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Abstract

The influence of temperature on zootechnical performances and physiology was assessed in giant gourami (*Osphronemus goramy*) larvae. Larvae aged ten days post-hatching were reared at five temperature treatments (22.5, 25.0, 27.5, 30.0 and 32.5 °C, three replicated per treatment) in indoor closed recirculating systems until they reached, at least, the commercial size of 2.5 cm in total length (i.e. rearing time of 21 to 42 d depending on the temperature). Samples of larvae were collected every week. Survival, growth, and feed intake were used as main indicators of zootechnical performances. In addition, concentration of glucose and cortisol and proximate composition of the young life-stages giant gourami were compared to assess effects of temperature on their physiology. Results showed that temperature strongly affected growth of giant gourami larvae with significantly increased observed at the increasing tested temperatures (from 57.2 ± 9.3 mg to 431.9 ± 64.2 mg of body weight after three weeks, $p < 0.05$). The increased growth can be related to changes in metabolism, feed intake and feed use efficiency. Based on cortisol and glucose values, we recommend to maintain rearing temperature at 30.0°C for both optimizing growth and avoid physiological impairments in giant gourami larvae. The consequences of larval rearing at stable temperature (30.0°C) should be further investigated in the nursery and grow-out outdoor phases.

Keywords: Fish larval rearing, Temperature, Survival, Growth, Cortisol, Glucose

1. Introduction

Giant gourami (*Osphronemus goramy*; Lacepède, 1801) is one of the most popular freshwater fish species in Southeast Asia and particularly in Indonesia, the main producing country in aquaculture (113,396 tons in 2015; FAO, 2017). Over the past 15 years, the Indonesian giant gourami aquaculture production has grown exponentially with an annual increase of 16% (FAO, 2017). As for other freshwater species, Indonesian giant gourami aquaculture is mainly ensured by micro and small-scale farms (more than 100,000 fish farmers mainly in Java Island; BPS, 2013; Maskur et al., 2013). Currently, there are still some obstacles to the production of gourami related to gaps of knowledge on the biology of this species (Arifin et al., in press). One of the main impediments in giant gourami aquaculture is ensuring availability of fry for fish farmers through the improvement of larval rearing methods (Amornsakun et al., 2014a, 2014b).

Commercial scale propagation of giant gourami in hatcheries is yet to be standardized (Amornsakun et al., 2014a). Thus, although Indonesian National Standard recommends indoor larval rearing allowing to limit variations of environmental conditions (SNI, 2000), an on-farm inquiry carried out on about 40 small-scale farms of the West Java province revealed that the current practices for giant gourami larval production consisted to rear larvae for the first days post-hatching (dph) in plastic basins and then transferred to outdoor small ponds. In such practices, larvae have to deal with fluctuations of environmental conditions that could not be controlled, especially for water temperature changes. For these reasons, the impacts of environmental conditions on giant gourami larval rearing, especially temperature, is thus become limiting factor for fish farmers.

Water temperature is one of main environmental factors affecting physiology of aquatic ectotherm organisms such as fish even before hatching (Kamler, 2002; Pauly and Pullin, 1988; Teletchea et al., 2009). Temperature changes affect physiological conditions, such as oxygen consumption, metabolism, digestion, growth, and reproduction (Wendelaar Bonga, 1997). When temperature tolerance ranges are exceeded, it results negative impacts, such as physiological disturbances, increased sensitivity to disease, and potentially mortality (Singh et al., 2013). Temperature is therefore a key environmental parameter in aquaculture contributing for successful outcome of larvae production (Blaxter, 1991; Pankhurst and King, 2010; Réalis-Doyelle et al., 2016). Effects of temperature on the larval and fry rearing have been extensively studied in freshwater fish (e.g. Desai and Singh, 2009; El-Gamal, 2009; Pandit and Nakamura, 2010; Rodkhum et al., 2011; Singh et al., 2009; Zeng et al., 2018).

Effects of temperature in fish larval and fry rearing may be diverse. As an example, Desai and Singh (2009) have shown that growth and food efficiency of common carp *Cyprinus carpio* fry was significantly increased by higher temperature (32°C vs. 28°C). Nevertheless, feed efficiency and protein efficiency ratio decreased at the highest temperature as already demonstrated for Asian catfish *Clarias batrachus* fry (Singh et al., 2009). Temperature can also affect the sensibility of fish to pathogens (Rodkhum et al., 2011) through, in particular, effects the ontogeny of mucous cells (El-Gamal, 2009). In addition, temperature affects deformity rate during fish larval rearing (Wang and Tsai, 2000). Such results emphasize the importance of assessing temperature effects on giant gourami larvae.

The objectives of this study were therefore (1) to assess the zootechnical performances of giant gourami (*O. goramy*) larvae reared until they reached, at least, the commercial size of 2.5 cm in total length (i.e. rearing time of 21 to 42 d depending on the temperature) in closed aquaculture recirculating systems (RAS) at five rearing temperatures (22.5-32.5°C according to preliminary tests and current practices) through their survival, growth and food intake and (2) measure the impacts of temperature changes on the physiology of the larvae through the measurements of glucose and cortisol concentrations and their chemical composition.

2. Materials and methods

2.1. Origin of larvae

Giant gourami larvae used in this experiment came from the same broodfish pair at the same natural spawning event. The 3-4 years old broodfish ("Galunggung" strain, Arifin et al., 2018) were reared in an outdoor pond at the Research Installation of Germplasm Freshwater Aquaculture (RIFAFE, Cijeruk, West Java, Indonesia). Broodfish were fed leaves of giant taro (*Alocasia macrorrhiza*) and commercial feeds (floating pellets: 32% proteins, 5% lipids) at a feeding rate of 2% and 1% of fish biomass per day, respectively. Bamboo nest supports and palm tree fibres were provided for nest building. The buoyant eggs were then incubated in the experimental room for 20 hours (20-L plastic basin; daily water change, temperature: 29.0 ± 0.6°C; light:dark cycle: 12:12 h). After hatching, larvae were kept unfed in the incubation basin (following fish farming practices), until the

beginning of the experiment, which was 10 dph (i.e. postflexion larva, approx. 6 d after the mouth opening, Morioka et al., 2013).

2.2. Live prey maintenance

According to the current practices (SNI, 2000) and Lucas et al. (2015) who demonstrated the benefits of this feed for survival rate and growth of giant gourami larvae, in this study, fish were fed tubifex worms (*Tubifex tubifex*) throughout the experiment. 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. Proximate analyses of tubifex worms were conducted to obtain nutritional quality based on the procedures described in AOAC (1999) giving the following results (dry matter basis): 52.87% crude proteins, 22.09% crude lipids, 4.09% ash, 1.23% crude fibre and 19.72% nitrogen free extract (NFE).

2.3. Temperature experiment

2.3.1. Experimental design

Zootechnical performances and physiology of giant gourami *O. goramy* larvae were studied under five temperature conditions (22.5, 25.0, 27.5, 30.0 and 32.5°C). Experiment was carried out in five identical indoor RAS (1 per temperature condition) under natural photoperiod (light:dark cycle: 12:12 h, daylight intensity: 60-4500 lux). At 10 dph, larvae were individually counted and measured (mean body weight: 12.4 ± 1.4 mg; mean total length: 9.8 ± 0.3 mm)

134 then randomly assigned to the experimental tanks (n=3 par temperature condition,
135 stocking density of 2.4 larvae L^{-1}) placed in RAS.
136 Larvae were acclimated into targeted temperatures by gradual temperature
137 changes (2.5°C h^{-1}). The experimental tanks were 30-L glass aquaria 40 × 30 × 30
138 cm (L × W × H), aerated by bubbling, with sides covered by isolating black
139 polyethylene foam and top closed by transparent polycarbonate sheet in order to
140 limit temperature exchanges. The experiment continued until the larvae reached,
141 at least, 2.5 cm of total length (i.e. after 21 to 42 days of rearing depending on the
142 temperature condition). The choice of this experimental protocol was made in
143 accordance with the "BPPSIGN" Centre (West Java Centre for the Development
144 of Giant Gourami Culture) which mentioned 2.5 cm as one of segmentation
145 market size for local fish farmers (called "Nguku"; Adida, 2014).
146
147 2.3.2. Feeding protocol and water quality monitoring
148 Larvae were fed every day, except on the sampling days, in large excess for all the
149 temperature treatments (9.1 ± 0.6 g $aquarium^{-1}$ day^{-1} throughout the experiment)
150 in order to ensure non-limiting food conditions for larvae and facilitate the
151 accurate estimation of ingestion. Thus, the same quantity of live tubifex worms
152 was spread in the bottom of each aquarium twice a day at 8:00 and 16:00. Prior to
153 distribution to giant gourami larvae, tubifex worms were collected, rinsed and
154 drained on a 50-µm mesh and weighed (nearest 0.1 g). In order to define food
155 intake, unconsumed tubifex worms were collected in each aquarium and weighed
156 before the addition of the new ration of tubifex. Water inlet flow into rearing
157 tanks was maintained at 33 L h^{-1} for the first 5 days of the experiment and then at

158 78 L h^{-1}. Temperature was monitored in each aquarium three times a day (at
159 08:00, 12:00 and 16:00) while water quality was checked once a week with direct
160 measurements using a multi-parameter probe (HI 9829 Hanna) for pH, dissolved
161 oxygen (DO) and conductivity. In addition, alkalinity, Total ammoniacal nitrogen
162 (TAN), NO_2^- and NO_3^- were measured by spectrophotometry analysis (n=6-15 per
163 treatment). NH_3 concentrations were calculated from TAN, temperature and pH
164 values. Results are provided in Table 1.
165
166 2.4. Observations and measurements of larvae
167 Fish (n=20 for each aquarium) were sampled every seven days in each aquarium,
168 transferred in a plastic basin containing water from their aquarium in order to
169 keep them in the same water temperature. The sampling frequency was selected in
170 order to limit stress for the larvae caused by handlings. Larvae were anaesthetized
171 (Eugenol, 50 µL L^{-1}) and their size (total body length, TL, mm) was measured
172 under a stereomicroscope with a micrometre (accuracy ranging from 0.05 to 0.1
173 mm, depending on fish size and magnification). Meanwhile, body weight (BW,
174 mg) was measured using a digital scale with an accuracy of 0.1 mg. After
175 individual measurements, fish were returned into their respective rearing
176 structures. No mortality was observed following samplings.
177 At the end of the experiment (i.e. after 21 to 42 d depending on the temperature
178 treatment, see Section 2.3.1), for each treatment, the aquaria were emptied and
179 remaining living larvae were counted allowing survival rate determination. Two
180 times during the experiment (after 4 h and at the end), fish from each treatment
181 were sampled to quantify whole-body cortisol and glucose concentrations. Whole-

182 body cortisol level was analysed by anesthetizing and grinding the larvae or
183 juveniles (n=3 pools of 20 fish). Afterwards, the samples were centrifuged
184 (10,000 rpm for 10 min at 25°C) and cortisol levels were analysed in the
185 supernatants by using Cortisol ELISA kit (DRG International, Inc., Germany) and
186 microplate photometer (Biosan HiPo MPP-96, Latvia). At the beginning of the
187 experiment, samplings for glucose analyses were carried out as described above
188 (n=3 pools of 20 fish). Methodology was adjusted for juveniles at the end of the
189 experiment. Thus, one drop of blood was collected on each anesthetised fish (n=3
190 fish). After collection, the blood samples were put on glucose reader (Accu-Chek
191 Active, Roche, Germany) for analysis. At the end of the experiment, 35-40 fish
192 from each treatment were collected for the whole-body proximate analyses (i.e.
193 protein, lipid, ash, and NFE). Anesthetizing fish from each treatment were taken
194 and pooled (n=3), autoclaved, homogenized, and dried to a constant weight at
195 105°C and then analysed following the methods described by AOAC (1999).
196
197 2.5. Data treatment and statistical analysis
198 Temperature effects on survival, growth and feed intake were determined by
199 calculating the following parameters for each experimental treatment. Survival
200 rates (SR), expressed as a percentage, were calculated by comparing the final
201 number (N_f) with the initial number of larvae (N_i): SR (%) = (N_f / N_i) x 100.
202 The specific growth in body weight ($SGR_{BW,}$ %) was calculated according to the
203 following equation: SGR_{BW} = (ln BW_f - ln BW_i) / ED) x 100, where BW_i and
204 BW_f are the initial and final body weight of larvae (mg) respectively, and ED is
205 the duration of the experiment in days.

206 The specific growth in total length (SGR_{TL}, %) was calculated using the same
207 calculation by substituting BW with LT: $SGR_{TL} = (\ln LT_f - \ln LT_i) / ED) \times 100$.
208 Heterogeneity of larval size (in body weight or total length) was assessed using
209 the coefficient of variation (CV, %) calculated as: $CV_{BW} = SD_{BW} / BW$ and
210 $CV_{TL} = SD_{TL} / TL$, where SD is the standard deviation and BW is the average body
211 weight (mg) and TL the average body length (mm).
212 The Fulton's condition factor (K) was calculated according to the relationship $K =$
213 BW_f / TL_f^3 (Froese, 2006). The equation was multiplied by 100 to bring the value
214 close to one.
215 Food intake per treatment (FI_{total}, %) was calculated as follows: FI_{total} = (Food
216 distributed in g - Food remaining in g) / Food distributed in g) x 100.
217 Ingestion rates (IR) of fish (expressed as g g^{-1} d^{-1}) exposed to the different
218 temperature were calculated according to the equation: $IR = TFi / (N_f BW_f - N_i$
219 $BW_i) / ED$, where TFi is the total food consumed by the fish (g) during the
220 duration of the experiment (ED, d).
221 Feed conversion ratio (FCR) was calculated using the following equation: $FCR =$
222 $F / (N_f BW_f - N_i BW_i)$, where F is the total quantity of food intake in wet weight
223 during the whole rearing period. F was determined as the total amount of uneaten
224 food subtracted from the total amount of food provided (g).
225 Protein efficiency ratio (PER) was calculated for each experimental treatment
226 according to the following equation: $PER = (N_f BW_f - N_i BW_i) / PI$ where PI is the
227 total protein ingested by the larvae (g).
228 Statistical comparisons were done for each parameter described above. Data were
229 first assessed to confirm normality (Shapiro's test) and homogeneity of variances

(Levene's test) and, where necessary, data expressed were arcsine-square root prior to analysis. One-way ANOVA following by Tukey's test were used to determine significant differences among temperature treatments. When assumptions of normality and homogeneity of variances were unable to be achieved, data were analyzed using Kruskal-Wallis and Siegel and Castellan non-parametric tests.

The growth kinetics of the larvae, both for body weight (BW) and total length (TL), reared at the five different temperature were best fitted using exponential model: BW or TF = BWi or TFi x e^{at} where a was the growth rate (d^{-1}) and t the time (d). Model constants were estimated by iterative adjustment of the model using the least square regression method. In order to statistically assess the effects of temperature on larval growth, for each experimental treatment, growth data (BW and TL) were linearized using natural logarithmic transformation and ANCOVA following by Tukey's test were then applied to identify differences between the regression slopes. The level of significance for statistical analyses was always set to $\alpha = 0.05$. All statistics were performed using R freeware version 3.3.0 (R Development Core Team, 2016).

3. Results

3.1. Water parameters

The targeted temperatures were kept constant throughout the experiment (Table 1) and significant differences were confirmed between each treatment ($\chi^2 = 1100$, $p < 0.001$, Table 1). Regular monitoring indicated that water quality was similar between each treatment. Dissolved oxygen saturation was maintained above 85%

254 in all treatments (χ^2 = 4.015, p = 0.400) with average DO concentration of 6.21 to
255 7.40 mg L^{-1}. Total ammoniacal nitrogen was not significantly different between
256 the five experimental treatments (χ^2 = 5.207, p = 0.267) and NH_3 was kept very
257 lower 0.05 mg L^{-1}.
258
259 3.2. Survival
260 The larval survival rates measured at the end of the experiment ranged from 61.6
261 ± 15.4% (treatment 22.5°C) to 75.5 ± 5.6% (treatment 32.5°C) without any
262 significant differences between the five temperatures tested (χ^2 = 4.196, p = 0.380,
263 Table 2).
264
265 3.3. Growth and size heterogeneity
266 The growth of giant gourami larvae reared at five different temperatures is
267 indicated in Table 2. At the end of the experiment (i.e. after 21 to 42 days
268 depending on the temperature), the average body weight (BW) and total length
269 (TL) of larvae ranged from 270.2 ± 95.4 mg and 24.7 ± 2.6 mm and 431.9 ± 64.2
270 mg and 30.3 ± 1.4 mm when they reared at 22.5°C and 32.5°C respectively (Fig. 1
271 and Table 2). Growth significantly increase with an increase of temperature (F =
272 568.3, p < 0.001 and F = 496.0, p < 0.001 for BW and TL respectively), with the
273 lowest growth observed for the larvae reared at 22.5°C (Fig. 1 and Table 2) and
274 the highest growth observed for the larvae reared at 32.5°C. No significant
275 difference was found for the larvae reared at intermediate temperature (i.e. 27.5°C
276 and 30.0°C). This trend was confirmed by the specific growth rate calculated from
277 body weight (SGR_{BW}) and total length (SGR_{TL}). Significant increases in SGR_{BW}

278 ($F = 360.4$, $p < 0.001$) and SRG_{TL} ($F = 351.5$, $p < 0.001$) were observed when
279 temperature increased, with values varying from 7.3 ± 0.6% for SGR_{BW} and 2.2 ±
280 0.2% for SRG_{TL} at 22.5°C to 17.0 ± 0.2% for SGR_{BW} and 5.4 ± 0.1% SRG_{TL} at
281 32.5°C (Table 2).
282 Interpolation from exponential growth curves (Table 3) indicated that, in this
283 experiment, the fry commercial size (i.e. 2.5 cm in total length) was reached after
284 17 days (i.e. 27 dph) of rearing larvae reared at 32.5°C while 42 days (i.e. 52 dph)
285 are needed for larvae reared at 22.5°C to reach similar size (Fig. 1).
286 Size heterogeneity of larvae as a function of temperature was assessed through the
287 calculation of coefficients of variation for body weight (CV_{BW}) and total length
288 (CV_{TL}) at the end of experiment for each temperature. Thus, CV_{BW} ranged from
289 12.1 ± 1.9 (treatment 25.0°C) to 24.2 ± 10.4% (treatment 22.5°C) and CV_{TL}
290 ranged from 3.8 ± 0.7% (treatment 25.0°C) to 8.0 ± 3.4% (treatment 22.5°C). For
291 CV_{BW} and CV_{TL}, no significant differences were found between the five tested
292 temperatures ($F = 2.3$, $p = 0.128$ and $F = 2.7$, $p = 0.089$ for CV_{BW} and CV_{TL}
293 respectively, Table 2). Statistical analysis revealed a slight significant decrease in
294 K with increased temperature ($F = 88.7$, $p < 0.001$) with K ranging from 1.7 ± 0.1
295 at 22.5°C and 1.5 ± 0.1 at 32.5°C (Table 2).
296
297 3.4. Food intake and feed efficiency
298 The proportion of the total distributed tubifex worms effectively ingested in each
299 aquarium was affected by temperature ($F = 105.7$, $p < 0.0001$) with higher feed
300 intake (45-59%) observed in the highest temperatures (27.5-32.5°C) compared to
301 low temperature treatments (22.5°C and 25.0°C) where ingestion was 26-30%

(Table 4). FCR and ingestion rate (IR) were not affected by the temperature (FCR: $\chi^2 = 9.459$, $p = 0.051$ and IR: $\chi^2 = 6.767$, $p = 0.149$) but remained more variable in the larvae reared at the lowest temperature (i.e. 22.5°C, FCR = 11.1 ± 5.6 and IR = 0.26 ± 0.13 g g^{-1} d^{-1}). Regarding protein use efficiency, PER was significantly lower at 22.5°C (3.1 ± 1.4) compared to the higher temperature treatments ($F=7.4$, $p = 0.005$, Table 4).

3.5. Chemical composition

The results presented in Table 5 showed that the moisture content in fish is stable and does not differ between different temperatures. However, chemical composition (% dry matter basis) of giant gourami juveniles was significantly affected by the rearing temperature especially for the lowest temperature treatment (i.e. 22.5°C). Indeed, crude lipid content was clearly significantly higher at 22.5°C with values of 26.05 ± 1.06% ($F = 198.0$, $p < 0.001$; Table 5). Nevertheless, significant, but less pronounced differences were also observed in the other temperature treatments with minimal values of crude lipid content of 15.88 ± 0.22% observed at 27.5°C. Meanwhile, NFE content was lowest at 22.5°C (6.88 ± 0.85%) and maximal values (14.17 ± 0.32% and 13.86 ± 0.28%) were observed at 25.0°C and 27.5°C respectively ($F = 96.1$, $p < 0.001$). Crude protein increased significantly up to 30.0°C and were lower for the treatment at 32.5°C ($F = 165.6$, $p < 0.001$; Table 5). Ash content was significantly lower for the treatment at 22.5°C and tended to be stable for the other temperature treatments although some significant differences were observed ($F = 92.8$, $p < 0.001$). For crude fibre

content, slight significant increase was observed between the five tested temperatures ($F = 4.5$, $p = 0.025$; Table 5).

3.6. Glucose and cortisol blood concentrations

Glucose and cortisol blood concentrations were measured in giant gourami at the beginning and at the end of each temperature treatments. Initial concentrations of glucose were not significantly different in all the treatments ($F = 0.4$, $p = 0.817$) with values ranging from 31.0 ± 14.0 mg dL^{-1} to 39.0 ± 3.6 mg dL^{-1} (Fig. 2). At the end of each treatment, no change was observed in glucose concentrations for juveniles reared at 22.5°C. Nevertheless, a significant increase was observed for juveniles reared in higher temperature conditions (i.e. from 25.0°C to 32.5°C) with values ranging from 96.0 ± 12.2 mg dL^{-1} to 133.7 ± 52.5 mg dL^{-1} ($F = 5.6$, $p = 0.013$) up to 32.5°C where a decrease of glucose concentration was observed (Fig. 2). Cortisol was not detected in larvae at the beginning of the experiment (Fig. 3). After the larval rearing period, cortisol was detected in juveniles coming from each temperature conditions. Significant higher cortisol concentrations ($F = 67.7$, $p < 0.001$) were observed in the intermediate temperatures (25.0°C and 27.5°C). Indeed, cortisol concentrations reached 72.0 ± 5.8 ng mL^{-1} and 45.9 ± 8.0 ng mL^{-1} at 25.0°C and 27.5°C respectively, while for other treatments, cortisol concentrations ranged from 12.3 ± 4.2 ng mL^{-1} to 17.3 ± 3.4 ng mL^{-1} (Fig. 3).

4. Discussion

4.1. Water quality

348 Aerobic metabolism predominates in fish, so dissolved oxygen (DO) can be a
349 limiting environmental factor in fish rearing (Fry, 1971), especially at high
350 temperature (Jobling, 1997). In the present study, DO was kept high with values
351 never under 6 mg L^{-1} and saturation >85%. Such conditions prevent effects on fish
352 health, growth and feed intake (Kestemont and Baras, 2001). Depending on
353 temperature, oxygen and pH, non-lethal concentrations of NH_3 may cause toxic
354 effects on fish especially for young-life stages (El-Greisy et al., 2016) and affect
355 their feeding behaviour (Kestemont and Baras, 2001). Although some statistical
356 differences have been highlighted between the five experimental treatments, NH_3
357 concentrations was always very lower 0.05 mg L^{-1} following the
358 recommendations of Francis-Floyd et al. (2009). Based on these findings, we can
359 reasonably assume that the results obtained in this study are solely related to
360 temperature and not to water quality degradation.
361
362 4.2. Effects of temperature on survival
363 The present study provides evidence for the effects of rearing temperature on
364 larvae production in the giant gourami (*O. goramy*). The range of temperatures
365 used in this study was based on prior measurements done in the larval rearing
366 structures of fish farms in the West Java province showing that temperatures may
367 vary from 22.9-33.1°C although pikes up to 42.1°C were observed (data not
368 shown). Furthermore, preliminary experimental observations performed over 15 d
369 from hatching have shown that despite a relatively high survival rate (80%), the
370 proportion of deformed larvae was very high above 34°C (77.6% with
371 deformation of vertebral axis, particularly lordosis). Under 21°C, the proportion

of deformed or abnormal larvae reached 64.1% (anomaly of vitellus aspect, haemorrhagic areas at vitellus surface, pericardial oedema). The targeted temperatures were thus selected in order to avoid the occurrence of abnormality in larvae. In this experiment, we demonstrated that temperatures ranging from 22.5°C to 32.5°C did not significantly affect the survival of giant gourami. Observed survival rates of 62-76% are similar (Verawati et al., 2015) or lower (Sarah et al., 2009; Arifin et al. in press) to other experimental studies on this species. We found a higher heterogeneity in the survival rates of the larvae reared at 22.5°C (61.6 ± 15.4%). Based on these findings, we reasonably assumed that 22.5°C seems to be close to the low tolerance limit of the young life-stages of giant gourami.

4.3. Effects of temperature on growth

Successful larval rearing of fish species in aquaculture depends on several factors, among them, the temperature and feed are the most significant ones (Kolman et al., 2018). In the present study, the growth of giant gourami larvae stocked at different temperatures was estimated from weekly measurements of body weight (BW), total length (TL) and specific growth rate (SGR) until the larvae reached the commercial size of 2.5-cm total length (called “Nguku”; Adida, 2014). For all the experimental conditions, exponential growth were observed. At comparable temperatures (29.0 ± 0.6°C vs. 30.01 ± 0.31°C) and at the same stocking density, the growth performances were similar to another recent study (i.e. 288.8 ± 19.3 vs. 354.32 ± 70.19 mg after 21 d, Arifin et al. in press). We found that increasing temperatures up to 32.5°C have positive effects on the growth of giant gourami

larvae. Previous studies have also highlighted the positive influence of increasing temperatures, within their tolerance limits in the growth of several tropical fish young life-stages, such as African catfish *Clarias gariepinus* (Britz and Hecht, 1987), blue tilapia *Oreochromis aureus* (Soderberg, 1990), Nile tilapia *O. niloticus* (Azaza et al., 2008; El-Sayed and Kawanna, 2008; Pandit and Nakamura, 2010) and stripped catfish *Pangasianodon hypophthalmus* (Baras et al., 2011). In this study, giant gourami larvae reared at 32.5°C reached the size of "Nguku" after only 17 days while between 30-98 days are needed in traditional fish farms suggesting that better temperature control provides significant gains in larval production of giant gourami.

4.4. Effects of temperature on food intake and chemical composition

Brett (1979) stated that the temperature influence on growth depends on food consumption and metabolic scope. The energy amount from feeding is used to cover metabolic cost for somatic growth. Following this pattern, larvae exposed to the highest temperature treatment (32.5°C) is likely to have higher feed consumption and metabolism compared with lower temperature treatments (22.5-30.0°C), which resulted on faster growth. Indeed, we found that increasing temperatures led to an increase in the feed consumption with highest ingestions observed in the fish reared at 32.5°C. Interestingly, no significant difference was observed in the IR and FCR among the five temperatures tested. Nevertheless, young life-stages of giant gourami reared at 22.5°C have more variable value on FCR and significant lower PER compared to the other experimental treatments. Such findings are in accordance with previous studies on the African catfish *C.*

gariepinus (Degani et al., 1989) and the hybrid Red Florida tilapia (Watanabe et al., 1993). These findings point out that the less efficient feed utilization on giant gourami larvae occurred at 22.5°C, which resulted in the slowest growth observed at this temperature.

In addition, in the present study, we found that proximate composition of giant gourami juveniles changed with temperature. Various effects of temperature on proximate composition have been shown by previous studies carried out in several fish species. Indeed, in some cases, no influence of temperature on proximate composition was observed (e.g. Martinez-Palacios et al., 1996). Conversely, other authors found temperature-dependent changes in proximate body composition especially for protein content (e.g. Hidalgo et al., 1987; Van Ham et al., 2003). The latter findings are in accordance with the statement of Jobling (1997) who mentioned that changes in proximate composition at high temperatures could be associated with increasing metabolism until it reaches the upper tolerance limits in poikilotherm organisms such as fish. In the present study, we found a higher lipid and a lower carbohydrate (i.e. NFE) contents in the fish exposed to the lowest temperature treatment. We assume that such results can be related to the lowest metabolism in fish reared at 22.5°C. Indeed, most freshwater fish species do not attempt to maintain a body temperature which is different from their environment. When water temperature declines, body temperature of the fish also declines and metabolic rate is reduced and, as consequence, the energy requirements is reduced too (FAO, 1980). Thus, excessive carbohydrates, a non-negligible energy source for fish can be stored as form of lipids (Tacon, 1987).

444 4.5. Physiological responses to temperature

445 Changes in temperatures can be a source of stress for fish larvae, affecting larval
446 rearing performances, and imply different physiological responses (Blaxter, 1991).
447 In this study, glucose and whole-body cortisol levels, recognized as primary
448 indicators of stress in young-life stages fish (Rudneva, 2013), were measured at
449 the beginning of the experiment and when the giant gourami larvae from each
450 temperature treatments reached the commercial size of "Nguku". Interestingly, we
451 found similar effects of rearing temperatures on blood glucose and plasma cortisol
452 levels in giant gourami larvae. Indeed, both for glucose and cortisol,
453 concentrations measured were higher at intermediate temperatures (25°C and
454 27.5-30.0°C) and significantly lower at the extreme temperatures (22.5°C and
455 30.0-32.5°C). Several studies have shown contrasting results regarding the effects
456 of temperature on glucose and cortisol concentrations in other tropical fish species
457 such as blue tilapia *O. aureus* (Chen et al., 2002), common carp *Cyprinus carpio*
458 (Tanck et al., 2000), Mozambique tilapia *O. mossambicus* (Zaragoza et al., 2008),
459 zebrafish *Danio rerio* (Long et al., 2012), and Tapah *Wallago leeri* (Tang et al.,
460 2017). Most previous studies, however, agree that cold/heat temperature exposure
461 that exceeds the tolerance limits modified blood glucose or plasma cortisol levels.
462 In giant gourami fingerlings, Hastuti et al. (2003) reported that cold shock to 26-
463 27°C, 23-24°C, and 20-21°C led to changes in blood glucose level with highest
464 values observed at 20-21°C (~80 mg dL^{-1}). In fish, Selye (1974) and Barton and
465 Iwama (1991) stated that the exposure of low or high temperatures beyond the
466 tolerance limits lead to negative impact on their physiology depending on each
467 species tolerance level. In the present study, we found that glucose concentrations

are the highest at the intermediate temperatures (25.0, 27.5 and 30.0°C) and decreased at the extreme rearing temperatures (22.5 and 32.5°C). Based on our main findings, we can reasonably assume that the extreme temperatures tested are closed to the tolerance limit of this species and affected the metabolism of the larvae. Thus, although, the most efficient temperature for larval growing (i.e. 32.5°C) does not cause serious impairment in larvae, a slightly lower rearing temperature (~30.0°C) should be maintained as a precaution.

The physiological responses measured in this study, in addition to the zootechnical performances observed, reinforce the interests of an optimization of the giant gourami larval rearing based on a better control of the temperature. Such improvements are currently required to support micro- and small-scale fish farmers who provide the bulk of gourami production in Indonesia.

5. Conclusion

We highlighted that temperature strongly affects the larval growth. Thus, we observed that 10-dph larvae reared at 22.5°C needed 42 days of production before reaching the commercial size of "Nguku" while only 17 days is needed at 32.5°C. Based on all our findings, maintaining a stable temperature at 30°C seems to be the best compromise to significantly improve the zootechnical and economic performance of giant gourami larval rearing. Nevertheless, control of lower temperatures is not always easy in traditional fish farming due to the limited access to electricity and its cost. Thus, further investigations are needed to (1) determine the performances of larvae reared at constant temperatures on the

nursery and grow-out outdoor phases and (2) assess effects of daily thermal fluctuations on the performances of giant gourami larvae.

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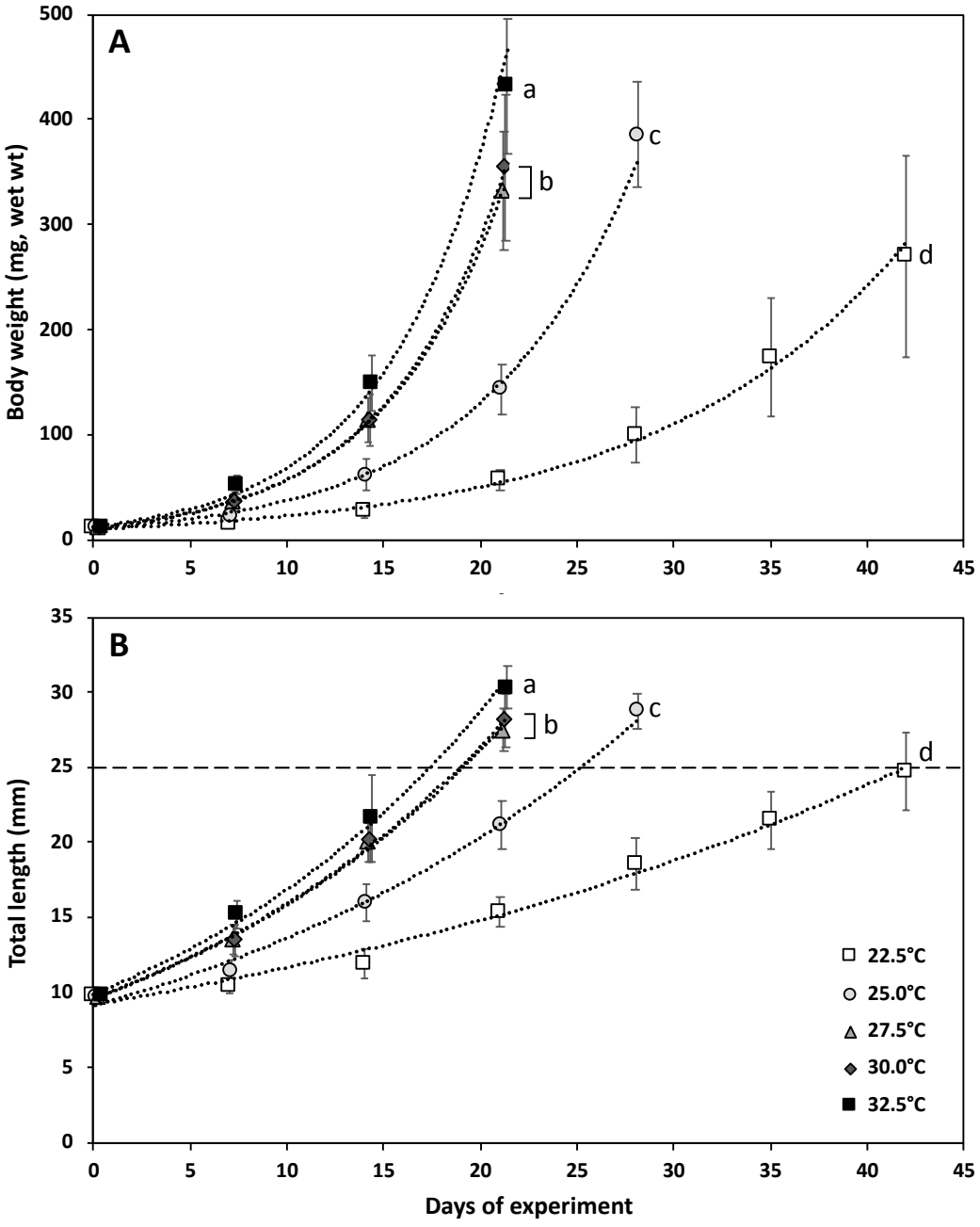
Captions to figures

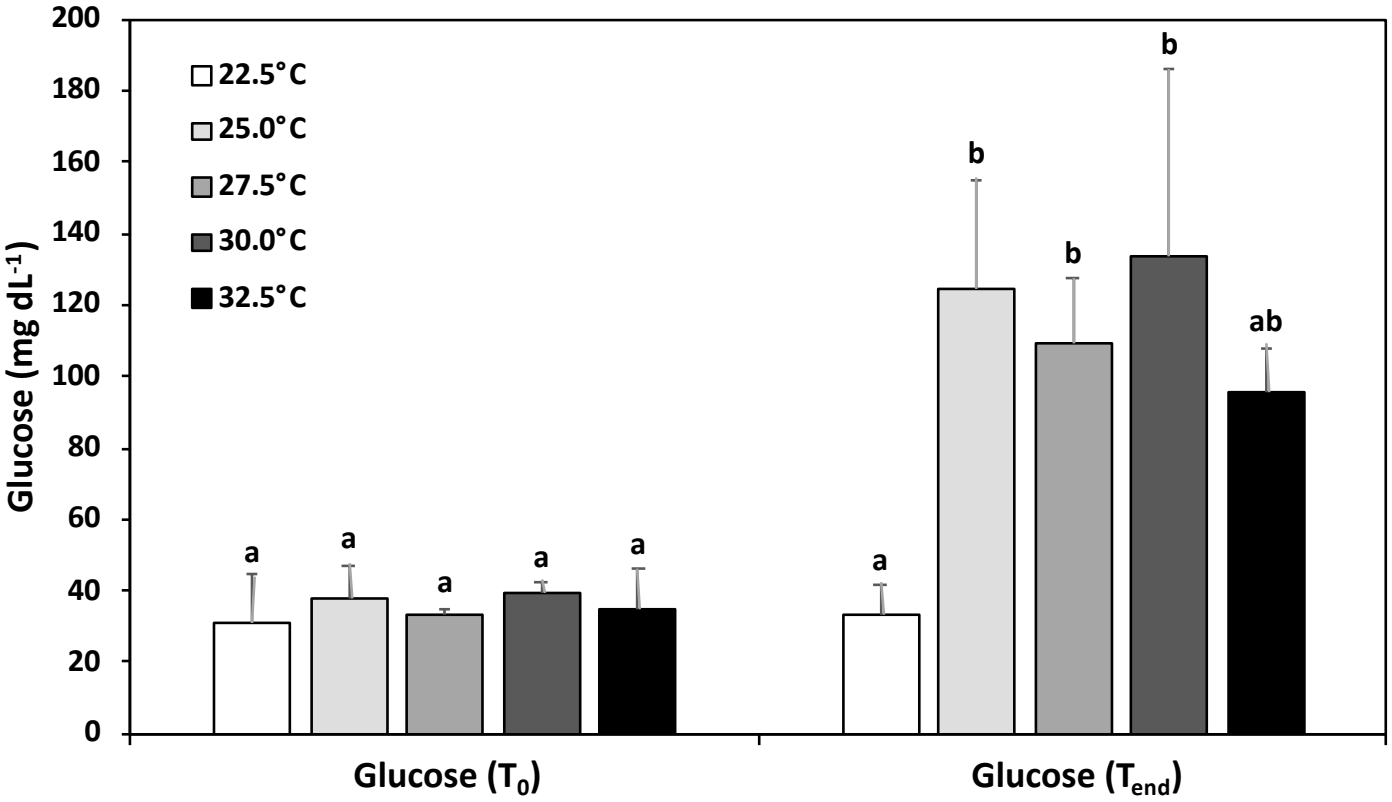
Figure 1. Growth in (A) body weight and (B) total length of giant gourami (*O. goramy*) larvae (n=60) reared at five temperatures from 8 dph. The horizontal dotted line represents the commercial size (i.e. called "Nguku", 2.5 cm of total length). Values are Means ± SD. Letters denote significant differences ($p < 0.05$) between treatments.

Figure 2. Concentration of glucose in the blood of giant gourami (*O. goramy*) larvae (n=3 pools of 20 larvae) reared at five temperatures from 10 dph (T_0) up to their reached 2.5-cm total length (T_{end}). Values are Means ± SD. For Letters denote significant differences ($p < 0.05$) between treatments.

Figure 3. Concentration of cortisol in the blood of giant gourami (*O. goramy*) larvae (n=20) reared at five temperatures from 10 dph (T_0) up to their reached 2.5-cm total length (T_{end}). Values are Means ± SD. Letters denote significant differences ($p < 0.05$) between treatments.


A
Body weight (mg, wet wt)
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
a
b
c
d
B
Total length (mm)
35
30
25
20
15
10
5
0
Days of experiment
22.5°C
25.0°C
27.5°C
30.0°C
32.5°C

698 Figure 1


200
180
160
140
120
100
80
60
40
20
0
Glucose (mg dL-1)
22.5°C
25.0°C
27.5°C
30.0°C
32.5°C
a
a
a
a
a
a
b
b
b
ab
Glucose (T0)
Glucose (Tend)

699 Figure 2

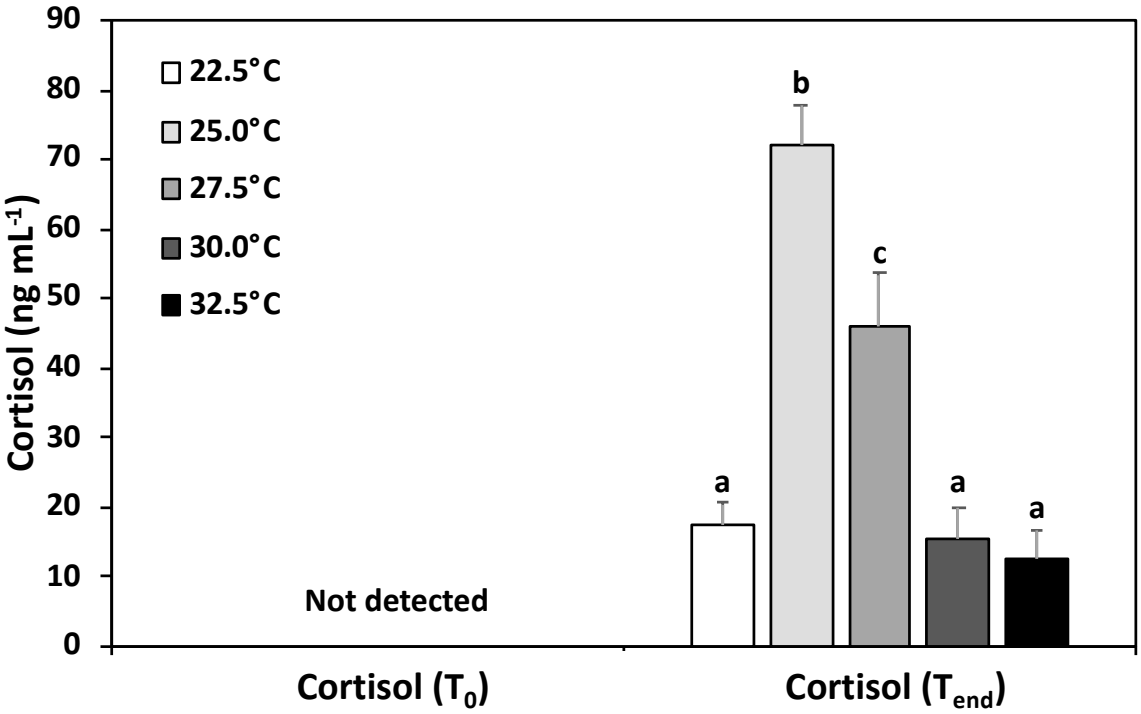

90
80
70
60
50
40
30
20
10
0
Cortisol (ng mL-1)
22.5°C
25.0°C
27.5°C
30.0°C
32.5°C
Not detected
a
b
c
a
a
Cortisol (T0)
Cortisol (Tend)

Figure 3

701 Table 1. Summary of water quality parameters measured for the five temperature
702 conditions during the experiment. Values are Means ± SD. For each parameter,
703 letters denote significant differences ($p < 0.05$) between treatments.

Parameters	Experimental treatment				
	22.5°C	25.0 °C	27.5°C	30.0°C	32.5°C
Temperature (°C)	22.57 ± 0.43^{a}	25.24 ± 0.53^{b}	28.17 ± 0.68^{c}	30.01 ± 0.31^{d}	32.45 ± 0.74^{e}
DO (mg L^{-1})	7.40 ± 0.37^{a}	7.05 ± 0.39^{ab}	6.67 ± 0.25^{b}	6.55 ± 0.15^{b}	6.21 ± 0.18^{c}
O_2 saturation (%)	85.6 ± 4.3^{a}	85.7 ± 4.7^{a}	85.5 ± 3.2^{a}	86.7 ± 2.0^{a}	85.7 ± 2.5^{a}
pH	6.58 ± 0.19^{a}	6.77 ± 0.21^{b}	7.04 ± 0.05^{b}	6.98 ± 0.28^{b}	7.05 ± 0.04^{b}
Conductivity (µS cm^{-1})	208.8 ± 13.3^{a}	263.9 ± 15.2^{b}	215.0 ± 6.1^{a}	327.0 ± 16.2^{c}	347.0 ± 7.2^{c}
Alkalinity (mg L^{-1})	130.4 ± 45.2^{a}	124.1 ± 23.4^{a}	135.4 ± 19.1^{a}	124.6 ± 26.0^{a}	142.2 ± 15.2^{a}
TAN (mg L^{-1})	0.12 ± 0.08^{a}	0.12 ± 0.06^{a}	0.09 ± 0.05^{a}	0.07 ± 0.02^{a}	0.12 ± 0.03^{a}
NH_3 (mg L^{-1})	0.0003 ± 0.0003^{a}	0.0011 ± 0.0019^{ab}	0.0002 ± 0.0001^{ac}	0.0007 ± 0.0003^{abc}	0.0015 ± 0.0004^{bd}
NO_2^- (mg L^{-1})	0.08 ± 0.138^{a}	0.02 ± 0.02^{a}	0.01 ± 0.003^{a}	0.02 ± 0.004^{a}	$0.02 \pm 0.0.017^{a}$
NO_3^- (mg L^{-1})	4.25 ± 3.15^{a}	5.48 ± 2.85^{a}	4.15 ± 0.75^{a}	12.30 ± 2.46^{a}	7.54 ± 2.01^{a}

704 DO: dissolved oxygen; TAN: total ammoniacal nitrogen

Table 2. Growth and survival of giant gourami larvae reared at five temperature conditions (22.5, 25.0, 27.5; 30.0, 32.5°C) in a closed recirculating system for 21 to 42 days (i.e. until they reached, at least, the commercial size of 2.5 cm of total length). See Section 2.5 for details of the parameters. Values are means ± SD. For each parameter, letters denote significant differences ($p < 0.05$) between treatments.

Parameters	Experimental treatment				
	22.5°C	25.0°C	27.5°C	30.0°C	32.5°C
Rearing duration (d)	42	28	21	21	21
BW_i (mg)	12.3 ± 1.5^{a}	12.1 ± 1.5^{a}	12.4 ± 1.4^{a}	11.8 ± 1.1^{a}	12.2 ± 1.4^{a}
BW_{21d} (mg)	57.2 ± 9.3^{a}	143.7 ± 23.6^{b}	332.0 ± 56.0^{c}	354.3 ± 70.2^{c}	431.9 ± 64.2^{d}
BW_f (mg)	270.2 ± 95.4^{a}	385.8 ± 50.3^{b}	332.0 ± 56.0^{c}	354.3 ± 70.2^{bc}	431.9 ± 64.2^{d}
CV_{BW} (%)	24.2 ± 10.4^{a}	12.1 ± 1.9^{a}	16.1 ± 2.1^{a}	19.9 ± 3.7^{a}	14.7 ± 3.3^{a}
CV_{TL} (%)	8.0 ± 3.4^{a}	3.8 ± 0.7^{a}	4.8 ± 1.0^{a}	6.37 ± 1.2^{a}	4.5 ± 0.8^{a}
K (mg mm^{-3} x 100)	1.7 ± 0.1^{a}	1.6 ± 0.1^{b}	1.6 ± 0.1^{bc}	1.6 ± 0.1^{c}	1.5 ± 0.1^{c}
SGR_{BW} (% d^{-1})	7.3 ± 0.6^{a}	12.4 ± 0.3^{b}	15.7 ± 0.3^{c}	16.2 ± 0.2^{cd}	17.0 ± 0.2^{d}
SGR_{TL} (% d^{-1})	2.2 ± 0.2^{a}	3.9 ± 0.1^{b}	4.9 ± 0.2^{c}	5.0 ± 0.1^{cd}	5.4 ± 0.1^{d}
SR (%)	61.6 ± 15.4^{a}	68.5 ± 5.3^{a}	66.7 ± 6.4^{a}	75.0 ± 4.8^{a}	75.5 ± 5.6^{a}
TL_i (mm)	9.8 ± 0.4^{a}	9.7 ± 0.3^{ab}	9.8 ± 0.4^{a}	9.9 ± 0.19^{ac}	9.8 ± 0.3^{a}
TL_{21d} (mm)	15.4 ± 1.0^{a}	21.2 ± 1.6^{b}	27.5 ± 1.4^{c}	28.2 ± 1.8^{c}	30.3 ± 1.4^{d}
TL_f (mm)	24.7 ± 2.6^{a}	28.8 ± 1.2^{b}	27.5 ± 1.4^{c}	28.2 ± 1.8^{bc}	30.3 ± 1.4^{d}

BW: body weight, BW_i: initial body weight, BW_f: final body weight, CV: coefficient of variation, TL: total length, SGR: specific growth rate, SR: survival rate, TL_i: initial total length, TL_f: final total length.

Table 3. Parameters (Mean ± SE, n = 20 at each time) of the exponential growth in body weight (BW, mg) and total length (TL, mm) of giant gourami (*O. goramy*) larvae reared at five different temperatures. Model parameters: a: growth rate (d^{-1}). R^2: determination coefficient.

Treatments	a ± SE	R^2
Body weight (BW)		
22.5°C	0.074 ± 0.001***	0.90
25.0°C	0.123 ± 0.001***	0.98
27.5°C	0.157 ± 0.001***	0.97
30.0°C	0.162 ± 0.001***	0.96
32.5°C	0.170 ± 0.001***	0.98
Total length (TL)		
22.5°C	0.022 ± 0.001***	0.96
25.0°C	0.038 ± 0.001***	0.98
27.5°C	0.050 ± 0.001***	0.99
30.0°C	0.050 ± 0.001***	0.99
32.5°C	0.054 ± 0.001***	0.97

*** Probability of the model adjustment: $p < 0.001$

719 Table 4. Feed utilization of gourami reared larvae rearing in closed recirculating
720 system at five temperature conditions. Values are means ± SD. For each
721 parameter, letters denote significant differences ($p < 0.05$) between treatments.

Parameters	Experimental treatment				
	22.5°C	25.0°C	27.5°C	30.0°C	32.5°C
FI_{total} (%)	25.7 ± 2.1^{a}	30.4 ± 1.5^{a}	45.3 ± 2.2^{b}	51.0 ± 3.6^{b}	58.6 ± 1.6^{c}
IR ($g\ g^{-1}\ d^{-1}$)	0.26 ± 0.13^{a}	0.17 ± 0.02^{a}	0.26 ± 0.02^{a}	0.24 ± 0.01^{a}	0.25 ± 0.01^{a}
FCR	11.1 ± 5.6^{a}	4.7 ± 0.6^{a}	5.4 ± 0.4^{a}	5.1 ± 0.2^{a}	5.2 ± 0.2^{a}
PER	3.1 ± 1.4^{a}	6.3 ± 0.8^{b}	5.4 ± 0.4^{b}	5.7 ± 0.2^{b}	5.6 ± 0.3^{b}

722 FI: food intake, IR: ingestion rate, FCR: feed conversion ratio, PER: protein
723 efficiency ratio.

724 Table 5. Moisture (%), dry matter (%) and proximate composition (% dry matter
725 basis) of giant gourami juveniles reared in closed recirculating system at five
726 temperature conditions. Values are means ± SD. For each parameter, letters
727 denote significant differences ($p < 0.05$) between treatments.

Composition	Experimental treatment				
	22.5°C	25.0°C	27.5°C	30.0°C	32.5°C
Moisture	77.80 ± 1.17^{a}	78.38 ± 0.10^{a}	79.02 ± 0.11^{a}	78.79 ± 0.29^{a}	78.31 ± 0.39^{a}
Dry matter	22.20 ± 1.17^{a}	21.62 ± 0.10^{a}	20.98 ± 0.11^{a}	21.21 ± 0.29^{a}	21.69 ± 0.39^{a}
Crude protein	57.90 ± 0.08^{a}	58.19 ± 0.10^{b}	59.23 ± 0.11^{c}	59.86 ± 0.09^{d}	58.90 ± 0.12^{e}
Crude lipid	26.05 ± 1.06^{a}	17.13 ± 0.23^{b}	15.88 ± 0.22^{c}	17.50 ± 0.35^{b}	18.91 ± 0.24^{d}
Ash	8.75 ± 0.19^{a}	10.09 ± 0.06^{b}	10.58 ± 0.11^{c}	10.26 ± 0.09^{bc}	10.27 ± 0.15^{bd}
Crude fibre	0.41 ± 0.02^{a}	0.42 ± 0.02^{a}	0.45 ± 0.02^{ab}	0.46 ± 0.03^{ab}	0.48 ± 0.01^{b}
NFE	6.88 ± 0.85^{a}	14.17 ± 0.32^{b}	13.86 ± 0.28^{b}	11.93 ± 0.38^{c}	11.45 ± 0.14^{c}

728 NFE: Nitrogen Free Extract.