stocking densities, we confirmed the findings of Sarah et al. (2009), who found a linear decrease of *FCR* in giant gourami larvae with increasing stocking density ranging from 2.5 to 10 fish L^{-1}. Because ingested food quantities remained stable (61-67%) across experimental treatments, the decrease in *FCR* can be explained by the significant increase in biomass gain (BG) observed for the highest stocking densities.

Effects of stocking density on *FCR* in fish vary greatly. For instance, in marbled spinefoot *S. rivulatus* juveniles fed commercial pellets, no significant effect of stocking density on *FCR* was found (Saoud et al., 2008). However, Niazie et al. (2013) and Keer et al. (2018) reported significant increases of *FCR* at higher stocking densities with juvenile Reba carp and goldfish *Carassius auratus* fed compounded feed. Such findings suggest that effects of stocking density are species-dependent. In addition, in the studies mentioned above, fish were fed using calculated food rations throughout the experiment, but not until satiation. Furthermore, in most of the studies, *FCR* calculations are based on the quantity of food distributed and not on actual consumption (Saoud et al., 2008; Niazie et al., 2013; Keer et al., 2018); it can be difficult to accurately estimate consumption using live prey or small inert food particles. Nevertheless, indirect food consumption estimates can lead to experimental bias for the quantification of *FCR* (Slembrouck et al., 2009) that may potentially explain the differences observed regarding the effects of stocking density on the *FCR* and more generally the food intake in fish larvae.

In fish, decreased food intake often is associated with increased stress (Saoud et al. 2008; Moradyan et al., 2012). When food is present in excess, water quality is affected, indirectly causing the decrease in the growth and survival rates of farmed fish (e.g., Werner and Blaxter, 1980; Puvanendran and Brown, 1999). In the present study, although larvae were voluntarily maintained in non-limiting, surplus-food conditions, water quality of the recirculating aquaculture system remained satisfactory throughout the experiment. Our results suggest that the water renewal (110 to 260% per hour) was sufficient to ensure no experimental bias due to poor water quality. Although tubifex worms were constantly available, they clustered on the bottom of the aquaria, which can limit food intake for some species of fish due the potential difficulty of engulfing such large quantities of food, as was shown for walking catfish *Clarias batrachus* (Dey et al., 2016). However, giant gourami larvae and juveniles have relatively small mouths and ingest tubifex worms one by one; they did not appear to be bothered by the clusters of tubifex worms and showed no aggressive behaviour. For these reasons, we assume that there was no stress regarding food accessibility, contrary to reports in rainbow trout *Oncorhynchus mykiss* (Ellis et al., 2002; North et al., 2006). Thus, in our experiment, increasing the number of fish per unit volume led to the reduction of space availability for each individual and likely acted as a direct stressor for larvae, limiting their ingestion of food. Nevertheless, measurements of physiological indicators of stress (e.g., haematocrit, lysozyme activity or plasma cortisol; Ellis et al., 2002; North et al., 2006) are needed to test this assumption.

Interestingly, we observed a drastic drop in *FCR* when the lowest density doubled from 0.6 to 1.2 fish L^{-1}. Similar findings were highlighted by in Nile tilapia *Oreochromis niloticus* fry fed experimental feed to apparent satiation (El-Sayed, 2002). In that study, *FCR* was not significantly affected by stocking density, except at the lowest densities, suggesting that the decrease in *FCR* may have been due to: (1) the lack of competition for food, or (2) the difficulties in catching food particles that were flushed out with the water outflow (thus leading to biased estimation of *FCR*). However, in the present study, no evidence of competition for food was shown at any stocking density. In addition, the *FCR* values calculated in the present study were based on real consumption of food by larvae because tubifex worms aggregated on the bottom of the aquarium, thereby avoiding loss of prey with water outflow. In our case, the difference in biomass gain (BG) observed between the two lowest stocking density conditions (± 60%) caused the drastic drop in *FCR*. Nevertheless, the reasons explaining this remarkable difference in gain in biomass remain uncertain. Further investigations are needed to better understand the growth dynamics of giant gourami larvae raised at low stocking densities, which is likely to affect the commercial production of juveniles.

5. Conclusion

The production of giant gourami fry is highly segmented, and the "Nguku" stage is the best-selling, although there are at least three intermediate stages that are also traded locally. Currently, there are no clear and standardized production methods. This study provides new information regarding the effects of stocking density on giant gourami larval production. The experiment was performed in a recirculating aquaculture system, ensuring constant water quality, easier control of feeding, and hence better control over the seedstock production process than traditional practices. We showed that stocking density has no effect on larval survival during the 8-29 dph period. Nevertheless, growth was strongly affected by stocking density. Thus, for a given surface area, although larvae production at low density limits the number of saleable fish, it reduces the time necessary to reach commercial size. On the other hand, higher densities produced increased numbers of fish, but lengthened the duration of larval rearing. Stocking density is therefore a key factor to take into account in the production of giant gourami juveniles. Further investigations are necessary to determine: (1) the effects of these strategies on the nursery and grow-out phases, and (2) why FCR decreases at low stocking densities in order to provide objective recommendations for fish farmers.

Acknowledgements

The authors acknowledge Imam Akhir Abdullah, technician at the Cijeruk research station, for his technical assistance throughout the experiment and Marc Legendre (IRD-ISEM) for his constructive input on this manuscript. This is publication ISEM 2019-090.

References

Allen, H., Sabetian, A., Bader, M., Rowlands, T., 2018. Influence of age, size and condition factor on lipid concentration in the snapper *Chrysophrys auratus*. New Zeal. J. Mar. Fresh. 52, 295-307.

Amornsakun, T., Kullai, S., Hassan, A., 2014a. Some aspects in early life stage of giant gourami, *Osphronemus goramy* (Lacepède) larvae. Songklanakarin J. Sci. Tech. 36, 493-498.

Amornsakun, T., Kullai, S., Hassan, A., 2014b. Feeding behavior of giant gourami, *Osphronemus goramy* (Lacepède) larvae. Songklanakarin J. Sci. Tech. 36, 261-264.

Arifin, O.Z., Imron, I., Muslim, N., Hendri, A., Aseppendi, A., Yani, A., 2017. Karakteristik fenotipe dan genotipe ikan gurami, *Osphronemus goramy*, strain galunggung hitam, galunggung putih, dan hibridanya. Jurnal Riset Akuakultur 12, 99-110.

Arifin, O.Z., At-thar, M.H.F., Nafiqoh, N., 2013. Pengaruh induk dan heterosis karakter pertumbuhan hasil persilangan intraspesifik gurame bastar dan bleuesafir (*Osphronemus goramy*). Prosiding Forum Inovasi Teknologi akuakultur, Lombok, Indonesia. (in Indonesian)

Aryani, N., Mardiah, A., Syandri, H., 2017. Influence of feeding rate on the growth, feed efficiency and carcass composition of the giant gourami (*Osphronemus goramy*). Pak. J. Zool. 49, 1775-1781.

Badan Pusat Statistik, 2013. Jumlah rumah tangga usaha budidaya bukan ikan hias menurut wilayah dan jenis ikan utama. https://st2013.bps.go.id/dev2/index.php/site/tabel?tid=57&wid=3200000000 (accessed 01 May 2018). (in Indonesian)

Baras, E., Kestemont, P., Mélard, C., 2003. Effect of stocking density on the dynamics of cannibalism in sibling larvae of *Perca fluviatilis* under controlled conditions. Aquaculture 219, 241-255.

Bardach, J.E., Ryther, J.H., McLarney, W.O., 1972. Culture of true eels (*Anguilla* sp.), in: Bardach, J.E., Ryther, J.H., McLarney, W.O. (Eds.), Aquaculture: The Farming and Husbandry of Freshwater and Marine Organisms. Wiley-Interscience, New-York, pp. 385-395.

Budi, D.S., Suprayudi, M.A., 2015. Growth response and feed utilization of giant gourami (*Osphronemus goramy*) juvenile feeding different protein levels of the diets supplemented with recombinant growth hormone. HAYATI J. Biosci. 22, 12-19.

Cowan, J.H., Rose, K.A., DeVries, D.R., 2000. Is density-dependent growth in young-of-the-year fishes a question of critical weight? Rev. Fish Biol. Fisher. 10, 61-89.

Cunniff, P. 1999. Official Methods of Analysis of AOAC International 16th ed.. AOAC, Washington, DC.

Cuvier, G., Valenciennes, A., 1831. Histoire naturelle des poisons : Tome septième, Levrault FG, Paris, France. (in French)

Dey, A., Ghosh, K., Hazra, N., 2016. Evaluation of preference of dry feed, bio-encapsulated and bio-encapsulated live feed and survival of the walking catfish *Clarias batrachus* (L.) juveniles. Int. J. Fish Aquat. Stud. 4, 545-549.

Ebrahimi, M.H., Imanpour, M.R., Adlou, M.N., 2010. Effects of stocking density on growth factors, survival and blood and tissue parameters, in giant gourami (*Osphronemus goramy* Lacepède, 1801). Journal of Fisheries 14, 97-105.

Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D., 2002. The relationships between stocking density and welfare in farmed rainbow trout. J. Fish Biol. 6, 493-531.

El-Sayed, A.F.M., 2002. Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. Aquac. Res. 33, 621-626.

Etoh, S., Putra, H.G.P., Carman, O., 2011. Growth and survival of giant gourami juvenile immersed in different doses of recombinant growth hormone. Jurnal Akuakultur Indonesia 10, 99-105.

FAO, 2017. FishStatJ: software for fishery statistical time series. Food and Agriculture Organization, Rome, Italy.

Froese, R., 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22, 241-253.

Huang, W.-B., Chiu, T.-S., 1997. Effects of stocking density on survival, growth, size variation, and production of Tilapia fry. Aquac. Res. 28, 165-173.

Jin, S., Yan, X., Zhang, H., Fan, W., 2015. Weight–length relationships and Fulton’s condition factors of skipjack tuna (*Katsuwonus pelamis*) in the western and central Pacific Ocean. PeerJ 3, e758.

Kaiser, H., Weyl, O., Hecht, T., 1995. The effect of stocking density on growth, survival and agonistic behaviour of African catfish. Aquacult. Int. 3, 217-225.

Keer, N.R., Datta, M.K., Patel, A.B., Priyanka, R., Rathor, M.K., Das, S., 2018. Effect of stocking density on growth and survival of *Cirrhinus reba* (Hamilton, 1822) during spawn to fry nursing (outdoor). J. Entomol. Zool. Stud. 6, 640-645.

Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., Baras E., 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: iotic and abiotic influences. Aquaculture 227, 333-356.

Król, J., Zieliński, E., 2015. Effects of stocking density and weaning age on cannibalism, survival and growth in European perch *Perca fluviatilis* larvae. Pol. J. Natur. Sc. 30, 403-415.

Le Thanh, H., Tam, B.M., Cacot, P., Lazard, J., 1999. Larval rearing of the Mekong catfish, *Pangasius bocourti* (Pangasiidae, Siluroidei): Substitution of *Artemia nauplii* with live and artificial feed. Aquat. Living Resour. 12, 229-232.

Lucas, W.G.F., Kalesaran, O.J., Lumenta, C., 2015. Pertumbuhan dan kelangsungan hidup larva gurami (*Osphronemus gouramy*) dengan pemberian beberapa jenis pakan. Jurnal Budidaya Perairan 3, 19-28. (in Indonesian)

Malla S., Banik S., 2015. Production and application of live food organisms for freshwater ornamental fish larviculture. Adv. Biores. 6, 159-167.

McDonald, J.H. 2009. Handbook of Biological Statistics Second Edition. Sparky House Publishing, Baltimore, MD.

Morioka, S., Vongvichith, B., Phommachan, P., Chantasone, P., 2013. Growth and morphological development of laboratory-reared larval and juvenile giant gourami *Osphronemus goramy* (Perciformes: Osphronemidae). Ichthyol. Res. 60, 209-217.

Moradyan, H., Karimi, H., Gandomkar, H.A., Sahraeian, M.R., Ertefaat, S., Sahafi, H.H., 2012. The effect of stocking density on growth parameters and survival rate of rainbow trout alevins (*Oncorhynchus mykiss*). World J. Fish Mar. Sci. 4, 480-485.

Nafiqoh, N., Nugroho, E., 2013. Performa pertumbuhan ikan gurame pada tahap pendederan di hatcheri tertutup. Prosiding Forum Inovasi Teknologi Akuakultur, Lombok, Indonesia. (in Indonesian)

Niazie, E.H.N., Imanpoor, M., Taghizade, V., Zadmajid, V., 2013. Effects of density stress on growth indices and survival rate of goldfish (*Carassius auratus*). Glob. Vet. 10, 365-371.

North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J., Bromage, N.R., 2006. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 255, 466-479.

Pouil, S., Samsudin, R., Slembrouck, J., Sihabuddin, A., Sundari, G., Khazaidan, K., Kristanto, A.H., Pantjara, B., Caruso, D., 2019. Nutrient budgets in a small-scale freshwater fish pond system in Indonesia. Aquaculture 504, 267-274.

Puvanendran, V., Brown, J.A., 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture 175, 77-92.

Ravichandra Reddy, S., Shakuntala, K., Rajagopal, K.V., 1977. Preliminary studies on the conversion of *Tubifex tubifex* as food by elvers of *Anguilla nebulosa* (Gray and Hardwicke). J. Fish Biol. 11, 279-281.

R Development Core Team, 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sahoo, S.K., Giri, S.S., Sahu, A.K., 2004. Effect of stocking density on growth and survival of *Clarias batrachus* (Linn.) larvae and fry during hatchery rearing. J. Appl. Ichthyol. 20, 302-305.

Saoud, I.P., Ghanawi, J., Lebbos, N., 2008. Effects of stocking density on the survival, growth, size variation and condition index of juvenile rabbitfish *Siganus rivulatus*. Aquacult. Int. 16, 109-116.

Sarah, S., Widanarni, W., Sudrajat, A.O., 2009. Pengaruh padat penebaran terhadap pertumbuhan dan kelangsungan hidup benih ikan gurame (*Osphronemus goramy* Lac.) (in indonesian). Jurnal Akuakultur Indonesia 8, 199-207.

SNI, 2000. SNI 01-6485.3-2000: Produksi benih ikan gurame (*Osphronemus goramy*, Lac) kelas benih sebar. Badan Standardisasi Nasional (SNI), Jakarta, Indonesia. (in Indonesian)

Slater, M., D'Abramo, L., Engle, C.R., 2018. Aquaculture research priorities for the next decade: A global perspective. J. World Aquac. Soc. 49, 3-6.

Slembrouck, J., Baras, E., Subagja, J., Hung, L.T., Legendre, M., 2009. Survival, growth and food conversion of cultured larvae of *Pangasianodon hypophthalmus*, depending on feeding level, prey density and fish density. Aquaculture 294, 52-59.

Slembrouck, J., Samsudin, R., Pantjara, B., Sihabuddin, A., Legendre, M., Caruso, D., 2018. Choosing floating macrophytes for ecological intensification of small-scale fish farming in tropical areas: A methodological approach. Aquat. Living Resour. 31:1-9.

Svobododá, Z., Lloyd, R., Máchová, J., Vykusová, B., 1993. Water quality and fish health. European Inland Fisheries Advisory Commission (EIFAC), Food and Agriculture Organization (FAO), Technical Paper, Roma, Italy.

Szkudlarek, M., Zakęś, Z., 2007. Effect of stocking density on survival and growth performance of pikeperch, *Sander lucioperca* (L.), larvae under controlled conditions. Aquacult. Int. 15, 67-81.

Verawati, Y., Muarifl, M., Mumpunil, F.S., 2015. Pengaruh perbedaan padat penebaran terhadap pertumbuhan dan kelangsungan hidup benih ikan gurami *(Osphronemus gouramy)* pada sisteivi resirkulasi. Jurnal Mina. Sains. 1, 6-12. (in Indonesian)

Werner, R.G., Blaxter, J.H.S., 1980. Growth and survival of larval herring (*Clupea harengus*) in relation to prey density. Can. J. Fish Aquat. Sci. 37, 1063-1069.

Figure captions

Figure 1. Total food intake (g $fish^{-1}$ wet wt) by giant gourami larvae reared at six different stocking densities A-F (A=0.6, B=1.2, C=2.4, D=4.8, E=9.6, and F=19.2 fish L^{-1}; i.e. A=150, B=300, C=600, D=1200, E=2400, and F=4800 fish m^{-2}, n=4) for 21 days. Bars show the proportion of tubifex worms eaten by the larvae (white) and the uneaten fraction (black). Values are means ± SD. Different letters denote significant differences between fish stocking densities ($p < 0.05$).

Figure 2. Total length (a) and body weight (b) of giant gourami larvae (n=40-240) at 15, 22 and 29 days post-hatching (dph) in the six stocking density treatments A=0.6, B=1.2, C=2.4, D=4.8, E=9.6, and F=19.2 fish L^{-1}; i.e., A=150, B=300, C=600, D=1200, E=2400, and F=4800 fish m^{-2}). Box-plots show the interquartile range, median (horizontal line), minimum and maximum values (whiskers). Different letters denote significant differences between fish stocking densities ($p < 0.05$).

Figure 3. Relationship between feed conversion ratio (*FCR*) and stocking densities of giant gourami larvae at 29 days post-hatching (dph) reared at six stocking density treatments (A=0.6, B=1.2, C=2.4, D=4.8, E=9.6, and F=19.2 fish L^{-1}; i.e., A=150, B=300, C=600, D=1200, E=2400, and F=4800 fish m^{-2}, n=4). Values are means ± SD.

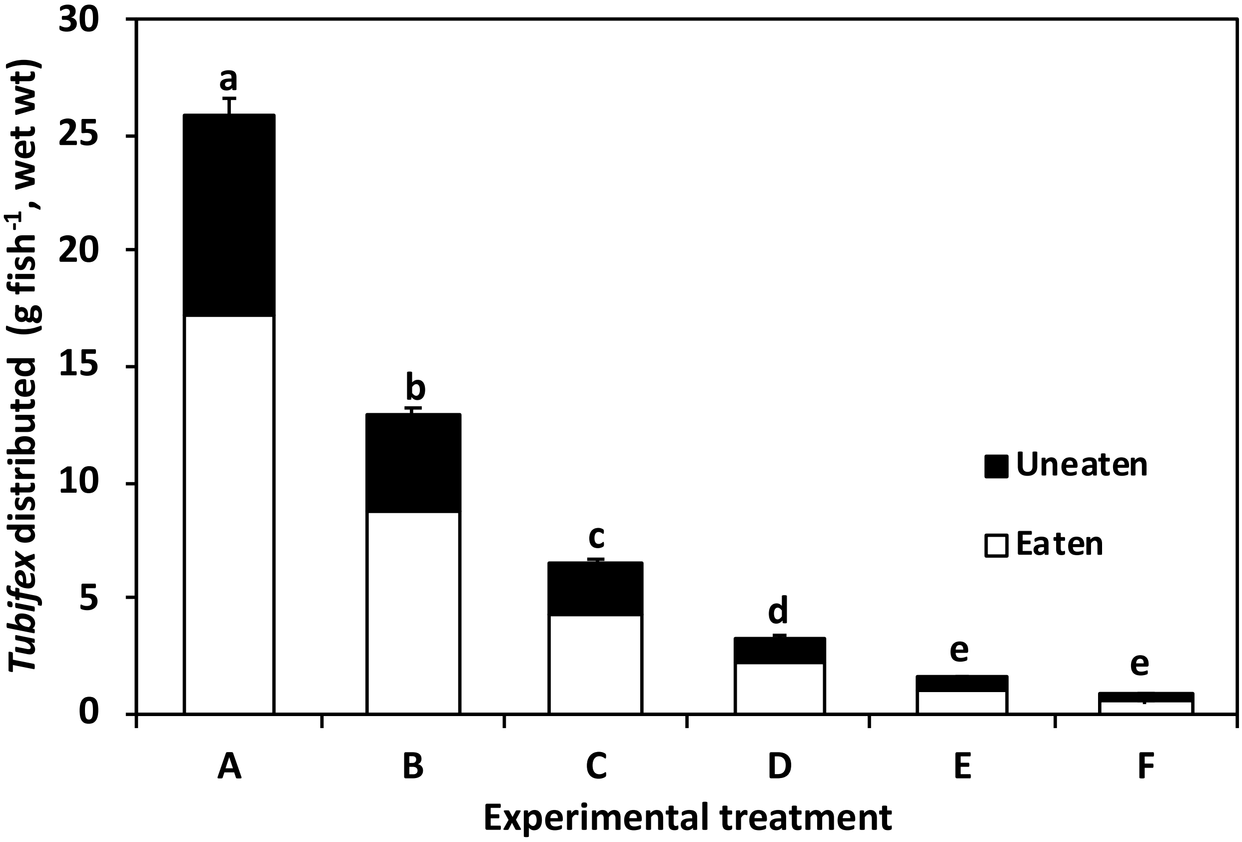
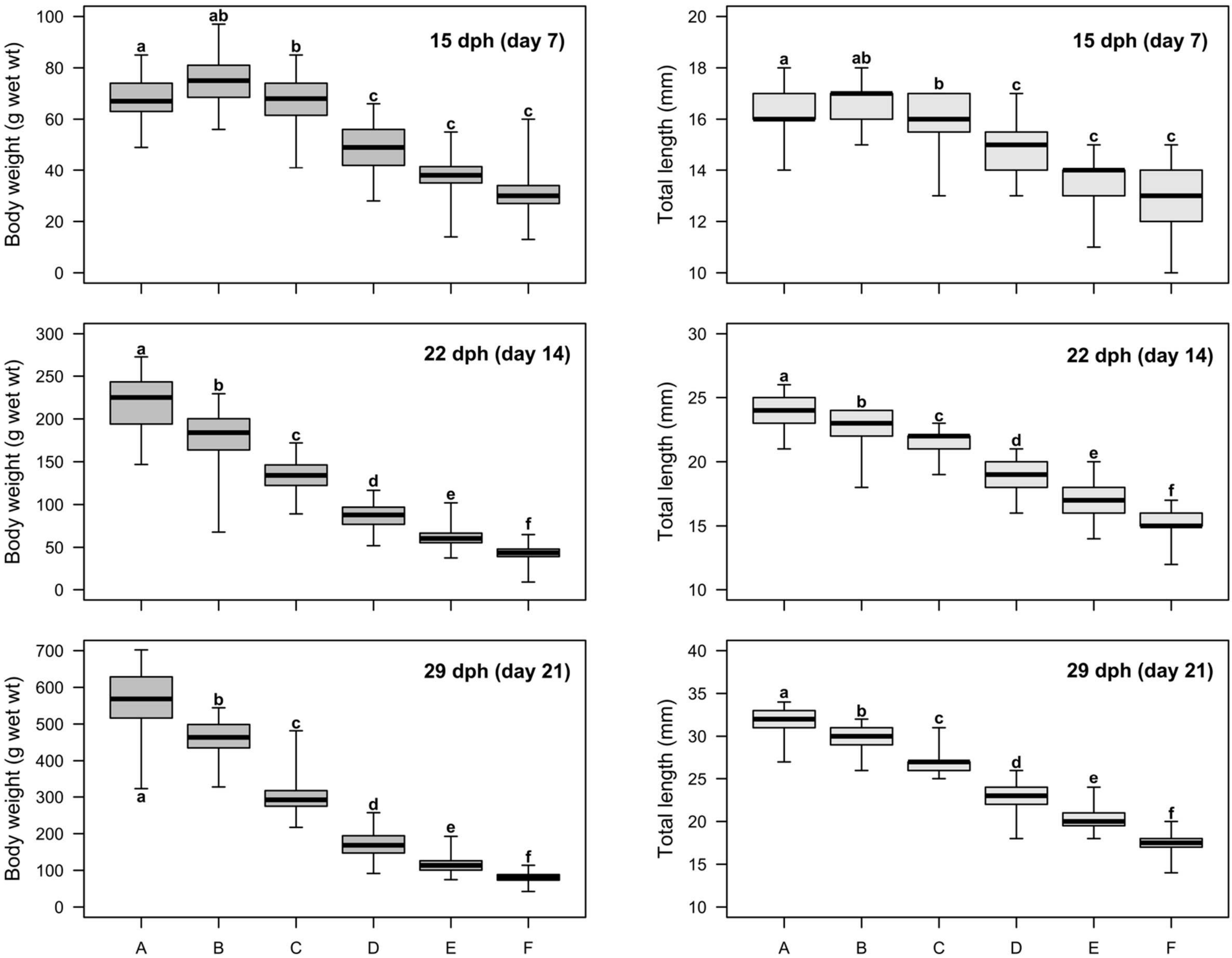
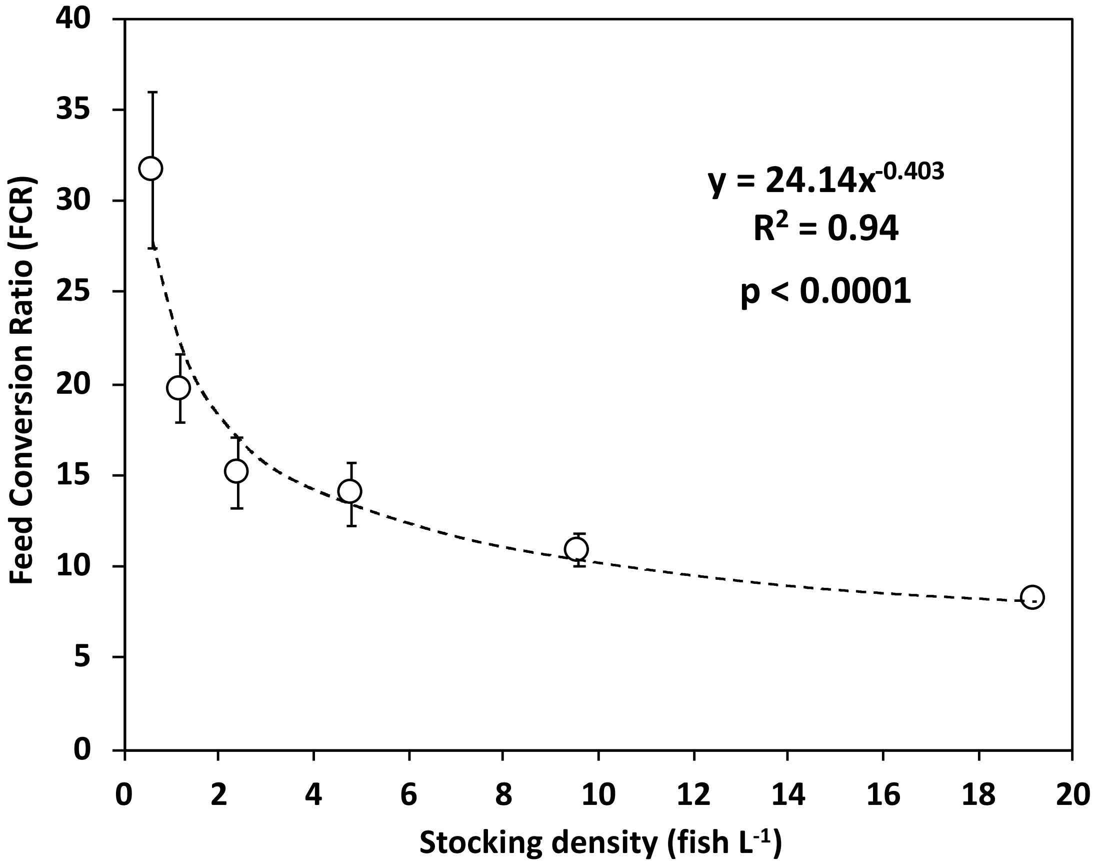

30
25
20
15
10
5
0
Tubifex distributed (g fish-1, wet wt)
a
b
c
d
e
e
Uneaten
Eaten
A
B
C
D
E
F
Experimental treatment

Figure 1



582 **Figure 2**

583



40
35
30
25
20
15
10
5
0
Feed Conversion Ratio (FCR)
y = 24.14x^-0.403
R^2 = 0.94
p < 0.0001
0 2 4 6 8 10 12 14 16 18 20
Stocking density (fish L^-1)

584 **Figure 3**

Table 1. Proximate composition of tubifex worms throughout the 21-day experiment. Except for water content, data are expressed as percentage of dry matter (n=3). Values are means ± SD.

Component	Value (%)
Water content	83.1 ± 1.1
Crude protein	54.0 ± 3.9
Crude lipid	23.5 ± 3.2
Ash	5.6 ± 2.8
Crude fibre	1.1 ± 0.1
NFE[1]	15.8 ± 2.5

[1]NFE: Nitrogen-free extract.

Table 2. Stocking densities of giant gourami larvae in the six experimental treatments.

Experimental treatment	Stocking density		Total number of larvae per aquarium
	Larvae L^{-1}	Larvae m^{-2}	
A	0.6	150	18
B	1.2	300	36
C	2.4	600	72
D	4.8	1200	144
E	9.6	2400	288
F	19.2	4800	576

Table 3. Summary of water quality parameters measured in the aquaria during experiment.

Parameters[1]	Mean ± SD	Range
Temperature (°C)	29.0 ± 0.6	28.3-30.0
DO (mg L^{-1})	6.1 ± 0.9	4.7-7.1
pH	8.4 ± 0.3	7.8-8.8
TDS (mg L^{-1})	78.4 ± 1.2	77-81
Turbidity (NTU)	0.1 ± 0.0	0.1-0.3
N-NH_4^+ (mg L^{-1})	0.23 ± 0.11	0.09-0.41
N-NO_2^- (mg L^{-1})	0.03 ± 0.03	0.00-0.09
N-NO_3^- (mg L^{-1})	3.1 ± 2.5	0.4-7.4

[1]DO: dissolved oxygen, TDS: total dissolved solids

Table 4. Effects of stocking density (A-F; see Table 2) on growth and survival of giant gourami larvae reared in a closed recirculating system from 8 to 29 days post-hatching. Values are means ± SD. Letters denote significant differences ($p < 0.05$) between treatments.

Parameters[1]	A	B	C	D	E	F
BG (g L^{-1})	0.33 ± 0.04^{a}	0.53 ± 0.03^{b}	0.68 ± 0.07^{c}	0.74 ± 0.07^{c}	0.94 ± 0.07^{d}	1.28 ± 0.04^{e}
BW_i (mg)	14.4 ± 0.8	14.4 ± 0.8	14.4 ± 0.8	14.4 ± 0.8	14.4 ± 0.8	14.4 ± 0.8
BW_f (mg)	563.2 ± 90.3^{a}	461.0 ± 53.7^{b}	301.0 ± 49.9^{c}	170.2 ± 35.7^{d}	113.6 ± 18.8^{e}	81.9 ± 12.3^{f}
CV_{BW} (%)	12.8 ± 4.2^{a}	10.5 ± 3.0^{a}	13.2 ± 6.8^{a}	19.9 ± 6.5^{a}	15.7 ± 2.0^{a}	14.5 ± 3.0^{a}
CV_{TL} (%)	3.9 ± 1.6^{a}	3.9 ± 1.9^{a}	4.5 ± 1.9^{a}	6.0 ± 2.0^{a}	4.9 ± 0.7^{a}	5.7 ± 1.1^{a}
K	1.75 ± 0.13^{a}	1.70 ± 0.09^{a}	1.56 ± 0.11^{b}	1.48 ± 0.14^{bc}	1.37 ± 0.08^{d}	1.52 ± 0.11^{bc}
SGR_{BW} (% day^{-1})	17.5 ± 0.5^{a}	16.5 ± 0.3^{b}	14.5 ± 0.5^{c}	11.8 ± 0.4^{d}	9.8 ± 0.3^{e}	8.3 ± 0.1^{f}
SGR_{TL} (% day^{-1})	5.5 ± 0.2^{a}	5.2 ± 0.1^{a}	4.6 ± 0.2^{b}	3.8 ± 0.2^{c}	3.3 ± 0.1^{d}	2.6 ± 0.0^{e}
SR (%)	100.0 ± 0.0^{a}	99.3 ± 1.4^{a}	98.6 ± 1.1^{a}	99.5 ± 0.7^{a}	99.2 ± 0.5^{a}	99.0 ± 1.1^{a}
TL_i (mm)	10.1 ± 0.4	10.1 ± 0.4	10.1 ± 0.4	10.1 ± 0.4	10.1 ± 0.4	10.1 ± 0.4
TL_f (mm)	31.7 ± 1.6^{a}	30.0 ± 1.3^{b}	26.8 ± 1.3^{c}	22.5 ± 1.6^{d}	20.2 ± 1.1^{e}	17.5 ± 1.0^{f}

[1]BG: biomass gain (n=4), BW_i: initial body weight (n=30), BW_f: final body weight (n=40-240), CV_{BW}: coefficient of variation for body weight (n=4), CV_{TL}: coefficient of variation for total length (n=4), K: condition factor (n=40-240), SGR_{BW}: specific growth rate for body weight (n=4), SGR_{TL}: specific growth rate for total length (n=4), SR: survival rate (n=4), TL_i: initial total length (n=30), TL_f: final total length (n=40-240).

Table 5. Food consumption expressed as total food ingested per treatment (FI_{total}, %, n=4), individual food intake during the 21-day experiment (FI_{fish}, g ind^{-1}, n=4) and feed conversion ratio (*FCR*) of gourami larvae reared in recirculating aquaculture system at six stocking densities. Values are means ± SD. For each parameter, different letters denote significant differences ($p < 0.05$) between treatments.

Parameters	A	B	C	D	E	F
FI_{fish} (g ind^{-1})	17.2 ± 0.82^a	8.8 ± 0.29^b	4.3 ± 0.25^c	2.1 ± 0.11^d	1.1 ± 0.02^e	0.6 ± 0.02^e
FI_{total} (%)	62.0 ± 0.03^a	63.1 ± 0.03^a	61.3 ± 0.04^a	62.4 ± 0.05^a	62.3 ± 0.02^a	66.6 ± 0.03^a
FCR	31.6 ± 4.3^a	19.7 ± 1.8^b	15.1 ± 2.0^c	14.0 ± 1.8^c	10.9 ± 0.9^d	8.3 ± 0.3^e