



HAL
open science

New insights into giant gourami (*Osphronemus goramy*) reproductive biology and egg production control

Otong Zenal Arifin, Jacques Slembrouck, Jojo Subagja, Simon Pouil, Akhmad
Yani, Asependi Asependi, Anang Hari Kristanto, Marc Legendre

► To cite this version:

Otong Zenal Arifin, Jacques Slembrouck, Jojo Subagja, Simon Pouil, Akhmad Yani, et al.. New insights into giant gourami (*Osphronemus goramy*) reproductive biology and egg production control. *Aquaculture*, 2020, 519, pp.734743. 10.1016/j.aquaculture.2019.734743 . hal-03163364

HAL Id: hal-03163364

<https://hal.inrae.fr/hal-03163364>

Submitted on 7 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

17 **Abstract**

18 Reliable production of giant gourami *Osphronemus goramy* Lacepède (1801) fry is one of the
19 main impediments hindering the aquaculture development of this species. The main objective
20 of the present study was to experimentally assess the influence of various biotic or abiotic
21 factors on the egg production of giant gourami. Egg production (egg-laying frequency,
22 quantity and quality of the eggs produced) was compared across several strategies including
23 different pond settings (open vs. compartmentalized ponds, number of nest supports, size of
24 compartments), as well as broodfish management (sex ratio, duration of egg production
25 period). A total of 705 spawns and more than 2.2 million eggs were collected from the 533
26 broodfish of giant gourami monitored throughout this study. The dissection of fully sexually
27 mature broodfish showed that giant gourami males are oligospermic with a GSI <0.1%. In
28 mature females, the GSI was <5%, and the absolute fecundity (number of post-vitellogenic
29 oocytes present in the ovaries) varied between 7,800 and 15,200 eggs (mean relative fecundity
30 of $4,011 \pm 287$ eggs per female kg). The best egg production was found in the production
31 system consisting of a pond divided into compartments of 8 m² with one nest support
32 provided and in which the broodfish were maintained at a sex ratio of 1:1 during egg
33 production periods of 6-7 months separated by resting/reconditioning periods of 1 month. The
34 results showed that a same male can fertilize eggs at 2 to 4 day-intervals and, in females, the
35 minimum lapse of time found between two successive spawns is 20 days. At best, the number
36 of eggs found in nests averaged $1,410 \pm 101$ eggs per female kg, which, compared with the
37 potential fecundity of the species, indicates that egg losses at the time of spawning generally
38 remain high. Based on these findings, recommendations are made for optimizing giant
39 gourami egg production in aquaculture conditions.

40 Keywords: Broodstock management, Fish reproduction, Freshwater fish, Indonesia, Pond
41 aquaculture

42 **1. Introduction**

43 One of the prerequisites for fish domestication and sustainable aquaculture production is the
44 capacity to control reproductive processes in captivity (Migaud et al., 2013; Teletchea and
45 Fontaine, 2014). Most farmed fish undergo and complete their gametogenesis in captivity.
46 Nevertheless, for most species, adequate spawning stimuli are lacking in the aquaculture
47 environment, making hormonal treatment necessary to induce final oocyte maturation and
48 ovulation of females (see Woynarovich and Horvath, 1980; Harvey and Carolsfeld, 1993;
49 Legendre et al., 1996; Mylonas et al., 2010; Migaud et al., 2013). However, some fish species
50 are able to breed spontaneously in the rearing facilities (e.g. Nile tilapia *Oreochromis*
51 *niloticus* and Channel catfish *Ictalurus punctatus*), as long as environmental and social cues
52 and triggers are present to complete final maturation, ovulation, and spawning (Lazard and
53 Legendre, 1996; Tucker and Hargreaves, 2004). In fish reproducing spontaneously in
54 captivity, the spawning rate and success can be artificially modulated according to a series of
55 abiotic and biotic factors.

56 Among abiotic factors, the effects of photoperiod, temperature, rainfall, and water salinity on
57 reproduction efficiency has been particularly documented (Harvey and Carolsfeld, 1993;
58 Bromage et al., 2001; Campos-Mendoza et al., 2004; Bobe and Labbé, 2010). However, other
59 factors are also known to strongly influence the spawning or gamete quality in fishes, such as
60 spawning substrate availability (Woynarovich and Horvath, 1980; Joseph et al., 2017),
61 broodfish feeding (Izquierdo et al., 2001), size of rearing structures (Mylonas et al., 2010), or
62 the spatial organization of the rearing environment (Duponchelle and Legendre, 2001). The
63 biotic factors are related to the broodfish themselves and fish social interactions. They include
64 the respective size of the broodfish, their stocking density or the sex ratio, and their influence
65 has been documented in some of the main farmed species (Legendre and Trébaol, 1996;
66 Salama, 1996; Siddiqui and Al-Harbi, 1997; Bhugel, 2000; Tsadik and Bart, 2007). However,

67 the egg production of many spontaneous spawners still remains limited due to a lack of
68 knowledge on the nature of the main factors influencing their reproduction in captivity and
69 their optimal conditions.

70 The giant gourami *Osphronemus goramy* Lacepède (1801), native to Southeast Asia, is one of
71 the main freshwater commodities of economic importance in Indonesia owing to its high price
72 and its high local demand (Rimmer et al., 2013). Its annual production in Indonesia has grown
73 exponentially over the past 15 years, reaching over 113,000 tons in 2015 (FAO, 2017).
74 Although giant gourami spontaneously spawns in captivity, subject to the availability of
75 suitable laying substrates, fry supply remains one of the main concerns for the development of
76 its aquaculture (Amornsakun et al., 2014; Arifin et al., 2019). In this respect, the Indonesian
77 National Standard (SNI) has issued guidelines for giant gourami production (SNI, 2000), but
78 the defined framework remains relatively limited and rarely supported by experimental
79 evidence. There are only few accessible scientific publications on the reproduction of giant
80 gourami, most of them being descriptive (Bhimachar et al., 1944; Woynarovich and Horvath,
81 1980; Wijayanti et al., 2009; Tanjung and Pilo, 2015; Ramadhani et al., 2018). A survey
82 conducted on fish farmers in the province of West Java revealed that giant gourami egg and
83 fry production practices vary greatly from one farm to another in almost every aspect (see
84 Kristanto et al., 2019) reflecting the lack of clear and standardized production guidelines.

85 The main objective of the present study was to experimentally assess the influence of
86 different factors on giant gourami egg production. All the experiments presented in this study
87 were carried out at the West Java Center for the Development of Giant Gourami Culture
88 (BPPSIGN), the largest giant gourami fry production center in West Java Province. We
89 experimentally tested different alternatives to the giant gourami production methods currently
90 used at BPPSIGN and mainly based on national standards (SNI). Thus, the reproduction
91 characteristics (egg-laying frequency, quantity and quality of the eggs produced) were

92 compared using different pond management strategies (open or compartmentalized), and
93 across different egg production periods, number of nest supports provided, sizes of the
94 spawning compartments, and broodfish sex ratios.

95

96 **2. Materials and methods**

97 **2.1. General information and procedures**

98 *2.1.1. Location and experimental site*

99 This study was carried out at the BPPSIGN Tasikmalaya Center (7°19'37.992"N,
100 108°6'101.155"E, altitude 489 m), a regional center for fish farmer training and giant gourami
101 fry production. A series of experiments on the spontaneous reproduction of giant gourami was
102 performed over a period of 20 months from May 2016 to January 2018. Experimental
103 structures were earthen flat bottom ponds with vertical concrete banks varying between 200
104 and 870 m² in surface area with an average water depth of 0.6 m. The ponds were supplied by
105 spring water brought by a channel coming from the Galunggung Mountain located less than
106 10 km from the BPPSIGN Center. The ponds' water inflow varied from 3 to 12 m³ h⁻¹
107 depending on water available for irrigation in the local area.

108

109 *2.1.2. Pond preparation and water quality monitoring*

110 Before starting the experiments and between egg production periods, the spawning ponds
111 were dried, cleaned, rid of all other species, and limed before being refilled. Pond water
112 temperature was monitored continuously using data logger (Onset HOBO) placed at the
113 center of the pond at 20 cm depth. Throughout the study, the instantaneous water temperature
114 varied between 22.6 °C and 32.7 °C. Water pH, dissolved oxygen, conductivity, and turbidity
115 were measured before each fish sampling between 07:00 and 08:00 AM with direct
116 measurements using a multi-parameter probe (HI 9829 Hanna); the corresponding range of

117 these parameters were 7.5-7.9, 3.8-7.3 mg L⁻¹, 171-188 µS cm⁻¹ and 9-13 NTU, respectively.
118 Water concentration in ammonia (NH₃), nitrite (NO₂⁻) and nitrate (NO₃⁻) was measured at
119 quarterly intervals using spectrophotometry analysis (Hanna HI83399); corresponding value
120 ranges were <0.0065 mg NH₃ L⁻¹, 0.006-0.020 mg NO₂⁻ L⁻¹ and 0.26-0.59 mg NO₃⁻ L⁻¹.
121 Although the minimum oxygen concentration was slightly low, < 4 mg L⁻¹, this did not affect
122 the broodfish because giant gourami is capable of aerial respiration (Helfman et al., 2009). All
123 other parameter values indicated that the pond water quality remained within appropriate
124 standards for tropical freshwater fish (Colt, 2006).

125

126 ***2.1.3. Broodfish characteristics and evaluation of sexual maturity***

127 All mature broodfish used in the experiments were descendants of giant gourami belonging to
128 the “Galunggung” strain selected since about 10 years from local fish farms in the
129 Tasikmalaya area (Arifin et al., 2017). A total of 533 sexually mature broodfish of giant
130 gourami were used in this study. The females (n = 399) were 3-5 years old and weighed 2,774
131 ± 22 g (mean ± SE), whereas males (n = 134) were 5-7 years old and weighed 3,749 ± 55 g.
132 For reproduction, according to current practices at BPPSIGN, males were consistently
133 selected larger than females with a mean body mass ratio of 1.3 (range: 1.1-1.4).

134 At the beginning of each egg production period, all broodfish were individually weighed with
135 a digital scale (nearest 10 g) and their sex was determined as presented in Slembrouck et al.
136 (2019). The sexual maturity of females was assessed from oocytes sampled by intraovarian
137 biopsies using a flexible polyethylene catheter (“*pipelle de Cornier*”; Cacot et al., 2002;
138 Slembrouck et al., 2004). Oocyte diameter was measured at the edge of the ponds using graph
139 paper. The largest oocyte diameter varied from less than 0.5 mm (beginning of vitellogenesis)
140 to more than 2.5 mm (pre-spawning stage) depending on the individual. A simple maturity
141 scale with seven stages based on 0.5 mm classes of the largest observed oocyte diameter was

142 used to characterize the sexual stage of each female; stage 1 corresponded to cases in which
143 no oocyte could be collected at biopsy, stage 2 corresponded to oocytes ≤ 0.5 mm, and stage 7
144 to oocytes > 2.5 mm. The sexual maturity of males was evaluated on the basis of the quantity
145 of milt emission following gentle abdominal massage. However, in most cases, the volume of
146 semen collected by stripping was small (a few drops), if any was emitted. After sampling and
147 sexing, broodfish were arbitrarily placed in the spawning structures.

148 Giant gourami broodfish are valuable resources, thus a limited number of fish was used to
149 perform dissections. Five males and four females, determined as fully sexually mature
150 broodfish by the emission of sperm at stripping or presence of stage 7 oocytes after
151 intraovarian biopsy, were anesthetized (Eugenol, 0.05 mL L^{-1}), then killed by an overdose of
152 anesthetic (Eugenol, 0.5 mL L^{-1}) and dissected to examine the gonads and determine the
153 gonado-somatic index [GSI = gonad mass/body mass $\times 100$]. Subsamples of ovaries were
154 weighed (nearest 0.01 g) and placed in 5% formalin to determine the size-frequency
155 distribution of oocytes and fish fecundity. Fecundity is defined here as the number of oocytes
156 to be released at the next spawn and correspond to absolute fecundity. In pre-spawning
157 females, it is estimated by the number of oocytes belonging to the largest diameter modal
158 group (Nuñez and Duponchelle, 2009). Oocyte counting and diameter measurements were
159 performed using IMAGE J freeware (Abramoff and Magalhaes, 2004).

160

161 ***2.1.4. Spawning conditions***

162 Two types of ponds were used: open communal ponds (OPs) or compartmentalized ponds
163 (CPs). In OPs, the broodfish were stocked together and had free access to the entire pond (Fig.
164 1a). In CPs, compartments of 2 to 24 m^2 in surface were delimited with nets embedded in the
165 sediment and held vertically on bamboo poles (Fig. 1b). Unless otherwise stated, the
166 broodfish were stocked in the spawning ponds at a density of 1 fish for $4\text{-}5 \text{ m}^2$, using a male-

167 to-female (M:F) ratio of 1:3, as recommended by SNI (2000). The giant gourami is a nest
168 builder and nest support and nesting material were provided in the ponds and compartments
169 for spawning (Fig 1).

170 For all experiments during the egg production periods, which lasted for 3 to 7 months
171 depending on trials, the fish were fed giant taro leaves (*Alocasia macrorrhizos*) and
172 commercial extruded pellets (32% proteins, 5% lipids) distributed at a daily feeding rate of
173 2% and 1% of fish biomass, respectively. Between two successive egg production periods,
174 males and females were separated into different ponds for a resting/reconditioning period,
175 lasting for 4-5 weeks. During these reconditioning periods, broodfish were also fed giant taro
176 and commercial extruded pellets, but with reverse daily feeding rates (1% of giant taro leaves
177 and 2% of floating pellets).

178

179 ***2.1.5. Egg collection and estimation of reproductive efficiency***

180 During the egg production periods, all nest supports were examined every two days and
181 closed nests containing eggs were collected, transferred to the hatchery, then immersed in a
182 water tank to release the eggs by carefully opening the palm fiber material (Fig. 1d). Due to
183 the presence of a very large oil globule, giant gourami eggs float (Baras et al., 2018). Their
184 manual separation from the nesting substrate is therefore very simple. All the eggs from each
185 nest were manually counted, separating live (transparent/clear) eggs from dead
186 (opaque/white) eggs. After counting, the clear eggs from each spawn were incubated in the
187 hatchery (100 L glass aquarium; 4-5 eggs cm²) for fry production.

188 In every trial, the efficiency of reproduction was estimated using three parameters: (1)
189 spawning frequency, defined as the number of spawns per female per month; (2) the total
190 number of eggs in a spawn (i.e., all eggs found in a nest); and (3) egg quality, assessed by the
191 proportion of clear eggs in each spawn. In some instances, the eggs were newly hatched at the

192 moment of nest collection; however, this had no or little incidence on the evaluation of
193 progeny number and spawn quality because white (dead) eggs take more than one day to
194 break up and newly hatched larvae, unable to swim, remain inside the nest.

195

196 **2.2. Experimental trials**

197 *2.2.1. Pond management strategies: open communal (OP) or compartmentalized (CP)* 198 *ponds*

199 To test the influence of pond management strategy, the efficiency of giant gourami
200 reproduction was characterized and compared between open communal ponds (OPs) and
201 compartmentalized ponds (CPs) over a total experimental period of 339 to 409 days
202 depending on the pond. Two OPs and two CPs of equivalent surface area (about 550 m²) were
203 stocked with broodfish at the same stocking density and sex ratio of 1:3 (see § 2.1.4). In the
204 OPs, 24 to 28 nest supports (equiv. 1 nest support per male) were installed 2 m from the banks
205 and about 2 m from each other (Fig. 1a). In the two CPs, the nest supports were placed in
206 corner of each of the 52 compartments (Fig. 1b). Broodfish were reconditioned for 1 month
207 after each continuous egg production period of 3 to 7 months (see § 2.2.2).

208

209 *2.2.2. Duration of egg production periods*

210 The effects of the duration of egg production periods on the efficiency of giant gourami egg
211 production was tested in the four ponds (2 CPs and 2 OPs) described in §2.1.1. The timing of
212 egg production and resting/reconditioning periods in the different ponds is detailed in Figure
213 2. In each CP, egg production periods (EPP) were organized either as (1) two successive 3-
214 month periods separated by an intermediate reconditioning (IR) period of 1 month or (2) one
215 continuous 7-month period without IR. These two EPP durations were tested simultaneously
216 in 50% of compartments of each CP, arbitrarily chosen. This experimental scheme was

217 repeated a second time after a general reconditioning period (GR) of 1 month for all broodfish
218 in the pond. The total duration of this study was therefore approximately 15 months (Fig. 2).
219 During IR, males from half the compartments were caught and placed together in another
220 pond, while the females remained in their respective compartments. In contrast, during GR,
221 all broodfish were removed from the experimental ponds and males and females were
222 separated and placed in different reconditioning ponds. Meanwhile, the experimental pond
223 was dried, cleaned and limed before being refilled.

224 In the two OPs, different EPP durations could not be tested at the same time in the same pond.
225 Because the fish were raised communally, all of them had to be reconditioned simultaneously
226 for a general reconditioning of 1 month, males and females being separated in distinct ponds.
227 Therefore, to avoid bias resulting from a possible seasonal incidence, the two replicated
228 communal ponds were phase shifted, alternating two EPPs of 3 months and one EPP of 6
229 months, each being separated by a GR period (Fig. 2).

230

231 ***2.2.3. Number of nest supports***

232 With a sex ratio of 1:3, it was uncertain if providing one nest support was sufficient. If
233 females participate in building the nests, then providing more supports may increase the
234 spawning frequency. To test this hypothesis, half of the 20 m² compartments of two ponds (n
235 = 26) were arbitrarily chosen and equipped with one nest support each, whereas the other half
236 (n = 26) was equipped with three nest supports each. The reproductive efficiency of giant
237 gourami in these two treatments was monitored simultaneously during about 15 months.
238 Complementary information regarding nest construction is available in the Supplementary
239 Material.

240

241 ***2.2.4. Broodfish sex ratio***

242 The effects of broodfish sex ratio on the reproductive efficiency of giant gourami was
243 evaluated for 195 days. Experimental structures consisted in 15 spawning compartments of 24
244 m² (three nest supports per compartment) implemented in a 870 m² pond. Five sex ratios (i.e.
245 1 male for 1, 2, 3, 5 or 7 females) were tested in triplicate.

246

247 ***2.2.5. Broodfish living space in the spawning compartments***

248 The incidence of the spawning compartment surface area on the reproductive characteristics
249 of giant gourami was evaluated for 179 days in 15 compartments implemented in a pond of
250 200 m² total area. In this experiment, considering the results of the previous sex ratio trial (see
251 § 2.2.4), broodfish pairs (sex ratio 1:1) were placed in compartments of 2, 4, 8 and 16 m² with
252 three replicates per treatment. Three additional 16 m² compartments implemented in the same
253 pond and stocked with one male and three females were monitored as a reference treatment.
254 Each compartment was equipped with one nest support.

255

256 **2.3. Statistical analysis**

257 Unless specified otherwise, values are means \pm standard error (SE). Data were first assessed
258 to confirm normality and homogeneity of variance. When these assumptions were met, one-
259 or two-way parametric ANOVAs were used to determine significant differences among
260 treatments. Scheffe's multiple range test was then performed on significant factors identified
261 in the ANOVA. When the conditions of normality and homogeneity of variance were not met,
262 non-parametric Kruskal-Wallis one-way ANOVA and Siegel & Castellan tests were used. All
263 tests were carried out using Statistica 10 software. Null hypotheses were rejected at $p < 0.05$.

264

265 **3. Results**

266 **3.1. Broodfish sexing, mortality, and overall egg production**

267 Overall, the mortality of broodfish remained low in all trials carried out and averaged $1.0 \pm$
268 0.3% per month. Repeated examination of females and males at intervals of a few months
269 confirmed the absence of sexing error when fish gender was determined using the cannulation
270 method. In total, 705 spawns and more than 2.2 million giant gourami eggs were collected
271 from the 533 broodfish used during the present series of experiments.

272

273 **3.2. Open communal (OP) vs. compartmentalized (CP) ponds**

274 The results, consistent across pond replications, are summarized in Table 1. With the same
275 number of broodfish (sex ratio 1:3), the number of spawns collected in CPs was more than
276 double that collected in OPs (see § 2.2.1). The monthly spawning frequency was significantly
277 higher in CPs than in OPs (0.16 ± 0.01 and 0.09 ± 0.01 spawn female⁻¹ month⁻¹, respectively;
278 $p < 0.001$). Given that the mean female body mass was homogeneous in the four ponds ($p >$
279 0.05), clutch size (i.e. number of eggs in a spawn) could be compared directly, irrespective of
280 female size. Clutch size was significantly higher in CPs than in OPs ($3,973 \pm 137$ and $2,380 \pm$
281 152 eggs spawn⁻¹ respectively; $p < 0.001$). Egg quality was also better in CPs than in OPs
282 (88.6 ± 1.0 and $83.9 \pm 1.4\%$ of clear eggs, respectively; $p < 0.001$). Altogether, the results
283 showed that the average monthly production of viable eggs, calculated as the number of
284 spawns month⁻¹ x average number of eggs in the nests x the proportion of viable eggs (see
285 Table 1), was more than three times higher in CPs (about 41,000 eggs) than in OPs (about
286 13,000 eggs).

287

288 **3.3. Incidence of initial maturity of broodfish**

289 In CPs (see § 2.2.1), the initial sexual maturity stage of the three females introduced into each
290 compartment was determined from intra-ovarian biopsies. We found that the time necessary

291 to obtain the first spawn in a compartment was highly dependent on the initial maturity of the
292 females. Thus, the higher the initial stage of maturation of the most advanced female, the
293 faster the first spawn occurred ($n = 116$, $r^2 = 0.479$, $F = 88.37$, $p < 0.001$). Nevertheless, there
294 was high variability between compartments for the time elapsed between the broodfish
295 stocking and the occurrence of the first spawning. Between 41 and 134 days (83 ± 16 d) were
296 needed for the first spawn in females starting at a low maturity stage (stage 2), whereas only 4
297 to 67 days (19 ± 3 d) were needed for the most mature females (stage 7; Fig. 3). In contrast,
298 no relationship was found between the initial maturity stages of males, as evaluated from
299 observed milt upon gentle fish abdominal massage, and the time to the first spawn. About 80
300 % of males could not be stripped for their sperm at the moment of broodfish stocking and
301 some of them were able to fertilize eggs as soon as 4 days after examination.

302

303 ***3.4. Duration of reproductive periods***

304 Because no pond effect was detected for any variable, the data relating to the same
305 reproduction period duration (see § 2.2.2) were pooled for analyses. For CPs, the reproductive
306 characteristics are summarized in Table 2. Over the total duration of experiment (about 15
307 months), spawns were obtained in all compartments with one exception in the “3 month”
308 treatment. The mean spawn number and egg production characteristics (quantity and quality)
309 were similar between spawning compartments managed over reproductive periods of either 4
310 x 3 months or 2 x 7 months (Table 2). On average, the number of viable eggs collected
311 monthly per compartment was similar when the broodfish were reconditioned every 3 months
312 ($1,794 \pm 157$) or every 7 months ($1,887 \pm 171$; $p > 0.05$). Therefore, the extension of the egg
313 production period (EPP) from 3 to 7 months did not impair the egg production of giant
314 gourami in CPs.

315 Nevertheless, using a sex ratio of 1:3, the mean time elapsed between two successive spawns
316 occurring in a given spawning compartment was significantly shorter during the first 3
317 months (30.7 ± 2.5 d) than during the last 3 months (60.5 ± 6.0 d) of a 7-month EPP (Fig. 4).
318 The lapse of time between two successive spawns was equivalent during the 3-month EPP
319 and during the first 3 months of the 7-month EPP (Fig. 4). A significant positive relationship
320 was found between the lapse of time separating successive spawns and the time spent by
321 broodfish in the spawning compartment ($n = 122$, $r^2 = 0.232$, $F = 36.48$; $p < 0.0001$; Fig. 5).
322 Altogether, these results show that the longer the broodfish remained in their compartment,
323 the longer was the lapse of time between two successive spawns. However, in terms of egg
324 production, this was compensated in the “7 month” treatment by the supplementary spawns
325 that were collected during the time corresponding to the intermediate reconditioning (IR)
326 periods between two successive EPPs in the “3 month” treatment. On average, the total
327 number of viable eggs produced per compartment over the whole experiment (calculated from
328 data synthesized in Table 2) was $21,652 \pm 1,896$ and $27,122 \pm 2,453$ for the “3 month” and “7
329 month” treatments, respectively ($p > 0.05$).

330 Similarly, in OPs, except for the lapse of time between successive spawns, which tended to
331 increase with time as in CPs, no significant differences were found in the egg production
332 parameters as a function of the duration of the reproduction period (3 vs. 6 months; data not
333 shown).

334

335 ***3.5. Nest construction and number of nest supports***

336 Our observations, based on examination of all nest supports every two days (§ 2.2.3), indicate
337 that the nest can be built rapidly. The shortest intervals observed in our study between two
338 consecutive nest collections in the same compartment were 2 days (2 observations), 4 days (n
339 = 3) and 5 days ($n = 7$). Although giant gourami males were predominantly involved in the

340 nest preparation and progeny guarding, females also participated in these activities. In pond
341 compartments in which males were absent and contained three females during IR periods, a
342 nest was constructed by females in 25% of cases (10 out of 40 compartments in which palm
343 fibers remained available during the temporary absence of males). These nests constructed by
344 females in mono-sex rearing never contained eggs. In mixed groups with a sex ratio of 1:3
345 (see § 2.2.3), the number of spawns collected was equivalent in the 52 compartments set up
346 for half of them with one or three nest supports (156 vs. 157 spawns, respectively). There was
347 no significant difference in the mean number of spawn per female per month (0.15 ± 0.01 vs.
348 0.15 ± 0.01), mean number of eggs collected per spawn ($3,835 \pm 204$ vs. $4,110 \pm 183$), or
349 mean egg quality ($88.5 \pm 1.3\%$ vs. $88.7 \pm 1.3\%$) in the compartments equipped with one or
350 three nest supports, respectively (see Supplementary Material for additional information).

351

352 **3.6. Broodfish sex ratio**

353 The effect of the male-to-female sex ratio (1:1, 1:2, 1:3, 1:5 or 1:7) on the reproductive
354 characteristics of giant gourami were evaluated over more than six consecutive months in
355 spawning compartments of 24 m² (see § 2.2.4). The broodfish sex ratio had no influence ($p \geq$
356 0.05) on the number of eggs collected per spawn (varying between $2,630 \pm 459$ and $3,515 \pm$
357 592 eggs spawn⁻¹ with sex ratios of 1:5 and 1:1, respectively), or on egg quality (varying
358 between $84.5 \pm 8.0\%$ and $93.0 \pm 1.9\%$ with sex ratios of 1:7 and 1:2, respectively; Fig. 6).
359 Similarly, despite a trend for a higher spawning activity with a sex ratio of 1:3, there was no
360 significant difference in the mean number of spawns collected per compartment as a function
361 of broodfish sex ratio (from 3.0 ± 0.6 to 4.7 ± 1.5 spawns compartment⁻¹ with sex ratios of 1:7
362 and 1:3, respectively; Fig. 6). In contrast, a progressive and significant decrease in the
363 spawning activity of each female was observed when the sex ratio was modified in favor of
364 females. The spawning frequency decreased progressively from 0.56 ± 0.14 spawn female⁻¹

365 month⁻¹ with a sex ratio of 1:1 to 0.07 ± 0.01 spawn female⁻¹ month⁻¹ with sex ratio of 1:7
366 (Fig. 6). The highest spawning frequency (i.e. 0.8 spawn month⁻¹ over 6 months), occurred in
367 a compartment stocked with a single pair of broodfish (sex ratio 1:1). Although the mating
368 frequency of males tended to be higher with a sex ratio of 1:3, the increase was slight in
369 comparison with the 1:1 sex ratio. In contrast, the reproductive activity of females was clearly
370 stimulated at the 1:1 sex ratio and impaired when the number of females was increased.

371

372 ***3.7. Living space in the spawning compartments***

373 The effects of living space available for the broodfish in the spawning compartments (surface
374 areas of 2, 4, 8 or 16 m²) on the reproductive characteristics of giant gourami were evaluated
375 over six consecutive months using a sex ratio of 1:1 (see § 2.2.5). Additional compartments of
376 16 m² with a sex ratio of 1:3 were used as a reference. The comparison of the total number of
377 spawns collected and the female spawning frequency between the 16 m² compartments with
378 sex ratios of 1:3 and 1:1 (Fig. 7) confirmed the trend observed in the experiment performed to
379 test the effect of sex ratio on fish reproductive activity (see § 3.6). No significant incidence of
380 living space was demonstrated on any of the reproductive characteristics under scrutiny
381 (spawning frequency, egg quantity or quality). Nevertheless, a progressive and marked trend
382 for reduced spawning frequency was observed when the broodfish living space was restricted
383 to 4 m² or 2 m². In contrast, reducing the living space of broodfish pairs from 16 m² to 8 m²
384 had no negative effects on fish spawning frequency or egg production (0.45 ± 0.11 vs. $0.40 \pm$
385 0.14 spawn female⁻¹ month⁻¹ and $8,788 \pm 2,438$ vs. $6,345 \pm 2,921$ eggs spawn⁻¹ in the 8 m² and
386 16 m² spawning compartments, respectively; Fig. 7). These results presented in Fig. 7, when
387 normalized to an equivalent surface of 16 m² spawning compartments, indicate that the egg
388 production over six months would be higher in two compartments of 8 m² (1:1 sex ratio,

389 about 17,500 eggs) than in a single 16 m² spawning compartment with 1 male and 3 females
390 (about 15,000 eggs).

391

392 ***3.8. Maintenance of the reproductive effort over time***

393 Altogether, results of the sex ratio and living space experiments confirmed that females have a
394 higher reproductive activity and egg production at a balanced sex ratio (1:1) than when the
395 sex ratio is biased in favor of females. Nevertheless, females associated in pairs with single
396 males showed no sign of exhaustion in their reproductive activity over a six-month period.
397 During this period, 2 to 6 spawns were collected per fish pair. No significant decrease in the
398 number of eggs per spawn or in spawning frequency (number of days between two successive
399 spawns) were observed with time in the successive reproduction events of the same fish pairs
400 (Fig. 8).

401

402 ***3.9. Gonad development, egg production, and spawning efficiency***

403 Overall, using data pooled from all experiments presented here above involving CPs (at sex
404 ratios of 1:3 or 1:1), no significant relationship was found between the number of eggs in a
405 spawn and female body mass (n = 352). The number of eggs collected in the nests varied
406 greatly, from about 100 to more than 14,000 (Fig. 9), despite the relatively narrow range of
407 body mass of female broodfish used in our experiments.

408 The four mature females that were dissected had a body mass of between 2.5 and 3.5 kg and a
409 GSI of between 3.7 and 4.6%. The analysis of egg-size frequency distributions in ovaries
410 showed oocytes at all stages of development, with an important group of oocytes at the end of
411 vitellogenesis (Fig. 10). These oocytes have an ovoid shape with a large diameter of 2.6 ± 0.2
412 mm and a small diameter of 2.2 ± 0.2 mm. The absolute fecundity (number of oocytes
413 belonging to the mode with the highest diameter) varied for these females from 7,800 eggs for

414 an individual of 2.5 kg to 15,172 eggs for an individual of 3.5 kg. These fecundities match the
415 number of eggs found in certain nests collected in spawning compartments stocked with
416 females of equivalent body mass (Fig. 9). The mean corresponding relative fecundity
417 estimated from the dissected females was $4,011 \pm 287$ eggs kg^{-1} ($n = 4$), a value about three
418 times greater than that estimated from eggs found in nests of compartments either with a sex
419 ratio of 1:1 ($1,410 \pm 101$, $n = 39$) or 1:3 ($1,360 \pm 112$, $n = 50$).

420 The testes of the five mature broodfish (body mass range 2.8-4.0 kg) that were dissected
421 contained intra-testicular sperm. These five individuals had a GSI comprised between 0.02 %
422 and 0.04 % underlining the oligospermic nature of males in the giant gourami.

423 After placing the broodfish in their respective spawning compartments, the first spawn
424 occurred after a similar lapse of time with sex ratios of 1:1 or 1:3 (60 ± 10 vs. 45 ± 6 d, $p >$
425 0.05 ; ranges of 10-130 and 4-167 d respectively). The mean lapse of time between two
426 successive spawns in the compartments stocked for reproductive periods of 6-7 months was of
427 46 ± 3 d when using a sex ratio of 1:3 (range: 2-194 d, $n = 124$) and remained similar with a
428 sex ratio of 1:1 with 42 ± 4 days (range: 20-97 d, $n = 23$). These extreme values indicate that,
429 in the presence of three females, a given male is able to fertilize two successive spawns within
430 an interval of 2 days and a given female with one male is able to produce a new spawn a
431 minimum of 20 days after the previous spawn.

432

433 **4. Discussion**

434 Although the life cycle of the giant gourami has been successfully completed in captivity for
435 nearly a century, knowledge on its reproduction remains limited. The rearing practices, most
436 often based on empirical knowledge, are currently unable to fully meet the demand for eggs
437 and fry of this species and thus limit the growth of its aquaculture. The present study, based

438 on the collection of more than 700 spawns, sheds new light on giant gourami reproductive
439 biology and egg production control under pond conditions.

440

441 ***4.1. Gonad development and evaluation of sexual maturity***

442 The dissection of fully mature broodfish shows that males of giant gourami are oligospermic
443 as indicated by particularly low GSI values ($< 0.1\%$). This characteristic is similar to that
444 reported in the euryhaline tilapia, *Sarotherodon melanotheron*, a mouthbrooding species in
445 which male GSI rarely exceeds 0.3% (Peters, 1963; Legendre et al., 2016). In both species,
446 sperm is released in the immediate vicinity of ova during mating, probably related to the fact
447 that low quantities of sperm suffice to fertilize the eggs. In *S. melanotheron*, the low number
448 of spermatozoa produced may be compensated by a particularly long duration of sperm
449 motility (>2 h; Legendre et al., 2016). To date, similar information on the duration of sperm
450 motility in the giant gourami is lacking. The difficulty of collecting sperm from mature giant
451 gourami males by abdominal massage (Slembrouck et al., 2019) is most likely related to the
452 oligospermic nature of the species. Although sperm release is clearly proof of sexual
453 readiness, the absence of sperm upon stripping is not necessarily related to sexual resting. For
454 example, some males in which sperm could not be stripped manually were able to reproduce
455 and fertilize ova efficiently in the spawning compartment as soon as five days after
456 examination. Therefore, evaluating the sexual maturity of males remains difficult. In this
457 study, the minimum delay between two successive mating events of a given male placed in a
458 pond compartment with several females was two days.

459 In females having reached full sexual maturity, GSI values of 4-5 % also indicated a relatively
460 modest development of the ovaries and correspond to values reported by Wijayanti et al.
461 (2009). The ovaries of mature giant gourami females contain oocytes at all developmental
462 stages, with a large unimodal group of post-vitellogenic oocytes (Fig. 10). From a histological

463 study, Wijayanti et al. (2009) also observed the simultaneous presence of oocytes at all stages
464 of vitellogenesis in giant gourami ovaries. This continuous recruitment of oocytes for
465 secondary growth is generally found in species with high oocyte growth (Ganias and
466 Lowerre-Barbieri, 2018). The oocyte size frequency distribution similar to that observed in
467 giant gourami is not rare in tropical freshwater species with an extended or continuous
468 reproductive period, e.g. the African catfishes *Clarias gariepinus* (Richter and Van den Hurk,
469 1982) and *Heterobranchus longifilis* (Legendre, 1992), and the clown loach *Chromobotia*
470 *macracanthus* (Legendre et al., 2012). In these latter species, oocyte maturation and ovulation
471 could be hormonally induced at intervals of 3 to 6 weeks without reduction in the number of
472 oocytes collected. This inter-spawning interval roughly corresponds to our observations,
473 whereby the shortest interval observed between two successive spontaneous spawns of a same
474 giant gourami female was 20 days. As in the three above-cited species, the unimodal oocyte
475 distribution found in mature giant gourami females (Fig. 10) strongly suggests that the
476 oocytes belonging to this advanced modal group (diameter from about 2 to 3 mm) are
477 ovulated and released, possibly in several successive batches, during the same spawning
478 event. Wijayanti et al. (2009) confirmed by histological analysis, the post-vitellogenic stage of
479 oocytes of diameter ≥ 2 mm in giant gourami females. Fish urogenital cannulation is a reliable
480 method for sexing giant gourami broodfish (Slembrouck et al., 2019). Intra-ovarian biopsy is
481 also a useful tool for evaluating the sexual maturity of female from the diameter of oocytes
482 sampled and gives a rough estimate, for a given female, of the delay that can be expected
483 before spawning (Fig. 3).

484

485 ***4.2. Fish fecundity and number of eggs collected from nests***

486 Estimated for the first time in the giant gourami by counting the number of post-vitellogenic
487 oocytes in dissected ovaries, the absolute fecundity varied between 7,800 and 15,172 eggs for

488 females (n = 4) weighing 2.5-3.5 kg. These values corresponded to a mean relative fecundity
489 of about 4,000 eggs per female kg. The number of eggs collected from the nests displayed
490 high variability, ranging from about 100 to 14,000. The highest number of eggs found in nests
491 (Fig. 9) clearly indicate that the full potential of giant gourami fecundity can be expressed in
492 some conditions. In giant gourami, Ellepola and Fernando (1966) also reported high
493 variability in egg number, with variation between 100 and 7,400 eggs in 159 nests collected
494 over five years in a hatchery in Ceylon. Overall, in our study, the mean number of eggs
495 collected from the nests was about two to three times lower than the potential fecundity of the
496 species. This large discrepancy may be attributed to (1) partial spawning of females and/or (2)
497 significant egg losses during spawning events in the pond. The first hypothesis would require
498 more detailed investigations. Nevertheless, partial spawning seems quite unlikely considering
499 the mono-modal distribution of intra-ovarian oocyte diameters and, based on our
500 observations, egg losses during spawning event is more likely. Due to the presence of a very
501 large lipid globule (about 40% of the total volume of the egg), giant gourami eggs are buoyant
502 (Baras et al., 2018). On two occasions during fish spawning, we observed large quantities of
503 eggs floating on the surface. Some were recovered in the fish parents' mouths, possibly to put
504 them back in the nest, as is the case in other gourami species (Hall and Miller, 1968; Kramer,
505 1973). Nevertheless, large amounts of eggs were either dispersed by wind and lost or predated
506 by other fish present in the ponds, such as Nile tilapia (*Oreochromis niloticus*), or bonylip
507 barb (*Osteochilus vittatus*). The fact that the eggs "miss" the nest and rise to the surface
508 during mating may be due to a poorly made nest or an inappropriate positioning of the
509 broodfish relative to the nest. However, in spawning compartments, this should not be due to
510 disturbance by other broodfish, because the variability in the number of eggs collected was
511 very similar at sex ratios of 1:1 and 1:3 (Fig. 9). Better knowledge of the breeding behavior of

512 the giant gourami would be useful to find ways of improving the shape and positioning of nest
513 supports to limit the loss of eggs during mating.

514

515 ***4.3. Nest construction and number of nest supports***

516 Despite an assertion still commonly found in various documents (e.g. Frimodt, 1995, cited by
517 www.fishbase.org), the giant gourami is not a bubble nester it builds immersed nests made of
518 plant fibers. As stated early by Bhimachar et al. (1944), this inconsistency originates from a
519 species misidentification. In most gourami species (e.g. *Trichogaster* spp.), the nest is
520 constructed by the male (Cole et al., 1999). In giant gourami, we observed that both sexes
521 participate in nest construction. This shared participation corroborates the observations of
522 Tanjung and Pilo (2015). When no males were in the spawning compartment, the nests could
523 be built by females alone. Nevertheless, providing more than one nest support in the spawning
524 compartments stocked with one male and three females did not lead to an increase in
525 broodfish spawning frequency. This suggests that the male has a leading role in nest
526 preparation before mating. Bhimachar et al. (1944) reported that giant gourami broodfishes
527 take about 8 to 10 days to build the nest. Our observations, based on examination of all nest
528 supports every two days, indicate that the nest can be built much more rapidly (2 to 5 days).

529

530 ***4.4. Pond management***

531 Overall, the number of eggs produced by the same number of broodfish, using a sex ratio of
532 1:3, was more than three times greater in CPs than in OPs. This resulted from both a lower
533 spawning frequency and a reduced number of eggs per spawn in OPs compared with CPs
534 (Table 1). The lower spawning frequency in OPs was very likely explained by social
535 interactions between large numbers of broodfish raised communally, especially the frequent
536 fights between males, which are prevented by separating males in different compartments.

537 The aggressive nature of giant gourami broodfish is well known (Woynarovitch and Horvath,
538 1980; Kristanto et al., 2019). The lower clutch size in OPs than in CPs may arise from, on the
539 one hand, a less efficient positioning of eggs in nests due to disturbance by other broodfish
540 during mating and, on the other hand, from higher predation by other fish species, free to
541 access eggs from the whole pond in OPs by the broodfish spawning. Similarly, the quality of
542 eggs was slightly, but significantly lower in OPs than in CPs, perhaps attributable to lower
543 fertilization success due to disturbance by other fish.

544

545 ***4.5. Length of reproductive period***

546 Our results showed that, in the second half of a 6-7-month egg production period, the time
547 lapse between two spawns tends to increase, thus potentially reducing egg production (Fig. 4
548 and 5). Therefore, over time, a certain "tiredness" or "lassitude" of the giant gourami
549 broodfish may justify frequent reconditioning of broodfish (e.g. every 3 months).
550 Nevertheless, we demonstrated on several successive breeding cycles that the global egg
551 production remained equivalent or even slightly higher when the egg production period was
552 continuous over 7 months instead of two successive 3-month periods separated by a 1 month
553 resting/reconditioning period. Indeed, in this latter treatment, no eggs were produced during
554 fish reconditioning. To avoid bias caused by potential seasonal variation in reproductive
555 activity, both durations of egg production periods (3 or 7 months) were run and compared
556 simultaneously in the CPs and phases were shifted (3 or 6 months) in two OPs.
557 Supplementary arguments in favor of the 6-7-month treatment are (1) reduced stress and
558 mortality of fish due to pond management and fish handling, (2) lower manpower, and (3) the
559 need, after such long time periods, to clean the pond of accumulated mud and filamentous
560 algae and eliminate colonizing fish species (i.e. potential predators on giant gourami eggs).
561 Therefore, egg production periods of 6-7 months represent the best compromise between the

562 constraints inherent to giant gourami reproduction and management of the spawning ponds.
563 The duration (1 month) and the conditions of the reconditioning periods (sexes separated;
564 specific broodfish feeding, mixing giant taro leaves and commercial pellets) as applied in the
565 present study are those generally used at the BPPSIGN Center and have not been tested here.
566 The reconditioning of broodfish proved efficient with regard to egg production results.
567 Nevertheless, resting period conditions would require more detailed investigations,
568 particularly with regard to broodfish feeding because information on this topic remains scarce
569 in the literature on giant gourami (Masrizal et al., 2015).

570

571 ***4.6. Broodfish sex ratio and size of spawning compartments***

572 Females associated in pairs with a male (1:1) in spawning compartments tended to spawn
573 more frequently than females maintained at a sex ratio of 1:3. This trend was observed in two
574 independent experiments. In contrast, the mating frequency of males was not substantially
575 increased when more than one female was present in the spawning compartment. In giant
576 gourami, the male is involved in nest construction, but is also strongly involved in guarding
577 the nest and its progeny after mating (Bhimachar et al., 1944; Woynarovitch and Horvath,
578 1980). In fish species with males strongly involved in parental care of eggs and fry, a 1:1 sex
579 ratio is generally recommended for egg production in captive conditions as for *S.*
580 *melanotheron*, a species in which oral incubation is done by the male (Legendre and Trébaol,
581 1996). In practice, farmers often believe that a sex ratio of 1:3 instead of 1:1 for egg
582 production in giant gourami may limit the risk of choosing immature females. However, we
583 demonstrated that urogenital cannulation is a very efficient technique to prevent this risk.
584 Moreover, in the present study, we also observed that the male may be the limiting factor in a
585 spawning compartment with three females. During no-spawn periods of several weeks,
586 changing the male often resulted in a spawning within a few days. Another concern for using

587 a 1:1 sex ratio is that the female may be sexually exhausted quite rapidly, which would indeed
588 impair egg production. However, using a 1:1 sex ratio, we demonstrated that, there was no
589 significant decrease in the number of eggs per spawn and spawning frequency in females
590 reproducing several times successively (up to 6 spawns over 6 months; Fig. 8).

591 Using pairs of broodfish, egg production was maintained in spawning compartments of 8 m²
592 and tended to be reduced in smaller ones. This is in agreement with the SNI (2000)
593 recommendations of using a stocking density of one giant gourami broodfish per 4-5 m². We
594 showed that egg production is greater when using two 8 m² spawning compartments, each
595 stocked with a pair of broodfish, than using one 16 m² compartment classically stocked with
596 one male and three females. This gap in egg production would be even greater when
597 considering three 8 m² compartments with a 1:1 sex ratio compared with one 24 m²
598 compartment with a 1:3 sex ratio, as often used in giant gourami farms.

599 In addition, the use of a balanced (1:1) sex ratio for egg production may lead to a
600 simplification of the production chain of future broodstock, because it would be no longer
601 necessary to eliminate males as it is the case with a sex ratio in favor of females. Furthermore,
602 the 1:1 sex ratio allows for accurate and individualized monitoring of each pair of broodfish
603 and would be favorable in the perspective of genetic selection program.

604

605 **5. Conclusion**

606 In this study we tested some aspects of the reproductive biology and egg production in the
607 giant gourami. Based on our findings, we recommend the following conditions to improve
608 egg production in this species:

- 609 • Maintenance of broodfish at a 1:1 sex ratio in pond spawning compartments of 8 m²
610 providing one nest support per compartment;

- 611 • Egg production periods of 6 to 7 months separated by a 1 month
612 resting/reconditioning period.

613 Altogether, the mean spawning frequency and mean number of eggs collected per nest can be
614 increased from about 0.09 spawn female⁻¹ month⁻¹ and 2,300 eggs spawn⁻¹ to > 0.40 spawn
615 female⁻¹ month⁻¹ and > 3,500 eggs spawn⁻¹, respectively, when changing from the traditional
616 communal open pond system with a 1:3 sex ratio to practices involving compartmentalized
617 ponds with a 1:1 sex ratio. Nevertheless, the mean number of eggs collected in nests remains
618 about three times lower than the potential fecundity of females, indicating that egg losses at
619 the time of spawning remains generally high. In the future, complementary investigations
620 should be carried out more particularly on the spawning behavior (nest preparation, fish
621 pairing, spawning act) of giant gourami to further improve broodfish management and design
622 of nest supports. Similarly, the conditions for broodfish reconditioning and feeding and their
623 incidence on gametogenesis and egg quality, not investigated here, remain important issues
624 requiring further research.

625

626 **Acknowledgements**

627 The authors acknowledge Maman Sudirman and the technicians of the West Java Center for
628 the Development of Giant Gourami Culture (BPPSIGN) for sharing their experience and for
629 their assistance throughout this study. This is a publication ISEM n° XXX-XXX.

630

631 **References**

632 Abramoff, M.D., Magalhaes, P.J., 2004. Image processing with imageJ. *Biophoton. Internat.*
633 11, 36-42.

634 Amornsakun, T., Kullai, S., Hassan, A., 2014. Some aspects in early life stage of giant
635 gourami, *Osphronemus goramy* (Lacepede) larvae. Songklanakar J. Sci. Technol.
636 36, 493-498.

637 Arifin, O.Z., Imron, I., Aseppendi, A., Hendri, A., Muslim, N., Yani, A., 2017. Hibridisasi
638 intraspesifik antar dua populasi ikan gurami Galunggung (*Osphronemus goramy*,
639 Lacepede, 1801). J. Ris. Akuakultur 12, 315-323 (In Indonesian).

640 Arifin, O.Z., Prakoso, V.A., Kristanto, A.H., Pouil, S., Slembrouck, J., 2019. Effect of
641 stocking density on growth, food intake and survival of giant gourami (*Osphronemus*
642 *goramy*, Lacepede) larvae. Aquaculture, 509, 159-166.

643 Baras, E., Arifin, O.Z., Slembrouck, J., Subagja, J., Kristanto, A.H., Legendre, M., 2018. Oil
644 globule size in fish eggs: a matter of biome and reproductive strategy. Fish Fish. 19,
645 996-1002.

646 Bhimachar, B.S., David, A., Muniappa, B., 1944. Observations on the acclimatization, nesting
647 habits and early development of *Osphronemus gorami* (Lacepede). Proc. Natl. Acad.
648 Sci. India Sect. B Biol. Sci. 20, 88-101.

649 Bhugel, R.C., 2000 A review of strategies for the management of Nile tilapia (*Oreochromis*
650 *niloticus*) broodfish in seed production systems, especially hapa-based systems.
651 Aquaculture 181, 37-59.

652 Bobe J., Labbé C., 2010. Egg and sperm quality in fish. Gen. Comp. Endocr. 165, 535-548.

653 Bromage, N., Porter, M., Randall, C., 2001. The environmental regulation of maturation in
654 farmed finfish with special reference to the role of photoperiod and melatonin.
655 Aquaculture 197, 63-98.

656 Cacot, P., Legendre, M., Dan, T.Q., Tung, L.T., Liem, P.T., Mariojous, C., Lazard, J., 2002.
657 Induced ovulation of *Pangasius bocourti* (Sauvage, 1880) with a progressive hCG
658 treatment. Aquaculture 213, 199-206.

659 Campos-Mendoza, A., McAndrew, B.J., Coward, K., Bromage, N., 2004. Reproductive
660 response of Nile tilapia (*Oreochromis niloticus*) to photoperiodic manipulation; effects
661 on spawning periodicity, fecundity and egg size. *Aquaculture* 231, 299-314.

662 Cole, B., Tamaru, C.S., Bailey, R., Brown, C., 1999. A manual for commercial production of
663 the gourami, *Trichogaster trichopterus*, a temporary paired spawner. Center for
664 Tropical and Subtropical Aquaculture, Hawaiï, USA.

665 Colt J., 2006. Water quality requirements for reuse systems. *Aquacult. Eng.* 34, 143-156.

666 Duponchelle F., Legendre M., 2001. Rapid phenotypic changes of reproductive traits in
667 response to modification of spatial structure in Nile tilapia, *Oreochromis niloticus*.
668 *Aquat. Living Resour.* 14, 145-152.

669 Ellepola, W.B., Fernando, C.H., 1966. Hatching and rearing of gourami, (*Osphronemus*
670 *goramy*, Lacepede) in the Polonnaruwa nursery. *Bulletin of the Fisheries Research*
671 *Station, Ceylon* 19, 5-9.

672 FAO, 2017. FishStatJ: Software for fishery statistical time series. Food and Agriculture
673 Organization, Rome, Italy.

674 Frimodt, C., 1995. Multilingual illustrated guide to the world's commercial warmwater fish.
675 (Fishing News Books), Wiley-Blackwell, Oxford, UK.

676 Ganas, K., Lowerre-Barbieri, S., 2018. Oocyte recruitment and fecundity type in fishes:
677 Refining terms to reflect underlying processes and drivers. *Fish Fish.* 19, 563-572.

678 Hall, D.D., Miller, R.J., 1968. A qualitative study of courtship and reproductive behavior in
679 the pearl gourami, *Trichogaster leeri* (Bleeker). *Behaviour* 32, 70–84.

680 Harvey, B., Carolsfeld, J., 1993, Induced breeding in tropical fish culture. International
681 Development Research Center, Ottawa, Canada.

682 Helfman, G.S., Collette, B.B., Facey, F.E., Bowen, B.W., 2009. The diversity of fishes:
683 Biology, evolution and ecology, 2nd edition. John Wiley & Sons Ltd, Chichester, UK.

684 Izquierdo, M.S., Fernández-Palacios, H., Tacon, A.G.J., 2001. Effect of broodstock nutrition
685 on reproductive performance of fish. *Aquaculture*, 197, 25-42.

686 Joseph, L.M.V., Ohs, C.L., DiMaggio, M.A., Broach, J.S., 2017. Effects of brood density and
687 spawning substrate type and orientation on the reproductive output of seminole
688 killifish in tanks. *North Am. J. Aquac.* 79, 267-274.

689 Kramer, D.L., 1973. Parental behaviour in the blue gourami *Trichogaster trichopterus*
690 (Pisces, Belontiidae) and its Induction during exposure to varying numbers of
691 conspecific eggs. *Behaviour* 47, 14-32.

692 Kristanto, A.H., Slembrouck, J., Subagja, J., Pouil, S., Arifin, O.Z., Prakoso, V.A., Legendre
693 M., 2019. Egg and fry production of giant gourami (*Osphronemus goramy*): Rearing
694 practices and recommendations for future research. *J. World Aquacult. Soc.*

695 Lazard, J., Legendre, M., 1996. The spontaneous reproduction of tilapia: An opportunity or a
696 handicap for the development of African aquaculture? In: Pullin, R.S.V., Lazard, J.,
697 Legendre, M., Amon Kothias, J.B., Pauly, D. (Eds), *The Third International*
698 *Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings, ICLARM,*
699 *Manila, Philippines, pp. 77-91.*

700 Legendre M., 1992. Potentialités aquacoles des Cichlidae (*Sarotherodon melanotheron*,
701 *Tilapia guineensis*) et Clariidae (*Heterobranchus longifilis*) autochtones des lagunes
702 de Côte d'Ivoire. Editions de l'ORSTOM, Paris, France (In French).

703 Legendre, M., Trébaol, L., 1996. Mouthbrooding efficiency and spawning frequency of
704 *Sarotherodon melanotheron* (Rüppel, 1852) in culture environments (Ebrié Lagoon,
705 Côte d'Ivoire). In: Pullin, R.S.V., Lazard, J., Legendre, M., Amon Kothias, J.B., Pauly,
706 D. (Eds), *The Third International Symposium on Tilapia in Aquaculture. ICLARM*
707 *Conference Proceedings, ICLARM, Manila, Philippines, pp. 339-348.*

708 Legendre, M., Linhart, O., Billard, R., 1996, Spawning and management of gametes, fertilized
709 eggs and embryos in Siluroidei. *Aquat. Living Resour.* 9, 59-80.

710 Legendre, M., Satyani, D., Subandiyah, S., Sudarto, Pouyaud, L., Baras, E., Slembrouck J.,
711 2012. Biology and culture of the clown loach *Chromobotia macracanthus*
712 (Cypriniformes, Cobitidae): 1 - Hormonal induced breeding, unusual latency response
713 and egg production in two populations from Sumatra and Borneo Islands. *Aquat.*
714 *Living Resour.* 25, 95-108.

715 Legendre M., Alavi S. M. H., Dzyuba B., Linhart O., Prokopchuk G., Cochet C., Dugué R.,
716 Cosson J., 2016. Adaptations of semen characteristics and sperm motility to harsh
717 salinity: Extreme situations encountered by the euryhaline tilapia *Sarotherodon*
718 *melanotheron heudelotii* (Dumeril, 1859). *Theriogenology*, 86, 1251-1267.

719 Masrizal, Udin, Z., Zein, M., Bulanin, U., 2015. Effects of energy, lipid and protein content in
720 broodstock diets on spawning fecundity and eggs quality of giant gourami
721 (*Osphronemus gouramy* Lac). *Pak. J. Nutr.* 14, 412-416.

722 Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herraez, M.P., Carrillo,
723 M., 2013. Gamete quality and broodstock management in temperate fish. *Rev.*
724 *Aquacult.* 5, S194–S223.

725 Mylonas, C.C., Fostier, A., Zanuy, S., 2010. Broodstock management and hormonal
726 manipulations of fish reproduction. *Gen. Comp. Endocrinol., Fish Reproduction* 165,
727 516–534.

728 Nuñez, J., Duponchelle, F., 2009. Towards a universal scale to assess sexual maturation and
729 related life history traits in oviparous teleost fishes. *Fish Physiol. Biochem.* 35,
730 167–180.

731 Peters, H.M., 1963. Eizal, Eigewicht und Geleheentwicklung in der Gattung Tilapia
732 (Cichlidae, Teleostei). *Int. Revue ges. Hydrobiol.* 48, 547-576 (In German)

733 Ramadhani, A.W., Samik, A., Madyawati, S.P., 2018. Vitellogenesis of giant gourami
734 (*Osphronemus goramy*) examined by the measurement of Estradiol-17 β , vitellogenin
735 concentration and the size of ovary. *Aquaculture Studies* 18, 113-125.

736 Richter, C.J.J., Van den Hurk, R., 1982. Effects of 1 l-desoxycorticosterone-acetate and carp
737 pituitary suspension on follicle maturation in the ovaries of the African catfish,
738 *Clarias lazera* (C. & V.). *Aquaculture* 29, 53-66.

739 Rimmer, M.A., Sugama, K., Rakhmawati, D., Rofiq, R., Habgood, R.H., 2013. A review and
740 SWOT analysis of aquaculture development in Indonesia. *Rev. Aquac.* 5, 255-279.

741 Salama, M.E., 1996. Effects of sex ratio and feed quality on mass production of Nile tilapia,
742 *Oreochromis niloticus* (L.), fry. *Aquacult. Res.*, 27: 581-585.

743 Slembrouck, J., Arifin, O.Z., Pouil, S., Subagja, J., Yani, A., Kristanto, A.H., Legendre, M.,
744 2019. Gender identification in farmed giant gourami (*Osphronemus goramy*): A
745 methodology for better broodstock management. *Aquaculture* 498, 388-395.

746 Slembrouck, J., Komarudin, O.M.A N., Maskur, L.M., 2004. Technical manual for the
747 artificial propagation of the Indonesian catfish, *Pangasius djambal*. IRD-BRKP, Paris,
748 Jakarta.

749 Siddiqui, A.Q., Al-Harbi, A.H., 1997. Effects of sex ratio, stocking density and age of hybrid
750 tilapia on seed production in concrete tanks in Saudi Arabia. *Aquac. Int.* 5, 207-216.

751 SNI, 2000. SNI 01-6485.3-2000: Produksi benih ikan gurame (*Osphronemus goramy*, Lac)
752 kelas benih sebar. Jakarta, Indonesia: Badan Standardisasi Nasional (BSN), Jakarta,
753 Indonesia (In Indonesian).

754 Tanjung, L.R., Pilo, J., 2015. Gurami padang dan teknik budi daya Jhonly Pilo. LIPI Press,
755 Jakarta, Indonesia (In Indonesian).

756 Teletchea, F., Fontaine, P., 2014. Levels of domestication in fish: Implications for the
757 sustainable future of aquaculture. *Fish Fish.* 15, 181-195.

- 758 Tsadik, G.G., Bart, A.N., 2007. Effects of feeding, stocking density and water-flow rate on
759 fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus*
760 (L.). *Aquaculture* 272, 380-388.
- 761 Tucker, C.S., Hargreaves, J.A., 2004. The biology and culture of channel catfish, 1st edition.
762 Elsevier, Amsterdam, Netherlands.
- 763 Wijayanti, G.E., Soeminto S., Simanjuntak, B.I., 2009. Reproductive hormone profiles and
764 gametogenesis in female giant gourami (*Osphronemus gouramy*). *Jurnal Akuakultur*
765 *Indonesia* 8, 77-89. (In Indonesian).
- 766 Woynarovich, E., Horvath, L., 1980. The artificial propagation of warm-water finfishes: A
767 manual for extension. Food and Agriculture Organization, Rome, Italy.

768 **Captions to figures**

769

770 **Figure 1.** Experimental ponds and devices used for spontaneous reproduction in giant
771 gourami: (a) open communal pond, (b) compartmentalized pond, (c) nest supports positioned
772 in compartments during preparation of ponds and (d) giant gourami (*Osphronemus goramy*)
773 eggs in a palm fiber nest upon collection. Nest supports (c) were baskets made of braided
774 bamboo strips (total length: 25 cm, including an opening of about 30 cm in diameter),
775 attached to bamboo stakes, and placed about 15 cm below the water surface with the opening
776 positioned slightly downward (angle of about 30 degrees). Plant fibers from palm trees
777 (*Arenga* sp.) were piled on a bamboo table positioned at the surface of the water (a and b), so
778 that the broodfish could easily grasp the fibers with their mouths and build their nest. Note the
779 presence (a and b) of giant taro (*Alocasia macrorrhizos*) planted on the banks of the ponds
780 and used to feed giant gourami broodfish.

781

782 **Figure 2.** Diagram of the protocol used to test the effects of the duration of the egg
783 production period (EPP) of giant gourami (*Osphronemus goramy*) in two compartmentalized
784 ponds (CP) and two open communal ponds (OP). In the compartmentalized ponds, an
785 intermediate broodfish resting/reconditioning period (IR) was applied in half of compartments
786 after 3 months of egg production, and a general reconditioning (GR) period, during which all
787 fish were removed from the pond and the pond cleaned, occurred every 7 months. In the open
788 communal ponds, general reconditioning of all fish in the ponds was carried out after each
789 reproduction period. Half of the compartments of each compartmentalized pond and the two
790 replicated communal ponds were phase-shifted, inverting long and short reproduction periods,
791 to avoid any possible bias that may result from seasonality.

792

793 **Figure 3.** Relationship between the initial stage of sexual maturity of the most advanced giant
794 gourami (*Osphronemus goramy*) female, as evaluated from intra-ovarian biopsies, and the
795 time from the moment of fish examination to the occurrence of the first spawn in a spawning
796 compartment ($n = 116$, $r^2 = 0.479$, $p < 0.001$).

797

798 **Figure 4.** Comparison of the time (mean \pm SE) between two successive spawns during the
799 first 3 months (“7-1”) and during the last 3 months (“7-2”) of a seven-month egg production
800 period in 24 m² spawning compartments using a sex ratio of 1:3. The spawning intervals
801 observed during simultaneous three-month egg production periods (“3-1” and “3-2”,
802 respectively) are also given for comparison. Data are means \pm SE. Different letters denote
803 significant differences ($p < 0.001$).

804

805 **Figure 5.** Relationship between the time separating two successive spawns (spawning
806 interval) and the time giant gourami (*Osphronemus goramy*) broodfish stayed in the 24 m²
807 spawning compartments after the beginning of the egg production period ($n = 122$, $r^2 = 0.232$,
808 $p < 0.0001$).

809

810 **Figure 6.** Number of spawns per compartment during the sex ratio experiment (195 days),
811 number of eggs in a spawn, proportion of clear (live) eggs, and spawning frequency (number
812 of spawns per female per month) in giant gourami (*Osphronemus goramy*) with respect to the
813 sex ratio of broodfish in compartmentalized ponds. In this experiment, the surface area of
814 every spawning compartment is 24 m². Data are means \pm SE. Letters denote significant
815 differences ($p < 0.01$).

816

817 **Figure 7.** Number of spawns per compartment during the living space experiment (179 days),
818 number of eggs in a spawn, proportion of clear (live) eggs and spawning frequency (number
819 of spawns per female per month) of giant gourami (*Osphronemus goramy*) with respect to the
820 surface area of the spawning compartment using a broodfish sex ratio of 1:1. The reference
821 situation corresponds to a spawning compartment of 16 m² using the usual sex ratio of 1:3.
822 Data are means ± SE. Letters denote significant differences ($p < 0.05$).

823

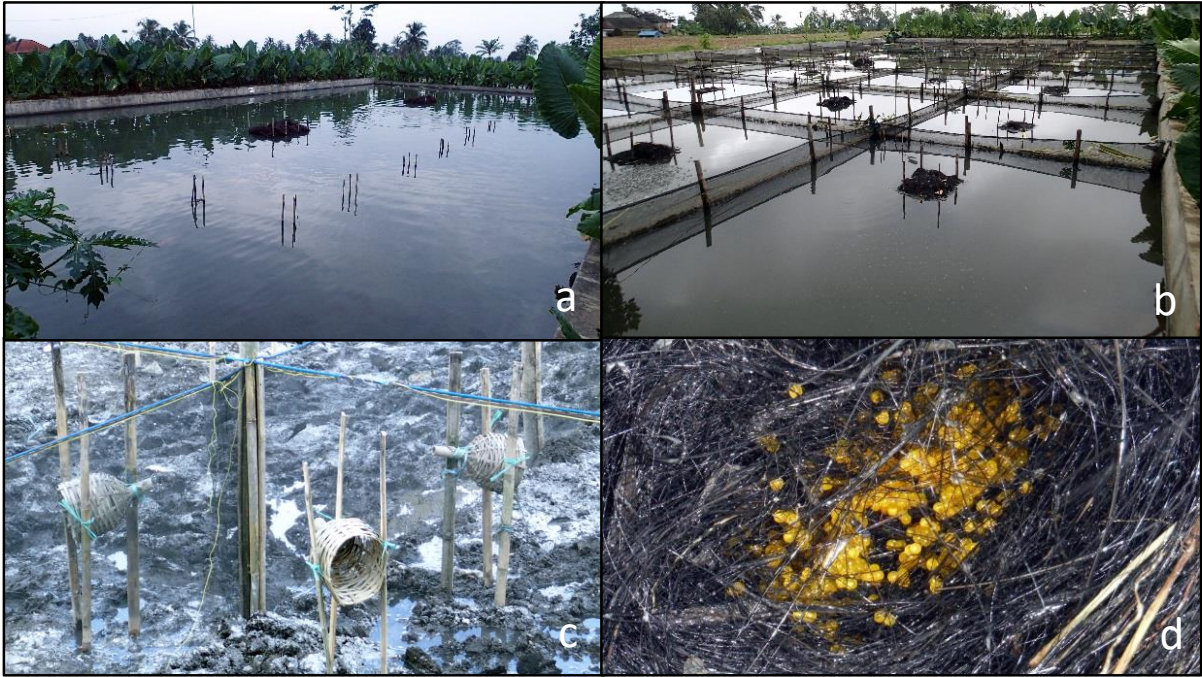
824 **Figure 8.** Number of eggs in spawns collected in nests (dark gray bars or circles) and number
825 of days since the previous spawn (light gray bars or circles) with respect to the temporal rank
826 of the spawn for a given female of giant gourami (*Osphronemus goramy*) maintained with a
827 given male in a spawning compartment (only compartments of surface area ≥ 8 m² are
828 considered here; duration of trial, from 179 to 195 days depending on spawning
829 compartment). In each category, the central horizontal line is the median (P50) of the data
830 range, the box is the interquartile (P25-P75) range, and whiskers encompass the entire data
831 range. The number of observations in each rank of successive spawns is given in parentheses.

832

833 **Figure 9.** Frequency distribution of the number of eggs found in nests collected in the
834 spawning compartments from all experiments in the study. The histogram refers to spawns (n
835 = 313) collected in spawning compartments with 1 male and 3 females. Number of eggs
836 collected in spawning compartments with a 1:1 sex ratio ($n = 39$) are superimposed for
837 comparison. Giant gourami (*Osphronemus goramy*) fecundity as estimated from direct
838 counting of post-vitellogenic oocytes after dissection of the ovaries from mature females ($n =$
839 4) is also indicated.

840

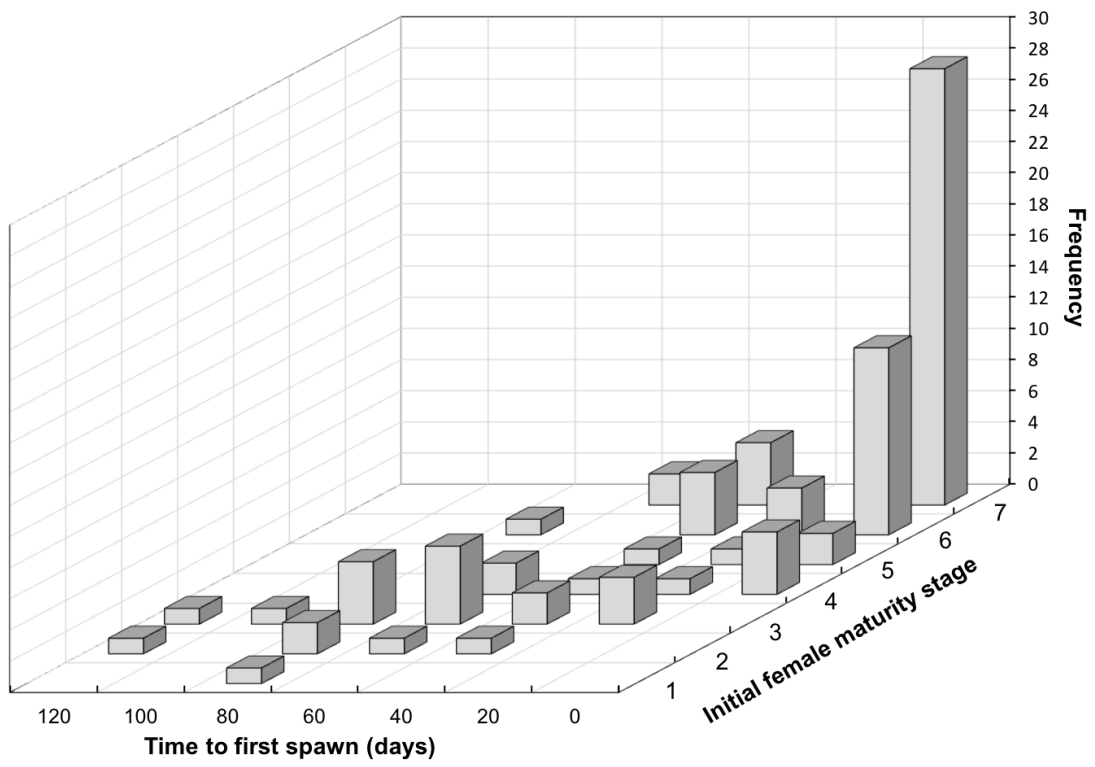
841 **Figure 10.** Typical size-frequency distribution of oocytes (n = 1059) from a mature female of
842 giant gourami (*Osphronemus goramy*) (GSI = 4.5%).



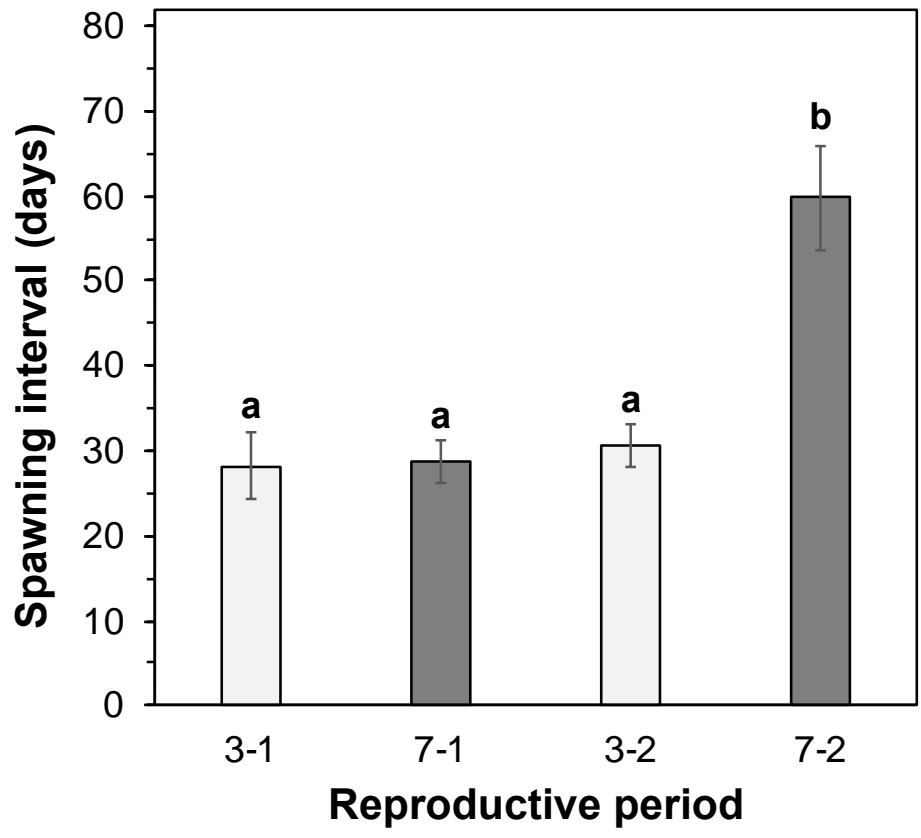
843 **Figure 1**

	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
CP (same protocol for the 2 ponds)	GR	EPP			IR	EPP			GR	EPP						GR	
		EPP								EPP			IR	EPP			
	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
OP (pond 1)	GR	EPP			GR	EPP			GR	EPP						GR	
OP (pond 2)	GR	EPP						GR	EPP			GR	EPP				GR

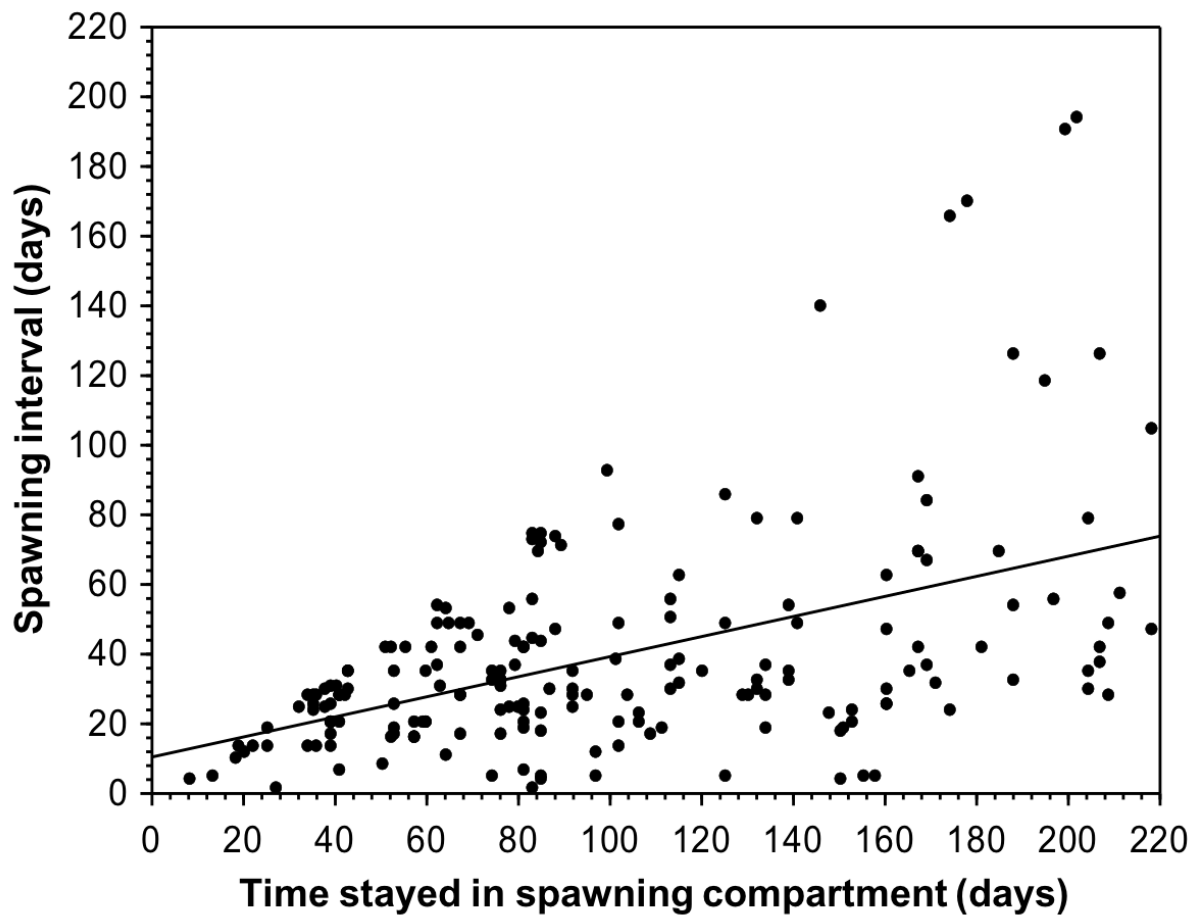
844 **Figure 2**



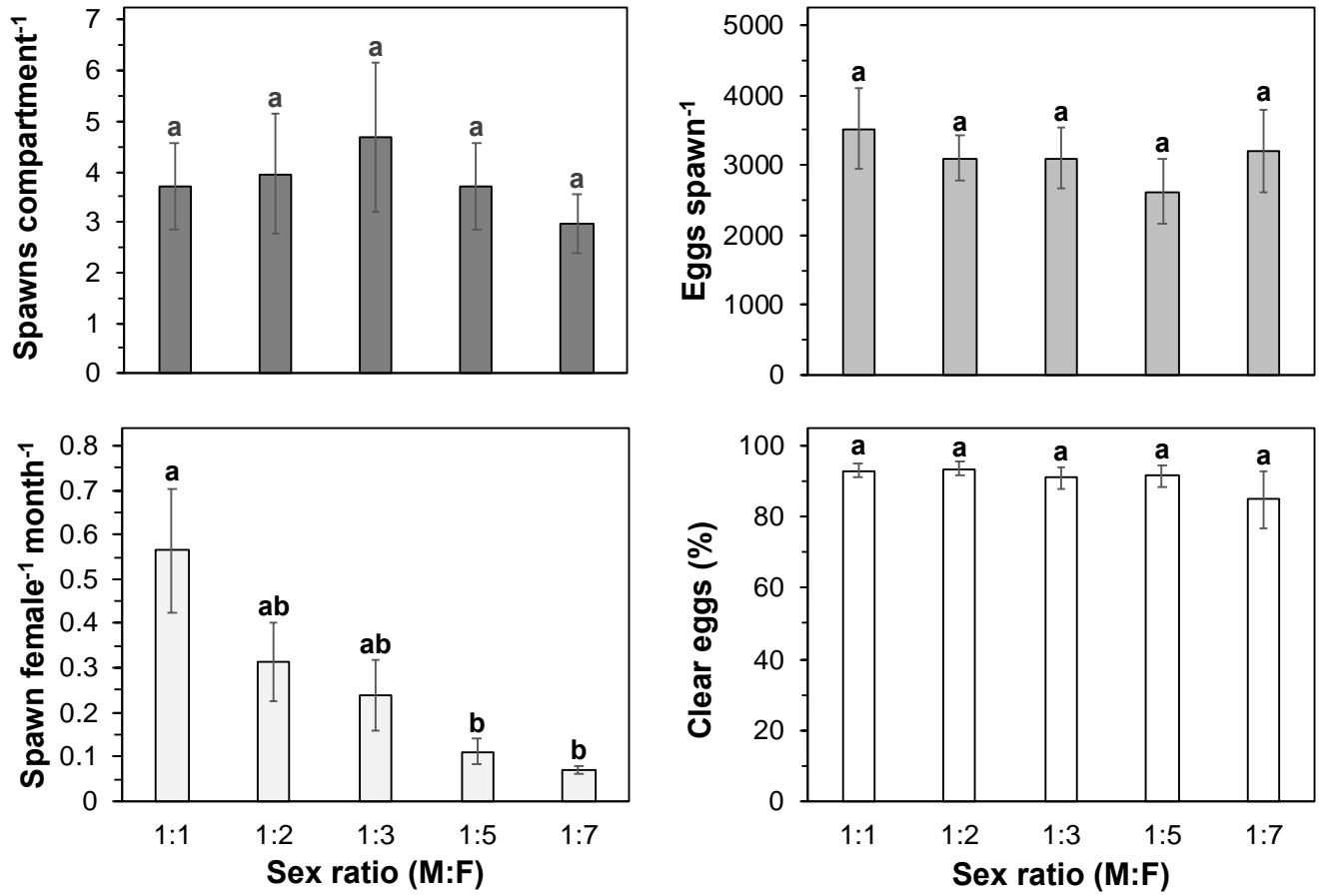
845 **Figure 3**



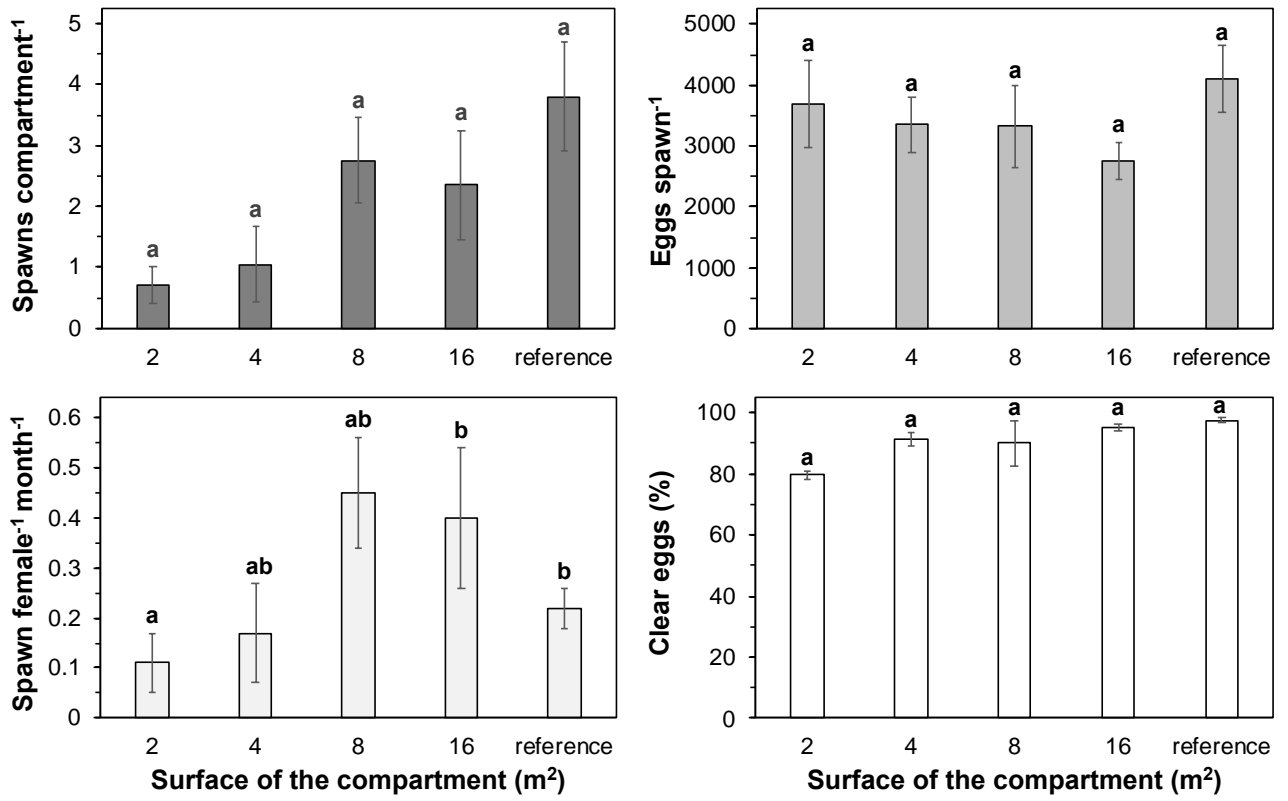
846 **Figure 4**



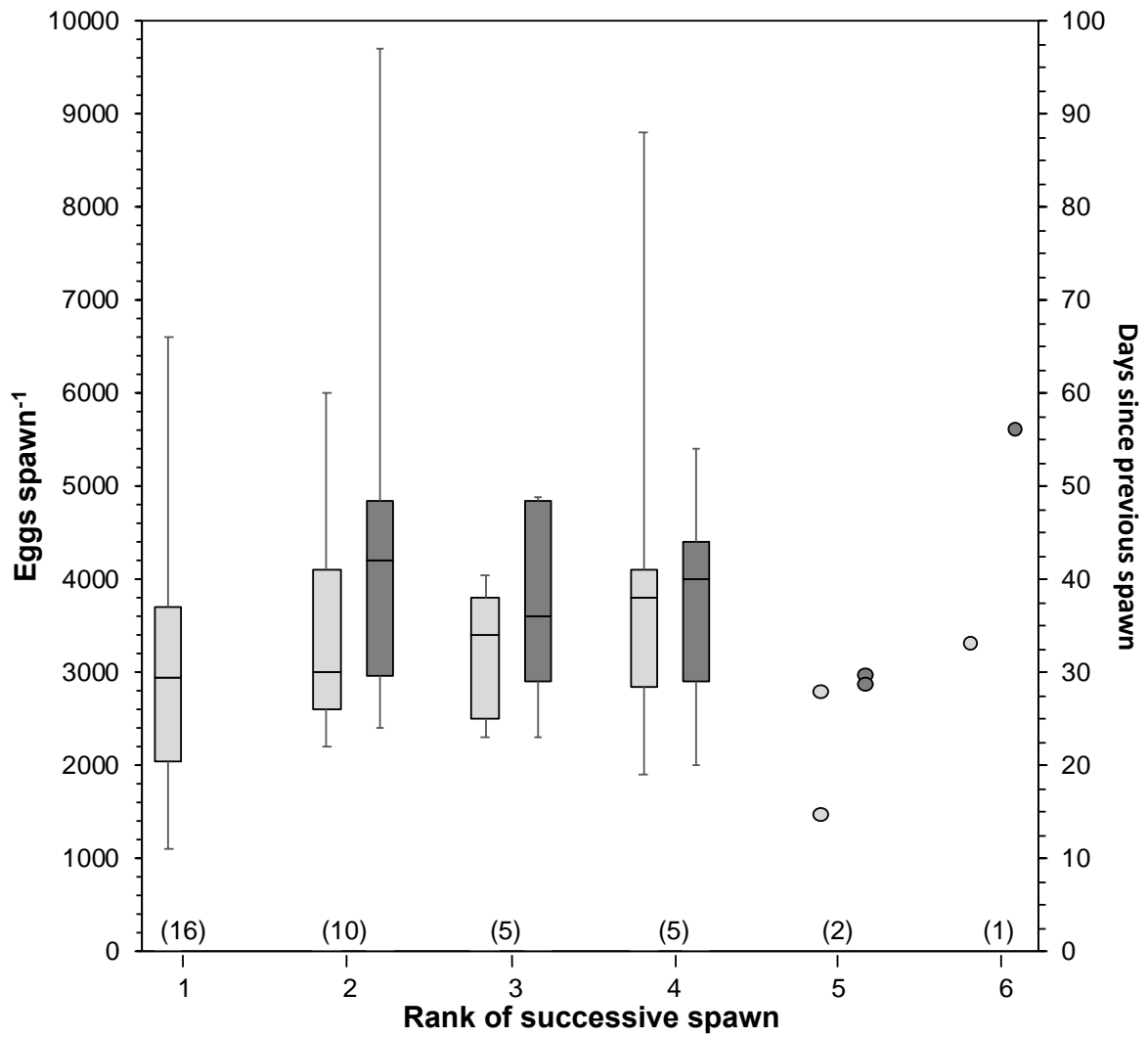
847 **Figure 5**



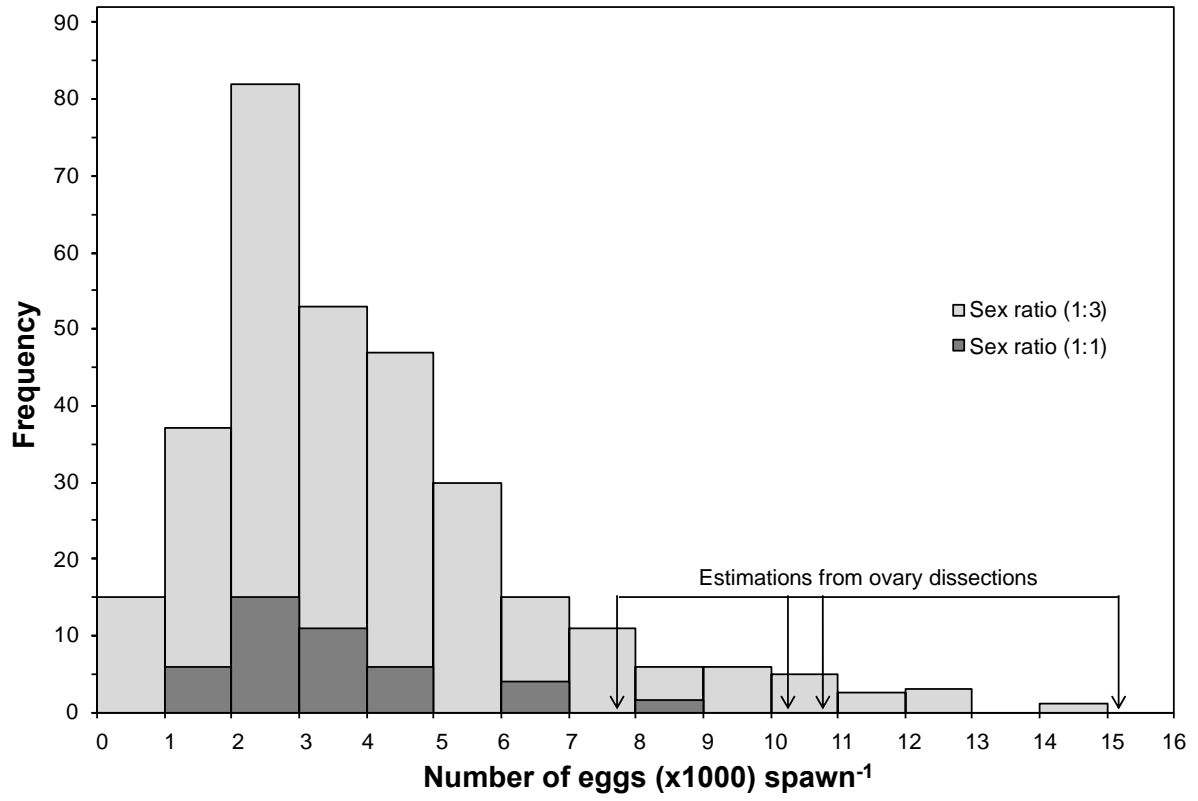
848 **Figure 6**



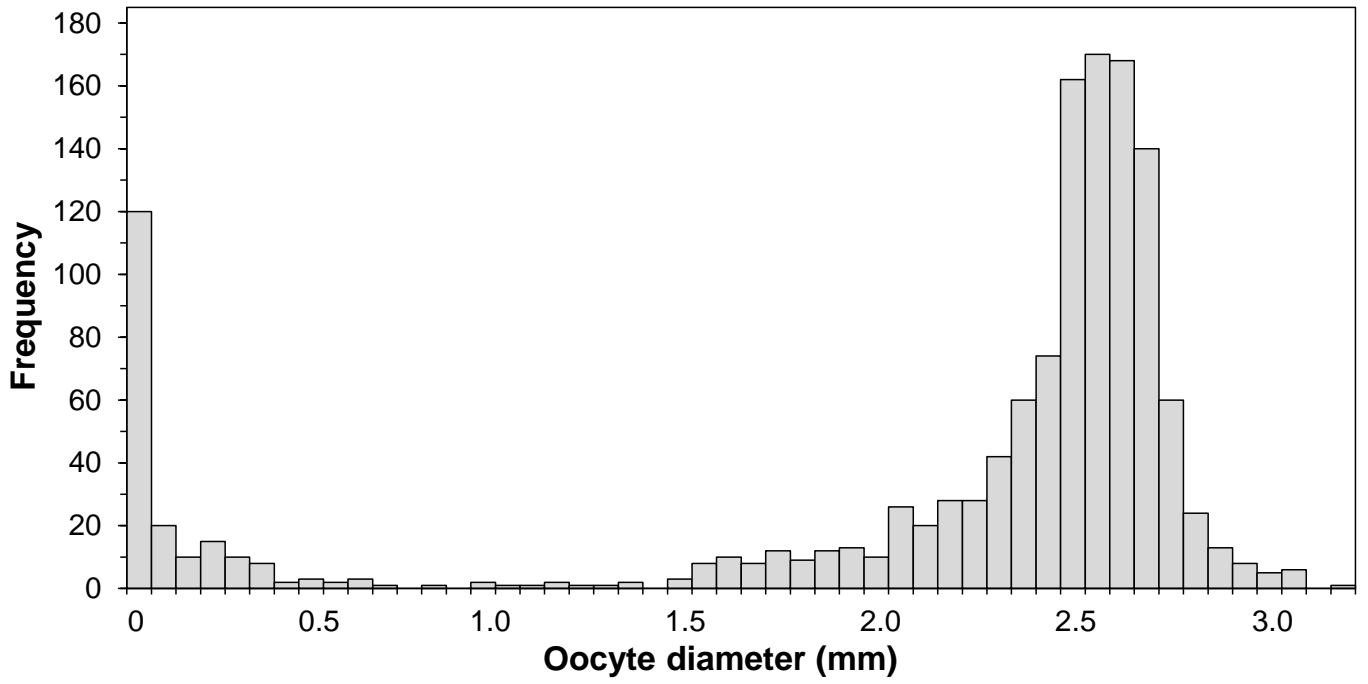
849 **Figure 7**



850 **Figure 8**



851 **Figure 9**



852 **Figure 10**

Table 1. Initial broodfish body mass (BM), spawning frequency, number of eggs per spawn and egg quality in the giant gourami (*Osphronemus goramy*) according to pond management: compartmentalized (CP, 20 m² spawning compartments) or open communal ponds (OP) using a sex ratio of 1 male for 3 females. Data are means \pm SE. The statistics of the tests (*F* or *H*), degrees of freedom (*df*) and the *p*-values are also given: $p \geq 0.05$ (NS), $p < 0.05$ (*) and $p < 0.001$ (***). Different letters denote significant differences between ponds ($p < 0.05$).

Variable	CP		OP		F or H	df	p
	Pond A	Pond B	Pond C	Pond D			
Sex ratio (M:F)	1:3	1:3	1:3	1:3	-	-	-
Number of females	72	84	84	72	-	-	-
Trial duration (days)	384	409	388	339	-	-	-
Female BM (g)	2,837 \pm 77 ^a	2,901 \pm 60 ^a	2,794 \pm 61 ^a	2,921 \pm 68 ^a	1.13	309	NS
Male BM (g)	3,954 \pm 138 ^{ab}	4,264 \pm 95 ^a	3,771 \pm 103 ^b	4,112 \pm 84 ^{ab}	4.69	101	*
Total number of spawns collected	147	166	72	79	-	-	-
Number of spawns female ⁻¹ month ⁻¹	0.16 \pm 0.02 ^a	0.16 \pm 0.02 ^a	0.07 \pm 0.01 ^b	0.11 \pm 0.02 ^b	14.75	56	***
Number of eggs spawn ⁻¹	4,179 \pm 187 ^a	3,791 \pm 176 ^a	2,389 \pm 258 ^b	2,371 \pm 255 ^b	* 62.67	469	***
Egg quality (% viable eggs)	87.7 \pm 1.4 ^{ab}	89.3 \pm 1.3 ^a	84.2 \pm 2.0 ^b	83.6 \pm 2.0 ^b	* 27.97	463	***
Number of viable eggs month ⁻¹ *	43,904	39,517	11,198	13,858	-	-	-

* Calculated as the number of spawns month⁻¹ x average number of eggs in the nests x the proportion of viable eggs.

Table 2. Initial broodfish body mass (BM, combining all egg production periods), spawning frequency, number of eggs per spawn and egg quality for giant gourami (*Osphronemus goramy*) with respect to the duration of the egg production period in compartmentalized ponds (1 male and 3 females per 24 m² spawning compartment) between two successive fish reconditioning events. Spawn number and egg production data are integrated and compared over four successive three-month reproductive periods vs. two successive seven-month reproductive periods. Data are means \pm SE. Extreme values of reproductive characteristics are given in parentheses. The statistics of the tests (*F*), degrees of freedom (*df*) and *p*-values are given: $p \geq 0.05$ (NS).

Variable	Duration of egg production period (month)		<i>F</i>	<i>df</i>	<i>p</i>
	3	7			
Number of reproductive periods	4	2	-	-	-
Total effective duration of trials (days)	362	431	-	-	-
Number of spawning compartments	25	26	-	-	-
Female BM (g)	2,981 \pm 33 ^a	2,972 \pm 46 ^a	0.03	469	NS
Male BM (g)	4,133 \pm 64 ^a	4,195 \pm 88 ^a	0.33	152	NS
Number of spawns compartment ⁻¹ over experiment	5.6 \pm 0.5 ^a (2-11)	6.7 \pm 0.5 ^a (2-13)	2.63	49	NS
Number of spawns female ⁻¹ month ⁻¹	0.15 \pm 0.01 ^a (0.05-0.29)	0.16 \pm 0.01 ^a (0.05-0.30)	0.00	49	NS
Number of eggs in a spawn	3,889 \pm 206 ^a (193-14,398)	4,040 \pm 184 ^a (109-12,803)	0.36	311	NS
Egg quality (clear eggs %)	87.8 \pm 1.4 ^a (12.6-99.8)	89.2 \pm 1.2 ^a (1.0-99.9)	0.54	308	NS
Number of viable eggs compartment ⁻¹ month ⁻¹ during egg production periods	1,794 \pm 157 ^a (716-3,414)	1,887 \pm 171 ^a (480-3,579)	0.16	49	NS

The following Supplementary Material accompanies the article

**New insights into giant gourami (*Osphronemus goramy*) reproductive
biology and egg production control**

Otong Zenal Arifin, Jacques Slembrouck*, Jojo Subagja, Simon Pouil, Akhmad Yani,
Asependi Asependi, Anang Hari Kristanto, Marc Legendre

*Corresponding author: Jacques.slembrouck@ird.fr

1. Nest construction and number of eggs

After the eggs had been separated, all of the palm fibers composing each nest were spread out in a thin layer, sundried for one day, then weighed (nearest 1 g) to obtain the weight of plant fibers used to build the nest.

We found that the number of eggs found in the nests was not proportional to the weight of nesting material. The quantity of palm fiber material used by fish to build the nests varied between 50 and 700 g. The mean weight of a palm fiber nest containing eggs was 346 ± 100 g ($n = 254$) and no significant effect of experimental conditions (pond management, number of nest supports, fish reconditioning) on nest construction were revealed. This situation contrasts with that observed in the blue gourami, *Trichogaster trichopterus*, in which a positive correlation between nest size and number of eggs and larvae in nest was reported (Degani, 1989).

In experiments carried out involving three nests supports per compartments and a 1:3 sex ratio, compartments found on the same day with nests containing eggs in two different nest supports were rarely observed (0.9 % of spawns collected). In these nests, the eggs were fertilized and of good quality (> 95 % of clear eggs), but relatively low in number (ranging

from 1,500 to 3,973). Therefore, taking into account that giant gourami fecundity may be much larger (up to about 14,000 eggs spawn⁻¹, see Section 3.9 of the article), it is uncertain if eggs found the same day in different nests from the same compartment were laid by one or several females.

2. Reference

Degani, G., 1989. The effect of temperature, light, fish size and container size on breeding of *Trichogaster trichopterus*. *Isr. J. Aquac.* 41, 67-73.