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1     **Effects of temperature on the zootechnical performances and physiology of**  
2                                    **giant gourami (*Osphronemus goramy*) larvae**

3

4     Vitas Atmadi Prakoso<sup>a\*</sup>, Simon Pouil<sup>b</sup>, Muhammad Naufal Ibrahim Prabowo<sup>c</sup>, Sri  
5             Sundari<sup>a</sup>, Otong Zenal Arifin<sup>a</sup>, Jojo Subagja<sup>a</sup>, Ridwan Affandi<sup>c</sup>, Anang Hari  
6                                    Kristanto<sup>a</sup>, and Jacques Slembrouck<sup>b</sup>

7

8     <sup>a</sup> Research Institute for Freshwater Aquaculture and Fisheries Extension  
9     (RIFAFE), Bogor, Indonesia

10    <sup>b</sup> ISEM, IRD, Université de Montpellier, CNRS, EPHE, Montpellier, France

11    <sup>c</sup> Department of Aquatic Resources Management, Fisheries and Marine Science  
12    Faculty, Bogor Agriculture University (IPB), Bogor, Indonesia

13

14    Corresponding author: [vitas.atmadi@gmail.com](mailto:vitas.atmadi@gmail.com)

15    Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE),  
16    Jl. Sempur No. 1, Bogor, West Java, Indonesia 16129

17    Phone: + 62 251 8313200

18 **Abstract**

19 The influence of temperature on zootechnical performances and physiology was  
20 assessed in giant gourami (*Osphronemus goramy*) larvae. Larvae aged ten days  
21 post-hatching were reared at five temperature treatments (22.5, 25.0, 27.5, 30.0  
22 and 32.5 °C, three replicated per treatment) in indoor closed recirculating systems  
23 until they reached, at least, the commercial size of 2.5 cm in total length (i.e.  
24 rearing time of 21 to 42 d depending on the temperature). Samples of larvae were  
25 collected every week. Survival, growth, and feed intake were used as main  
26 indicators of zootechnical performances. In addition, concentration of glucose and  
27 cortisol and proximate composition of the young life-stages giant gourami were  
28 compared to assess effects of temperature on their physiology. Results showed  
29 that temperature strongly affected growth of giant gourami larvae with  
30 significantly increased observed at the increasing tested temperatures (from  $57.2 \pm$   
31  $9.3$  mg to  $431.9 \pm 64.2$  mg of body weight after three weeks,  $p < 0.05$ ). The  
32 increased growth can be related to changes in metabolism, feed intake and feed  
33 use efficiency. Based on cortisol and glucose values, we recommend to maintain  
34 rearing temperature at 30.0°C for both optimizing growth and avoid physiological  
35 impairments in giant gourami larvae. The consequences of larval rearing at stable  
36 temperature (30.0°C) should be further investigated in the nursery and grow-out  
37 outdoor phases.

38

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

40 **1. Introduction**

41 Giant gourami (*Osphronemus goramy*; Lacepède, 1801) is one of the most  
42 popular freshwater fish species in Southeast Asia and particularly in Indonesia,  
43 the main producing country in aquaculture (113,396 tons in 2015; FAO, 2017).  
44 Over the past 15 years, the Indonesian giant gourami aquaculture production has  
45 grown exponentially with an annual increase of 16% (FAO, 2017). As for other  
46 freshwater species, Indonesian giant gourami aquaculture is mainly ensured by  
47 micro and small-scale farms (more than 100,000 fish farmers mainly in Java  
48 Island; BPS, 2013; Maskur et al., 2013). Currently, there are still some obstacles  
49 to the production of gourami related to gaps of knowledge on the biology of this  
50 species (Arifin et al., in press). One of the main impediments in giant gourami  
51 aquaculture is ensuring availability of fry for fish farmers through the  
52 improvement of larval rearing methods (Amornsakun et al., 2014a, 2014b).  
53 Commercial scale propagation of giant gourami in hatcheries is yet to be  
54 standardized (Amornsakun et al., 2014a). Thus, although Indonesian National  
55 Standard recommends indoor larval rearing allowing to limit variations of  
56 environmental conditions (SNI, 2000), an on-farm inquiry carried out on about 40  
57 small-scale farms of the West Java province revealed that the current practices for  
58 giant gourami larval production consisted to rear larvae for the first days post-  
59 hatching (dph) in plastic basins and then transferred to outdoor small ponds. In  
60 such practices, larvae have to deal with fluctuations of environmental conditions  
61 that could not be controlled, especially for water temperature changes. For these  
62 reasons, the impacts of environmental conditions on giant gourami larval rearing,  
63 especially temperature, is thus become limiting factor for fish farmers.

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

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

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

97

## 98 **2. Materials and methods**

### 99 2.1. Origin of larvae

100 Giant gourami larvae used in this experiment came from the same broodfish pair  
101 at the same natural spawning event. The 3-4 years old broodfish (“Galunggung”  
102 strain, Arifin et al., 2018) were reared in an outdoor pond at the Research  
103 Installation of Germplasm Freshwater Aquaculture (RIFAFE, Cijeruk, West Java,  
104 Indonesia). Broodfish were fed leaves of giant taro (*Alocasia macrorrhiza*) and  
105 commercial feeds (floating pellets: 32% proteins, 5% lipids) at a feeding rate of  
106 2% and 1% of fish biomass per day, respectively. Bamboo nest supports and palm  
107 tree fibres were provided for nest building. The buoyant eggs were then incubated  
108 in the experimental room for 20 hours (20-L plastic basin; daily water change,  
109 temperature:  $29.0 \pm 0.6^{\circ}\text{C}$ ; light:dark cycle: 12:12 h). After hatching, larvae were  
110 kept unfed in the incubation basin (following fish farming practices), until the

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

113

## 114 2.2. Live prey maintenance

115 According to the current practices (SNI, 2000) and Lucas et al. (2015) who  
116 demonstrated the benefits of this feed for survival rate and growth of giant  
117 gourami larvae, in this study, fish were fed tubifex worms (*Tubifex tubifex*)  
118 throughout the experiment. Live tubifex worms were purchased weekly and stored  
119 in the experimental room (100-L aquarium; daily water change, temperature: 29.0  
120  $\pm$  0.6°C; light:dark cycle: 12:12 h) and kept unfed. Proximate analyses of tubifex  
121 worms were conducted to obtain nutritional quality based on the procedures  
122 described in AOAC (1999) giving the following results (dry matter basis): 52.87%  
123 crude proteins, 22.09% crude lipids, 4.09% ash, 1.23% crude fibre and 19.72%  
124 nitrogen free extract (NFE).

125

## 126 2.3. Temperature experiment

### 127 2.3.1. Experimental design

128 Zootechnical performances and physiology of giant gourami *O. goramy* larvae  
129 were studied under five temperature conditions (22.5, 25.0, 27.5, 30.0 and  
130 32.5°C). Experiment was carried out in five identical indoor RAS (1 per  
131 temperature condition) under natural photoperiod (light:dark cycle: 12:12 h,  
132 daylight intensity: 60-4500 lux). At 10 dph, larvae were individually counted and  
133 measured (mean body weight: 12.4  $\pm$  1.4 mg; mean total length: 9.8  $\pm$  0.3 mm)

134 then randomly assigned to the experimental tanks (n=3 per temperature condition,  
135 stocking density of 2.4 larvae L<sup>-1</sup>) placed in RAS.

136 Larvae were acclimated into targeted temperatures by gradual temperature  
137 changes (2.5°C h<sup>-1</sup>). 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<sup>-1</sup> day<sup>-1</sup> 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<sup>-1</sup> for the first 5 days of the experiment and then at

158 78 L h<sup>-1</sup>. 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<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were measured by spectrophotometry analysis (n=6-15 per  
163 treatment). NH<sub>3</sub> 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<sup>-1</sup>) 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 ( $N_i$ ):  $SR (\%) = (N_f / N_i) \times 100$ .

202 The specific growth in body weight ( $SGR_{BW}$ , %) was calculated according to the  
203 following equation:  $SGR_{BW} = (\ln BW_f - \ln BW_i) / ED \times 100$ , 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  $distributed\ in\ g - Food\ remaining\ in\ g) / Food\ distributed\ in\ g) \times 100$ .  
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

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

236 The growth kinetics of the larvae, both for body weight (BW) and total length  
237 (TL), reared at the five different temperature were best fitted using exponential  
238 model:  $BW$  or  $TF = BW_i$  or  $TF_i \times e^{at}$  where  $a$  was the growth rate ( $d^{-1}$ ) and  $t$  the  
239 time (d). Model constants were estimated by iterative adjustment of the model  
240 using the least square regression method. In order to statistically assess the effects  
241 of temperature on larval growth, for each experimental treatment, growth data  
242 (BW and TL) were linearized using natural logarithmic transformation and  
243 ANCOVA following by Tukey's test were then applied to identify differences  
244 between the regression slopes. The level of significance for statistical analyses  
245 was always set to  $\alpha = 0.05$ . All statistics were performed using R freeware version  
246 3.3.0 (R Development Core Team, 2016).

247

### 248 **3. Results**

#### 249 3.1. Water parameters

250 The targeted temperatures were kept constant throughout the experiment (Table 1)  
251 and significant differences were confirmed between each treatment ( $\chi^2 = 1100$ ,  $p$   
252  $< 0.001$ , Table 1). Regular monitoring indicated that water quality was similar  
253 between each treatment. Dissolved oxygen saturation was maintained above 85%

254 in all treatments ( $\chi^2 = 4.015$ ,  $p = 0.400$ ) with average DO concentration of 6.21 to  
255 7.40 mg L<sup>-1</sup>. Total ammoniacal nitrogen was not significantly different between  
256 the five experimental treatments ( $\chi^2 = 5.207$ ,  $p = 0.267$ ) and NH<sub>3</sub> was kept very  
257 lower 0.05 mg L<sup>-1</sup>.

258

### 259 3.2. Survival

260 The larval survival rates measured at the end of the experiment ranged from 61.6  
261  $\pm$  15.4% (treatment 22.5°C) to 75.5  $\pm$  5.6% (treatment 32.5°C) without any  
262 significant differences between the five temperatures tested ( $\chi^2 = 4.196$ ,  $p = 0.380$ ,  
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  $\pm$  95.4 mg and 24.7  $\pm$  2.6 mm and 431.9  $\pm$  64.2  
270 mg and 30.3  $\pm$  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  $7.3 \pm 0.6\%$  for  $SGR_{BW}$  and  $2.2 \pm$   
280  $0.2\%$  for  $SRG_{TL}$  at  $22.5^\circ\text{C}$  to  $17.0 \pm 0.2\%$  for  $SGR_{BW}$  and  $5.4 \pm 0.1\%$   $SRG_{TL}$  at  
281  $32.5^\circ\text{C}$  (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  $32.5^\circ\text{C}$  while 42 days (i.e. 52 dph)  
285 are needed for larvae reared at  $22.5^\circ\text{C}$  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  $12.1 \pm 1.9$  (treatment  $25.0^\circ\text{C}$ ) to  $24.2 \pm 10.4\%$  (treatment  $22.5^\circ\text{C}$ ) and  $CV_{TL}$   
290 ranged from  $3.8 \pm 0.7\%$  (treatment  $25.0^\circ\text{C}$ ) to  $8.0 \pm 3.4\%$  (treatment  $22.5^\circ\text{C}$ ). 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  $1.7 \pm 0.1$   
295 at  $22.5^\circ\text{C}$  and  $1.5 \pm 0.1$  at  $32.5^\circ\text{C}$  (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 ( $27.5$ - $32.5^\circ\text{C}$ ) compared to  
301 low temperature treatments ( $22.5^\circ\text{C}$  and  $25.0^\circ\text{C}$ ) where ingestion was 26-30%

302 (Table 4). FCR and ingestion rate (IR) were not affected by the temperature (FCR:  
303  $\chi^2 = 9.459$ ,  $p = 0.051$  and IR:  $\chi^2 = 6.767$ ,  $p = 0.149$ ) but remained more variable in  
304 the larvae reared at the lowest temperature (i.e. 22.5°C, FCR =  $11.1 \pm 5.6$  and IR  
305 =  $0.26 \pm 0.13 \text{ g g}^{-1} \text{ d}^{-1}$ ). Regarding protein use efficiency, PER was significantly  
306 lower at 22.5°C ( $3.1 \pm 1.4$ ) compared to the higher temperature treatments ( $F=7.4$ ,  
307  $p = 0.005$ , Table 4).

308

### 309 3.5. Chemical composition

310 The results presented in Table 5 showed that the moisture content in fish is stable  
311 and does not differ between different temperatures. However, chemical  
312 composition (% dry matter basis) of giant gourami juveniles was significantly  
313 affected by the rearing temperature especially for the lowest temperature  
314 treatment (i.e. 22.5°C). Indeed, crude lipid content was clearly significantly higher  
315 at 22.5°C with values of  $26.05 \pm 1.06\%$  ( $F = 198.0$ ,  $p < 0.001$ ; Table 5).  
316 Nevertheless, significant, but less pronounced differences were also observed in  
317 the other temperature treatments with minimal values of crude lipid content of  
318  $15.88 \pm 0.22\%$  observed at 27.5°C. Meanwhile, NFE content was lowest at 22.5°C  
319 ( $6.88 \pm 0.85\%$ ) and maximal values ( $14.17 \pm 0.32\%$  and  $13.86 \pm 0.28\%$ ) were  
320 observed at 25.0°C and 27.5°C respectively ( $F = 96.1$ ,  $p < 0.001$ ). Crude protein  
321 increased significantly up to 30.0°C and were lower for the treatment at 32.5°C ( $F$   
322 =  $165.6$ ,  $p < 0.001$ ; Table 5). Ash content was significantly lower for the treatment  
323 at 22.5°C and tended to be stable for the other temperature treatments although  
324 some significant differences were observed ( $F = 92.8$ ,  $p < 0.001$ ). For crude fibre

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

327

### 328 3.6. Glucose and cortisol blood concentrations

329 Glucose and cortisol blood concentrations were measured in giant gourami at the  
330 beginning and at the end of each temperature treatments. Initial concentrations of  
331 glucose were not significantly different in all the treatments ( $F = 0.4$ ,  $p = 0.817$ )  
332 with values ranging from  $31.0 \pm 14.0$  mg dL<sup>-1</sup> to  $39.0 \pm 3.6$  mg dL<sup>-1</sup> (Fig. 2). At  
333 the end of each treatment, no change was observed in glucose concentrations for  
334 juveniles reared at 22.5°C. Nevertheless, a significant increase was observed for  
335 juveniles reared in higher temperature conditions (i.e. from 25.0°C to 32.5°C)  
336 with values ranging from  $96.0 \pm 12.2$  mg dL<sup>-1</sup> to  $133.7 \pm 52.5$  mg dL<sup>-1</sup> ( $F = 5.6$ ,  $p$   
337  $= 0.013$ ) up to 32.5°C where a decrease of glucose concentration was observed  
338 (Fig. 2). Cortisol was not detected in larvae at the beginning of the experiment  
339 (Fig. 3). After the larval rearing period, cortisol was detected in juveniles coming  
340 from each temperature conditions. Significant higher cortisol concentrations ( $F =$   
341  $67.7$ ,  $p < 0.001$ ) were observed in the intermediate temperatures (25.0°C and  
342 27.5°C). Indeed, cortisol concentrations reached  $72.0 \pm 5.8$  ng mL<sup>-1</sup> and  $45.9 \pm 8.0$   
343 ng mL<sup>-1</sup> at 25.0°C and 27.5°C respectively, while for other treatments, cortisol  
344 concentrations ranged from  $12.3 \pm 4.2$  ng mL<sup>-1</sup> to  $17.3 \pm 3.4$  ng mL<sup>-1</sup> (Fig. 3).

345

## 346 **4. Discussion**

### 347 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<sup>-1</sup> 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<sub>3</sub> 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<sub>3</sub>  
357 concentrations was always very lower 0.05 mg L<sup>-1</sup> 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

372 of deformed or abnormal larvae reached 64.1% (anomaly of vitellus aspect,  
373 haemorrhagic areas at vitellus surface, pericardial oedema). The targeted  
374 temperatures were thus selected in order to avoid the occurrence of abnormality in  
375 larvae. In this experiment, we demonstrated that temperatures ranging from  
376 22.5°C to 32.5°C did not significantly affect the survival of giant gourami.  
377 Observed survival rates of 62-76% are similar (Verawati et al., 2015) or lower  
378 (Sarah et al., 2009; Arifin et al. in press) to other experimental studies on this  
379 species. We found a higher heterogeneity in the survival rates of the larvae reared  
380 at 22.5°C ( $61.6 \pm 15.4\%$ ). Based on these findings, we reasonably assumed that  
381 22.5°C seems to be close to the low tolerance limit of the young life-stages of  
382 giant gourami.

383

#### 384 4.3. Effects of temperature on growth

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

396 larvae. Previous studies have also highlighted the positive influence of increasing  
397 temperatures, within their tolerance limits in the growth of several tropical fish  
398 young life-stages, such as African catfish *Clarias gariepinus* (Britz and Hecht,  
399 1987), blue tilapia *Oreochromis aureus* (Soderberg, 1990), Nile tilapia *O.*  
400 *niloticus* (Azaza et al., 2008; El-Sayed and Kawanna, 2008; Pandit and Nakamura,  
401 2010) and striped catfish *Pangasianodon hypophthalmus* (Baras et al., 2011). In  
402 this study, giant gourami larvae reared at 32.5°C reached the size of “Nguku”  
403 after only 17 days while between 30-98 days are needed in traditional fish farms  
404 suggesting that better temperature control provides significant gains in larval  
405 production of giant gourami.

406

#### 407 4.4. Effects of temperature on food intake and chemical composition

408 Brett (1979) stated that the temperature influence on growth depends on food  
409 consumption and metabolic scope. The energy amount from feeding is used to  
410 cover metabolic cost for somatic growth. Following this pattern, larvae exposed to  
411 the highest temperature treatment (32.5°C) is likely to have higher feed  
412 consumption and metabolism compared with lower temperature treatments (22.5-  
413 30.0°C), which resulted on faster growth. Indeed, we found that increasing  
414 temperatures led to an increase in the feed consumption with highest ingestions  
415 observed in the fish reared at 32.5°C. Interestingly, no significant difference was  
416 observed in the IR and FCR among the five temperatures tested. Nevertheless,  
417 young life-stages of giant gourami reared at 22.5°C have more variable value on  
418 FCR and significant lower PER compared to the other experimental treatments.  
419 Such findings are in accordance with previous studies on the African catfish *C.*

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

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

443

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<sup>-1</sup>). 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

468 are the highest at the intermediate temperatures (25.0, 27.5 and 30.0°C) and  
469 decreased at the extreme rearing temperatures (22.5 and 32.5°C). Based on our  
470 main findings, we can reasonably assume that the extreme temperatures tested are  
471 closed to the tolerance limit of this species and affected the metabolism of the  
472 larvae. Thus, although, the most efficient temperature for larval growing (i.e.  
473 32.5°C) does not cause serious impairment in larvae, a slightly lower rearing  
474 temperature (~30.0°C) should be maintained as a precaution.

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

480

## 481 **5. Conclusion**

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

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

493

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499

#### 500 **References**

501 Adida, A., 2014. Efisiensi Pemasaran Benih Ikan Gurami (*Osphronemus*  
502 *gouramy*) Ukuran ‘Nguku’ ditinjau dari Keragaan Pasar di Kelurahan Duren  
503 Mekar dan Duren Seribu, Depok Jawa Barat (in Indonesian). J. Manaj.  
504 Perikan. Dan Kelaut. 1(1), 1-9.

505 Amornsakun, T., Kullai, S., Hassan, A., 2014a. Some aspects in early life stage of  
506 giant gourami, *Osphronemus goramy* (Lacepede) larvae. Songklanakarin J.  
507 Sci. Technol. 36(5), 493-498.

508 Amornsakun, T., Kullai, S., Hassan, A., 2014b. Feeding behavior of giant  
509 gourami, *Osphronemus gouramy* (Lacepede) larvae. Songklanakarin J. Sci.  
510 Technol. 36(3), 261-264.

511 AOAC, 1999. Official methods of analysis of AOAC International. Association of  
512 Official Analytical Chemists, Maryland, USA.

- 513 Arifin, O.Z., Imron, I., Asependi, A., Hendri, A., Muslim, N., Yani, A., 2018.  
514 Hibridisasi intraspesifik antar dua populasi ikan gurami Galunggung  
515 (*Osphronemus goramy*, Lacepede, 1801) (in Indonesian). J. Ris. Akuakultur  
516 12, 315-323.
- 517 Arifin, O.Z., Prakoso, V.A., Kristanto, A.H., Pouil, S., Slembrouck, J. in press.  
518 Effect of stocking density on growth, food intake and survival of giant  
519 gourami (*Osphronemus goramy*, Lacepède) larvae. Aquaculture.
- 520 Azaza, M.S., Dhraief, M.N., Kraiem, M.M., 2008. Effects of water temperature on  
521 growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus*  
522 (Linnaeus) reared in geothermal waters in southern Tunisia. J. Therm.  
523 Biol. 33(2), 98-105.
- 524 Baras, E., Raynaud, T., Slembrouck, J., Caruso, D., Cochet, C., Legendre, M.,  
525 2011. Interactions between temperature and size on the growth, size  
526 heterogeneity, mortality and cannibalism in cultured larvae and juveniles of  
527 the Asian catfish, *Pangasianodon hypophthalmus* (Sauvage). Aquac. Res.  
528 42(2), 260-276.
- 529 Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in  
530 aquaculture with emphasis on the response and effects of corticosteroids.  
531 Annu. Rev. Fish Dis. 1, 3-26.
- 532 Blaxter, J.H.S., 1991. The effect of temperature on larval fishes. Neth. J. Zool. 42,  
533 336-357.
- 534 BPS, 2013. Jumlah rumah tangga usaha budidaya bukan ikan hias menurut  
535 wilayah dan jenis ikan utama (in Indonesian).

536 <https://st2013.bps.go.id/dev2/index.php/site/tabel?tid=57&wid=3200000000>  
537 (accessed 29 November 2018).

538 Brett, J.R., 1979. Environmental factors and growth. in: Hoar, W.S., Randall, D.J.,  
539 Brett, J.R. (Eds.), Fish Physiology VIII. Academic Press, London, pp. 599–  
540 667.

541 Britz, P. J., Hecht, T., 1987. Temperature preferences and optimum temperature  
542 for growth of African sharptooth catfish (*Clarias gariepinus*) larvae and  
543 postlarvae. Aquaculture 63(1-4), 205-214.

544 Chen, W.H., Sun, L.T., Tsai, C.L., Song, Y.L., Chang, C.F., 2002. Cold-stress  
545 induced the modulation of catecholamines, cortisol, immunoglobulin M, and  
546 leukocyte phagocytosis in tilapia. Gen. Comp. Endocr. 126(1), 90-100.

547 Degani, G., Ben-Zvi, Y., Levanon, D., 1989. The effect of different protein levels  
548 and temperatures on feed utilization, growth and body composition of  
549 *Clarias gariepinus* (Burchell 1822). Aquaculture 6(3-4), 293-301.

550 Desai, A.S., Singh, R.K., 2009. The effects of water temperature and ration size  
551 on growth and body composition of fry of common carp, *Cyprinus carpio*. J.  
552 Therm. Biol. 34, 276–280.

553 El-Gamal, A.E.E., 2009. Effect of temperature on hatching and larval  
554 development and mucin secretion in common carp, *Cyprinus carpio*  
555 (Linnaeus, 1758). Glob. Vet. 3, 80–90.

556 El-Greisy, Z.A.E.-B., Elgamal A.E.E., Ahmed, N.A.M., 2016. Effect of prolonged  
557 ammonia toxicity on fertilized eggs, hatchability and size of newly hatched

558 larvae of Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat. Res. 42, 215-  
559 222.

560 El-Sayed, A.F.M., Kawanna, M., 2008. Optimum water temperature boosts the  
561 growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a  
562 recycling system. Aquac. Res. 39(6), 670-672.

563 FAO, 1980. Fish Feed Technology. Food and Agriculture Organization, Roma,  
564 Italy.

565 FAO, 2017. FishStatJ: software for fishery statistical time series. Roma, Italy.

566 Francis-Floyd, R., Watson, C., Petty, D., Pouder, D. B., 2009. Ammonia in  
567 aquatic systems. University of Florida IFAS Extension Publication, Florida,  
568 USA.

569 Froese, R., 2006. Cube law, condition factor and weight–length relationships:  
570 history, meta-analysis and recommendations. J. Appl. Ichthyol. 22, 241-  
571 253.

572 Fry, F.E.J., 1971. The effects of environmental factors on the physiology of fish.  
573 in: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology XI, Academic Press,  
574 New York, pp. 1–98.

575 Hastuti, S., Supriyono, E, Mokoginta, I., Subandiyono., 2003. Blood glucose  
576 response of giant gouramy (*Osphronemus gouramy*, Lac.) to the stress of  
577 environmental temperature changes. Jurnal Akuakultur Indonesia 2(2), 73-  
578 77.

- 579 Hidalgo, F., Alliot, E., Thebault, H., 1987. Influence of water temperature on food  
580 intake, food efficiency and gross composition of juvenile sea bass,  
581 *Dicentrarchus labrax*. *Aquaculture* 64(3), 199-207.
- 582 Jobling, M., 1997. Temperature and growth: modulation of growth rate via  
583 temperature change. in: Wood, C.M., McDonald, D.G. (Eds.), *Global*  
584 *Warming: Implications for Freshwater and Marine Fish*. Cambridge  
585 University Press, Cambridge, pp. 225-254.
- 586 Kamler, E. 2002. Ontogeny of yolk-feeding fish: an ecological perspective. *Rev.*  
587 *Fish Bio. Fisher.* 12, 79-103.
- 588 Kestemont, P., Baras, E. 2001. Environmental factors and feed intake:  
589 mechanisms and interactions. In: Houlihan, D., Boujard, T., Jobling, M.  
590 (Eds.), *Feed Intake in Fish*, Blackwell Science, Oxford, pp. 131-156.
- 591 Kolman, R., Khudiyi, O., Kushniryk, O., Khuda, L., Prusinska, M., Wiszniewski,  
592 G. 2018. Influence of temperature and *Artemia* enriched with  $\alpha$ -3 PUFAs on  
593 the early ontogenesis of Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill,  
594 1815. *Aquac. Res.* 49, 1740-1751.
- 595 Long, Y., Li, L., Li, Q., He, X., Cui, Z., 2012. Transcriptomic characterization of  
596 temperature stress responses in larval zebrafish. *PLOS ONE* 7(5), e37209.
- 597 Lucas, W.G.F., Kalesaran, O.J., Lumenta, C., 2015. Pertumbuhan dan  
598 kelangsungan hidup larva gurami (*Osphronemus gouramy*) dengan  
599 pemberian beberapa jenis pakan (in Indonesian). *Jurnal Budidaya Perairan*  
600 3, 19-28.

601 Martinez-Palacios, C.A., Chavez-Sanchez, M.C., Ross, L.G., 1996. The effects of  
602 water temperature on food intake, growth and body composition of  
603 *Cichlasoma urophthalmus* (Günther) juveniles. *Aquac. Res.* 27, 455-461.

604 Maskur, M., Rina, R., Hamid, M.A., 2013. Small-scale freshwater aquaculture  
605 extension development in Indonesia. [https://enaca.org/?id=189&title=small-](https://enaca.org/?id=189&title=small-scale-aquaculture-extension-in-indonesia)  
606 [scale-aquaculture-extension-in-indonesia](https://enaca.org/?id=189&title=small-scale-aquaculture-extension-in-indonesia) (accessed 29 November 2018).

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

611 Pandit, N.P., Nakamura, M., 2010. effect of high temperature on survival, growth  
612 and feed conversion ratio of Nile tilapia, *Oreochromis niloticus*. *Our Nat.* 8,  
613 219–224.

614 Pankhurst, N.W., King, H.R., 2010. Temperature and salmonid reproduction:  
615 implications for aquaculture. *J. Fish Biol.* 76, 69–85.

616 Pauly, D., Pullin, R.S.V., 1988. Hatching time in spherical, pelagic, marine fish  
617 eggs in response to temperature and egg size. *Environ. Biol. Fishes* 22, 261–  
618 271.

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

621 Réalis-Doyelle, E., Pasquet, A., Charleroy, D.D., Fontaine, P., Teletchea, F., 2016.  
622 Strong effects of temperature on the early life stages of a cold stenothermal  
623 fish species, brown trout (*Salmo trutta* L.). *PLOS ONE* 11, e0155487.

- 624 Rodkhum, C., Kayansamruaj, P., Pirarat, N., 2011. Effect of water temperature on  
625 susceptibility to *Streptococcus agalactiae* serotype Ia infection in Nile  
626 tilapia (*Oreochromis niloticus*). Thai J. Vet. Med. 41, 309–314.
- 627 Rudneva, I. 2013. Biomarkers for Stress in Fish Embryos and Larvae. CRC Press,  
628 Boca Raton, FL, USA.
- 629 Sarah, S., Widanarni W., Sudrajat, A.O., 2009. Pengaruh padat penebaran  
630 terhadap pertumbuhan dan kelangsungan hidup benih ikan gurame  
631 (*Osphronemus goramy* Lac.) (in Indonesian). Jurnal Akuakultur Indonesia  
632 8(2), 199-207.
- 633 Selye, H., 1974. Stress without distress. McClelland Stewart, Toronto.
- 634 Singh, R.K., Desai, A.S., Chavan, S.L., Khandagale, P.A., 2009. Effect of water  
635 temperature on dietary protein requirement, growth and body composition  
636 of Asian catfish, *Clarias batrachus* fry. J. Therm. Biol. 34, 8–13.
- 637 Singh, S.P., Sharma, J.G., Ahmad, T., Chakrabarti, R., 2013. Effect of water  
638 temperature on the physiological responses of Asian catfish *Clarias*  
639 *batrachus* (Linnaeus 1758). Asian Fish. Sci. 26, 26–38.
- 640 SNI, 2000. SNI: 01-6485.3-2000: Produksi benih ikan gurame (*Osphronemus*  
641 *goramy*, Lac) kelas benih sebar (in Indonesian). Badan Standardisasi  
642 Nasional (BSN), Jakarta, Indonesia.
- 643 Soderberg, R.W., 1990. Temperature effects on the growth of blue tilapia in  
644 intensive aquaculture. Prog. Fish-Cult., 52(3), 155-157.

- 645 Tacon, A.G.J., 1987. The nutrition and feeding of farmed fish and shrimp - a  
646 training manual: 1. The essential nutrients. Food and Agriculture  
647 Organization, Roma, Italy.
- 648 Tanck, M. W. T., Booms, G.H.R., Eding, E.H., Bonga, S.W., Komen, J., 2000.  
649 Cold shocks: a stressor for common carp. *J. Fish Biol.*, 57(4), 881-894.
- 650 Tang, U.M., Muchlisin, Z.A., Syawal, H., Masjudi, H., 2017. Effect of water  
651 temperature on the physiological stress and growth performance of tapah  
652 (*Wallago leeri*) during domestication. *Archives of Polish Fisheries* 25(3),  
653 165-171.
- 654 Teletchea, F., Gardeur, J.-N., Kamler, E., Fontaine, P., 2009. The relationship of  
655 oocyte diameter and incubation temperature to incubation time in temperate  
656 freshwater fish species. *J. Fish Biol.* 74(3), 652-668.
- 657 Van Ham, E.H., Berntssen, M.H., Imsland, A.K., Parpoura, A.C., Bonga, S.E.W.,  
658 Stefansson, S.O., 2003. The influence of temperature and ration on growth,  
659 feed conversion, body composition and nutrient retention of juvenile turbot  
660 (*Scophthalmus maximus*). *Aquaculture* 217(1-4), 547-558.
- 661 Verawati, Y., Muarifi, M., Mumpunil, F.S., 2015. Pengaruh perbedaan padat  
662 penebaran terhadap pertumbuhan dan kelangsungan hidup benih ikan  
663 gurami (*Osphronemus gouramy*) pada sisteivi resirkulasi (in Indonesian).  
664 *Jurnal Mina Sains* 1(1): 6-12.
- 665 Wang, L.H., Tsai, C.L., 2000. Effects of temperature on the deformity and sex  
666 differentiation of tilapia, *Oreochromis mossambicus*. *J. Exp. Zool.* 286, 534-  
667 537.

- 668 Watanabe, W.O., Ernst, D.H., Chasar, M.P., Wicklund, R.I., Olla, B.L., 1993. The  
669 effects of temperature and salinity on growth and feed utilization of juvenile,  
670 sex-reversed male Florida red tilapia cultured in a recirculating system.  
671 *Aquaculture* 12(4), 309-320.
- 672 Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.* 77, 591–  
673 625.
- 674 Zaragoza, O. D. R., Rodríguez, M. H., Bückle Ramirez, L. F., 2008. Thermal  
675 stress effect on tilapia *Oreochromis mossambicus* (Pisces: Cichlidae) blood  
676 parameters. *Mar. Freshw. Behav. Phy.* 41(2), 79-89.
- 677 Zeng, L.-Q., Fu, C., Fu, S.-J., 2018. The effects of temperature and food  
678 availability on growth, flexibility in metabolic rates and their relationships  
679 in juvenile common carp. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*  
680 217, 26–34.

681 **Captions to figures**

682

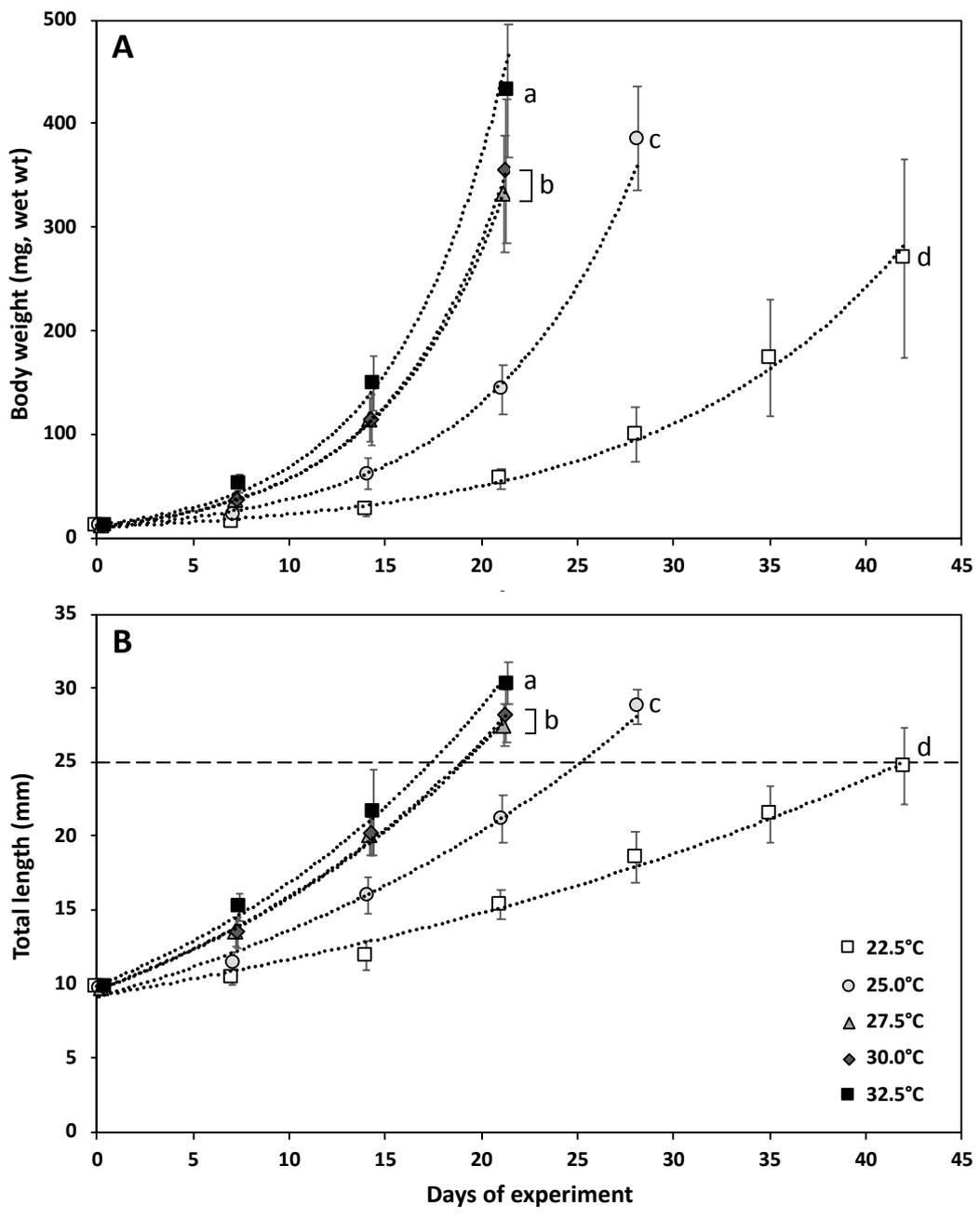
683 **Figure 1.** Growth in (A) body weight and (B) total length of giant gourami (*O.*  
684 *goramy*) larvae (n=60) reared at five temperatures from 8 dph. The horizontal  
685 dotted line represents the commercial size (i.e. called “Nguku”, 2.5 cm of total  
686 length). Values are Means  $\pm$  SD.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

688

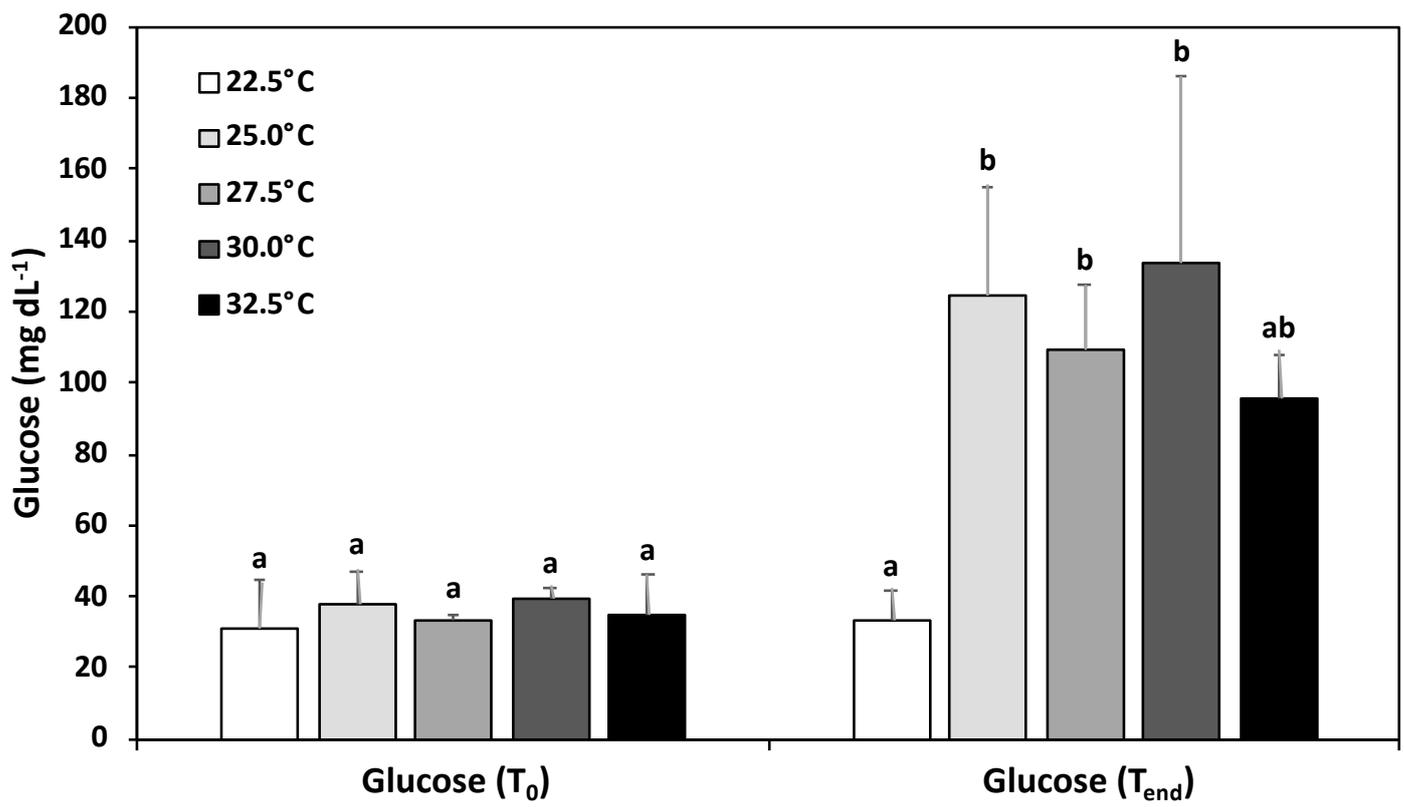
689 **Figure 2.** Concentration of glucose in the blood of giant gourami (*O. goramy*)  
690 larvae (n=3 pools of 20 larvae) reared at five temperatures from 10 dph ( $T_0$ ) up to  
691 their reached 2.5-cm total length ( $T_{end}$ ). Values are Means  $\pm$  SD. For Letters  
692 denote significant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

693

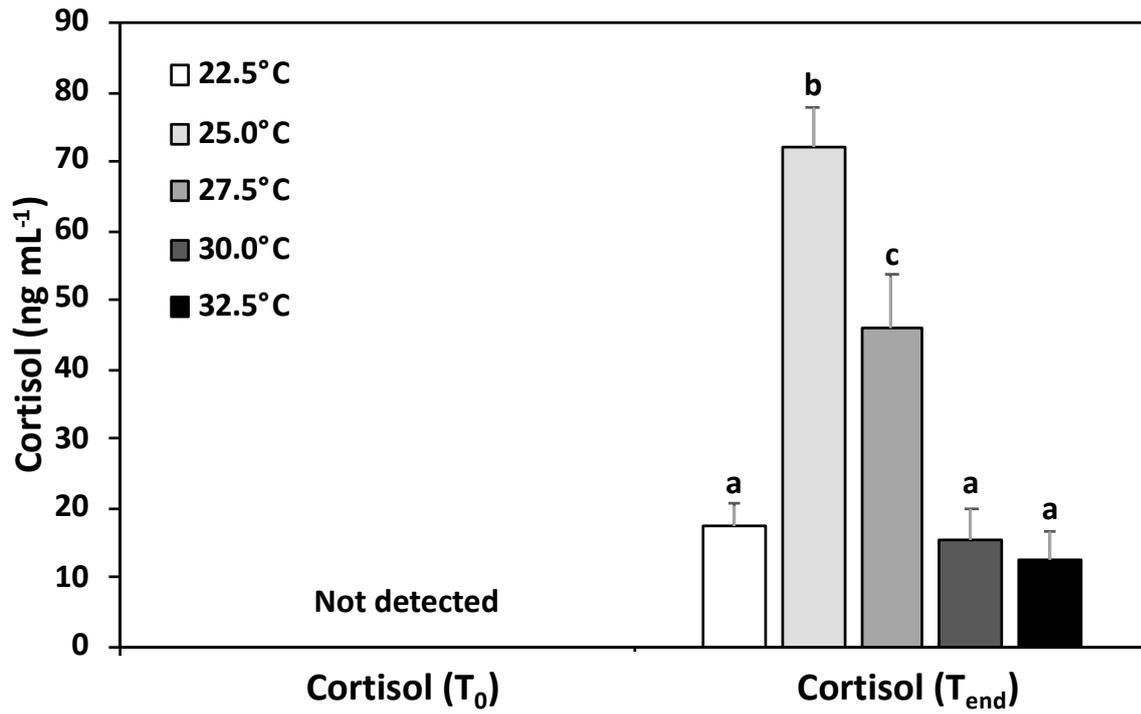
694 **Figure 3.** Concentration of cortisol in the blood of giant gourami (*O. goramy*)  
695 larvae (n=20) reared at five temperatures from 10 dph ( $T_0$ ) up to their reached 2.5-  
696 cm total length ( $T_{end}$ ). Values are Means  $\pm$  SD. Letters denote significant  
697 differences ( $p <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



698 Figure 1



699 Figure 2



700 Figure 3

701 Table 1. Summary of water quality parameters measured for the five temperature  
 702 conditions during the experiment. Values are Means  $\pm$  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 $\pm$ 0.43 <sup>a</sup>	25.24 $\pm$ 0.53 <sup>b</sup>	28.17 $\pm$ 0.68 <sup>c</sup>	30.01 $\pm$ 0.31 <sup>d</sup>	32.45 $\pm$ 0.74 <sup>e</sup>
DO (mg L <sup>-1</sup> )	7.40 $\pm$ 0.37 <sup>a</sup>	7.05 $\pm$ 0.39 <sup>ab</sup>	6.67 $\pm$ 0.25 <sup>b</sup>	6.55 $\pm$ 0.15 <sup>b</sup>	6.21 $\pm$ 0.18 <sup>c</sup>
O <sub>2</sub> saturation (%)	85.6 $\pm$ 4.3 <sup>a</sup>	85.7 $\pm$ 4.7 <sup>a</sup>	85.5 $\pm$ 3.2 <sup>a</sup>	86.7 $\pm$ 2.0 <sup>a</sup>	85.7 $\pm$ 2.5 <sup>a</sup>
pH	6.58 $\pm$ 0.19 <sup>a</sup>	6.77 $\pm$ 0.21 <sup>b</sup>	7.04 $\pm$ 0.05 <sup>b</sup>	6.98 $\pm$ 0.28 <sup>b</sup>	7.05 $\pm$ 0.04 <sup>b</sup>
Conductivity (μS cm <sup>-1</sup> )	208.8 $\pm$ 13.3 <sup>a</sup>	263.9 $\pm$ 15.2 <sup>b</sup>	215.0 $\pm$ 6.1 <sup>a</sup>	327.0 $\pm$ 16.2 <sup>c</sup>	347.0 $\pm$ 7.2 <sup>c</sup>
Alkalinity (mg L <sup>-1</sup> )	130.4 $\pm$ 45.2 <sup>a</sup>	124.1 $\pm$ 23.4 <sup>a</sup>	135.4 $\pm$ 19.1 <sup>a</sup>	124.6 $\pm$ 26.0 <sup>a</sup>	142.2 $\pm$ 15.2 <sup>a</sup>
TAN (mg L <sup>-1</sup> )	0.12 $\pm$ 0.08 <sup>a</sup>	0.12 $\pm$ 0.06 <sup>a</sup>	0.09 $\pm$ 0.05 <sup>a</sup>	0.07 $\pm$ 0.02 <sup>a</sup>	0.12 $\pm$ 0.03 <sup>a</sup>
NH <sub>3</sub> (mg L <sup>-1</sup> )	0.0003 $\pm$ 0.0003 <sup>a</sup>	0.0011 $\pm$ 0.0019 <sup>ab</sup>	0.0002 $\pm$ 0.0001 <sup>ac</sup>	0.0007 $\pm$ 0.0003 <sup>abc</sup>	0.0015 $\pm$ 0.0004 <sup>bd</sup>
NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.08 $\pm$ 0.138 <sup>a</sup>	0.02 $\pm$ 0.02 <sup>a</sup>	0.01 $\pm$ 0.003 <sup>a</sup>	0.02 $\pm$ 0.004 <sup>a</sup>	0.02 $\pm$ 0.0017 <sup>a</sup>
NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	4.25 $\pm$ 3.15 <sup>a</sup>	5.48 $\pm$ 2.85 <sup>a</sup>	4.15 $\pm$ 0.75 <sup>a</sup>	12.30 $\pm$ 2.46 <sup>a</sup>	7.54 $\pm$ 2.01 <sup>a</sup>

704 DO: dissolved oxygen; TAN: total ammoniacal nitrogen

705 Table 2. Growth and survival of giant gourami larvae reared at five temperature  
706 conditions (22.5, 25.0, 27.5; 30.0, 32.5°C) in a closed recirculating system for 21  
707 to 42 days (i.e. until they reached, at least, the commercial size of 2.5 cm of total  
708 length). See Section 2.5 for details of the parameters. Values are means  $\pm$  SD. For  
709 each parameter, letters denote significant differences ( $p < 0.05$ ) between  
710 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 <sub>i</sub> (mg)	12.3 $\pm$ 1.5 <sup>a</sup>	12.1 $\pm$ 1.5 <sup>a</sup>	12.4 $\pm$ 1.4 <sup>a</sup>	11.8 $\pm$ 1.1 <sup>a</sup>	12.2 $\pm$ 1.4 <sup>a</sup>
BW <sub>21d</sub> (mg)	57.2 $\pm$ 9.3 <sup>a</sup>	143.7 $\pm$ 23.6 <sup>b</sup>	332.0 $\pm$ 56.0 <sup>c</sup>	354.3 $\pm$ 70.2 <sup>c</sup>	431.9 $\pm$ 64.2 <sup>d</sup>
BW <sub>f</sub> (mg)	270.2 $\pm$ 95.4 <sup>a</sup>	385.8 $\pm$ 50.3 <sup>b</sup>	332.0 $\pm$ 56.0 <sup>c</sup>	354.3 $\pm$ 70.2 <sup>bc</sup>	431.9 $\pm$ 64.2 <sup>d</sup>
CV <sub>BW</sub> (%)	24.2 $\pm$ 10.4 <sup>a</sup>	12.1 $\pm$ 1.9 <sup>a</sup>	16.1 $\pm$ 2.1 <sup>a</sup>	19.9 $\pm$ 3.7 <sup>a</sup>	14.7 $\pm$ 3.3 <sup>a</sup>
CV <sub>TL</sub> (%)	8.0 $\pm$ 3.4 <sup>a</sup>	3.8 $\pm$ 0.7 <sup>a</sup>	4.8 $\pm$ 1.0 <sup>a</sup>	6.37 $\pm$ 1.2 <sup>a</sup>	4.5 $\pm$ 0.8 <sup>a</sup>
K (mg mm <sup>-3</sup> x 100)	1.7 $\pm$ 0.1 <sup>a</sup>	1.6 $\pm$ 0.1 <sup>b</sup>	1.6 $\pm$ 0.1 <sup>bc</sup>	1.6 $\pm$ 0.1 <sup>c</sup>	1.5 $\pm$ 0.1 <sup>c</sup>
SGR <sub>BW</sub> (% d <sup>-1</sup> )	7.3 $\pm$ 0.6 <sup>a</sup>	12.4 $\pm$ 0.3 <sup>b</sup>	15.7 $\pm$ 0.3 <sup>c</sup>	16.2 $\pm$ 0.2 <sup>cd</sup>	17.0 $\pm$ 0.2 <sup>d</sup>
SGR <sub>TL</sub> (% d <sup>-1</sup> )	2.2 $\pm$ 0.2 <sup>a</sup>	3.9 $\pm$ 0.1 <sup>b</sup>	4.9 $\pm$ 0.2 <sup>c</sup>	5.0 $\pm$ 0.1 <sup>cd</sup>	5.4 $\pm$ 0.1 <sup>d</sup>
SR (%)	61.6 $\pm$ 15.4 <sup>a</sup>	68.5 $\pm$ 5.3 <sup>a</sup>	66.7 $\pm$ 6.4 <sup>a</sup>	75.0 $\pm$ 4.8 <sup>a</sup>	75.5 $\pm$ 5.6 <sup>a</sup>
TL <sub>i</sub> (mm)	9.8 $\pm$ 0.4 <sup>a</sup>	9.7 $\pm$ 0.3 <sup>ab</sup>	9.8 $\pm$ 0.4 <sup>a</sup>	9.9 $\pm$ 0.19 <sup>ac</sup>	9.8 $\pm$ 0.3 <sup>a</sup>
TL <sub>21d</sub> (mm)	15.4 $\pm$ 1.0 <sup>a</sup>	21.2 $\pm$ 1.6 <sup>b</sup>	27.5 $\pm$ 1.4 <sup>c</sup>	28.2 $\pm$ 1.8 <sup>c</sup>	30.3 $\pm$ 1.4 <sup>d</sup>
TL <sub>f</sub> (mm)	24.7 $\pm$ 2.6 <sup>a</sup>	28.8 $\pm$ 1.2 <sup>b</sup>	27.5 $\pm$ 1.4 <sup>c</sup>	28.2 $\pm$ 1.8 <sup>bc</sup>	30.3 $\pm$ 1.4 <sup>d</sup>

711 BW: body weight, BW<sub>i</sub>: initial body weight, BW<sub>f</sub>: final body weight, CV:  
712 coefficient of variation, TL: total length, SGR: specific growth rate, SR: survival  
713 rate, TL<sub>i</sub>: initial total length, TL<sub>f</sub>: final total length.

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

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

718 \*\*\* 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  $\pm$  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 <sub>total</sub> (%)	25.7 $\pm$ 2.1 <sup>a</sup>	30.4 $\pm$ 1.5 <sup>a</sup>	45.3 $\pm$ 2.2 <sup>b</sup>	51.0 $\pm$ 3.6 <sup>b</sup>	58.6 $\pm$ 1.6 <sup>c</sup>
IR (g g <sup>-1</sup> d <sup>-1</sup> )	0.26 $\pm$ 0.13 <sup>a</sup>	0.17 $\pm$ 0.02 <sup>a</sup>	0.26 $\pm$ 0.02 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>a</sup>	0.25 $\pm$ 0.01 <sup>a</sup>
FCR	11.1 $\pm$ 5.6 <sup>a</sup>	4.7 $\pm$ 0.6 <sup>a</sup>	5.4 $\pm$ 0.4 <sup>a</sup>	5.1 $\pm$ 0.2 <sup>a</sup>	5.2 $\pm$ 0.2 <sup>a</sup>
PER	3.1 $\pm$ 1.4 <sup>a</sup>	6.3 $\pm$ 0.8 <sup>b</sup>	5.4 $\pm$ 0.4 <sup>b</sup>	5.7 $\pm$ 0.2 <sup>b</sup>	5.6 $\pm$ 0.3 <sup>b</sup>

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  $\pm$  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 $\pm$ 1.17 <sup>a</sup>	78.38 $\pm$ 0.10 <sup>a</sup>	79.02 $\pm$ 0.11 <sup>a</sup>	78.79 $\pm$ 0.29 <sup>a</sup>	78.31 $\pm$ 0.39 <sup>a</sup>
Dry matter	22.20 $\pm$ 1.17 <sup>a</sup>	21.62 $\pm$ 0.10 <sup>a</sup>	20.98 $\pm$ 0.11 <sup>a</sup>	21.21 $\pm$ 0.29 <sup>a</sup>	21.69 $\pm$ 0.39 <sup>a</sup>
Crude protein	57.90 $\pm$ 0.08 <sup>a</sup>	58.19 $\pm$ 0.10 <sup>b</sup>	59.23 $\pm$ 0.11 <sup>c</sup>	59.86 $\pm$ 0.09 <sup>d</sup>	58.90 $\pm$ 0.12 <sup>e</sup>
Crude lipid	26.05 $\pm$ 1.06 <sup>a</sup>	17.13 $\pm$ 0.23 <sup>b</sup>	15.88 $\pm$ 0.22 <sup>c</sup>	17.50 $\pm$ 0.35 <sup>b</sup>	18.91 $\pm$ 0.24 <sup>d</sup>
Ash	8.75 $\pm$ 0.19 <sup>a</sup>	10.09 $\pm$ 0.06 <sup>b</sup>	10.58 $\pm$ 0.11 <sup>c</sup>	10.26 $\pm$ 0.09 <sup>bc</sup>	10.27 $\pm$ 0.15 <sup>bd</sup>
Crude fibre	0.41 $\pm$ 0.02 <sup>a</sup>	0.42 $\pm$ 0.02 <sup>a</sup>	0.45 $\pm$ 0.02 <sup>ab</sup>	0.46 $\pm$ 0.03 <sup>ab</sup>	0.48 $\pm$ 0.01 <sup>b</sup>
NFE	6.88 $\pm$ 0.85 <sup>a</sup>	14.17 $\pm$ 0.32 <sup>b</sup>	13.86 $\pm$ 0.28 <sup>b</sup>	11.93 $\pm$ 0.38 <sup>c</sup>	11.45 $\pm$ 0.14 <sup>c</sup>

728 NFE: Nitrogen Free Extract.