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► To cite this version:

Vitas Atmadi Prakoso, Simon Pouil, Wahyulia Cahyanti, Sri Sundari, Otong Zenal Arifin, et al.. Fluctuating temperature regime impairs growth in giant gourami (*Osphronemus goramy*) larvae. *Aquaculture*, 2021, 539, 10.1016/j.aquaculture.2021.736606 . hal-03196727

HAL Id: hal-03196727

<https://hal.science/hal-03196727>

Submitted on 5 Sep 2023

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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 ± 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 × 30 × 30 cm) placed on a bench. At 8 dph, larvae
139 (mean body mass: 12.1 ± 1.2 mg; mean total length: 9.4 ± 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 (+/-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- μ 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), NH_3 , NH_4^+ , and 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 ¹, 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⁻¹ in
282 all treatments. TAN was not significantly different between the four experimental
283 treatments, and the NO₂⁻ and NH₃ concentrations were lower than 0.1 and 0.05 mg
284 L⁻¹, 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 ± 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 ± 0.92) and the highest FCR observed at 35
330 °C (10.58 ± 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 ± 6.21 ng
339 mL^{-1} at 30 °C to 28.46 ± 2.72 ng 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 ± 5.64 ng mL^{-1} and 12.56
342 ± 11.63 ng 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 ± 10.07 mg dL^{-1} to
345 54.17 ± 9.87 mg 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 ± 20 times
354 min^{-1} at $25 \text{ }^\circ\text{C}$ to 215 ± 8 times 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 ($25\text{-}35 \text{ }^\circ\text{C}$) 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 NH_4^+ (approx. 1 mg L^{-1}), were
368 observed at $25 \text{ }^\circ\text{C}$ than for the other temperature treatments, the 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 ± 0.1 °C), 178.0 mg at fluctuating regime (29.0 ± 3.4 °C), 276.7 mg
393 at constant 30 °C regime (29.9 ± 0.1 °C), and 262.9 mg at constant 35 °C regime
394 (34.8 ± 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⁻¹) 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⁻¹) 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 O₂ kg⁻¹ h⁻¹ at 25, 30, and 35 °C,
476 respectively; Sarma et al., 2010), common carp *Cyprinus carpio* (64, 71, and 99
477 mg O₂ kg⁻¹ h⁻¹ at 25, 30, and 35 °C, respectively; Chatterjee et al., 2004),
478 yellowtail catfish *Pangasius pangasius* (from 111, 232, 236 mg O₂ kg⁻¹ h⁻¹ at 30,
479 34, and 38 °C, respectively; Debnath et al., 2006), and Nile tilapia (from 22 to 95
480 mg O₂ kg⁻¹ h⁻¹ 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⁻¹ at 21.5 and 31.5 °C, respectively; Prakoso and Kurniawan,
484 2020), the pikeperch *Sander lucioperca* (from 8 to 31 times min⁻¹ 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⁻¹ 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 (Q₁₀) 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

514 **Acknowledgements**

515 The authors acknowledge Imam Akhir Abdullah, technician at the Cijeruk
516 research station, and Ahmad Sihabuddin, technician at IRD Indonesia, for their

517 technical assistance throughout the experiment. This is publication ISEM XXXX-
518 XXX.

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 \pm 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 \pm SD. Different letters
765 denote significant differences (p < 0.05) between temperature regimes.

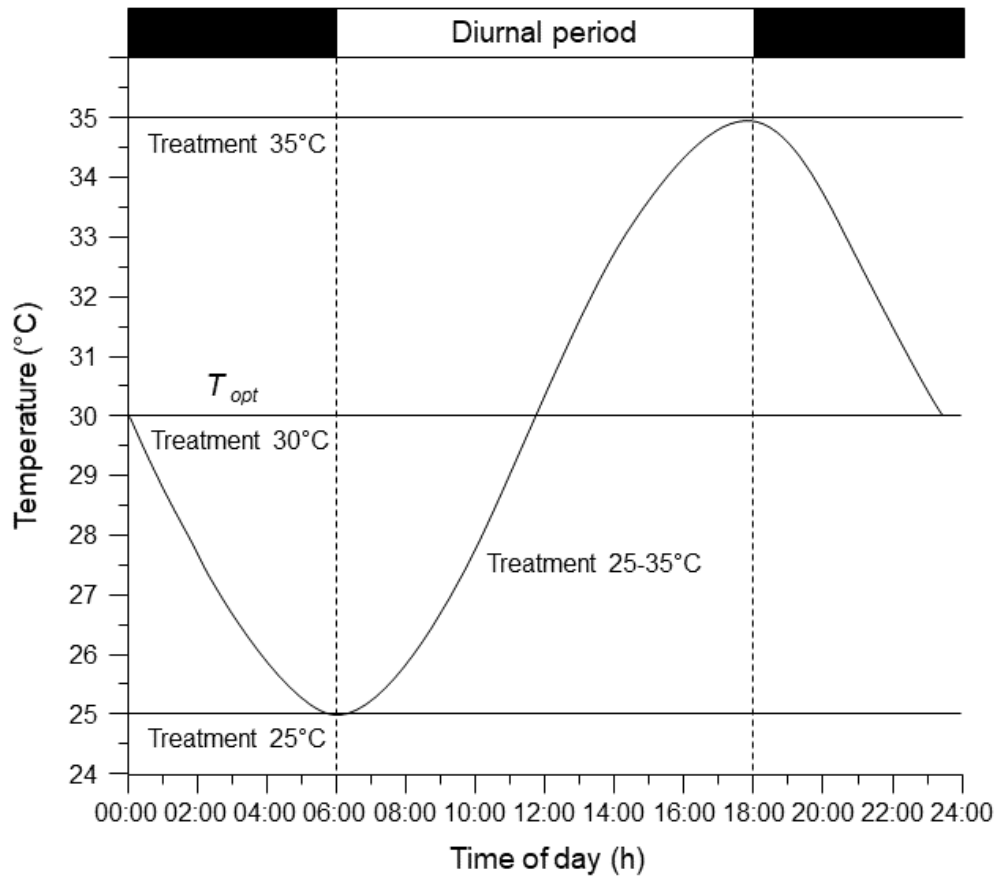
766

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

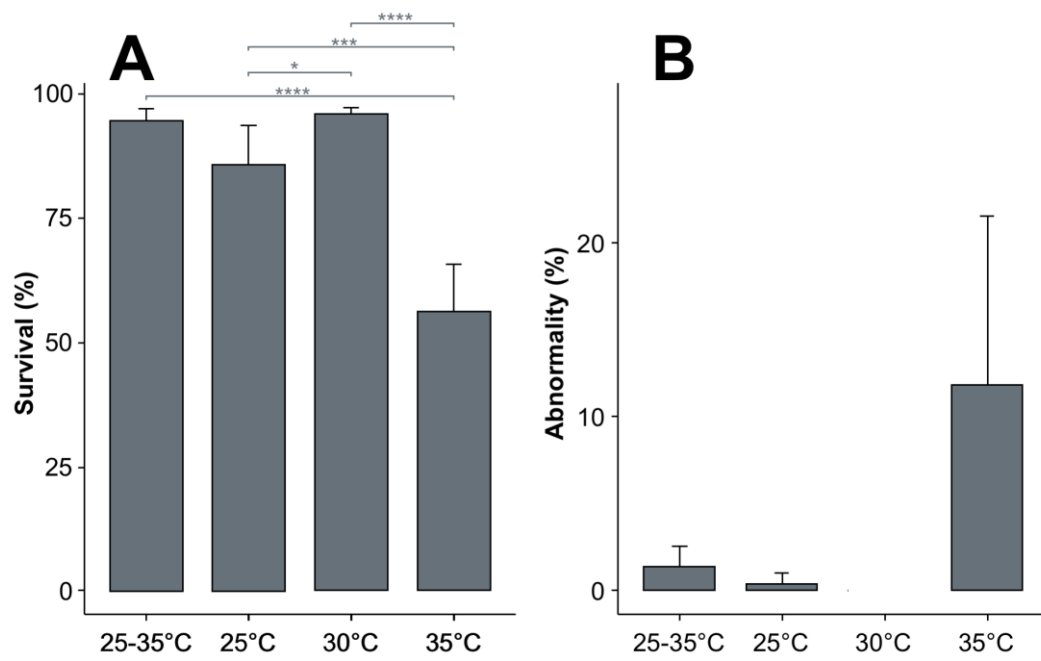
770 35 °C) temperature regimes for 21 days. Values are means \pm SD. Asterisks denote
771 significant differences: * ($p < 0.05$) between regimes.

772

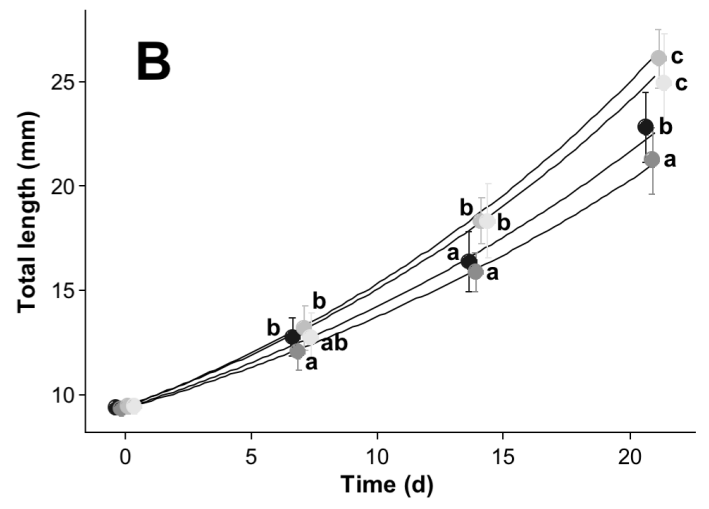
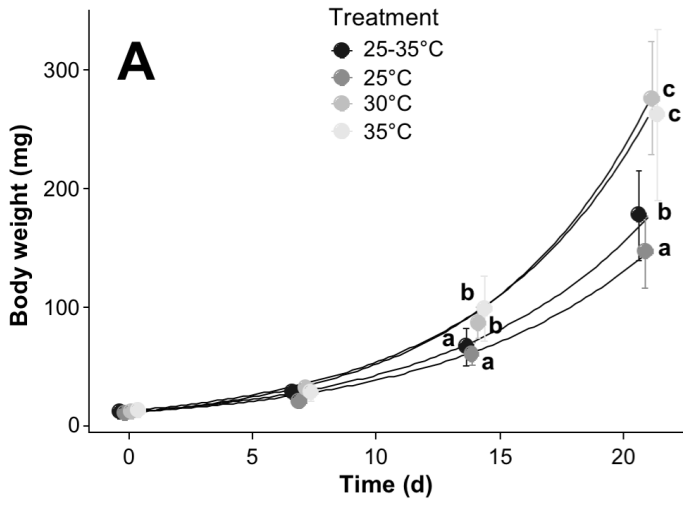
773 **Figure 5.** Oxygen consumption and ventilation rates ($n = 3$ batches of 21 larvae)
774 concentrations in the blood of giant gourami *Osphronemus goramy* larvae reared
775 at one daily fluctuating (25-35 °C) and three constant (25, 30, and 35 °C)
776 temperature regimes for 21 days. Values are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



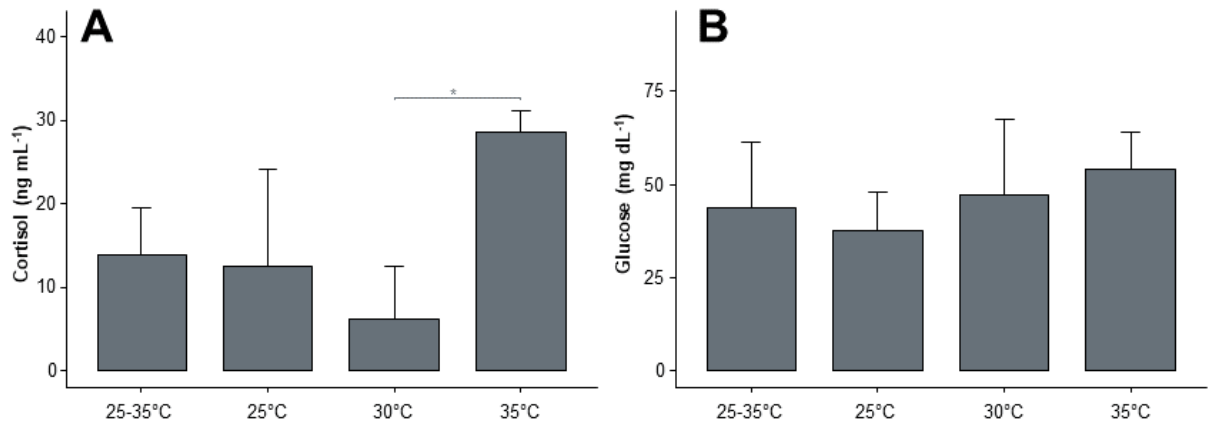
779 **Figure 1**



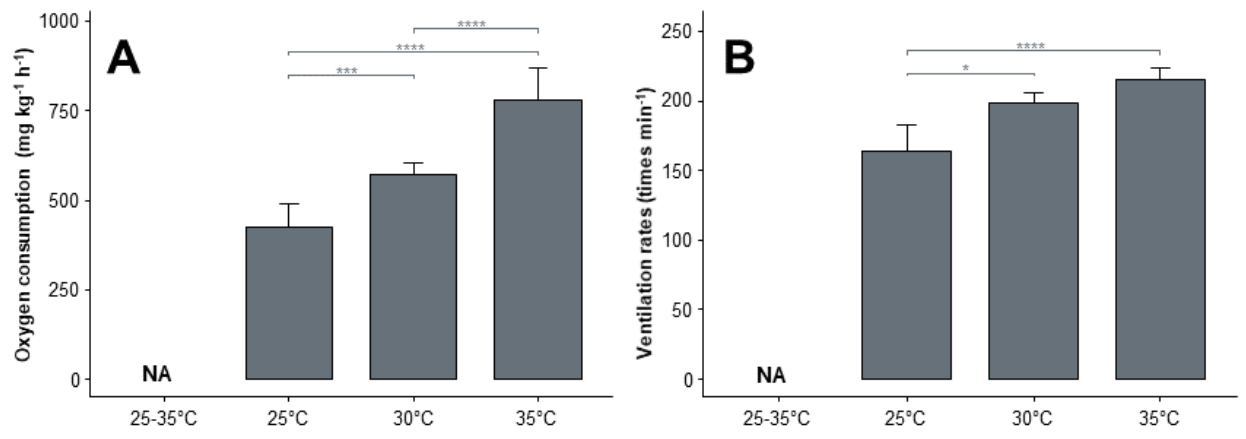
780 **Figure 2**



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

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 _i (mg)	4*	12.0 \pm 1.1 ^a	11.6 \pm 1.1 ^a	11.7 \pm 1.1 ^a	13.0 \pm 1.4 ^a
BM _f (mg)	4*	178.0 \pm 38.1 ^a	147.1 \pm 30.6 ^b	276.7 \pm 47.5 ^c	262.9 \pm 72.2 ^c
TL _i (mm)	4*	9.3 \pm 0.3 ^a	9.3 \pm 0.3 ^a	9.4 \pm 0.2 ^a	9.4 \pm 0.2 ^a
TL _f (mm)	4*	22.8 \pm 1.7 ^a	21.2 \pm 1.6 ^b	26.1 \pm 1.4 ^c	24.9 \pm 2.4 ^d
K (mg mm ⁻³ x 100)	4*	1.48 \pm 0.18 ^a	1.53 \pm 0.18 ^{ab}	1.54 \pm 0.09 ^b	1.67 \pm 0.28 ^c
SGR _{BM} (% d ⁻¹)	4	12.8 \pm 0.4 ^a	12.1 \pm 0.5 ^a	15.1 \pm 0.2 ^b	14.3 \pm 0.5 ^b
SGR _{TL} (% d ⁻¹)	4	4.3 \pm 0.1 ^a	3.9 \pm 0.2 ^b	4.9 \pm 0.1 ^c	4.6 \pm 0.2 ^c
CV _{BM} (%)	4	19.8 \pm 3.3 ^{ab}	18.1 \pm 1.2 ^{ab}	17.1 \pm 2.7 ^a	26.4 \pm 7.9 ^b
CV _{TL} (%)	4	7.1 \pm 1.9 ^a	6.6 \pm 1.5 ^a	5.5 \pm 0.8 ^a	9.3 \pm 3.2 ^a
BG (g)	4	11.1 \pm 0.7 ^{ab}	8.5 \pm 1.6 ^a	20.7 \pm 5.2 ^b	8.4 \pm 2.4 ^a
FCR	4	7.33 \pm 0.35 ^{ab}	8.47 \pm 1.64 ^{ac}	4.75 \pm 0.92 ^b	10.58 \pm 2.27 ^c

796 BG: biomass gain, BM: body mass, BM_i: initial body mass, BM_f: 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_i: initial total length, TL_f:
 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