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1 **Seasonal variation of giant gourami (*Osphronemus goramy*) spawning activity and egg**
2 **production in aquaculture ponds**

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18 **Abstract**

19 The giant gourami *Osphronemus goramy* Lacépède, 1801, spontaneously spawns in captivity,
20 making nests for egg deposition when suitable nest supports and nesting material are made
21 available in rearing facilities. The seasonal variation in reproductive performance of the giant
22 gourami was characterized during a 20-month experiment in two compartmentalized ponds
23 (about 550 m² each) at a fry production center in West Java, Indonesia. A total of 208 giant
24 gourami broodfish were introduced in the two experimental ponds, with a sex-ratio of one male
25 to three females in each of the 52 spawning compartments delimited by vertical nets. Nesting
26 supports in each spawning compartment were examined every two days for the presence of
27 eggs resulting from natural reproduction. Three performance indicators were quantified from
28 the collected nests: spawning frequency (monthly number of spawns per female), effective
29 fecundity (number of eggs per spawn) and egg quality (estimated from the proportion of clear
30 living eggs in each spawn). The seasonality of giant gourami reproduction was analyzed as a
31 function of seasonal variation in rainfall, rearing water temperature and day length. Based on a
32 total of 413 collected spawns, the multiple linear regression models did not show any significant
33 relationship between giant gourami spawning frequency and the three environmental variables
34 studied. In contrast, the number of eggs per spawn was highly and positively related to rainfall
35 ($p < 0.0001$), moderately and negatively related to water temperature ($p < 0.001$), and weakly
36 and negatively related to day length ($p < 0.01$). The proportion of clear eggs was a positively
37 related to day length ($p < 0.01$). Overall, this study represents one of the first comprehensive
38 datasets on the reproductive phenology of giant gourami, and as such will be helpful in
39 formulating broodfish management strategies for the species.

40

41 **Keywords:** Reproduction control; Breeding cycle; Natural spawning; Freshwater aquaculture;
42 Indonesia

43 **1. Introduction**

44 Native to Southeast Asia, notably from Indonesia, the Malay Peninsula, Thailand and the
45 Mekong basin, the giant gourami *Osphronemus goramy* Lacépède, 1801, has been introduced
46 to several other countries for aquaculture purposes (Welcomme, 1988). This fish is omnivorous,
47 with a strong vegetarian tendency, and shows relative robustness and adaptability to
48 unfavorable environmental conditions (aerial respiration), which make it a very attractive
49 candidate for low-input aquaculture (Caruso et al., 2019). In Indonesia, the giant gourami is
50 one of the main freshwater commodities of economic importance owing to its high price and
51 local demand (Rimmer et al., 2013). Since 2000, giant gourami aquaculture production has
52 grown by a factor of 10 in Indonesia, the main country farming this species (145,000 tons in
53 2017; FAO, 2019). The Indonesian national production is ensured by approximately 100,000
54 small-scale fish farmers mainly located on Java Island (79%; BPS, 2013). Although the giant
55 gourami has been reared for decades and has now reached a significant production level, there
56 are still important gaps in our knowledge on several aspects of its biology.

57 The production of giant gourami fry relies on the natural spawning of captive broodfish held in
58 ponds, but the commercial-scale propagation of the species still needs to be standardized
59 (Amornsakun et al., 2014a; Arifin et al., 2019; Prakoso et al., 2019). The overall recruitment of
60 this multiple spawning species (i.e. number of fry produced per broodfish and per spawn) in
61 the breeding ponds remains generally low, resulting from variable spawning frequency or
62 success, and variable egg number and quality (Azrita, 2015). Therefore, ensuring a regular and
63 sufficient supply of fry through reliable production methods remains a major challenge for the
64 expansion of gourami aquaculture (Amornsakun et al., 2014a, b; Slembrouck et al., 2019).
65 Among the various biotic and abiotic factors influencing the reproductive performance of
66 broodfish, the effects of pond management strategies, sex-ratio and duration of egg production
67 periods were specifically investigated in a recent study (Arifin et al., 2020). Nevertheless, the

68 seasonality of reproduction has also been mentioned as an important limitation to a regular
69 supply of giant gourami fry (e.g. Arfah et al., 2006; Kristanto et al., 2019; Wijayanti et al.,
70 2009). In some instances, this seasonality has prompted studies to test hormonal treatments that
71 induce oocyte maturation and ovulation of giant gourami broodfish (Arfah et al., 2006).
72 Nevertheless, to our knowledge, an accurate description of the seasonal variation in
73 reproductive performance of captive giant gourami broodfish is still lacking in the scientific
74 literature. Such information would help to rationalize and optimize hatchery operations, as in
75 many intensively farmed finfish species (Migaud et al., 2013). Because there are considerable
76 interspecific variations in the patterns of reproductive seasonality (Wootton and Smith, 2014;
77 Paugy et al. 2017), such investigation would also be useful to further document the influence
78 of the environment on the reproductive phenology of freshwater tropical fishes for which, with
79 the exception of some families (mostly cichlids and clariids), availability of accurate datasets
80 remains limited.

81 The objective of the present study was to assess seasonal fluctuations in the reproduction of
82 giant gourami (spawning frequency, number of eggs in a spawn and egg quality), based on
83 nearly two years of follow-up in ponds at the Tasikmalaya Center for the Development of Giant
84 Gourami Culture (BPPSIGN), the largest giant gourami fry production center in the West Java
85 Province, Indonesia. The seasonality of giant gourami reproduction was analyzed in function
86 of seasonal variation in rainfall, rearing water temperature and day length, three environmental
87 parameters considered as major cues for the reproductive phenology of most tropical fishes
88 (Lowe-McConnell, 1979; Legendre and Jalabert, 1988; Paugy et al., 2017).

89 **2. Materials and Methods**

90 **2.1. Experimental facilities and pond water quality**

91 This study was carried out at the BPPSIGN Tasikmalaya Center (7°19'37.992"N,
92 108°6'101.155"E; altitude 489 m). The experiment was performed over a period of 20 months
93 from May 2016 to December 2017. Experimental structures were two earthen-bottom ponds of
94 527 and 563 m² in surface area with vertical concrete banks and an average water depth of 0.6
95 m. The two ponds were divided into spawning compartments of about 20 m² each (52
96 compartments in total), delimited with nets embedded in the sediment and held vertically on
97 bamboo poles. Ponds were supplied from a conduit carrying spring water from the Galunggung
98 Mountain located less than 10 km from the BPPSIGN Center.

99 Before starting the experiment, the spawning ponds were dried, cleaned, rid of all other species
100 and limed before being refilled. Pond water pH, dissolved oxygen, conductivity, and turbidity
101 were measured at quarterly intervals between 07:00 and 08:00 AM with direct measurements
102 using a multi-parameter probe (HI 9829 Hanna). Water concentration in ammonia (NH₃), nitrite
103 (NO₂⁻) and nitrate (NO₃⁻) was measured at the same time using spectrophotometry analysis
104 (Hanna HI83399). The average and range of variation of these parameters are provided in
105 Table 1 and indicate that the pond water quality remained stable during the experiment and
106 within appropriate standards for tropical freshwater fish (Colt, 2006).

107

108 **2.2 Fish maintenance and assessment of reproductive performance**

109 The mature broodfish (n = 208) used in this study were descendants of giant gourami belonging
110 to the "Galunggung" strain (Arifin et al., 2018). The females were 3-5 years old and weighed
111 ~3 kg, whereas males were 5-7 years old and weighed ~4 kg. Identification of fish sex and
112 assessment of their sexual maturity were done on the basis of morphological criteria, milt
113 emission following gentle abdominal massage or examination of oocytes obtained by

114 intraovarian biopsies as described by Slembrouck et al. (2019). One sexually mature male and
115 three sexually mature females were placed in each of the 52 spawning compartments of the two
116 experimental ponds.

117 The giant gourami is a nest builder; therefore, nest supports and nesting material were provided
118 in each compartment for spawning. Nest supports were baskets made of braided bamboo strips
119 (total length: 25 cm, including an opening of about 30 cm in diameter), attached to bamboo
120 stakes, and placed about 15 cm below the water surface with the opening positioned slightly
121 downward (angle of about 30°). Plant fibers from palm trees (*Arenga* sp.) were piled on a
122 bamboo table positioned at the surface of the water in the middle of every compartment, so that
123 the broodfish could easily grasp the fibers with their mouths and build their nests (see Arifin et
124 al., 2020, for picture of the nest supports).

125 Every two days, all nest supports were examined and closed nests containing eggs were
126 collected, transferred to the hatchery, then immersed in a water tank to release the eggs by
127 carefully opening the palm fiber material as detailed in Arifin et al. (2020). All the eggs from
128 each nest were manually counted, separating live (transparent/clear) eggs from dead
129 (opaque/white) eggs. During the experiment, reproductive performance was assessed by
130 monitoring spawning frequency (the number of spawns per female per month), the number of
131 eggs in a spawn (i.e., numbers of eggs in a nest) and egg quality (estimated by the proportion
132 of clear eggs in each spawn). In some instances, the eggs were newly hatched at the moment of
133 nest collection; however, this had no or little incidence on the evaluation of progeny number
134 and spawn quality because white (dead) eggs take more than one day to break up and newly
135 hatched larvae, unable to swim, remain inside the nest.

136 During the egg production periods, the broodfish were fed with leaves of elephant ear plants
137 (*Alocasia macrorrhizos*) and commercial floating pellets (32% proteins, 5% lipids) distributed
138 at a daily feeding rate of 2% and 1% of fish biomass, respectively. The egg production periods

139 lasted for 3 or 7 months depending on spawning compartments, two durations that lead to
140 equivalent results in terms of egg production over the long term (Arifin et al., 2020). Between
141 two successive egg production periods, the broodfish were reconditioned for 1 month
142 (separating males and females in different ponds and using reverse daily feeding rates of
143 elephant ear leaves and floating pellets). The broodfish fish were examined every three months
144 and weighed with a digital scale for adjustment of feeding rations.

145

146 **2.3. Monitoring of environmental conditions**

147 Throughout the 20-month experiment, fluctuations of three environmental parameters were
148 considered: rainfall, pond water temperature and day length. Pond water temperature was
149 monitored continuously using a data logger (Onset HOBO) placed in the center of the ponds at
150 20 cm depth. A rain gauge was installed in the immediate vicinity of the ponds to record the
151 daily rainfall and the information related to day length was collected from Time and Date
152 (2019).

153

154 **2.4. Data treatment and statistical analysis**

155 Prior to statistical analysis, fish reproduction and environmental data were compiled and, for
156 each pond and each variable, monthly means of the recorded values were calculated. Statistical
157 analyses were then performed on these average monthly values.

158 The non-significance of the variable “pond”, initially included in general linear models (GLM)
159 with quantitative environmental variables was confirmed. Then, stepwise multiple linear
160 regression models (MLR) were used to test for relationships between female spawning
161 frequency, egg numbers or clear egg proportion in spawns and environmental variables
162 (rainfall, water temperature and day length). The assumptions underlying the MLR were
163 checked by visual observation of the model residuals and by statistical analysis (Goldfeld-

164 Quandt test, Rainbow test and Shapiro-Wilk test). Variance inflation factors (VIF) were used
165 to estimate collinearity of the explanatory variables, with VIF values close to 1 indicating the
166 absence of strong multi-collinearity (Hair et al., 2010). Statistical analyses were performed
167 using R freeware version 3.5.2 using the packages “car”, “lmtest” and “stats” (R Development
168 Core Team, 2019).

169

170 **3. Results**

171 At the BPPSIGN Tasikmalaya Center, day length varied annually between 11 h 41 min and 12
172 h 33 min, and pond water temperature ranged from 22.6°C to 32.7°C. The cumulative monthly
173 rainfall ranged from 5 to 528 mm during the experiment, from May 2016 to December 2017.
174 Overall, the monthly mean of the pond water temperature showed limited variation (between
175 25.4°C and 28.0°C), whereas the monthly rainfall was much lower during the second than
176 during the first half of the experimental period (from 331 to 528 mm vs. 0.5 to 288 mm,
177 respectively; Figure 1A).

178 During this 20-month period, as determined from the 413 spawns collected in the two
179 experimental ponds (206 and 207 spawns per pond), the reproduction of giant gourami occurred
180 continuously. Because no effect of the duration of egg production periods (successive periods
181 of 3 or 7 months) was detected for any variable (spawning frequency, egg number and quality),
182 the data were pooled for analyses. The mean monthly spawning frequency of giant gourami
183 varied between 0.07 and 0.26 spawn female⁻¹ month⁻¹ without a clear seasonal rhythm in the
184 intensity of spawning activity (Figure 1B). The mean monthly number of eggs per spawn
185 collected from the nests varied between 2020 and 5920 eggs spawn⁻¹ and peaked (more than
186 4000 eggs spawn⁻¹) during the period between July and November 2016 (Figure 1B). The mean
187 monthly proportion of clear (live) eggs in the spawns varied between 79 and 96% (Figure 1B).

188 The MLR did not reveal any significant relationship between giant gourami spawning
189 frequency and any of the environmental variables (Table 2). Observed monthly variation in
190 spawning frequency had no clear seasonal rhythm; it was not related to simultaneous variations
191 in rainfall, water temperature or day length. In contrast, variation in the number of eggs
192 produced per spawn was clearly related to variation in environmental variables (multiple $R^2 =$
193 0.71 ; $p < 0.0001$). Egg number was highly and positively related to rainfall (t-value = 7.777 , p
194 < 0.0001), moderately and negatively related to water temperature (t-value = -4.387 , $p = 0.001$),
195 and weaker and negatively related to day length (t-value = -2.983 , $p < 0.01$) (Table 2). For
196 egg quality, as evaluated through the proportion of clear eggs, there was a positive but relatively
197 weak relationship with day length (t-value = 3.126 , $p < 0.01$), but not with rainfall or water
198 temperature.

199

200 **4. Discussion**

201 In the present study, giant gourami clearly reproduced all year round in the ponds at the
202 BPPSIGN Tasikmalaya Center. No clear seasonal rhythm was revealed in the spawning
203 frequency of broodfish, which was not related to concurrent variations in rainfall, water
204 temperature or day length. Although the spawning activity of the giant gourami is often
205 considered as continuous all year round (Bhimachar et al., 1944), seasonal variations in the
206 spawning frequency have been noted, depending on the region or authors (Arfah et al., 2006;
207 Bhimachar et al., 1944; Wijayanti et al., 2009), but relationships between these variations and
208 environmental variables were not documented. In a recent study performed in the West Java
209 Province, targeted questionnaires and interviews also highlighted that fish farmers from Bogor
210 districts report seasonal variation in the reproductive intensity of their giant gourami broodfish,
211 with rain events (possibly together with associated variation in water temperature) leading to a
212 reduction in spawning frequency (Kristanto et al., 2019). In the same study, farmers from the

213 Tasikmalaya district observed no clear seasonality in giant gourami spawning activity, which
214 the present investigation confirms. In contrast to spawning frequency, the number of eggs
215 collected from the nests were strongly related to environmental factors; it was positively related
216 to rainfall, intermediately and negatively related to water temperature and weakly, and
217 negatively related to day length. To our knowledge, such temporal variation in egg number and
218 their strong relationship with rainfall and water temperature has not been reported previously
219 in the giant gourami. However, seasonal variation in fecundity is known to occur in several
220 tropical species reproducing all year round. In the African catfish *Heterobranchus longifilis*,
221 maximal egg production is observed during the rainy season, whereas in the lagoon tilapias
222 *Sarotherodon melanotheron* and *Tilapia guineensis*, fecundity is highest during the hot, dry
223 season (Legendre, 1992). In *Oreochromis niloticus*, the peak of fecundity corresponds with
224 maximum resource availability and flooding imminence (Duponchelle et al., 2000). Tropical
225 fish species are generally very plastic and can allocate resources to reproduction according to
226 rainfall regimes (Chellappa et al., 2009). In the present study, the giant gourami egg quality
227 was positively, although weakly, related to day length. Prayogo et al. (2018) claimed that
228 experimentally controlled long photoperiods (18L-6D) have a positive effect on the expression
229 levels of vitellogenin genes. It is well known that fish vitellogenins are critical for providing an
230 adequate nutritional reserve for embryonic development and are also involved in other complex
231 functions that influence egg quality (Reading et al., 2018). Nevertheless, further studies are
232 needed to better understand the mechanisms underlying the positive influence of day length
233 observed here on giant gourami egg quality.

234 As a whole, the present study provides the first detailed long-term investigation on the seasonal
235 variation of the spawning performance of captive giant gourami. We found no clear spawning
236 rhythms in this species in the environmental conditions of the Tasikmalaya area. On the same
237 location and using identical rearing procedures, it was observed that a same female spawns in

238 average every 5 months and that spawning frequency was strongly influenced by broodfish sex-
239 ratio (Arifin et al, 2020). In Tasikmalaya ponds, fluctuations of environmental parameters were
240 limited (particularly temperature) and, as a matter of fact, remained during the whole study in
241 a range favorable to giant gourami gametogenesis and spawning. Nevertheless, in other areas,
242 several studies (*op. cit.*) reported the occurrence of seasonal variation in giant gourami
243 reproduction, probably as a consequence of a locally less stable environment. Further
244 investigation conducted in such area with the same methodology as in the present study would
245 provide complementary view on the environmental control of giant gourami reproductive
246 phenology. By contrast to spawning frequency, the number of eggs per spawn showed greater
247 sensitivity to environmental changes and was strongly related to variations of environmental
248 variables (particularly rainfall and, to a lesser extent, water temperature and day length). Egg
249 quality was also affected by day length. These findings should be taken into consideration when
250 establishing egg production plans in commercial hatcheries. For instance, egg production
251 periods of 6–7 months separated by resting/reconditioning periods of 1 month are
252 recommended in the management of giant gourami spawning ponds (Arifin et al., 2020). As far
253 as possible, it is therefore recommended for optimizing egg production to synchronize the
254 broodfish resting/reconditioning periods with the periods of the year during which rainfall and
255 broodfish fecundity are at their lowest.

256

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261

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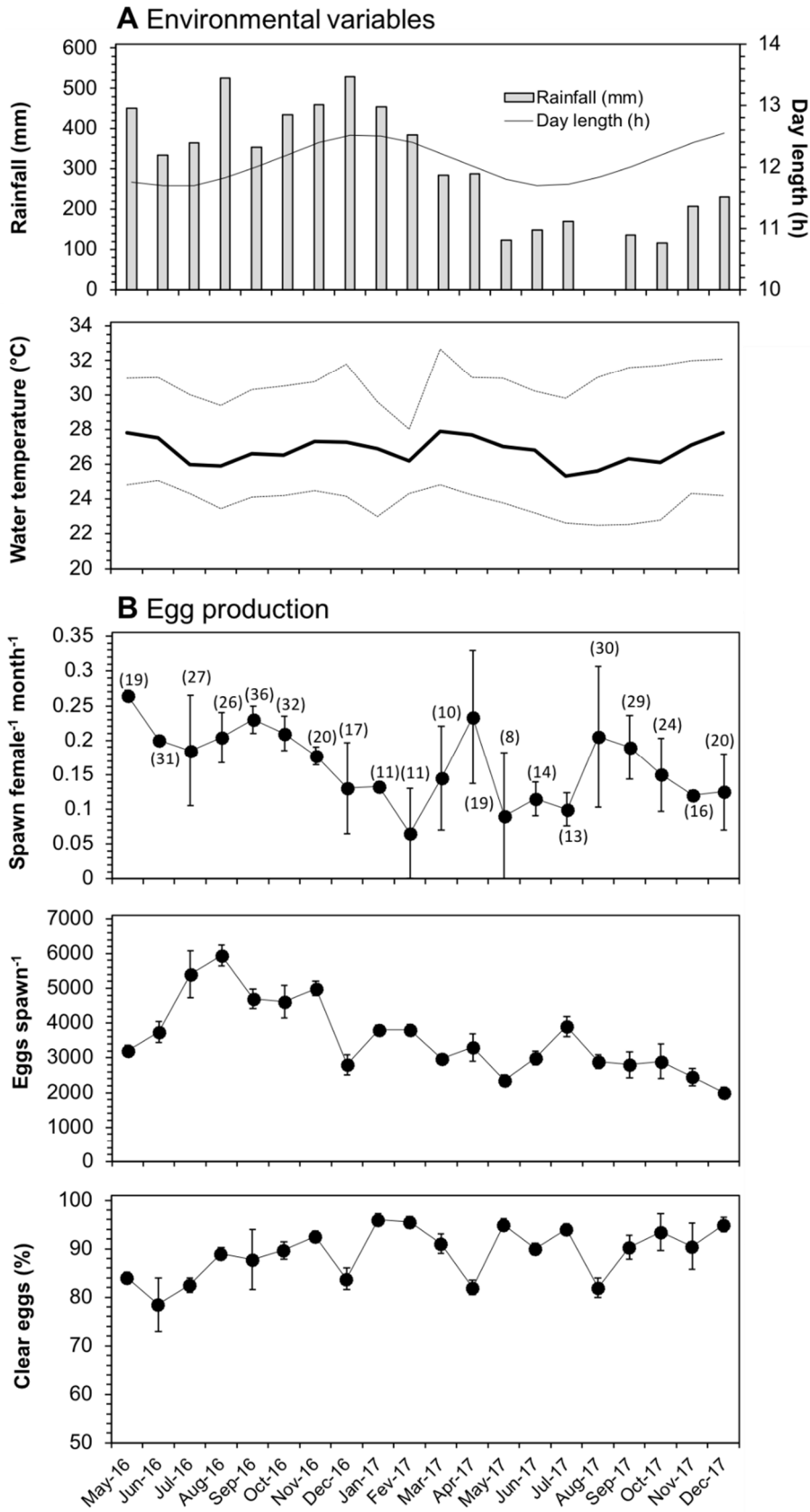
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352

353 **Captions to figure**

354

355 **Figure 1.** Mean monthly variation in environmental variables (A) and egg production
356 characteristics of giant gourami in ponds (B) at the BPPSIGN Tasikmalaya Center. A₁: rainfall
357 and day length, A₂: pond water temperature (mean ± extreme instantaneous temperature), B₁:
358 female spawning frequency, B₂: number of eggs in a spawn and B₃: egg quality according to
359 the proportion of clear eggs. In graphs B₁, B₂ and B₃, vertical bars indicate the range between
360 the means recorded in the two compartmentalized ponds. Due to the occurrence of periodic
361 reconditioning periods, the number of females actually involved in reproduction each month
362 was not constant and varied between 72 and 156 individuals (127 females per month on
363 average). The total number of spawns collected per month is given in parentheses (graph B₁).



364 **Figure 1**

365 **Table 1.** Summary of water quality parameters measured in the ponds during the 20-month
366 experiment.

Parameters ¹	Mean \pm SD (Range)
DO (mg L ⁻¹)	5.2 \pm 1.3 (3.8-7.3)
pH	7.6 \pm 0.2 (7.5-7.9)
Conductivity (μ S cm ⁻¹)	180 \pm 8 (171-188)
Turbidity (NTU)	9 \pm 3 (9-13)
NH ₃ (mg L ⁻¹)	<0.0065
NO ₂ ⁻ (mg L ⁻¹)	0.010 \pm 0.005 (0.006-0.020)
NO ₃ ⁻ (mg L ⁻¹)	0.41 \pm 0.12 (0.26-0.59)

367 ¹DO: dissolved oxygen

368

369 **Table 2.** Results of multiple linear regression models assessing the effects of environmental
 370 variables (i.e. daily rainfall, water temperature and day length) on the reproductive performance
 371 (i.e. female spawning frequency, egg number and egg quality) of captive giant gourami
 372 broodfish.

A - Spawning frequency				
Multiple R ² : 0.08	Estimate	SD	t-value	p-value
Intercept	0.7356	0.6163	1.194	0.241
Daily rainfall	0.0032	0.0027	1.183	0.245
Water temperature	0.0093	0.0180	0.520	0.606
Day length	-0.0707	0.0459	-1.541	0.132
B - Number of eggs per spawn				
Multiple R ² : 0.71	Estimate	SD	t-value	p-value
Intercept	34172.81	5151.12	6.634	< 0.0001
Daily rainfall	180.07	23.15	7.777	< 0.0001
Water temperature	-678.61	154.70	-4.387	< 0.001
Day length	-1172.40	393.08	-2.983	< 0.01
C - Proportion of clear eggs				
Multiple R ² : 0.24	Estimate	SD	t-value	p-value
Intercept	14.356	42.061	0.341	0.735
Daily rainfall	-0.217	0.189	-1.148	0.260
Water temperature	-1.678	1.263	-1.328	0.194
Day length	10.034	3.210	3.126	< 0.01

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