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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,

63 rearing conditions and age of the fish (Saoud et al., 2008). For example, some studies have
64 demonstrated that survival rate and growth are negatively affected by an increase in stocking
65 density (El-Sayed, 2002; Keer et al., 2018; Sahoo et al., 2004) while other studies (Kaiser et al.,
66 1995; Niazie et al., 2013) did not find any effects of stocking density on survival or growth rate.
67 These results highlight the importance of better characterising the effects of stocking density
68 on survival and growth of giant gourami larvae.
69 In this context, the objective of this study was to assess zootechnical performance through the
70 assessment of survival, food intake, and growth of the larvae of giant gourami reared in a closed
71 recirculating aquaculture system (RAS) at six stocking densities. The range of larval densities
72 (from 0.6 to 19.2 larvae L^{-1} i.e., 150 to 4600 larvae m^{-2}) was selected in accordance with the
73 recommendations of the "BPPSIGN" Centre (West Java Centre for the Development of Giant
74 Gourami Culture) for the lower densities (0.6 to 1.2 larvae L^{-1}, i.e. 150-300 larvae m^{-2}) and
75 extended according to a gradient of increasing production intensification.
76

77 **2. Materials and methods**

78 **2.1. Origin of larvae**

79 Giant gourami broodfish (3-4 years old), belonging to the local "Galunggung" strain (Arifin et
80 al., 2017), were reared in a 200-m^2 outdoor pond at the Research and Development Installation
81 of Germplasm for Freshwater Aquaculture (RIFAFE, Cijeruk, West Java, Indonesia). The
82 broodfish were fed leaves of giant taro (*Alocasia macrorrhiza*) and commercial floating fish
83 feed pellets (32% proteins, 5% lipids) distributed at a daily feeding rate of 1-2% of fish biomass,
84 respectively. The broodfish pond was divided by net into 10 compartments of 16 m^2. Each
85 compartment contained one male and three females. Bamboo nest supports and palm tree fibres
86 were provided so that broodfish could build nests. The natural spawning event occurs once the
87 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

138 the entire experiment, the total amount of tubifex worms distributed in each aquarium was 465
139 g. Since tubifex worms are benthic and live on the bottom of the aquaria, they were continuously
140 available for larvae without any degradation of water quality (see Table 3).
141
142 [Fig. 1 is here]
143
144 Prior to each larval feeding, tubifex worms were collected, rinsed and drained on a 50-µm mesh
145 and weighed (to the nearest 0.1 g). To determine food intake, unconsumed tubifex worms were
146 collected from each aquarium and weighed before the addition of the new ration of worms. The
147 quantity distributed was kept constant in each aquarium. In the RAS, the filtration system
148 consisted of filtration foams as the mechanical filtration medium and BioBall® carriers as
149 bacterial support. Water flow into the rearing tanks was maintained at 33 L h^{-1} for the first four
150 days of the experiment and then at 78 L h^{-1}. Water was added every day to compensate for
151 evaporation and losses when aquaria were cleaned (approx. 5-7% of volume). Water quality
152 was monitored in each aquarium once a week with direct measurements using a multi-parameter
153 probe (Hanna HI 9829) for dissolved oxygen (DO), pH, total dissolved solids (TDS) and
154 turbidity, and then by spectrophotometry analysis (Hanna HI 83399) for $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and
155 $N\text{-}NO_3^-$. Temperature was monitored twice a day (at 08:00 and 16:00). Since no statistical
156 differences were observed between the six experimental treatments for any of the parameters,
157 data were pooled and are presented in Table 3. All the values indicate that water quality did not
158 deteriorate and corresponded to appropriate rearing conditions for tropical freshwater fishes
159 (Svobodová et al., 1993; Aryani et al., 2017).
160
161 [Table 3 is here]
162

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): $SR\ (\%) = [(N_f / N_i) \times 100]$.

The specific growth in body weight (SGR_{BW}, %) was calculated according to the following equation: $SGR_{BW} = [(\ln BW_f - \ln BW_i) / 21) \times 100]$, 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 $SGR_{TL} = [(\ln LT_f - \ln LT_i) / 21] \times 100$.

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

213 (body weight and total length) and Fulton's condition factor. When significant differences were
214 detected, the Games-Howell test (McDonald, 2009) with Bonferroni correction was performed
215 to compare means.
216 For the other metrics used (see Section 2.4), data were first tested for normality (Shapiro's test)
217 and homogeneity of variance (Levene's test) and, where necessary, data were arcsine or log
218 transformed prior to analysis. One-way ANOVA was used to assess significance of differences
219 among treatments. When significant differences were detected, Tukey's test was performed to
220 compare means. The level of significance for statistical analyses was always set to $\alpha = 0.05$. All
221 statistics were performed using R freeware version 3.3 (R Development Core Team, 2016).
222
223 **3. Results**
224 **3.1. Survival rate**
225 The survival rates (*SR*) measured at the end of the experiment were very high (98.6-100 %,
226 Table 4) without any significant differences between the six stocking densities tested ($F = 1.31$,
227 $p = 0.304$). Regardless of the experimental stocking density, larvae exhibited no aggressive
228 behaviour throughout the experiment and no cannibalism was observed.
229
230 [Table 4 is here]
231
232 **3.2. Growth and size heterogeneity**
233 The growth of larvae reared at six different densities is indicated in Table 4. At the end of the
234 experiment, the average total length (TL_f) and body weight (BW_f) of larvae ranged from 17.5 ±
235 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
236 A (Fig. 2). Growth was significantly less with increased stocking density ($F = 833.7$, $p < 0.0001$
237 and $F = 1382$, $p < 0.0001$ for BW_f and TL_f respectively), with the lowest growth observed for

238 the larvae reared at the highest density (Fig. 2). This trend was quantified by the specific growth
239 rate calculated for both body weight (SGR_{BW}) and total length (SGR_{TL}). Significant decreases in
240 SGR_{BW} ($F = 425.3$, $p < 0.0001$) and SRG_{TL} ($F = 267.6$, $p < 0.0001$) were observed when stocking
241 densities increased, with values varying from 17.5 ± 0.5 % for SGR_{BW} and 5.5 ± 0.2 % for SRG_{TL}
242 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
243 (19.2 larvae L^{-1}). In this experiment, the commercial fry size ("Nguku", i.e., fish >2 cm in total
244 length) was reached after 14 days of rearing (22 dph) for larvae reared at the lowest stocking
245 densities (A: 24.0 ± 1.3 mm, B: 22.8 ± 1.24 mm and C: 21.5 ± 1.0 mm), whereas larvae from
246 treatments D and E reached fry commercial size only seven days later (TL_D: 22.5 ± 1.6 mm and
247 TL_E: 20.2 ± 1.1 mm). At the highest stocking density, the larvae had not reached the commercial
248 size (TL_F: 17.5 ± 1.0 mm) even after 21 days of culture (Fig. 2). Although growth was reduced
249 at high stocking densities, the biomass gain (BG), ranging from 0.33 ± 0.04 to 1.28 ± 0.04 g L^{-1},
250 increased significantly ($F = 155.3$, $p < 0.0001$) with increasing stocking density (Table 4).
251 Size heterogeneity as a function of stocking density was assessed at the end of the experiment
252 (i.e., 29 dph) by the coefficients of variation for body weight (CV_{BW}) and total length (CV_{TL}).
253 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
254 CV_{BW} and CV_{TL}, no significant differences were found between any of the groups ($F = 1.974$, p
255 $= 0.132$ and $F = 1.302$, $p = 0.307$) for CV_{BW} and CV_{TL} respectively (Table 4).
256 The Fulton's condition factors (K) estimated at the end of the experiment ranged from 1.38 to
257 1.77. Statistical analysis revealed significant decrease in K with increased stocking density (F
258 $= 131.18$, $p < 0.0001$). Nevertheless, no statistical difference was found between treatments C,
259 D and F (Table 4).
260 [Fig. 2 is here]
261
262 **3.3. Food intake**

263 The proportion of the total distributed tubifex worms (FI_{total}, %) effectively ingested in each
264 aquarium was not affected by the stocking density ($F = 0.627$, $p = 0.681$) and remained similar
265 in the six treatments (61-67%, Table 5). Furthermore, the minimum quantities of tubifex worms
266 remaining at the end of each feeding period were never less than 6%. On the other hand, the
267 total individual food intake (FI_{fish}, g ind^{-1}) during the 21 day the experiment was greatly affected
268 by the stocking density ($F=1857$, $p < 0.0001$; Table 5 and Fig. 1). For the entire experiment, the
269 highest individual food intake was 17.2 ± 0.82 g ind^{-1} for treatment A and only 0.60 ± 0.02 g
270 ind^{-1} for treatment F. Similarly, the feed conversion ratio (*FCR*) was the highest at the lowest
271 stocking density (i.e. treatment A, 31.6 ± 4.3) and decreased significantly ($F = 79.29$, $p <$
272 0.0001), with lowest values (8.3 ± 0.3) for the highest density treatment (F). The relationship
273 between *FCR* and stocking densities was fitted using a single-component exponential model
274 ($R^2=0.94$, $p < 0.0001$): $y = 24.14\ x^{-0.403}$ (Fig. 3).
275
276 [Fig. 3 is here]
277 [Table 5 is here]
278
279 **4. Discussion**
280 **4.1. Effects of stocking density on survival rate and growth**
281 There are few studies testing the influence of stocking density on survival and growth of giant
282 gourami larvae. Moreover, the density ranges tested were often narrow (e.g. 0.3 to 0.7 fish L^{-1},
283 Ebrahimi et al., 2010; and 2.5 to 10 fish L^{-1}, Sarah et al., 2009). The present study provides
284 quantification of the effects of stocking density on larvae considering a wider range of density
285 (0.6 to 19.2 fish L^{-1}). The lowest stocking density treatments (i.e., 150-300 larvae m^{-2} or 0.6-
286 1.2 fish L^{-1}) were based on the current recommendations from the "BPPSIGN" Centre.

287 Increasing intensification was applied until reaching densities 6-fold higher than what is
288 observed among the fish farmers.
289 First, we compare our results to larval production reported in an on-farm survey of 39 small-
290 scale farms and two training centers that produced "Nguku" in West Java province (mainly
291 located in Bogor and Tasikmalaya districts) carried out in November 2016. Overall, the growth
292 of the larvae reared in our recirculating aquaculture system (RAS) was higher than those
293 reported from small-scale farms, where 22 to 90 days were needed to reach the 2-cm
294 commercial size at stocking densities ranging from 111 to 714 larvae m^{-2} in small, stagnant
295 outdoor ponds based on the farmer's responses (n = 20, unpublished data). In addition, we
296 observed very high survival rates for all the stocking densities (>98%) much higher than those
297 reported by Javanese fish farmers (0-98% and 50% on average, n = 23 farmers) or in previous
298 experiments (e.g., Verawati et al., 2015). Regardless of the experimental stocking density,
299 larvae exhibited no aggressive behaviour throughout the experiment and no cannibalism was
300 observed.
301 Overall, we observed high growth rates for giant gourami. Indeed, although we found similar
302 results for higher stocking densities, Sarah et al. (2009) observed about 50% lower growth when
303 larvae were maintained at 5 fish L^{-1} compared to our findings. Altogether, our results suggest
304 that (1) larvae were reared under appropriate environmental conditions, and (2) RAS is a
305 suitable method for improving juvenile production in giant gourami aquaculture.
306 We found that increasing stocking density had negative effects on the growth of giant gourami
307 larvae. These results are in accordance with those of Sarah et al. (2009). Conversely, Ebrahimi
308 et al. (2010) reported that very low stocking densities (< 0.7 fish L^{-1}) had no effect on the growth
309 of young-stage giant gourami. These findings suggest that the lowest density tested in our study
310 (0.6 fish L^{-1}) was the minimum value to detect effects of stocking density.

311 Several interpretations have been offered to explain the effects of stocking density on growth
312 and survival in fish. In Reba carp *Cirrhinus reba* fry, lower survival rates observed at high
313 stocking densities were attributed to stronger competition for food and space as well as
314 increased stress (Keer et al., 2018). On the other hand, stocking density showed no negative
315 effect on survival and growth in marbled spinefoot *Siganus rivulatus* juveniles, a result credited
316 to the maintenance of water quality within the tolerance range for this species (Saoud et al.,
317 2008). European perch *Perca fluviatilis* and European seabass *Dicentrarchus labrax* showed
318 contrasting results for growth and survival depending on the life-stage considered (larvae and
319 post-larvae) with regard to the occurrence of cannibalism (Kestemont et al., 2003). In the
320 present study, water quality remained constant throughout the experiment and was not affected
321 by stocking density. Survival was very high and did not vary significantly between stocking
322 densities tested. Not surprisingly for a non-aggressive fish such as the giant gourami, no
323 cannibalism was observed, likely contributing to the homogeneity of larval size at each stocking
324 density (i.e. CV_{TL} = 4-6%; CV_{BW} = 13-20%). These results accord with those of a previous study
325 on European perch *P. fluviatilis* (Król and Zieliński, 2015) larvae. However, the effects of
326 stocking density on survival and growth are species-dependent (Huang and Chiu,1997;
327 Szkudlarek and Zakęś, 2007), and results can vary for a given species (e.g., Baras et al., 2003;
328 Król and Zieliński, 2015). At the end of the experiment, the Fulton's condition factors (*K*)
329 decreased significantly for the four highest densities tested, suggesting that the larvae stocked
330 in these experimental treatments were under poorer nutritional conditions. All together, these
331 results indicate that, in the giant gourami, the decrease in larval growth due to stocking density
332 is very likely related to lower food intake.
333

334 **4.2. Effects of density on food intake and FCR**

335 The effects of stocking density on food intake were assessed using tubifex worms as living
336 prey. This live food source is commonly used to feed various fish species (Ravichandra Reddy
337 et al., 1977; Le Thanh et al., 1999; Malla and Banik, 2015). Due to their high levels of protein
338 and lipids (Bardach et al., 1972; Table 1) and their aquatic lifestyle, tubifex worms ensure an
339 adequate nutritional intake for fish larvae without causing significant effects on water quality.
340 In the present study, we showed that the proportion of the total distributed tubifex worms
341 effectively ingested in each aquarium did not vary with stocking density, indicating a large
342 decrease in the quantity ingested by individual larvae with increasing stocking density
343 (Table 5). Nevertheless, despite a reduction in individual food intake and slower growth at the
344 highest stocking densities, we found a significant negative relationship between *FCR* and
345 stocking density, indicating that the higher the larval density, the lower the *FCR*. Using a wider
346 range of stocking densities, we confirmed the findings of Sarah et al. (2009), who found a linear
347 decrease of *FCR* in giant gourami larvae with increasing stocking density ranging from 2.5 to
348 10 fish L^{-1}. Because ingested food quantities remained stable (61-67%) across experimental
349 treatments, the decrease in *FCR* can be explained by the significant increase in biomass gain
350 (BG) observed for the highest stocking densities.
351 Effects of stocking density on *FCR* in fish vary greatly. For instance, in marbled spinefoot *S.*
352 *rivulatus* juveniles fed commercial pellets, no significant effect of stocking density on *FCR* was
353 found (Saoud et al., 2008). However, Niazie et al. (2013) and Keer et al. (2018) reported
354 significant increases of *FCR* at higher stocking densities with juvenile Reba carp and goldfish
355 *Carassius auratus* fed compounded feed. Such findings suggest that effects of stocking density
356 are species-dependent. In addition, in the studies mentioned above, fish were fed using
357 calculated food rations throughout the experiment, but not until satiation. Furthermore, in most
358 of the studies, *FCR* calculations are based on the quantity of food distributed and not on actual
359 consumption (Saoud et al., 2008; Niazie et al., 2013; Keer et al., 2018); it can be difficult to

360 accurately estimate consumption using live prey or small inert food particles. Nevertheless,
361 indirect food consumption estimates can lead to experimental bias for the quantification of *FCR*
362 (Slembrouck et al., 2009) that may potentially explain the differences observed regarding the
363 effects of stocking density on the *FCR* and more generally the food intake in fish larvae.
364 In fish, decreased food intake often is associated with increased stress (Saoud et al. 2008;
365 Moradyan et al., 2012). When food is present in excess, water quality is affected, indirectly
366 causing the decrease in the growth and survival rates of farmed fish (e.g., Werner and Blaxter,
367 1980; Puvanendran and Brown, 1999). In the present study, although larvae were voluntarily
368 maintained in non-limiting, surplus-food conditions, water quality of the recirculating
369 aquaculture system remained satisfactory throughout the experiment. Our results suggest that
370 the water renewal (110 to 260% per hour) was sufficient to ensure no experimental bias due to
371 poor water quality. Although tubifex worms were constantly available, they clustered on the
372 bottom of the aquaria, which can limit food intake for some species of fish due the potential
373 difficulty of engulfing such large quantities of food, as was shown for walking catfish *Clarias*
374 *batrachus* (Dey et al., 2016). However, giant gourami larvae and juveniles have relatively small
375 mouths and ingest tubifex worms one by one; they did not appear to be bothered by the clusters
376 of tubifex worms and showed no aggressive behaviour. For these reasons, we assume that there
377 was no stress regarding food accessibility, contrary to reports in rainbow trout *Oncorhynchus*
378 *mykiss* (Ellis et al., 2002; North et al., 2006). Thus, in our experiment, increasing the number
379 of fish per unit volume led to the reduction of space availability for each individual and likely
380 acted as a direct stressor for larvae, limiting their ingestion of food. Nevertheless, measurements
381 of physiological indicators of stress (e.g., haematocrit, lysozyme activity or plasma cortisol;
382 Ellis et al., 2002; North et al., 2006) are needed to test this assumption.
383 Interestingly, we observed a drastic drop in *FCR* when the lowest density doubled from 0.6 to
384 1.2 fish L^{-1}. Similar findings were highlighted by in Nile tilapia *Oreochromis niloticus* fry fed

385 experimental feed to apparent satiation (El-Sayed, 2002). In that study, *FCR* was not
386 significantly affected by stocking density, except at the lowest densities, suggesting that the
387 decrease in *FCR* may have been due to: (1) the lack of competition for food, or (2) the
388 difficulties in catching food particles that were flushed out with the water outflow (thus leading
389 to biased estimation of *FCR*). However, in the present study, no evidence of competition for
390 food was shown at any stocking density. In addition, the *FCR* values calculated in the present
391 study were based on real consumption of food by larvae because tubifex worms aggregated on
392 the bottom of the aquarium, thereby avoiding loss of prey with water outflow. In our case, the
393 difference in biomass gain (BG) observed between the two lowest stocking density conditions
394 (± 60%) caused the drastic drop in *FCR*. Nevertheless, the reasons explaining this remarkable
395 difference in gain in biomass remain uncertain. Further investigations are needed to better
396 understand the growth dynamics of giant gourami larvae raised at low stocking densities, which
397 is likely to affect the commercial production of juveniles.
398
399 **5. Conclusion**
400 The production of giant gourami fry is highly segmented, and the "Nguku" stage is the best-
401 selling, although there are at least three intermediate stages that are also traded locally.
402 Currently, there are no clear and standardized production methods. This study provides new
403 information regarding the effects of stocking density on giant gourami larval production. The
404 experiment was performed in a recirculating aquaculture system, ensuring constant water
405 quality, easier control of feeding, and hence better control over the seedstock production
406 process than traditional practices. We showed that stocking density has no effect on larval
407 survival during the 8-29 dph period. Nevertheless, growth was strongly affected by stocking
408 density. Thus, for a given surface area, although larvae production at low density limits the
409 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.

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560

561 **Figure captions**

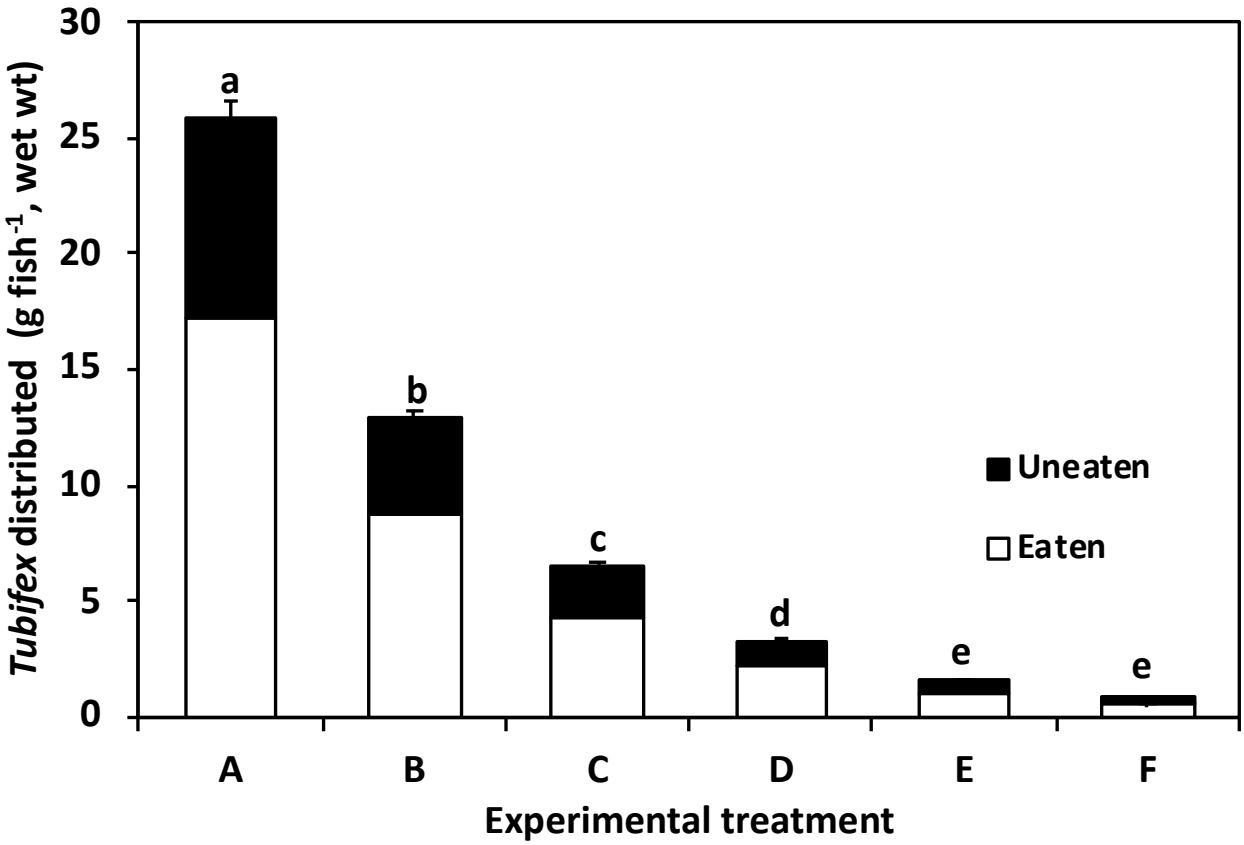
562 **Figure 1.** Total food intake (g $fish^{-1}$ wet wt) by giant gourami larvae reared at six different
563 stocking densities A-F (A=0.6, B=1.2, C=2.4, D=4.8, E=9.6, and F=19.2 fish L^{-1}; i.e. A=150,
564 B=300, C=600, D=1200, E=2400, and F=4800 fish m^{-2}, n=4) for 21 days. Bars show the
565 proportion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

568

569 **Figure 2.** Total length (a) and body weight (b) of giant gourami larvae (n=40-240) at 15, 22
570 and 29 days post-hatching (dph) in the six stocking density treatments A=0.6, B=1.2, C=2.4,
571 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
572 fish m^{-2}). Box-plots show the interquartile range, median (horizontal line), minimum and
573 maximum values (whiskers). Different letters denote significant differences between fish
574 stocking densities ($p < 0.05$).

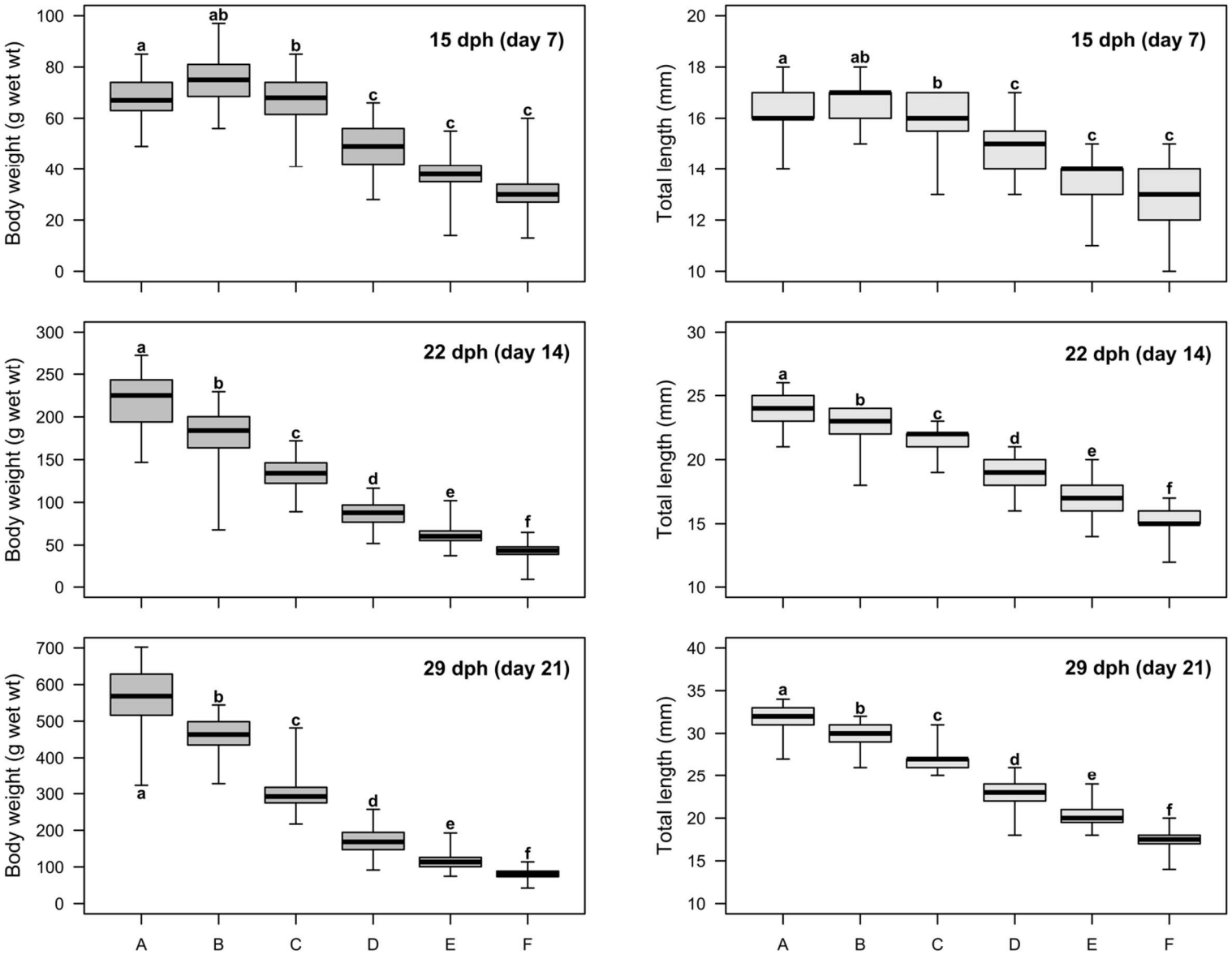
575

576 **Figure 3.** Relationship between feed conversion ratio (*FCR*) and stocking densities of giant
577 gourami larvae at 29 days post-hatching (dph) reared at six stocking density treatments (A=0.6,
578 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,
579 E=2400, and F=4800 fish m^{-2}, n=4). Values are means ± SD.



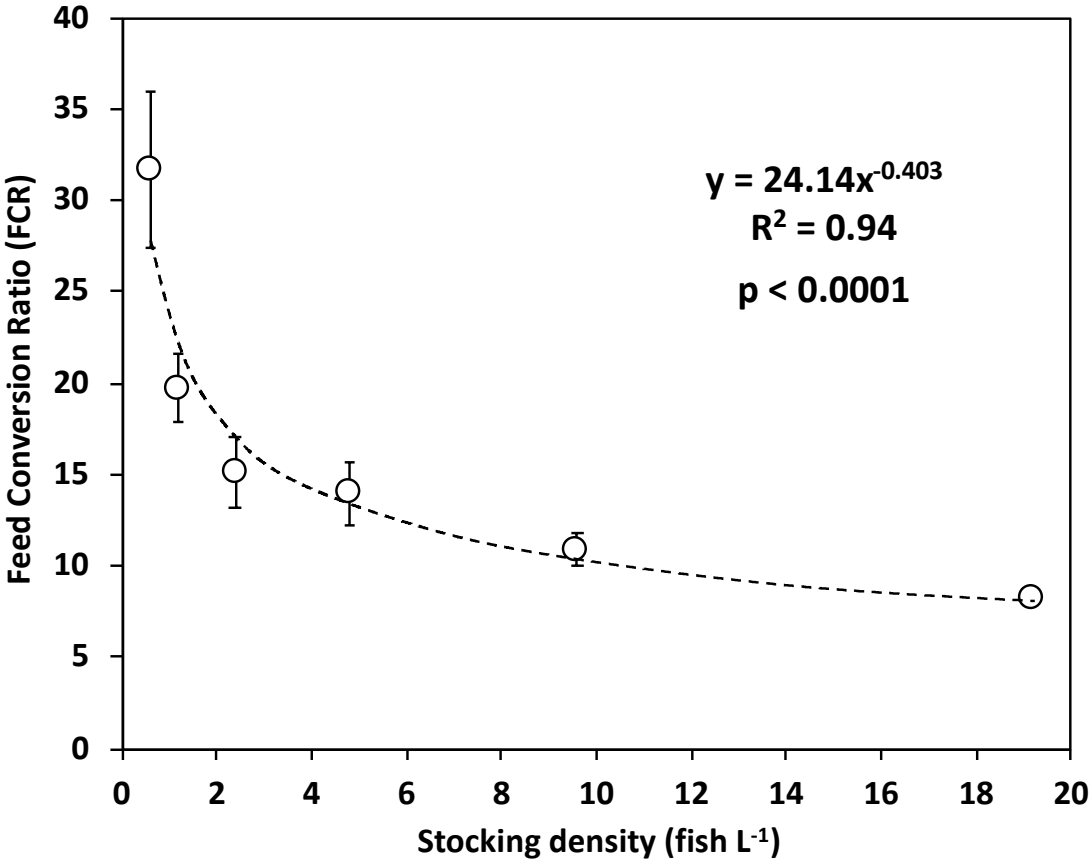
580 **Figure 1**

581



582 **Figure 2**

583


Feed Conversion Ratio (FCR)
Stocking density (fish L-1)
y = 24.14x-0.403
R2 = 0.94
p < 0.0001
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
14
16
18
20

584 **Figure 3**

585 **Table 1.** Proximate composition of tubifex worms throughout the 21-day experiment. Except
586 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

587 [1]NFE: Nitrogen-free extract.

588 **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

589

590 **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

591 [1]DO: dissolved oxygen, TDS: total dissolved solids

592 **Table 4.** Effects of stocking density (A-F; see Table 2) on growth and survival of giant gourami
593 larvae reared in a closed recirculating system from 8 to 29 days post-hatching. Values are means
594 ± 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}

595 [1]*BG*: biomass gain (n=4), BW_i: initial body weight (n=30), BW_f: final body weight (n=40-240),
596 CV_{BW}: coefficient of variation for body weight (n=4), CV_{TL}: coefficient of variation for total
597 length (n=4), *K*: condition factor (n=40-240), SGR_{BW}: specific growth rate for body weight
598 (n=4), SGR_{TL}: specific growth rate for total length (n=4), *SR*: survival rate (n=4), TL_i: initial
599 total length (n=30), TL_f: final total length (n=40-240).
600

601 **Table 5.** Food consumption expressed as total food ingested per treatment (FI_{total}, %, n=4),
602 individual food intake during the 21-day experiment (FI_{fish}, g ind^{-1}, n=4) and feed conversion
603 ratio (*FCR*) of gourami larvae reared in recirculating aquaculture system at six stocking
604 densities. Values are means ± SD. For each parameter, different letters denote significant
605 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}

606