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1           **Fluctuating temperature regime impairs growth in giant gourami**  
2                           *(Osphronemus goramy)* larvae  
3  
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21 **Abstract**

22 Growth and physiology were compared in giant gourami (*Osphronemus goramy*)  
23 larvae reared in constant (25, 30, and 35°C) and daily fluctuating (25-35°C)  
24 temperature regimes. Larvae aged eight days post-hatching were reared indoors  
25 for 21 days in four closed recirculating systems (four aquaria per system) in the  
26 different temperature regimes. Samples of larvae were collected every week from  
27 each temperature regime for growth measurements. At the end of the experiment,  
28 cortisol and glucose concentrations, as well as oxygen consumption and  
29 ventilation rates, were determined in fish from each temperature regime. Results  
30 showed that the fluctuating temperature regime did not affect survival or  
31 abnormality, but significantly impaired growth in giant gourami larvae.  
32 Physiological responses, assessed using cortisol and glucose levels and oxygen  
33 consumption, corroborate previous findings, suggesting that a stable temperature  
34 of 30°C is optimal for rearing giant gourami larvae. Our experimental approach  
35 demonstrated that temperature variations observed in larval rearing structures on  
36 small-scale fish farms are likely to impair the production of giant gourami fry.

37

38 **Keywords:** Growth, Larval rearing, Oxygen consumption, Physiology, Thermal  
39 fluctuation

40

41 **1. Introduction**

42 In aquatic environments, the thermal tolerance range of ectothermic organisms is  
43 largely determined by the amplitude of thermal variation in their natural habitat.  
44 Accordingly, the range of thermal tolerance is generally wider in temperate  
45 species than in those that inhabit colder, warmer, and more stable water  
46 temperatures (Pörtner, 2002; Souchon and Tissot, 2012). Fish growth usually  
47 increases with increasing temperature up to an optimal temperature, and then  
48 decreases at temperatures higher than this optimum (Árnason et al., 2019).  
49 Temperature changes that exceed the thermal limit for long durations may  
50 compromise fish survival (Whitney et al., 2016; Colchen et al., 2017; Mueller et  
51 al., 2017; Réalis-Doyelle et al., 2018). Thus, knowledge on thermal plasticity is  
52 important to understand how thermal regimes affect fish aquaculture production  
53 (Mommsen et al., 1999; Whitney et al., 2016).

54 The effects of constant and fluctuating thermal regimes on fish have been assessed  
55 in previous studies. Several studies performed on temperate species have revealed  
56 that exposure to fluctuating temperatures improves fish tolerance to high  
57 temperatures (e.g. Carveth et al., 2007; Eldridge et al., 2015; Pisano et al., 2019).  
58 In contrast, fluctuating temperatures confer no benefits compared with a constant  
59 temperature regime in Lahontan cutthroat trout *Oncorhynchus clarki henshawi*  
60 (Dickerson and Vinyard, 1999) or smallmouth bass *Micropterus dolomieu*  
61 (Eldridge et al., 2015).

62 The effects of temperature fluctuations on fish growth may be diverse. Although  
63 growth can be boosted by daily fluctuating temperatures (Konstantinov et al.,  
64 1990; Carveth et al., 2007; Azaza et al., 2010), other studies, mostly involving

65 temperate species, have shown slower growth than at constant thermal regimes  
66 (Meeuwig et al., 2004, Hevrøy et al., 2012; Eldridge et al., 2015; Tromp et al.,  
67 2016). Still other species show no significant differences, such as blue tilapia  
68 *Oreochromis aureus* (Baras et al., 2000) and Japanese medaka *Oryzias latipes*  
69 (Dhillon and Fox, 2007). These various studies highlight that the effects of  
70 temperature fluctuations are species-dependent and that there are few data on  
71 tropical species.

72 Fish metabolism and physiology are influenced by fluctuating temperatures,  
73 affecting in turn growth, health, and survival (Pérez-Casanova et al., 2008a,  
74 2008b; Singh et al., 2013; Tromp et al., 2016; Fu et al., 2018). In general,  
75 individuals respond directly to acute exposure to stressors by increasing cortisol  
76 and glucose levels to cope with stress (Iwama et al., 2005; Tromp et al., 2018).  
77 Oxygen consumption in fish also depends on temperatures and increases with  
78 increasing temperatures (Claësson et al., 2016; Remen et al., 2015). Such changes  
79 in physiology may affect growth and survival.

80 There are still gaps in knowledge on several aspects of the biology of some  
81 aquaculture tropical species. Among them, giant gourami (*Osphronemus goramy*  
82 Lacepède, 1801) is the main species being farmed in Indonesia and is in great  
83 demand for the food aquaculture industry (Amornsakun et al., 2014; Budi and  
84 Suprayudi, 2015). Currently, with an estimated production of more than 173,000 t  
85 in 2018, Indonesia is the world's highest giant gourami producer (>98% of world  
86 production; FAO, 2020). Giant gourami aquaculture activities in Indonesia are  
87 mainly driven by micro and small-scale farms, which are concentrated on Java  
88 Island (Maskur et al., 2013). Although the giant gourami is an interesting species

89 in regard to current Indonesian aquaculture guidance, its production has slowed  
90 for various reasons. One of the main impediments in giant gourami aquaculture is  
91 ensuring a regular supply of fry (Slembrouck et al., 2019). A recent survey on  
92 small-scale giant gourami farms in West Java identified irregular temperatures as  
93 the main limiting factor for egg and fry production (Kristanto et al., 2020).  
94 Current practices for raising giant gourami larvae still involve their rearing in  
95 plastic basins and being moved to small outdoor ponds (Kristanto et al., 2020).  
96 However, the Indonesian National Standard recommends indoor larval rearing to  
97 avoid exposure to the large variations encountered in environmental conditions  
98 (SNI, 2000). Temperatures fluctuate widely in outdoor rearing systems with  
99 observed variations ranging from 27 to 40 °C, and massive mortality events are  
100 frequently reported by farmers (Kristanto et al., 2020).  
101 In this context, the objectives of this study were (1) to assess the growth and  
102 survival of giant gourami (*O. goramy*) larvae reared in closed recirculating  
103 systems at four rearing temperature regimes (fluctuating: 25-35 °C and constant:  
104 25, 30, and 35 °C) and (2) to determine the effects of temperature fluctuation on  
105 larval physiology.

106

## 107 **2. Materials and methods**

### 108 2.1. Source of larvae

109 Giant gourami *O. goramy* larvae used in this experiment came from natural  
110 spawns of the same giant gourami broodfish pair. The broodfish, belonging to the  
111 “Galunggung” strain, were approximately 4-5 years old and spawned at the  
112 Tasikmalaya Center for the Development of Giant Gourami Culture (BPPSIGN)

113 where temperatures in broodfish ponds usually range from 25 to 32 °C (Kristanto  
114 et al., 2020). After spawning, eggs were collected from the nest and then  
115 transported to the Germplasm Freshwater Aquaculture Research Station  
116 (RIFAFE, Cijeruk, Bogor, West Java, Indonesia). Eggs were then placed in an  
117 incubation basin (20 L) inside an experiment room with a daily water change, a  
118 temperature of  $29.5 \pm 0.7$  °C, and light:dark cycle of 12:12 h, until the eggs  
119 hatched. After hatching, larvae were kept unfed in the incubation basin until they  
120 reached 8 days post-hatching (dph) for the experiment.

121

## 122 2.2. Live prey

123 Giant gourami larvae were fed with live tubifex worms (*Tubifex tubifex*) during  
124 the experiment based on current practices (SNI, 2000) for optimal survival and  
125 growth. Live tubifex worms, purchased regularly, were stored in a 100-L  
126 aquarium in the experiment room with a daily water change, temperature of  $29.5 \pm$   
127  $0.7$  °C, and a light:dark cycle of 12:12 h; they were kept unfed.

128

## 129 2.3. Temperature regime experiment

### 130 2.3.1. Experimental design

131 Zootechnical and physiological performances of giant gourami larvae were  
132 evaluated in four temperature treatments: one fluctuating temperature regime (25-  
133 35 °C) and three constant temperature regimes (25, 30, and 35 °C). The  
134 experiment was carried out in four identical indoor recirculating systems (RAS;  
135 one system for each temperature treatment) under a light:dark cycle of 12:12 h  
136 (daylight intensity: 60-4500 lux) including a sump with mechanical and biological

137 filtration and a buffer tank. PVC pipes connected each RAS to the experimental  
138 tanks (i.e. 30 L glass aquaria  $40 \times 30 \times 30$  cm) placed on a bench. At 8 dph, larvae  
139 (mean body mass:  $12.1 \pm 1.2$  mg; mean total length:  $9.4 \pm 0.3$  mm) were randomly  
140 distributed into the experimental tanks ( $n = 4$  tanks per temperature treatment;  
141 stocking density of 72 larvae per tanks or  $2.4$  larvae  $L^{-1}$ ) in each RAS. During the  
142 experiment, water inlet flow into rearing tanks was maintained at  $33 L h^{-1}$  for the  
143 first 5 days, and then increased to  $78 L h^{-1}$  until the end of the experiment.  
144 Prior to the experiment, giant gourami larvae in each treatment were kept in their  
145 respective experimental tanks at an initial temperature of  $28$  °C. Then, larvae were  
146 gradually acclimated to target temperatures ( $\pm 1$  °C  $h^{-1}$ ). Temperatures were kept  
147 stable at target temperatures, except for the fluctuating temperature treatment  
148 (Figure 1). Submersible heaters and chillers (when appropriate) regulated by  
149 thermostats and placed in the sump of each RAS were used to control temperature  
150 during the experiment. Six weeks prior to the experiment, preliminary tests were  
151 performed to evaluate the temperature in the RASs. Temperature was monitored  
152 regularly, especially in the fluctuating regime during the ascent and descent  
153 phases to ensure that the variations were gradual. The experiment started several  
154 days after all the temperature treatments were running properly and temperature  
155 was then checked as described Section 2.3.2. To prevent heat loss and temperature  
156 variations, the outside of the experimental tanks was insulated with plastic and  
157 extruded polystyrene foam and covered with a transparent polycarbonate sheet.  
158 Each experimental tank was equipped with an aeration system. The experiment  
159 was carried out for 21 days, the typical rearing time to reach market size.  
160



161 2.3.2. Feeding protocol and water quality monitoring

162 Giant gourami larvae were fed live tubifex worms every day, except on the  
163 sampling days. To ensure continuous and non-limiting feeding, live tubifex  
164 worms were placed on the bottom of each aquarium twice a day (at 8:00 and  
165 17:00), the quantities were given in excess and in an equivalent amount for each  
166 treatment during the whole experiment. Feeding in excess allows accurate  
167 estimation of feed intake by weighing the uneaten tubifex worms remaining on the  
168 bottom of each aquarium (Prakoso et al., 2019). Prior to their distribution, live  
169 tubifex worms were collected, washed, and drained on a 50- $\mu$ m mesh and weighed  
170 (to the nearest 0.1 g). Uneaten tubifex worms were removed from each aquarium  
171 before giving the new ration and weighed to calculate feed intake. The  
172 temperature in each experimental tank was observed three times a day (at 08:00,  
173 12:00, and 16:00). Meanwhile, water quality was directly measured for pH,  
174 dissolved oxygen (DO), and conductivity once a week using a portable  
175 multiparameter meter (HI 9829 Hanna Instruments). Additionally, total ammonia  
176 nitrogen (TAN),  $\text{NH}_3$ ,  $\text{NH}_4^+$ , and  $\text{NO}_2^-$  were measured using spectrophotometry (n  
177 = 12 per treatment). The results of water quality measurements are given in Table  
178 1.

179

180 2.4. Observations and measurements on larvae

181 Larvae (n = 22 for each aquarium, i.e. ~30% of the total number of larvae) were  
182 sampled every seven days. Prior to measurement, larvae were transferred to a  
183 plastic basin containing water from their respective aquaria to keep them in the  
184 same water temperature conditions. Larvae were anesthetized by using eugenol

185 and measured (total body length, TL, mm) under a stereomicroscope with a  
186 micrometer (accuracy ranging from 0.05 to 0.1 mm, depending on fish size and  
187 magnification). Meanwhile, body mass (BM, mg) was measured using a digital  
188 weighing scale (accuracy of 0.1 mg). After measurement, fish were returned to  
189 their respective experimental tanks.

190 At the end of the experiment, the living larvae in each aquarium were counted to  
191 determine the survival rate and were visually observed for anatomic abnormalities  
192 (i.e. morphological deformities). Additionally, cortisol and glucose levels were  
193 recorded from each treatment following Prakoso et al. (2019). Whole fish were  
194 collected, anesthetized, and ground (n = 4 pools of 10 fish). Subsequently, the  
195 samples were vortex-mixed (30 s) and centrifuged (10,000 rpm for 10 min at 25  
196 °C) to collect the supernatant before being frozen. Cortisol levels were determined  
197 using the Cortisol ELISA kit (DRG International Inc., Germany) and a microplate  
198 photometer (Biosan HiPo MPP-96, Latvia) according to the manufacturer's  
199 instructions. Inter-plate and intra-plate coefficients of variation were always less  
200 than 5%. Meanwhile, blood glucose samples were collected as described below (n  
201 = 4 pools of 10 fish). One drop of blood was collected from each anesthetized fish  
202 from each treatment (n = 10 fish) and was read on a blood glucose meter (Accu-  
203 Chek Active, Roche, Germany).

204

## 205 2.5. Oxygen consumption, ventilation rate, and $Q_{10}$ measurements

206 At the end of the rearing period, oxygen consumption was measured in giant  
207 gourami fry using a closed respirometer (1.4 L) connected to a dissolved oxygen  
208 meter (Trans Instrument HD3030, Singapore). After a 10 min acclimation period,

209 measurements were read for the three constant temperature treatments (25, 30, and  
210 35 °C). The fluctuating temperature treatment (25-35 °C) was not measured due to  
211 technical constraints. Twenty-one fish were combined for each measurement with  
212 three replicates for each constant temperature treatment. Dissolved oxygen  
213 concentrations were recorded every five minutes. Ventilation rates were also  
214 determined by counting opercular movements for 1 min with nine replications for  
215 each treatment. Furthermore, the measured oxygen consumption data were used to  
216 calculate  $Q_{10}$  (e.g. Hölker, 2006; see Section 2.6 for details).

217

## 218 2.6. Physiological and zootechnical parameters

219 Temperature effects on survival, growth, and feed intake were determined by  
220 calculating the following parameters for each experimental treatment.

221 Survival rates (SR), expressed as a percentage, were calculated by comparing the  
222 final number ( $N_f$ ) with the initial number of larvae ( $N_i$ ):  $SR (\%) = (N_f/N_i) \times 100$ .

223 Abnormal fish rates in % were calculated by comparing the number of deformed  
224 fish ( $N_{ab}$ ) with the final number of live fish ( $N_f$ ):  $(N_{ab}/N_f) \times 100$ .

225 Specific growth in body mass ( $SGR_{BM}$ , %) was calculated according to the  
226 following equation:  $SGR_{BM} = (\ln BM_f - \ln BM_i)/ED \times 100$ , where  $BM_i$  and  $BM_f$   
227 are the initial and final body mass of larvae (mg), respectively, and ED is the  
228 duration of the experiment in days.

229 Specific growth in total length ( $SGR_{TL}$ , %) was calculated using the same  
230 calculation by substituting BM for TL:  $SGR_{TL} = (\ln TL_f - \ln TL_i)/ED \times 100$ .

231 Heterogeneity of larval size (in body mass or total length) was assessed using the  
232 coefficient of variation (CV, %) calculated as  $CV_{BM} = SD_{BM}/BM$  and  $CV_{TL} =$

233  $SD_{TL}/TL$ , where SD is the standard deviation and BM is the average body mass  
234 (mg) and TL, the average body length (mm).

235 Fulton's condition factor (K) was calculated according to the relationship  
236  $K = BM_f/TL_f^3$ . The equation was multiplied by 100 to bring the value close to  
237 one.

238 Fish biomass gain (BG, g) was calculated following the equation:  $BG = (N_f BM_f -$   
239  $N_i BM_i)/1000$ , where  $BM_i$  and  $BM_f$  are the initial and the final average body mass  
240 (mg), respectively.

241 The feed conversion ratio (FCR) was calculated using the following equation:  
242  $FCR = F/(N_f BM_f - N_i BM_i)$ , where F is the total quantity of food intake in wet  
243 weight during the whole rearing period. F was determined as the total amount of  
244 uneaten food subtracted from the total amount of food provided (g).

245 Oxygen consumption was calculated by the following equation:  $OC = (DO_0 -$   
246  $DO_t) \times (V/(t \times W))$ , where OC is the oxygen consumption expressed as  $mg\ kg^{-1}\ h^{-1}$   
247 <sup>1</sup>,  $DO_0$  and  $DO_t$  are the dissolved oxygen concentrations at initial time and after t  
248 hours ( $mg\ L^{-1}$ ), t is the time elapsed for fish consuming the oxygen (h), V is water  
249 volume on respirometer (L), and W is the total weight of fish (kg).

250  $Q_{10}$  values were calculated using the following equation:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{t_2-t_1}\right)}$$

251 where  $R_1$  is the oxygen consumption rate at temperature  $t_1$ , and  $R_2$ , the oxygen  
252 consumption rate at temperature  $t_2$ .

253

254 2.7. Data analysis

255 Statistical comparisons were done for each parameter described in Section 2.6.  
256 Data were first assessed to confirm normality (Shapiro's test) and homogeneity of  
257 variances (Levene's test) and, where necessary, data were arcsine-square-root-  
258 transformed prior to analysis. One-way ANOVA followed by Tukey's test was  
259 used to determine significant differences among temperature treatments. When  
260 assumptions of normality and homogeneity of variances were not met, data were  
261 analyzed using the Kruskal-Wallis and Dunn's non-parametric tests.  
262 The growth kinetics of the larvae, both BM and total length TL, reared at the five  
263 different temperatures were best fitted using an exponential model:  $BM$  or  $TL =$   
264  $BM_i$  or  $TF_i \times e^{at}$  where  $a$  is the growth rate ( $d^{-1}$ ), and  $t$ , time (d). Model constants  
265 were estimated by iterative adjustment using the least squares regression method.  
266 Differences in growth (BM, TL) and body condition (K) over time were assessed  
267 using a repeated-measures ANOVA designed as a linear-mixed-effect model.  
268 "Temperature" and "Time" were used as fixed factors and "Tank" was used as a  
269 random factor to take into account a possible "tank effects" (Sswat et al., 2018).  
270 The models were fitted using means of body mass and total length per tank and  
271 sampling time (Sswat et al., 2018). The level of significance for statistical  
272 analyses was always set to  $\alpha = 0.05$ . All statistics were performed using R  
273 freeware version 3.6.3 (R Development Core Team, 2020).

274

### 275 **3. Results**

#### 276 3.1. Water quality parameters

277 The targeted temperature regimes were met throughout the experiment (Table 1)  
278 and significant differences were confirmed between each treatment ( $F = 381.93$ ,  $p$

279 < 0.001, Table 1). Regular monitoring indicated that pH was similar between  
280 treatments, but some differences were observed for conductivity ( $\chi^2 = 57.7$ ,  $p <$   
281 0.001, Table 1). Dissolved oxygen concentration remained above 6.50 mg L<sup>-1</sup> in  
282 all treatments. TAN was not significantly different between the four experimental  
283 treatments, and the NO<sub>2</sub><sup>-</sup> and NH<sub>3</sub> concentrations were lower than 0.1 and 0.05 mg  
284 L<sup>-1</sup>, respectively.

285

### 286 3.2. Survival and abnormality

287 The larval survival rate measured at the end of the experiment ranged from 56.6 ±  
288 9.2% (35 °C treatment) to 96.2 ± 1.3% (30 °C treatment), with significant  
289 differences in survival between treatments ( $F = 37.34$ ,  $p < 0.001$ ) except between  
290 the fluctuating temperature regime (i.e. 25-35 °C treatment) and the two lowest  
291 constant temperatures (i.e. 25 and 30 °C; Fig. 2A). The proportion of  
292 abnormalities, mainly malformation of the spinal column, in living larvae varied  
293 from 0 (30 °C treatment) to 11.8 ± 9.7% (35 °C treatment), but was not  
294 significantly different between experimental treatments (Fig. 2B).

295

### 296 3.3. Growth and size heterogeneity

297 Larval growth (BM and TL) increased over time and varied significantly between  
298 the temperature treatments ( $F = 64.58-72.46$ ,  $p < 0.001$ ) with a significant  
299 interaction between temperature and time ( $F = 70.10-146.849$ ,  $p < 0.001$ ),  
300 indicating an accentuation of the differences as the experiment progressed (Fig. 3).

301 At the end of the 21-day experiment, average BM and TL of larvae ranged from  
302 147.1 ± 30.6 mg and 21.2 ± 1.6 mm and 276.7 ± 47.5 mg and 26.1 ± 1.4 mm

303 when they were reared at 25 and 30 °C, respectively (Table 2). Growth  
304 significantly increased with increasing temperature up to 30 °C, with the lowest  
305 growth observed for larvae reared at 25 °C (Fig. 3 and Table 2), and a slight, but  
306 non-significant, decrease in growth was observed at the highest tested temperature  
307 (35 °C, Fig. 3 and Table 2). Intermediate growth was observed in fish reared in  
308 the fluctuating temperature regime (25-35 °C). This trend was confirmed by the  
309 specific growth rate: significant increases in  $SGR_{BM}$  ( $F = 37.10$ ,  $p < 0.001$ ) and  
310  $SRG_{TL}$  ( $F = 45.70$ ,  $p < 0.001$ ) were observed with increasing temperatures up to  
311 30 °C, with values varying from  $12.1 \pm 0.6\%$  for  $SGR_{BM}$  and  $3.9 \pm 0.2\%$  for  
312  $SRG_{TL}$  at 25 °C to  $15.1 \pm 0.2\%$  for  $SGR_{BM}$  and  $4.9 \pm 0.1\%$   $SRG_{TL}$  at 30 °C (Table  
313 2). No significant differences were observed in SGR between 30 and 35 °C for  
314 either length or weight (Table 2).

315 Size heterogeneity in larvae as a function of temperature was assessed based on  
316 calculation of the coefficients of variation for body mass ( $CV_{BM}$ ) and total length  
317 ( $CV_{TL}$ ) at the end of the experiment.  $CV_{BM}$  ranged from  $17.1 \pm 2.7\%$  (30 °C  
318 treatment) to  $26.4 \pm 7.9\%$  (35 °C treatment) and  $CV_{TL}$  ranged from  $5.5 \pm 0.8\%$   
319 (30 °C treatment) to  $9.3 \pm 3.2\%$  (35 °C treatment). Although no significant change  
320 was observed for  $CV_{TL}$ , a slight change was observed for  $CV_{BM}$  ( $F = 3.59$ ,  $p =$   
321  $0.046$ , Table 2) with significant differences between the 30 °C and 35°C  
322 treatments. There was a significant change in K for the different temperature  
323 regimes ( $\chi^2 = 195$ ,  $p < 0.001$ ), with final K ranging from  $1.48 \pm 0.18$  and  $1.67 \pm$   
324  $0.28$  for larvae reared at the fluctuant temperature regime (25-35 °C) and at the  
325 highest constant temperature (i.e. 35 °C, Table 2), respectively.

326

327 3.4. Feed efficiency

328 FCR was affected by the temperature regime ( $F = 10.73$ ,  $p = 0.001$ ), with the  
329 lowest FCR observed at 30 °C ( $4.75 \pm 0.92$ ) and the highest FCR observed at 35  
330 °C ( $10.58 \pm 2.27$ , Table 2). There were no significant differences between the  
331 fluctuating temperature regime (25-35 °C) and the lowest constant temperature  
332 treatments (25 and 30 °C).

333

334 3.5. Cortisol and blood glucose concentrations

335 Cortisol and blood glucose concentrations were measured in giant gourami at the  
336 end of the 21-day experiment for each temperature treatment. Cortisol  
337 concentrations were not significantly different among treatments except between  
338 30 °C and 35 °C ( $F = 4.98$ ,  $p = 0.031$ ), with values ranging from  $6.22 \pm 6.21$  ng  
339  $\text{mL}^{-1}$  at 30 °C to  $28.46 \pm 2.72$  ng  $\text{mL}^{-1}$  at 35 °C (Fig. 4). Intermediate cortisol  
340 values were observed for the fluctuating temperature regime (25-35 °C) and the  
341 lowest constant temperature regime (25 °C) with  $13.81 \pm 5.64$  ng  $\text{mL}^{-1}$  and  $12.56$   
342  $\pm 11.63$  ng  $\text{mL}^{-1}$ , respectively (Fig. 4). At the end of the experiment, no  
343 differences were observed in glucose concentrations in giant gourami reared at the  
344 different temperature regimes, with values ranging from  $37.83 \pm 10.07$  mg  $\text{dL}^{-1}$  to  
345  $54.17 \pm 9.87$  mg  $\text{dL}^{-1}$  at 25 °C and 35 °C, respectively (Fig. 4).

346

347 3.6. Oxygen consumption, ventilation rates, and  $Q_{10}$

348 Oxygen consumption and ventilation rates were not assessed for the fluctuating  
349 temperature regime (25-35 °C) due to technical constraints. Among the constant  
350 temperature treatments, oxygen consumption increased with temperature ( $F =$



351 62.20,  $p < 0.001$ ), with values ranging from  $422 \pm 70 \text{ mg kg}^{-1} \text{ h}^{-1}$  at  $25 \text{ }^\circ\text{C}$  to  $780 \pm$   
352  $90 \text{ mg kg}^{-1} \text{ h}^{-1}$  at  $35 \text{ }^\circ\text{C}$  (Fig. 5). Overall, the same trend was observed for  
353 ventilation rates ( $\chi^2 = 22.8$ ,  $p < 0.001$ ), with values ranging from  $163 \pm 20$  times  
354  $\text{min}^{-1}$  at  $25 \text{ }^\circ\text{C}$  to  $215 \pm 8$  times  $\text{min}^{-1}$  at  $35 \text{ }^\circ\text{C}$  (Fig. 5), although there were no  
355 significant differences between the two highest constant temperature regimes (i.e.  
356  $30$  and  $35 \text{ }^\circ\text{C}$ ). The temperature coefficient ( $Q_{10}$ ) of giant gourami larvae between  
357  $25$  and  $30 \text{ }^\circ\text{C}$  (the highest) was  $1.98$ , between  $25$  and  $35 \text{ }^\circ\text{C}$ , it was  $1.92$ , and  
358 between  $30$  and  $35 \text{ }^\circ\text{C}$ , it was  $1.91$ .

359

#### 360 **4. Discussion**

##### 361 4.1. Survival and abnormalities at the different temperature regimes

362 In the present study, daily temperature fluctuations ( $25$ - $35 \text{ }^\circ\text{C}$ ) did not  
363 significantly affect the mortality of giant gourami larvae compared with the  
364 constant temperature regimes at  $25$  and  $30 \text{ }^\circ\text{C}$ . However, mortality increased  
365 drastically at  $35 \text{ }^\circ\text{C}$ . Overall, our results corroborate those of previous studies on  
366 the same species (Sarah et al., 2009, Verawati et al. 2015, Arifin et al. 2019).  
367 Although higher TAN values, explained by  $\text{NH}_4^+$  (approx.  $1 \text{ mg L}^{-1}$ ), were  
368 observed at  $25 \text{ }^\circ\text{C}$  than for the other temperature treatments, the  $\text{NH}_3$  values  
369 remained within a non-toxic range for tropical fish (i.e.  $< 0.05 \text{ mg L}^{-1}$ ; Boyd and  
370 Tucker, 2012). Thus, we assumed that TAN did not explain the mortality  
371 observed at  $25 \text{ }^\circ\text{C}$ . The increase of mortality for the low temperature treatment  
372 was also observed by Prakoso et al. (2019). In the present study, there were no  
373 significant differences in the fish anatomic abnormality rate between the different  
374 temperature regimes or between fluctuating and constant temperatures.

375 Nevertheless, at the highest temperature treatment (35 °C), heterogeneity in the  
376 abnormality rate per aquarium was high, with abnormalities ranging from 0 to  
377 22%. Dead larvae were not checked for abnormalities, but our observations  
378 suggest that taking them into account could have increased the actual abnormality  
379 rate for the 35 °C treatment. Our findings showed once again that a constant 30 °C  
380 regime is optimal for survival in giant gourami larval rearing, as previously  
381 suggested (Prakoso et al. 2019); this temperature minimizes the fish abnormality  
382 rate. The high mortality found in this study (i.e. 30-51% at 35 °C) clearly shows  
383 that 35 °C is higher than the tolerance limit of giant gourami larvae. Our  
384 experimental approach demonstrated that daily thermal fluctuations do not impair  
385 survival.

386

#### 387 4.2. Effects of fluctuating temperatures on growth and feed conversion

388 The fluctuating temperature regime had a negative effect on giant gourami growth.  
389 Our results also showed that fluctuating temperatures tend to increase  
390 heterogeneity in size slightly. Interestingly, the final average body mass increased  
391 with average temperatures during the experiment: 147.1 mg at constant 25 °C  
392 regime ( $24.8 \pm 0.1$  °C), 178.0 mg at fluctuating regime ( $29.0 \pm 3.4$  °C), 276.7 mg  
393 at constant 30 °C regime ( $29.9 \pm 0.1$  °C), and 262.9 mg at constant 35 °C regime  
394 ( $34.8 \pm 0.1$  °C). In terms of growth, the difference between fluctuating  
395 temperatures and constant 30 °C was higher than between the fluctuating  
396 temperature regime and the lowest constant temperature (25°C), suggesting that  
397 minimizing fluctuations in temperature is a key factor in maximizing larval  
398 growth. Surprisingly, the effects of a fluctuating temperature regime have been

399 poorly investigated in the literature. Overall, fluctuating temperatures negatively  
400 affect fish growth. For example, at comparable developmental stages, spikedace  
401 (*Meda fulgida*) held at fluctuations of 24–34 °C and 30–34 °C show significantly  
402 lower growth than the constant temperature control (25 °C) (Carveth et al., 2007).  
403 Furthermore, although small juveniles of the Nile tilapia *Oreochromis niloticus*  
404 grow slightly faster when raised under fluctuating temperatures than under  
405 constant temperatures, thermal fluctuations become increasingly unsuitable in fish  
406 of increasing size (Azaza et al., 2010). A similar conclusion was drawn in a study  
407 on blue tilapia (Baras et al., 2000). In the present study, at constant temperatures,  
408 there was an overall trend of increased giant gourami larval growth with  
409 temperatures of up to 30 °C. From 30 to 35 °C, this trend was reversed. At  
410 comparable temperatures and stocking densities, growth performances were  
411 similar to those reported in recent studies on giant gourami larval rearing (Arifin  
412 et al., 2019; Sarah et al., 2009).

413 In regard to growth, the fluctuating temperature treatment showed significantly  
414 higher FCR values compared with the constant 30 °C treatment, but remained  
415 lower than the two extreme temperatures (i.e. 25 and 35 °C). These results are the  
416 consequence of the combined effects of temperature in feed consumption, food  
417 passage time, and enzyme activity in fish (Michalsen et al., 1998), and corroborate  
418 previous findings on giant gourami (Prakoso et al., 2019) and other tropical fish  
419 species (e.g. Degani et al., 1989; Watanabe et al., 1993). FCRs are generally high  
420 in giant gourami (Arifin et al., 2019; Prakoso et al., 2019), and our findings  
421 emphasize that controlling — and especially stabilizing — temperature during

422 larval rearing is the key to improving feed efficiency and minimizing production  
423 costs related to diet (Kristanto et al., 2020).

424

#### 425 4.3. Physiological responses to temperature fluctuation

426 Temperature changes are expected to stress aquatic organisms due to their  
427 influence on all biological processes (Iwama et al., 2005; Pörtner et al., 2006;  
428 Killen et al., 2013; Verhille et al., 2016; Tromp et al., 2018), which can be  
429 predicted by measuring glucose and whole-body cortisol as the main indicators of  
430 stress in fish larvae (Rudneva, 2013). Overall, there were no significant  
431 differences between fluctuating and constant temperatures regarding cortisol and  
432 glucose concentrations, except for cortisol between the constant temperature  
433 treatments of 30 and 35 °C. Most previous studies on tropical fish species report  
434 effects of temperatures on cortisol and glucose levels when temperature deviates  
435 from the tolerance limits specific to each species (Chen et al., 2002; Zaragoza et  
436 al., 2008; Long et al., 2012). Prakoso et al. (2019) found changes in cortisol levels  
437 over time (but not detected at the beginning of the experiment) in giant gourami  
438 10-dph larvae exposed to different temperatures for several weeks. The same  
439 authors reported limited effects of temperature on glucose concentrations (initial  
440 levels ranging from 30 to 40 mg dL<sup>-1</sup>) measured at the end of the experiment,  
441 except a decrease in glucose levels at the extreme temperatures tested (22.5 and  
442 32.5 °C), but cortisol levels were highest (i.e. up to 78 ng mL<sup>-1</sup>) at 25 and 27.5 °C.  
443 Our findings corroborate those of Ebrahimi et al. (2011), who did not show  
444 differences in glucose levels among giant gourami juveniles reared at  
445 temperatures of 28, 30 and 32 °C. In this study, cortisol and glucose levels were

446 measured only at the end of the experiment; therefore, we cannot highlight any  
447 changes during development. However, on larvae of the same age exposed for  
448 three weeks to different temperatures, we found that cortisol levels were the  
449 highest at 35 °C, indicating the occurrence of stress for larvae reared at this  
450 temperature regime as shown by Hanke et al. (2019) in juvenile milkfish *Chanos*  
451 *chanos* exposed to a gradual temperature increase of 1 °C per day in the range  
452 from 26 °C to 33 °C. This study on milkfish warns that plasma cortisol values may  
453 be biased by the fish-handling procedures. As described above, in the present  
454 study, whole fish were collected, anesthetized, and ground to analyze cortisol  
455 levels. Because all the fish were treated in the same way, the cortisol  
456 concentrations we measured are comparable between treatments. Based on these  
457 findings, we can reasonably assume that the highest constant temperature tested in  
458 this study negatively affected the physiological response of giant gourami.  
459 Interestingly, the fluctuating temperature regime tested in this study (25-35 °C)  
460 was less harmful for giant gourami larvae compared with the constant 35 °C  
461 rearing temperature. Nevertheless, as in Prakoso et al. (2019), we used larvae  
462 from the same pair of brooders from a production site located at 480 m above the  
463 sea level where the range of water temperature is restricted. Thus, further  
464 investigations based on other gourami strains are needed to confirm our results on  
465 a broader scale.

466

#### 467 4.4. Changes in oxygen consumption with temperature

468 Fish metabolism is influenced by water temperature, which is also related to  
469 health, aerobic swimming performance, stress response, growth, and survival

470 (Pérez-Casanova et al., 2008a, 2008b; McKenzie and Claireaux, 2010; Singh et al.,  
471 2013; Tromp et al., 2016; Fu et al., 2018). Giant gourami larvae responded to the  
472 increase in temperatures (i.e. 25, 30, and 35 °C) by increasing oxygen  
473 consumption and ventilation rates. The oxygen consumption pattern we observed  
474 is similar to previous reports on other freshwater tropical fish such as climbing  
475 perch *Anabas testudineus* (117, 126, and 198 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> at 25, 30, and 35 °C,  
476 respectively; Sarma et al., 2010), common carp *Cyprinus carpio* (64, 71, and 99  
477 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> at 25, 30, and 35 °C, respectively; Chatterjee et al., 2004),  
478 yellowtail catfish *Pangasius pangasius* (from 111, 232, 236 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> at 30,  
479 34, and 38 °C, respectively; Debnath et al., 2006), and Nile tilapia (from 22 to 95  
480 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> at 19 and 31 °C, respectively; Turker, 2011). Moreover, the pattern  
481 of ventilation rates in giant gourami fry we measured is consistent with previous  
482 observations on other fish species such as the barred loach *Nemacheilus fasciatus*  
483 (115 to 265 times min<sup>-1</sup> at 21.5 and 31.5 °C, respectively; Prakoso and Kurniawan,  
484 2020), the pikeperch *Sander lucioperca* (from 8 to 31 times min<sup>-1</sup> at 13 and 28 °C,  
485 respectively; Frisk et al., 2012), and the exquisite sand-goby *Favonigobius*  
486 *exquisitus* (from 31 to 160 times min<sup>-1</sup> at 15 and 35 °C, respectively; Ford et al.,  
487 2004). Each fish species has a specific range of temperature preferences, resulting  
488 in various oxygen consumption patterns for specific metabolic processes (Das et  
489 al., 2005; Majhi and Das, 2013). Here, we found that higher temperatures lead to  
490 higher metabolic rates for giant gourami larvae reared at temperatures of up to 35  
491 °C.

492 The lowest temperature coefficient (Q<sub>10</sub>) in giant gourami larvae was estimated  
493 between 30 and 35 °C (1.91). This result indicates that within this temperature

494 range, this species maintains homeostasis more efficiently (González et al. 2010).  
495 According to previous studies (e.g. Das et al., 2018; Prakoso and Kurniawan,  
496 2020), the value at which  $Q_{10}$  drops among temperature treatments indicates the  
497 ideal thermal range for growth. Such results corroborate a previous study showing  
498 that the optimal larval rearing temperature for giant gourami is 30 °C (Prakoso et  
499 al., 2019). All larvae we used have the same genetic background; similar studies  
500 on other strains/populations/geographic locations are needed to generalize our  
501 findings.

502

## 503 **5. Conclusion**

504 The present study demonstrated that a fluctuating temperature regime impairs  
505 growth in giant gourami larvae. Accordingly, we observed a >60% reduction in  
506 growth between larvae reared in optimal conditions (i.e. stable temperature, at  
507 30 °C) and larvae that undergo daily fluctuations in temperature (i.e. 25-35 °C).  
508 Additional, albeit less significant, impacts were observed for survival and  
509 physiological responses. Our experimental approach confirmed that daily  
510 temperature variations observed in larval rearing structures in small-scale fish  
511 farms (Kristanto et al. 2020) are likely to be an impediment in the production of  
512 giant gourami fry.

513

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519

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747 **Captions to figures**

748

749 **Figure 1.** Illustration of the experimental protocol used to test for the effect of the  
750 daily temperature fluctuations on the growth performance and physiology of giant  
751 gourami *Osphronemus goramy* (indoor recirculating systems, 12L:12D). The  
752 continuous lines show the daily fluctuating (25-35 °C) and three constant (25, 30  
753 and 35°C) temperature regimes.  $T_{opt}$  is the optimal temperature for giant gourami  
754 larval rearing (Prakoso et al., 2020).

755

756 **Figure 2.** Percentages of (A) mortality and (B) abnormality observed in giant  
757 gourami *Osphronemus goramy* larvae (n = 4) reared at one daily fluctuating (25-  
758 35 °C) and three constant (25, 30 and 35 °C) temperature regimes for 21 days.  
759 Values are means ± SD. Asterisks denote significant differences: \*(p < 0.05), \*\*\*  
760 (p < 0.001), \*\*\*\* (p < 0.0001) between treatments.

761

762 **Figure 3.** (A) Body mass (mg) and (B) total length (mm) of giant gourami  
763 *Osphronemus goramy* larvae (n = 4 pools of 22 larvae) reared at four temperature  
764 regimes from 8 days post-hatching. Values are means ± SD. Different letters  
765 denote significant differences (p < 0.05) between temperature regimes.

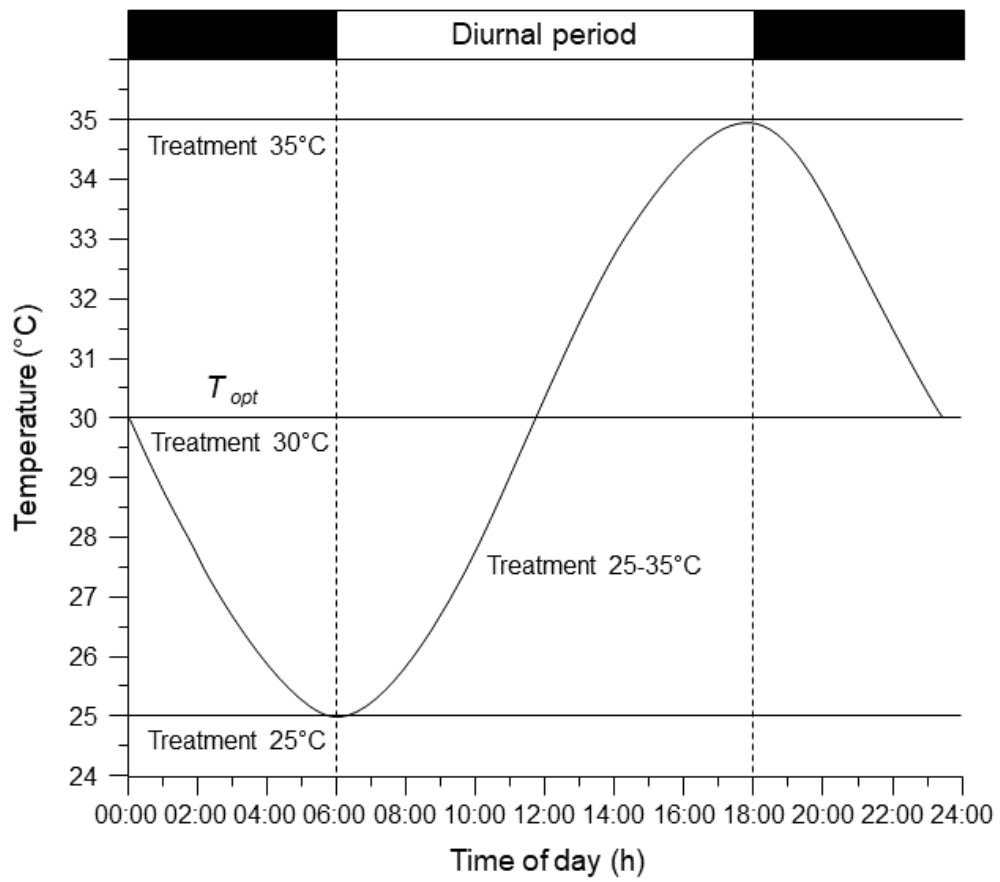
766

767 **Figure 4.** Concentration of cortisol and glucose (n = 4 pools of 10 fish)  
768 concentrations in the blood of giant gourami *Osphronemus goramy* larvae reared  
769 at one daily fluctuating temperature (25-35 °C) and three constant (25, 30 and

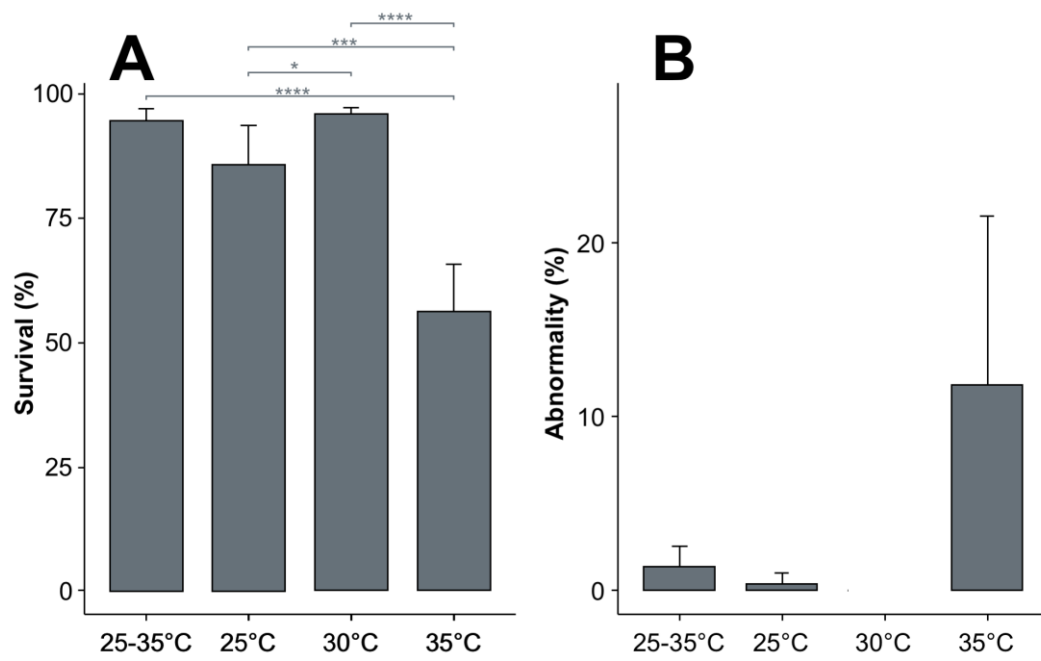
770 35 °C) temperature regimes for 21 days. Values are means  $\pm$  SD. Asterisks denote  
771 significant differences: \* ( $p < 0.05$ ) between regimes.

772

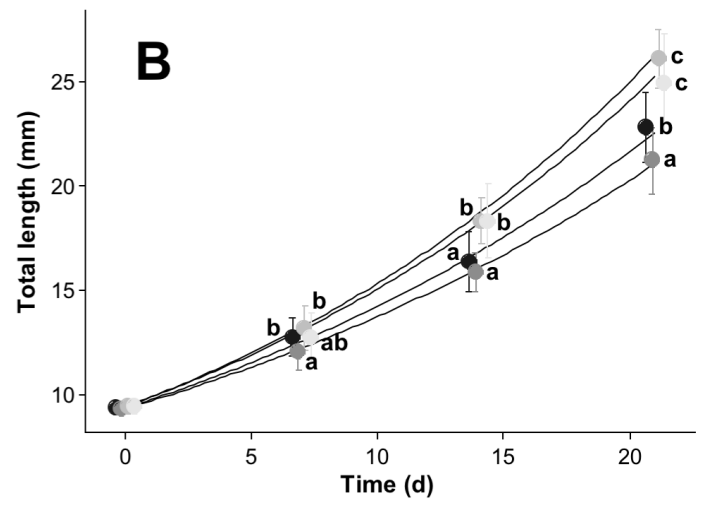
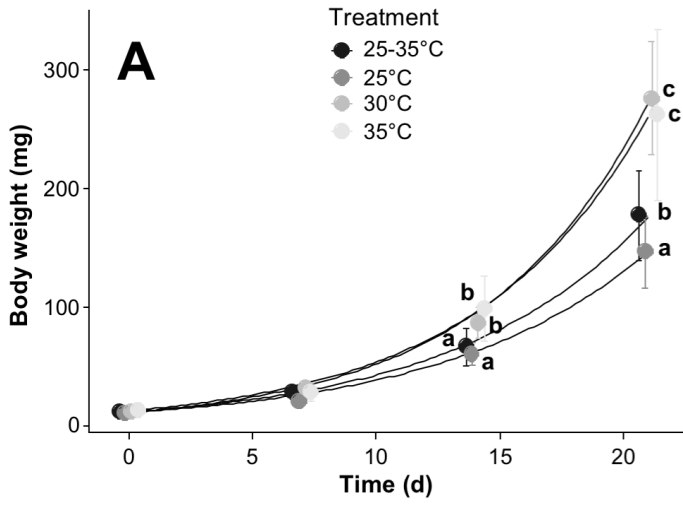
773 **Figure 5.** Oxygen consumption and ventilation rates ( $n = 3$  batches of 21 larvae)  
774 concentrations in the blood of giant gourami *Osphronemus goramy* larvae reared  
775 at one daily fluctuating (25-35 °C) and three constant (25, 30, and 35 °C)  
776 temperature regimes for 21 days. Values are means  $\pm$  SD. Asterisks denote  
777 significant differences: \* ( $p < 0.05$ ), \*\*\* ( $p < 0.001$ ), \*\*\*\* ( $p < 0.0001$ ) between  
778 treatments.



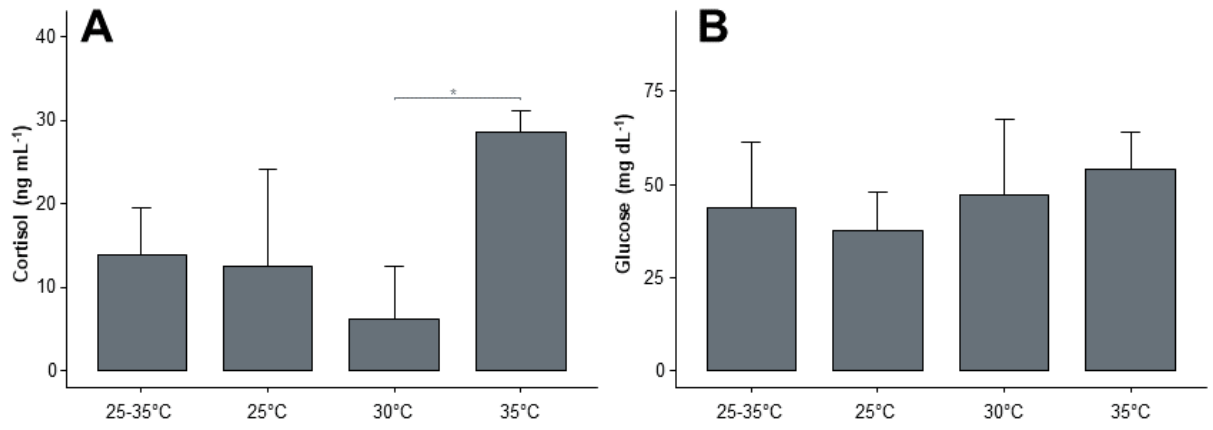
779 **Figure 1**



780 **Figure 2**

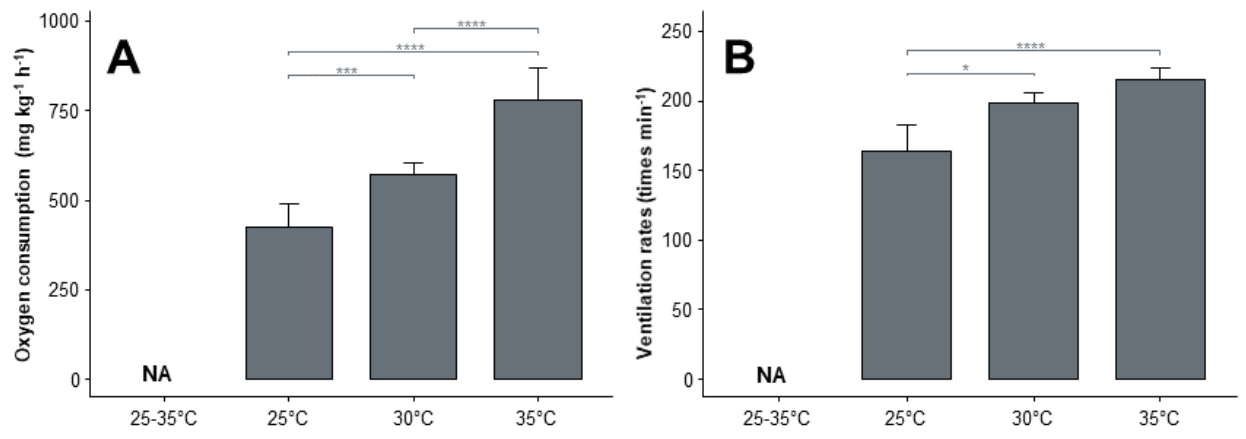


781 **Figure 3**



782 **Figure 4**





783 **Figure 5**

784 Table 1. Summary of water quality parameters measured for the one fluctuating  
 785 (25-35°C) and the three constant (25 °C, 30 °C and 35 °C) temperature regimes  
 786 during the experiment. Values are means  $\pm$  SD. For each parameter, values with  
 787 different letters are significantly different ( $p < 0.05$ ) between temperature  
 788 treatments.

Parameter	n	Temperature treatment			
		Fluctuating (25-35 °C)	Constant (25 °C)	Constant (30 °C)	Constant (35 °C)
Temperature (°C)	66	29.0 $\pm$ 3.4 <sup>a</sup>	24.8 $\pm$ 0.1 <sup>b</sup>	29.9 $\pm$ 0.1 <sup>c</sup>	34.8 $\pm$ 0.1 <sup>d</sup>
Temperature range (°C)	66	24.7-34.4 <sup>a</sup>	24.6-25.2 <sup>b</sup>	29.6-30.2 <sup>c</sup>	34.4-35.2 <sup>d</sup>
DO (mg L <sup>-1</sup> )	16	7.81 $\pm$ 0.25 <sup>a</sup>	7.77 $\pm$ 0.18 <sup>a</sup>	6.98 $\pm$ 0.16 <sup>b</sup>	6.59 $\pm$ 0.11 <sup>b</sup>
pH	16	7.32 $\pm$ 0.46 <sup>a</sup>	7.47 $\pm$ 0.38 <sup>a</sup>	7.67 $\pm$ 0.17 <sup>a</sup>	7.66 $\pm$ 0.51 <sup>a</sup>
Conductivity ( $\mu$ S cm <sup>-1</sup> )	16	158.3 $\pm$ 8.1 <sup>a</sup>	172.9 $\pm$ 3.6 <sup>ab</sup>	201.3 $\pm$ 7.8 <sup>c</sup>	186.5 $\pm$ 5.6 <sup>bc</sup>
TAN (mg L <sup>-1</sup> )	12	0.24 $\pm$ 0.08 <sup>a</sup>	0.95 $\pm$ 0.97 <sup>a</sup>	0.24 $\pm$ 0.11 <sup>a</sup>	0.25 $\pm$ 0.09 <sup>a</sup>
NH <sub>3</sub> (mg L <sup>-1</sup> )	12	0.006 $\pm$ 0.007 <sup>a</sup>	0.013 $\pm$ 0.010 <sup>a</sup>	0.007 $\pm$ 0.004 <sup>a</sup>	0.012 $\pm$ 0.001 <sup>a</sup>
NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	12	0.23 $\pm$ 0.08 <sup>a</sup>	0.93 $\pm$ 0.96 <sup>a</sup>	0.23 $\pm$ 0.11 <sup>a</sup>	0.23 $\pm$ 0.08 <sup>a</sup>
NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	12	0.022 $\pm$ 0.017 <sup>a</sup>	0.029 $\pm$ 0.01 <sup>a</sup>	0.024 $\pm$ 0.013 <sup>a</sup>	0.033 $\pm$ 0.025 <sup>a</sup>

789 DO: dissolved oxygen; TAN: total ammonia nitrogen

790 Table 2. Growth performances of giant gourami *Osphronemus goramy* larvae  
 791 reared at one fluctuating (25-35 °C) and three constant temperature (25 °C, 30 °C,  
 792 and 35 °C) for 21 days from 8 days post-hatching. See Materials and Methods for  
 793 details on the parameters. Values are means  $\pm$  SD. For each parameter, values  
 794 with different letters are significantly different ( $p < 0.05$ ) between temperature  
 795 treatments.

Parameter	n	Temperature treatment			
		Fluctuating (25-35 °C)	Constant (25 °C)	Constant (30 °C)	Constant (35 °C)
BM <sub>i</sub> (mg)	4*	12.0 $\pm$ 1.1 <sup>a</sup>	11.6 $\pm$ 1.1 <sup>a</sup>	11.7 $\pm$ 1.1 <sup>a</sup>	13.0 $\pm$ 1.4 <sup>a</sup>
BM <sub>f</sub> (mg)	4*	178.0 $\pm$ 38.1 <sup>a</sup>	147.1 $\pm$ 30.6 <sup>b</sup>	276.7 $\pm$ 47.5 <sup>c</sup>	262.9 $\pm$ 72.2 <sup>c</sup>
TL <sub>i</sub> (mm)	4*	9.3 $\pm$ 0.3 <sup>a</sup>	9.3 $\pm$ 0.3 <sup>a</sup>	9.4 $\pm$ 0.2 <sup>a</sup>	9.4 $\pm$ 0.2 <sup>a</sup>
TL <sub>f</sub> (mm)	4*	22.8 $\pm$ 1.7 <sup>a</sup>	21.2 $\pm$ 1.6 <sup>b</sup>	26.1 $\pm$ 1.4 <sup>c</sup>	24.9 $\pm$ 2.4 <sup>d</sup>
K (mg mm <sup>-3</sup> x 100)	4*	1.48 $\pm$ 0.18 <sup>a</sup>	1.53 $\pm$ 0.18 <sup>ab</sup>	1.54 $\pm$ 0.09 <sup>b</sup>	1.67 $\pm$ 0.28 <sup>c</sup>
SGR <sub>BM</sub> (% d <sup>-1</sup> )	4	12.8 $\pm$ 0.4 <sup>a</sup>	12.1 $\pm$ 0.5 <sup>a</sup>	15.1 $\pm$ 0.2 <sup>b</sup>	14.3 $\pm$ 0.5 <sup>b</sup>
SGR <sub>TL</sub> (% d <sup>-1</sup> )	4	4.3 $\pm$ 0.1 <sup>a</sup>	3.9 $\pm$ 0.2 <sup>b</sup>	4.9 $\pm$ 0.1 <sup>c</sup>	4.6 $\pm$ 0.2 <sup>c</sup>
CV <sub>BM</sub> (%)	4	19.8 $\pm$ 3.3 <sup>ab</sup>	18.1 $\pm$ 1.2 <sup>ab</sup>	17.1 $\pm$ 2.7 <sup>a</sup>	26.4 $\pm$ 7.9 <sup>b</sup>
CV <sub>TL</sub> (%)	4	7.1 $\pm$ 1.9 <sup>a</sup>	6.6 $\pm$ 1.5 <sup>a</sup>	5.5 $\pm$ 0.8 <sup>a</sup>	9.3 $\pm$ 3.2 <sup>a</sup>
BG (g)	4	11.1 $\pm$ 0.7 <sup>ab</sup>	8.5 $\pm$ 1.6 <sup>a</sup>	20.7 $\pm$ 5.2 <sup>b</sup>	8.4 $\pm$ 2.4 <sup>a</sup>
FCR	4	7.33 $\pm$ 0.35 <sup>ab</sup>	8.47 $\pm$ 1.64 <sup>ac</sup>	4.75 $\pm$ 0.92 <sup>b</sup>	10.58 $\pm$ 2.27 <sup>c</sup>

796 BG: biomass gain, BM: body mass, BM<sub>i</sub>: initial body mass, BM<sub>f</sub>: final body mass,  
 797 CV: coefficient of variation, FCR: feed conversion ratio, K: Fulton's condition  
 798 factor, TL: total length, SGR: specific growth rate, TL<sub>i</sub>: initial total length, TL<sub>f</sub>:  
 799 final total length.

800 \* For BM, TF and K, 22 larvae were sampled from each of the four tanks.

801 Table 3. Parameters (Mean  $\pm$  SE, n = 20 at each time) of exponential growth in  
 802 body mass (BM, mg) and total length (TL, mm) of giant gourami (*Osphronemus*  
 803 *goramy*) larvae reared in four different temperature treatments. Model parameters:  
 804 a: growth rate ( $d^{-1}$ ).  $R^2$ : determination coefficient. \*\*\* Probability of the model  
 805 adjustment:  $p < 0.001$

Temperature treatment	a $\pm$ SE	$R^2$
Body mass (BM)		
25-35 °C	0.128 $\pm$ 0.001***	0.95
25 °C	0.121 $\pm$ 0.001***	0.96
30 °C	0.150 $\pm$ 0.001***	0.97
35 °C	0.143 $\pm$ 0.001***	0.93
Total length (TL)		
25-35 °C	0.042 $\pm$ 0.001***	0.97
25 °C	0.039 $\pm$ 0.001***	0.98
30 °C	0.049 $\pm$ 0.001***	0.99
35 °C	0.047 $\pm$ 0.001***	0.96

806