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

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Abstract

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

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

1. Introduction

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

Among abiotic factors, the effects of photoperiod, temperature, rainfall, and water salinity on reproduction efficiency has been particularly documented (Harvey and Carolsfeld, 1993; Bromage et al., 2001; Campos-Mendoza et al., 2004; Bobe and Labbé, 2010). However, other factors are also known to strongly influence the spawning or gamete quality in fishes, such as spawning substrate availability (Woynarovich and Horvath, 1980; Joseph et al., 2017), broodfish feeding (Izquierdo et al., 2001), size of rearing structures (Mylonas et al., 2010), or the spatial organization of the rearing environment (Duponchelle and Legendre, 2001). The biotic factors are related to the broodfish themselves and fish social interactions. They include the respective size of the broodfish, their stocking density or the sex ratio, and their influence has been documented in some of the main farmed species (Legendre and Trébaol, 1996; Salama, 1996; Siddiqui and Al-Harbi, 1997; Bhugel, 2000; Tsadik and Bart, 2007). However, the egg production of many spontaneous spawners still remains limited due to a lack of knowledge on the nature of the main factors influencing their reproduction in captivity and their optimal conditions.

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

The main objective of the present study was to experimentally assess the influence of different factors on giant gourami egg production. All the experiments presented in this study were carried out at the West Java Center for the Development of Giant Gourami Culture (BPPSIGN), the largest giant gourami fry production center in West Java Province. We experimentally tested different alternatives to the giant gourami production methods currently used at BPPSIGN and mainly based on national standards (SNI). Thus, the reproduction characteristics (egg-laying frequency, quantity and quality of the eggs produced) were compared using different pond management strategies (open or compartmentalized), and across different egg production periods, number of nest supports provided, sizes of the spawning compartments, and broodfish sex ratios.

2. Materials and methods

2.1. General information and procedures

2.1.1. Location and experimental site

This study was carried out at the BPPSIGN Tasikmalaya Center (7°19′37.992″N, 108°6′101.155″E, altitude 489 m), a regional center for fish farmer training and giant gourami fry production. A series of experiments on the spontaneous reproduction of giant gourami was performed over a period of 20 months from May 2016 to January 2018. Experimental structures were earthen flat bottom ponds with vertical concrete banks varying between 200 and 870 m^2 in surface area with an average water depth of 0.6 m. The ponds were supplied by spring water brought by a channel coming from the Galunggung Mountain located less than 10 km from the BPPSIGN Center. The ponds' water inflow varied from 3 to 12 m^3 h^{-1} depending on water available for irrigation in the local area.

2.1.2. Pond preparation and water quality monitoring

Before starting the experiments and between egg production periods, the spawning ponds were dried, cleaned, rid of all other species, and limed before being refilled. Pond water temperature was monitored continuously using data logger (Onset HOBO) placed at the center of the pond at 20 cm depth. Throughout the study, the instantaneous water temperature varied between 22.6 °C and 32.7 °C. Water pH, dissolved oxygen, conductivity, and turbidity were measured before each fish sampling between 07:00 and 08:00 AM with direct measurements using a multi-parameter probe (HI 9829 Hanna); the corresponding range of these parameters were 7.5-7.9, 3.8-7.3 mg L^{-1}, 171-188 µS cm^{-1} and 9-13 NTU, respectively. Water concentration in ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) was measured at quarterly intervals using spectrophotometry analysis (Hanna HI83399); corresponding value ranges were <0.0065 mg NH_3 L^{-1}, 0.006-0.020 mg NO_2^- L^{-1} and 0.26-0.59 mg NO_3^- L^{-1}. Although the minimum oxygen concentration was slightly low, < 4 mg L^{-1}, this did not affect the broodfish because giant gourami is capable of aerial respiration (Helfman et al., 2009). All other parameter values indicated that the pond water quality remained within appropriate standards for tropical freshwater fish (Colt, 2006).

2.1.3. Broodfish characteristics and evaluation of sexual maturity

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

At the beginning of each egg production period, all broodfish were individually weighed with a digital scale (nearest 10 g) and their sex was determined as presented in Slembrouck et al. (2019). The sexual maturity of females was assessed from oocytes sampled by intraovarian biopsies using a flexible polyethylene catheter ("*pipelle de Cornier*"; Cacot et al., 2002; Slembrouck et al., 2004). Oocyte diameter was measured at the edge of the ponds using graph paper. The largest oocyte diameter varied from less than 0.5 mm (beginning of vitellogenesis) to more than 2.5 mm (pre-spawning stage) depending on the individual. A simple maturity scale with seven stages based on 0.5 mm classes of the largest observed oocyte diameter was used to characterize the sexual stage of each female; stage 1 corresponded to cases in which no oocyte could be collected at biopsy, stage 2 corresponded to oocytes ≤ 0.5 mm, and stage 7 to oocytes > 2.5 mm. The sexual maturity of males was evaluated on the basis of the quantity of milt emission following gentle abdominal massage. However, in most cases, the volume of semen collected by stripping was small (a few drops), if any was emitted. After sampling and sexing, broodfish were arbitrarily placed in the spawning structures.

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

2.1.4. Spawning conditions

Two types of ponds were used: open communal ponds (OPs) or compartmentalized ponds (CPs). In OPs, the broodfish were stocked together and had free access to the entire pond (Fig. 1a). In CPs, compartments of 2 to 24 m^2 in surface were delimited with nets embedded in the sediment and held vertically on bamboo poles (Fig. 1b). Unless otherwise stated, the broodfish were stocked in the spawning ponds at a density of 1 fish for 4-5 m^2, using a male-to-female (M:F) ratio of 1:3, as recommended by SNI (2000). The giant gourami is a nest builder and nest support and nesting material were provided in the ponds and compartments for spawning (Fig 1).

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

2.1.5. Egg collection and estimation of reproductive efficiency

During the egg production periods, all nest supports were examined every two days and closed nests containing eggs were collected, transferred to the hatchery, then immersed in a water tank to release the eggs by carefully opening the palm fiber material (Fig. 1d). Due to the presence of a very large oil globule, giant gourami eggs float (Baras et al., 2018). Their manual separation from the nesting substrate is therefore very simple. All the eggs from each nest were manually counted, separating live (transparent/clear) eggs from dead (opaque/white) eggs. After counting, the clear eggs from each spawn were incubated in the hatchery (100 L glass aquarium; 4-5 eggs cm^{-2}) for fry production.

In every trial, the efficiency of reproduction was estimated using three parameters: (1) spawning frequency, defined as the number of spawns per female per month; (2) the total number of eggs in a spawn (i.e., all eggs found in a nest); and (3) egg quality, assessed by the proportion of clear eggs in each spawn. In some instances, the eggs were newly hatched at the moment of nest collection; however, this had no or little incidence on the evaluation of progeny number and spawn quality because white (dead) eggs take more than one day to break up and newly hatched larvae, unable to swim, remain inside the nest.

2.2. Experimental trials

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

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

2.2.2. Duration of egg production periods

The effects of the duration of egg production periods on the efficiency of giant gourami egg production was tested in the four ponds (2 CPs and 2 OPs) described in §2.1.1. The timing of egg production and resting/reconditioning periods in the different ponds is detailed in Figure 2. In each CP, egg production periods (EPP) were organized either as (1) two successive 3-month periods separated by an intermediate reconditioning (IR) period of 1 month or (2) one continuous 7-month period without IR. These two EPP durations were tested simultaneously in 50% of compartments of each CP, arbitrarily chosen. This experimental scheme was repeated a second time after a general reconditioning period (GR) of 1 month for all broodfish in the pond. The total duration of this study was therefore approximately 15 months (Fig. 2). During IR, males from half the compartments were caught and placed together in another pond, while the females remained in their respective compartments. In contrast, during GR, all broodfish were removed from the experimental ponds and males and females were separated and placed in different reconditioning ponds. Meanwhile, the experimental pond was dried, cleaned and limed before being refilled.

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

2.2.3. Number of nest supports

With a sex ratio of 1:3, it was uncertain if providing one nest support was sufficient. If females participate in building the nests, then providing more supports may increase the spawning frequency. To test this hypothesis, half of the 20 m^2 compartments of two ponds (n = 26) were arbitrarily chosen and equipped with one nest support each, whereas the other half (n = 26) was equipped with three nest supports each. The reproductive efficiency of giant gourami in these two treatments was monitored simultaneously during about 15 months. Complementary information regarding nest construction is available in the Supplementary Material.

2.2.4. Broodfish sex ratio

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

2.2.5. Broodfish living space in the spawning compartments

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

2.3. Statistical analysis

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

3. Results

3.1. Broodfish sexing, mortality, and overall egg production

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

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

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

3.3. Incidence of initial maturity of broodfish

In CPs (see § 2.2.1), the initial sexual maturity stage of the three females introduced into each compartment was determined from intra-ovarian biopsies. We found that the time necessary to obtain the first spawn in a compartment was highly dependent on the initial maturity of the females. Thus, the higher the initial stage of maturation of the most advanced female, the faster the first spawn occurred (n = 116, $r^2 = 0.479$, F = 88.37, $p < 0.001$). Nevertheless, there was high variability between compartments for the time elapsed between the broodfish stocking and the occurrence of the first spawning. Between 41 and 134 days (83 ± 16 d) were needed for the first spawn in females starting at a low maturity stage (stage 2), whereas only 4 to 67 days (19 ± 3 d) were needed for the most mature females (stage 7; Fig. 3). In contrast, no relationship was found between the initial maturity stages of males, as evaluated from observed milt upon gentle fish abdominal massage, and the time to the first spawn. About 80 % of males could not be stripped for their sperm at the moment of broodfish stocking and some of them were able to fertilize eggs as soon as 4 days after examination.

3.4. Duration of reproductive periods

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

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

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

3.5. Nest construction and number of nest supports

Our observations, based on examination of all nest supports every two days (§ 2.2.3), indicate that the nest can be built rapidly. The shortest intervals observed in our study between two consecutive nest collections in the same compartment were 2 days (2 observations), 4 days (n = 3) and 5 days (n = 7). Although giant gourami males were predominantly involved in the nest preparation and progeny guarding, females also participated in these activities. In pond compartments in which males were absent and contained three females during IR periods, a nest was constructed by females in 25% of cases (10 out of 40 compartments in which palm fibers remained available during the temporary absence of males). These nests constructed by females in mono-sex rearing never contained eggs. In mixed groups with a sex ratio of 1:3 (see § 2.2.3), the number of spawns collected was equivalent in the 52 compartments set up for half of them with one or three nest supports (156 vs. 157 spawns, respectively). There was no significant difference in the mean number of spawn per female per month (0.15 ± 0.01 vs. 0.15 ± 0.01), mean number of eggs collected per spawn (3,835 ± 204 vs. 4,110 ± 183), or mean egg quality (88.5 ± 1.3% vs. 88.7 ± 1.3%) in the compartments equipped with one or three nest supports, respectively (see Supplementary Material for additional information).

3.6. Broodfish sex ratio

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

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 (Fig. 6). The highest spawning frequency (i.e. 0.8 spawn month^{-1} over 6 months), occurred in a compartment stocked with a single pair of broodfish (sex ratio 1:1). Although the mating frequency of males tended to be higher with a sex ratio of 1:3, the increase was slight in comparison with the 1:1 sex ratio. In contrast, the reproductive activity of females was clearly stimulated at the 1:1 sex ratio and impaired when the number of females was increased.

3.7. Living space in the spawning compartments

The effects of living space available for the broodfish in the spawning compartments (surface areas of 2, 4, 8 or 16 m^2) on the reproductive characteristics of giant gourami were evaluated over six consecutive months using a sex ratio of 1:1 (see § 2.2.5). Additional compartments of 16 m^2 with a sex ratio of 1:3 were used as a reference. The comparison of the total number of spawns collected and the female spawning frequency between the 16 m^2 compartments with sex ratios of 1:3 and 1:1 (Fig. 7) confirmed the trend observed in the experiment performed to test the effect of sex ratio on fish reproductive activity (see § 3.6). No significant incidence of living space was demonstrated on any of the reproductive characteristics under scrutiny (spawning frequency, egg quantity or quality). Nevertheless, a progressive and marked trend for reduced spawning frequency was observed when the broodfish living space was restricted to 4 m^2 or 2 m^2. In contrast, reducing the living space of broodfish pairs from 16 m^2 to 8 m^2 had no negative effects on fish spawning frequency or egg production (0.45 ± 0.11 vs. 0.40 ± 0.14 spawn female^{-1} month^{-1} and 8,788 ± 2,438 vs. 6,345 ± 2,921 eggs spawn^{-1} in the 8 m^2 and 16 m^2 spawning compartments, respectively; Fig. 7). These results presented in Fig. 7, when normalized to an equivalent surface of 16 m^2 spawning compartments, indicate that the egg production over six months would be higher in two compartments of 8 m^2 (1:1 sex ratio, about 17,500 eggs) than in a single 16 m^2 spawning compartment with 1 male and 3 females (about 15,000 eggs).

3.8. Maintenance of the reproductive effort over time

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

3.9. Gonad development, egg production, and spawning efficiency

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

The four mature females that were dissected had a body mass of between 2.5 and 3.5 kg and a GSI of between 3.7 and 4.6%. The analysis of egg-size frequency distributions in ovaries showed oocytes at all stages of development, with an important group of oocytes at the end of vitellogenesis (Fig. 10). These oocytes have an ovoid shape with a large diameter of 2.6 ± 0.2 mm and a small diameter of 2.2 ± 0.2 mm. The absolute fecundity (number of oocytes belonging to the mode with the highest diameter) varied for these females from 7,800 eggs for an individual of 2.5 kg to 15,172 eggs for an individual of 3.5 kg. These fecundities match the number of eggs found in certain nests collected in spawning compartments stocked with females of equivalent body mass (Fig. 9). The mean corresponding relative fecundity estimated from the dissected females was 4,011 ± 287 eggs kg^{-1} (n = 4), a value about three times greater than that estimated from eggs found in nests of compartments either with a sex ratio of 1:1 (1,410 ± 101, n = 39) or 1:3 (1,360 ± 112, n = 50).

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

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

4. Discussion

Although the life cycle of the giant gourami has been successfully completed in captivity for nearly a century, knowledge on its reproduction remains limited. The rearing practices, most often based on empirical knowledge, are currently unable to fully meet the demand for eggs and fry of this species and thus limit the growth of its aquaculture. The present study, based on the collection of more than 700 spawns, sheds new light on giant gourami reproductive biology and egg production control under pond conditions.

4.1. Gonad development and evaluation of sexual maturity

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

In females having reached full sexual maturity, GSI values of 4-5 % also indicated a relatively modest development of the ovaries and correspond to values reported by Wijayanti et al. (2009). The ovaries of mature giant gourami females contain oocytes at all developmental stages, with a large unimodal group of post-vitellogenic oocytes (Fig. 10). From a histological study, Wijayanti et al. (2009) also observed the simultaneous presence of oocytes at all stages of vitellogenesis in giant gourami ovaries. This continuous recruitment of oocytes for secondary growth is generally found in species with high oocyte growth (Ganias and Lowerre-Barbieri, 2018). The oocyte size frequency distribution similar to that observed in giant gourami is not rare in tropical freshwater species with an extended or continuous reproductive period, e.g. the African catfishes *Clarias gariepinus* (Richter and Van den Hurk, 1982) and *Heterobranchus longifilis* (Legendre, 1992), and the clown loach *Chromobotia macracanthus* (Legendre et al., 2012). In these latter species, oocyte maturation and ovulation could be hormonally induced at intervals of 3 to 6 weeks without reduction in the number of oocytes collected. This inter-spawning interval roughly corresponds to our observations, whereby the shortest interval observed between two successive spontaneous spawns of a same giant gourami female was 20 days. As in the three above-cited species, the unimodal oocyte distribution found in mature giant gourami females (Fig. 10) strongly suggests that the oocytes belonging to this advanced modal group (diameter from about 2 to 3 mm) are ovulated and released, possibly in several successive batches, during the same spawning event. Wijayanti et al. (2009) confirmed by histological analysis, the post-vitellogenic stage of oocytes of diameter ≥ 2 mm in giant gourami females. Fish urogenital cannulation is a reliable method for sexing giant gourami broodfish (Slembrouck et al., 2019). Intra-ovarian biopsy is also a useful tool for evaluating the sexual maturity of female from the diameter of oocytes sampled and gives a rough estimate, for a given female, of the delay that can be expected before spawning (Fig. 3).

4.2. Fish fecundity and number of eggs collected from nests

Estimated for the first time in the giant gourami by counting the number of post-vitellogenic oocytes in dissected ovaries, the absolute fecundity varied between 7,800 and 15,172 eggs for females (n = 4) weighing 2.5-3.5 kg. These values corresponded to a mean relative fecundity of about 4,000 eggs per female kg. The number of eggs collected from the nests displayed high variability, ranging from about 100 to 14,000. The highest number of eggs found in nests (Fig. 9) clearly indicate that the full potential of giant gourami fecundity can be expressed in some conditions. In giant gourami, Ellepola and Fernando (1966) also reported high variability in egg number, with variation between 100 and 7,400 eggs in 159 nests collected over five years in a hatchery in Ceylon. Overall, in our study, the mean number of eggs collected from the nests was about two to three times lower than the potential fecundity of the species. This large discrepancy may be attributed to (1) partial spawning of females and/or (2) significant egg losses during spawning events in the pond. The first hypothesis would require more detailed investigations. Nevertheless, partial spawning seems quite unlikely considering the mono-modal distribution of intra-ovarian oocyte diameters and, based on our observations, egg losses during spawning event is more likely. Due to the presence of a very large lipid globule (about 40% of the total volume of the egg), giant gourami eggs are buoyant (Baras et al., 2018). On two occasions during fish spawning, we observed large quantities of eggs floating on the surface. Some were recovered in the fish parents' mouths, possibly to put them back in the nest, as is the case in other gourami species (Hall and Miller, 1968; Kramer, 1973). Nevertheless, large amounts of eggs were either dispersed by wind and lost or predated by other fish present in the ponds, such as Nile tilapia (*Oreochromis niloticus*), or bonylip barb (*Osteochilus vittatus*). The fact that the eggs "miss" the nest and rise to the surface during mating may be due to a poorly made nest or an inappropriate positioning of the broodfish relative to the nest. However, in spawning compartments, this should not be due to disturbance by other broodfish, because the variability in the number of eggs collected was very similar at sex ratios of 1:1 and 1:3 (Fig. 9). Better knowledge of the breeding behavior of the giant gourami would be useful to find ways of improving the shape and positioning of nest supports to limit the loss of eggs during mating.

4.3. Nest construction and number of nest supports

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

4.4. Pond management

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

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

4.5. Length of reproductive period

Our results showed that, in the second half of a 6-7-month egg production period, the time lapse between two spawns tends to increase, thus potentially reducing egg production (Fig. 4 and 5). Therefore, over time, a certain "tiredness" or "lassitude" of the giant gourami broodfish may justify frequent reconditioning of broodfish (e.g. every 3 months). Nevertheless, we demonstrated on several successive breeding cycles that the global egg production remained equivalent or even slightly higher when the egg production period was continuous over 7 months instead of two successive 3-month periods separated by a 1 month resting/reconditioning period. Indeed, in this latter treatment, no eggs were produced during fish reconditioning. To avoid bias caused by potential seasonal variation in reproductive activity, both durations of egg production periods (3 or 7 months) were run and compared simultaneously in the CPs and phases were shifted (3 or 6 months) in two OPs. Supplementary arguments in favor of the 6-7-month treatment are (1) reduced stress and mortality of fish due to pond management and fish handling, (2) lower manpower, and (3) the need, after such long time periods, to clean the pond of accumulated mud and filamentous algae and eliminate colonizing fish species (i.e. potential predators on giant gourami eggs). Therefore, egg production periods of 6-7 months represent the best compromise between the constraints inherent to giant gourami reproduction and management of the spawning ponds. The duration (1 month) and the conditions of the reconditioning periods (sexes separated; specific broodfish feeding, mixing giant taro leaves and commercial pellets) as applied in the present study are those generally used at the BPPSIGN Center and have not been tested here. The reconditioning of broodfish proved efficient with regard to egg production results. Nevertheless, resting period conditions would require more detailed investigations, particularly with regard to broodfish feeding because information on this topic remains scarce in the literature on giant gourami (Masrizal et al., 2015).

4.6. Broodfish sex ratio and size of spawning compartments

Females associated in pairs with a male (1:1) in spawning compartments tended to spawn more frequently than females maintained at a sex ratio of 1:3. This trend was observed in two independent experiments. In contrast, the mating frequency of males was not substantially increased when more than one female was present in the spawning compartment. In giant gourami, the male is involved in nest construction, but is also strongly involved in guarding the nest and its progeny after mating (Bhimachar et al., 1944; Woynarovitch and Horvath, 1980). In fish species with males strongly involved in parental care of eggs and fry, a 1:1 sex ratio is generally recommended for egg production in captive conditions as for *S. melanotheron*, a species in which oral incubation is done by the male (Legendre and Trébaol, 1996). In practice, farmers often believe that a sex ratio of 1:3 instead of 1:1 for egg production in giant gourami may limit the risk of choosing immature females. However, we demonstrated that urogenital cannulation is a very efficient technique to prevent this risk. Moreover, in the present study, we also observed that the male may be the limiting factor in a spawning compartment with three females. During no-spawn periods of several weeks, changing the male often resulted in a spawning within a few days. Another concern for using a 1:1 sex ratio is that the female may be sexually exhausted quite rapidly, which would indeed impair egg production. However, using a 1:1 sex ratio, we demonstrated that, there was no significant decrease in the number of eggs per spawn and spawning frequency in females reproducing several times successively (up to 6 spawns over 6 months; Fig. 8).

Using pairs of broodfish, egg production was maintained in spawning compartments of 8 m^2 and tended to be reduced in smaller ones. This is in agreement with the SNI (2000) recommendations of using a stocking density of one giant gourami broodfish per 4-5 m^2. We showed that egg production is greater when using two 8 m^2 spawning compartments, each stocked with a pair of broodfish, than using one 16 m^2 compartment classically stocked with one male and three females. This gap in egg production would be even greater when considering three 8 m^2 compartments with a 1:1 sex ratio compared with one 24 m^2 compartment with a 1:3 sex ratio, as often used in giant gourami farms.

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

5. Conclusion

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

- Maintenance of broodfish at a 1:1 sex ratio in pond spawning compartments of 8 m^2 providing one nest support per compartment;

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

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

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Captions to figures

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

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

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

Figure 4. Comparison of the time (mean ± SE) between two successive spawns during the first 3 months ("7-1") and during the last 3 months ("7-2") of a seven-month egg production period in 24 m^2 spawning compartments using a sex ratio of 1:3. The spawning intervals observed during simultaneous three-month egg production periods ("3-1" and "3-2", respectively) are also given for comparison. Data are means ± SE. Different letters denote significant differences ($p < 0.001$).

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

Figure 6. Number of spawns per compartment during the sex ratio experiment (195 days), number of eggs in a spawn, proportion of clear (live) eggs, and spawning frequency (number of spawns per female per month) in giant gourami (*Osphronemus goramy*) with respect to the sex ratio of broodfish in compartmentalized ponds. In this experiment, the surface area of every spawning compartment is 24 m^2. Data are means ± SE. Letters denote significant differences ($p < 0.01$).

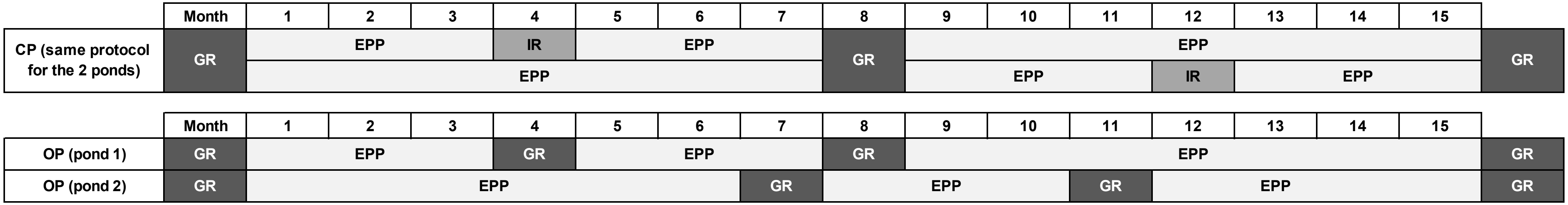
Figure 7. Number of spawns per compartment during the living space experiment (179 days), number of eggs in a spawn, proportion of clear (live) eggs and spawning frequency (number of spawns per female per month) of giant gourami (*Osphronemus goramy*) with respect to the surface area of the spawning compartment using a broodfish sex ratio of 1:1. The reference situation corresponds to a spawning compartment of 16 m^2 using the usual sex ratio of 1:3. Data are means ± SE. Letters denote significant differences ($p < 0.05$).

Figure 8. Number of eggs in spawns collected in nests (dark gray bars or circles) and number of days since the previous spawn (light gray bars or circles) with respect to the temporal rank of the spawn for a given female of giant gourami (*Osphronemus goramy*) maintained with a given male in a spawning compartment (only compartments of surface area ≥ 8 m^2 are considered here; duration of trial, from 179 to 195 days depending on spawning compartment). In each category, the central horizontal line is the median (P50) of the data range, the box is the interquartile (P25-P75) range, and whiskers encompass the entire data range. The number of observations in each rank of successive spawns is given in parentheses.

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

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

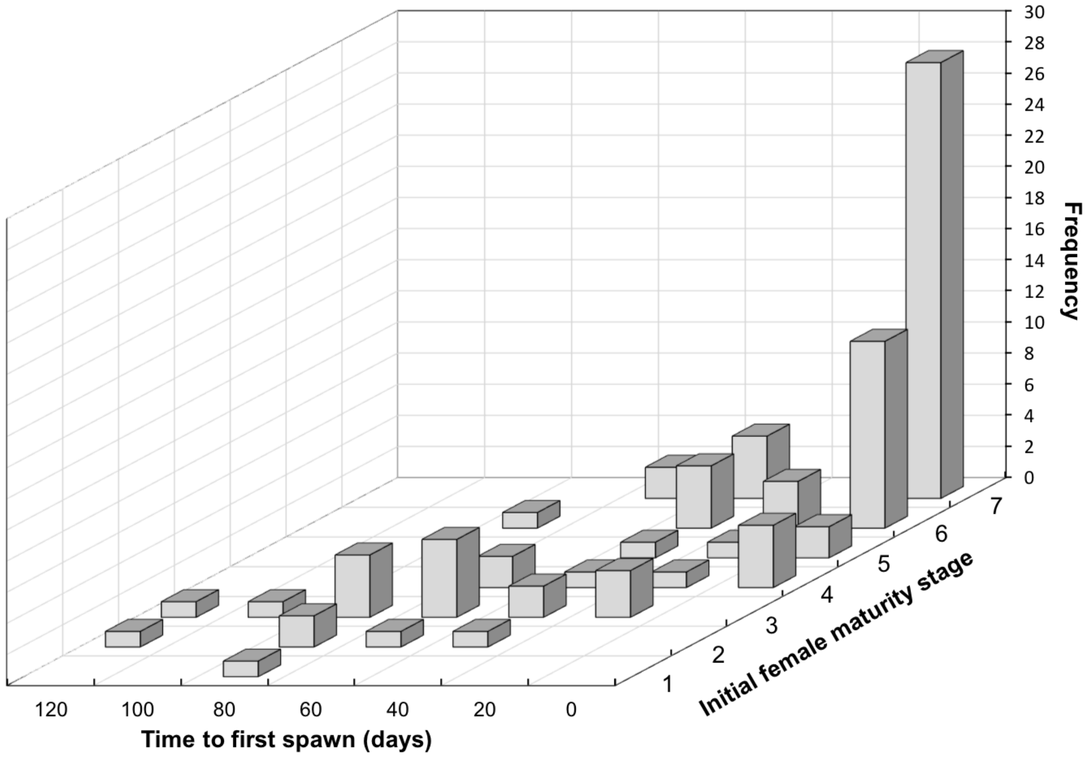
Figure 1



<table>
<tr><td></td><td>Month</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td><td>13</td><td>14</td><td>15</td><td></td></tr>
<tr><td rowspan="2">CP (same protocol for the 2 ponds)</td><td rowspan="2">GR</td><td colspan="3">EPP</td><td>IR</td><td colspan="3">EPP</td><td rowspan="2">GR</td><td colspan="7">EPP</td><td rowspan="2">GR</td></tr>
<tr><td colspan="7">EPP</td><td colspan="3">EPP</td><td>IR</td><td colspan="3">EPP</td></tr>
</table>

<table>
<tr><td></td><td>Month</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td><td>13</td><td>14</td><td>15</td><td></td></tr>
<tr><td>OP (pond 1)</td><td>GR</td><td colspan="3">EPP</td><td>GR</td><td colspan="3">EPP</td><td>GR</td><td colspan="7">EPP</td><td>GR</td></tr>
<tr><td>OP (pond 2)</td><td>GR</td><td colspan="6">EPP</td><td>GR</td><td colspan="3">EPP</td><td>GR</td><td colspan="4">EPP</td><td>GR</td></tr>
</table>

Figure 2



Frequency
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
120
100
80
60
40
20
0
Time to first spawn (days)
1
2
3
4
5
6
7
Initial female maturity stage

845 **Figure 3**

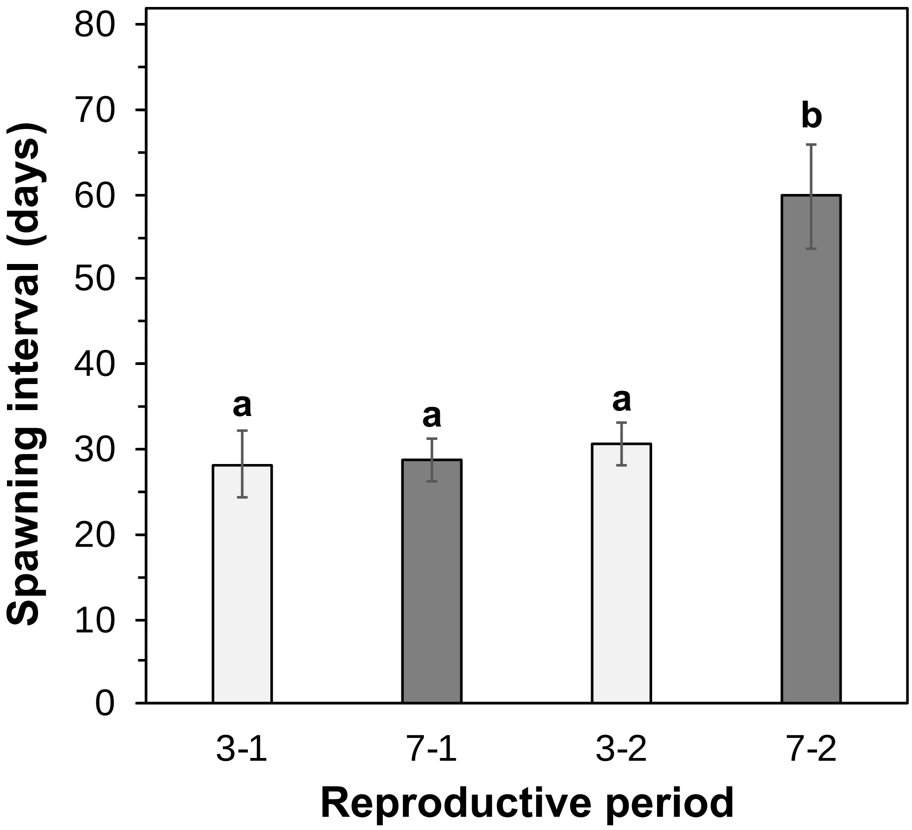
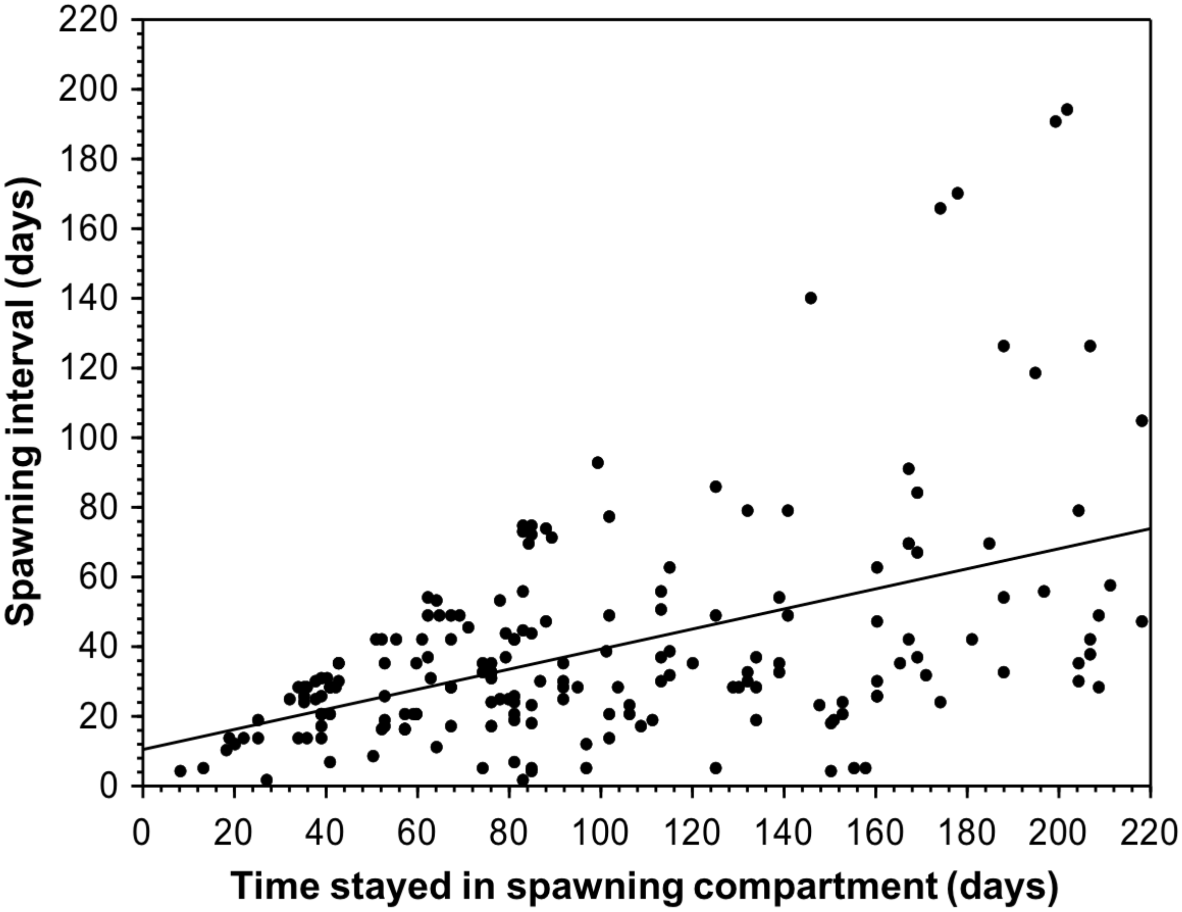
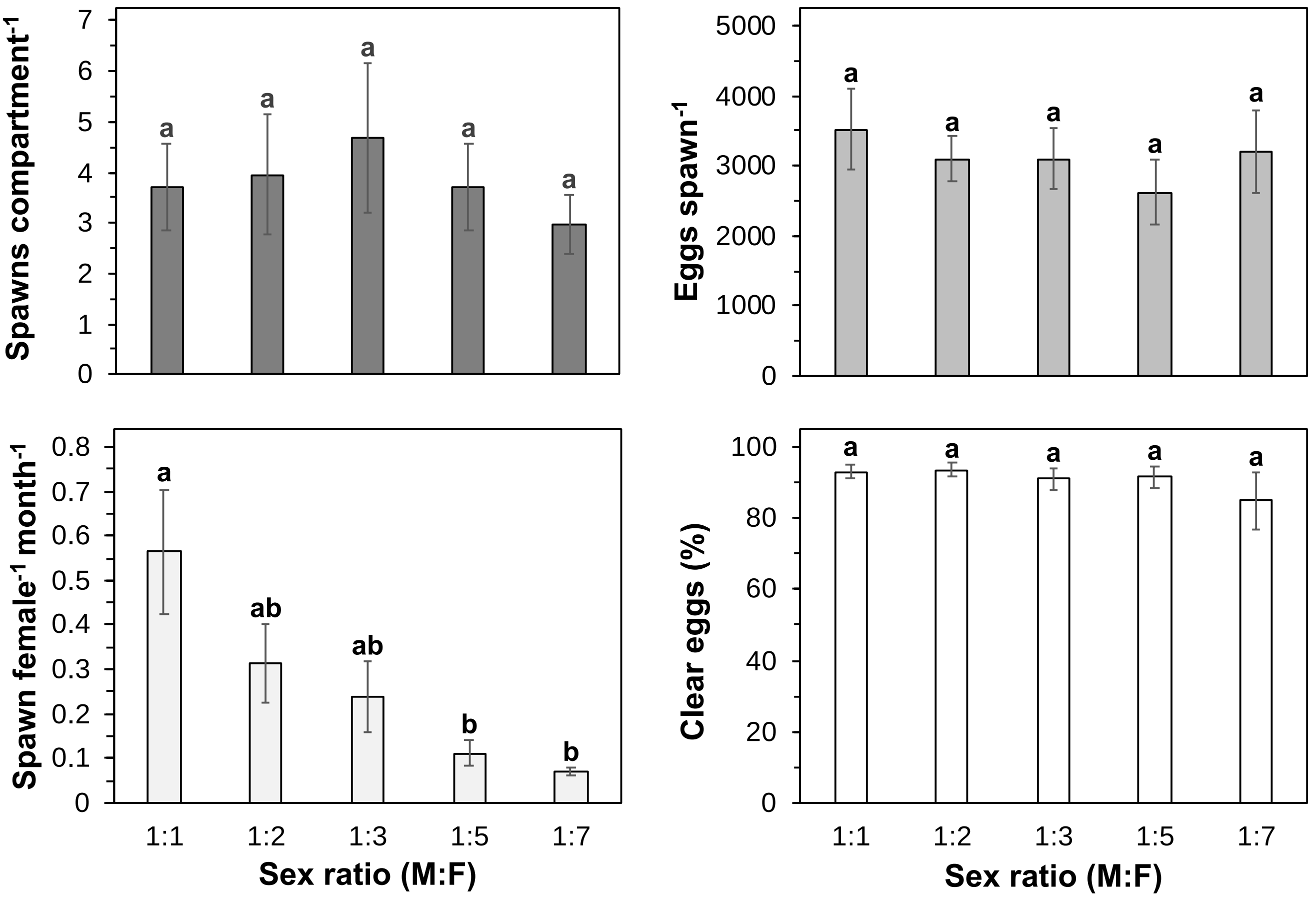

Spawning interval (days)
80
70
60
50
40
30
20
10
0
a
a
a
b
3-1
7-1
3-2
7-2
Reproductive period

Figure 4


Spawning interval (days)
0
20
40
60
80
100
120
140
160
180
200
220
Time stayed in spawning compartment (days)
0
20
40
60
80
100
120
140
160
180
200
220

847 **Figure 5**


Spawns compartment^{-1}
7
6
5
4
3
2
1
0
a
a
a
a
a
Eggs spawn^{-1}
5000
4000
3000
2000
1000
0
a
a
a
a
a
Spawn female^{-1} month^{-1}
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
ab
ab
b
b
Clear eggs (%)
100
80
60
40
20
0
a
a
a
a
a
1:1
1:2
1:3
1:5
1:7
Sex ratio (M:F)
1:1
1:2
1:3
1:5
1:7
Sex ratio (M:F)

848 **Figure 6**

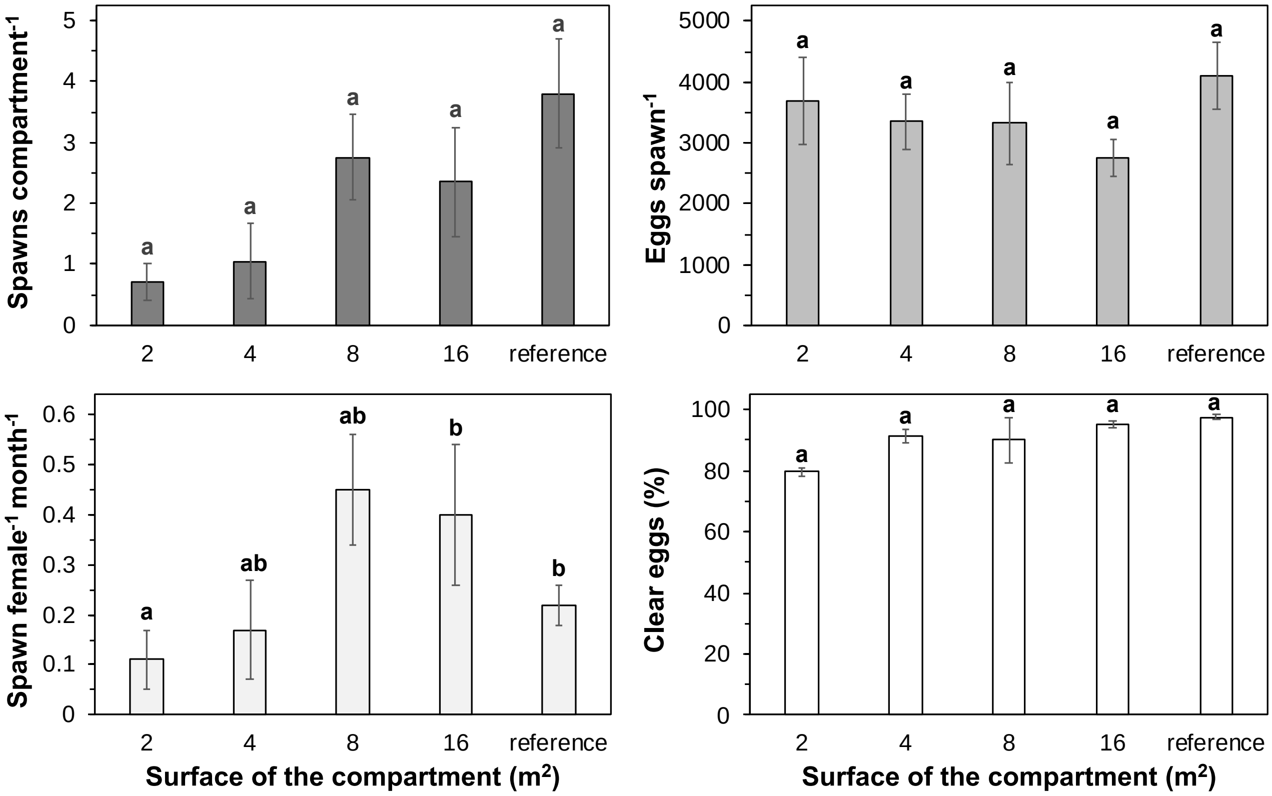
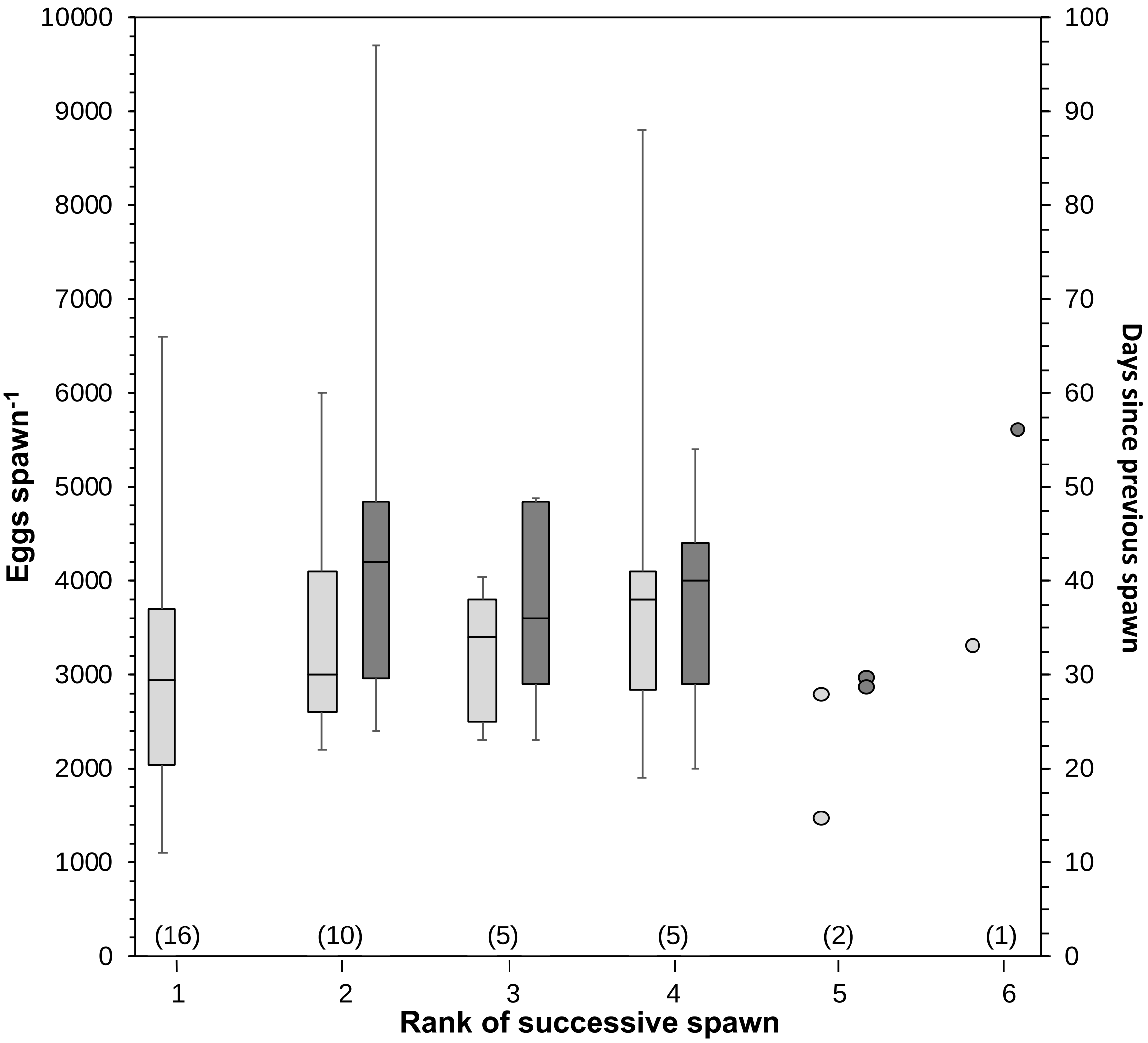
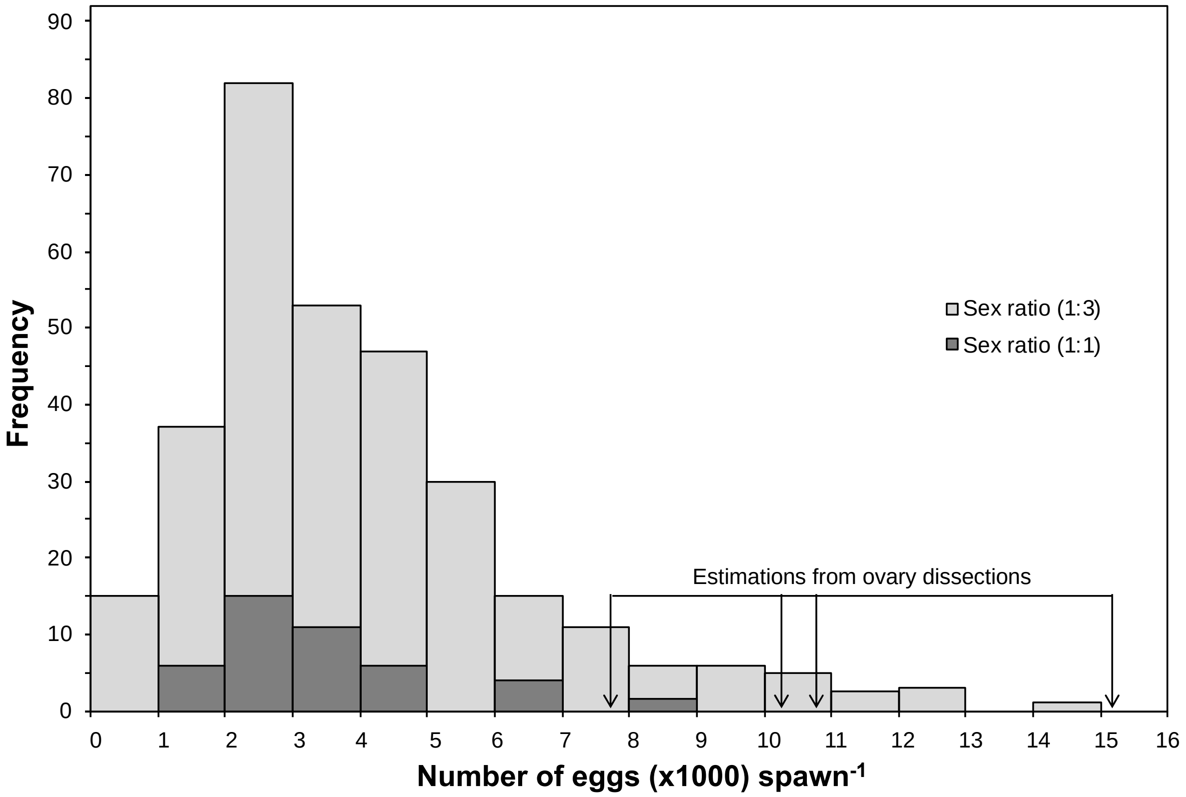

Spawns compartment-1
0
1
2
3
4
5
a
a
a
a
a
Eggs spawn-1
0
1000
2000
3000
4000
5000
a
a
a
a
a
2
4
8
16
reference
Spawn female-1 month-1
0
0.1
0.2
0.3
0.4
0.5
0.6
a
ab
ab
b
b
Clear eggs (%)
0
20
40
60
80
100
a
a
a
a
a
Surface of the compartment (m2)
Surface of the compartment (m2)

Figure 7


10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Eggs spawn^{-1}
100
90
80
70
60
50
40
30
20
10
0
Days since previous spawn
(16)
(10)
(5)
(5)
(2)
(1)
1
2
3
4
5
6
Rank of successive spawn

850 **Figure 8**


Frequency
0
10
20
30
40
50
60
70
80
90
Sex ratio (1:3)
Sex ratio (1:1)
Estimations from ovary dissections
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Number of eggs (x1000) spawn^{-1}

851 **Figure 9**

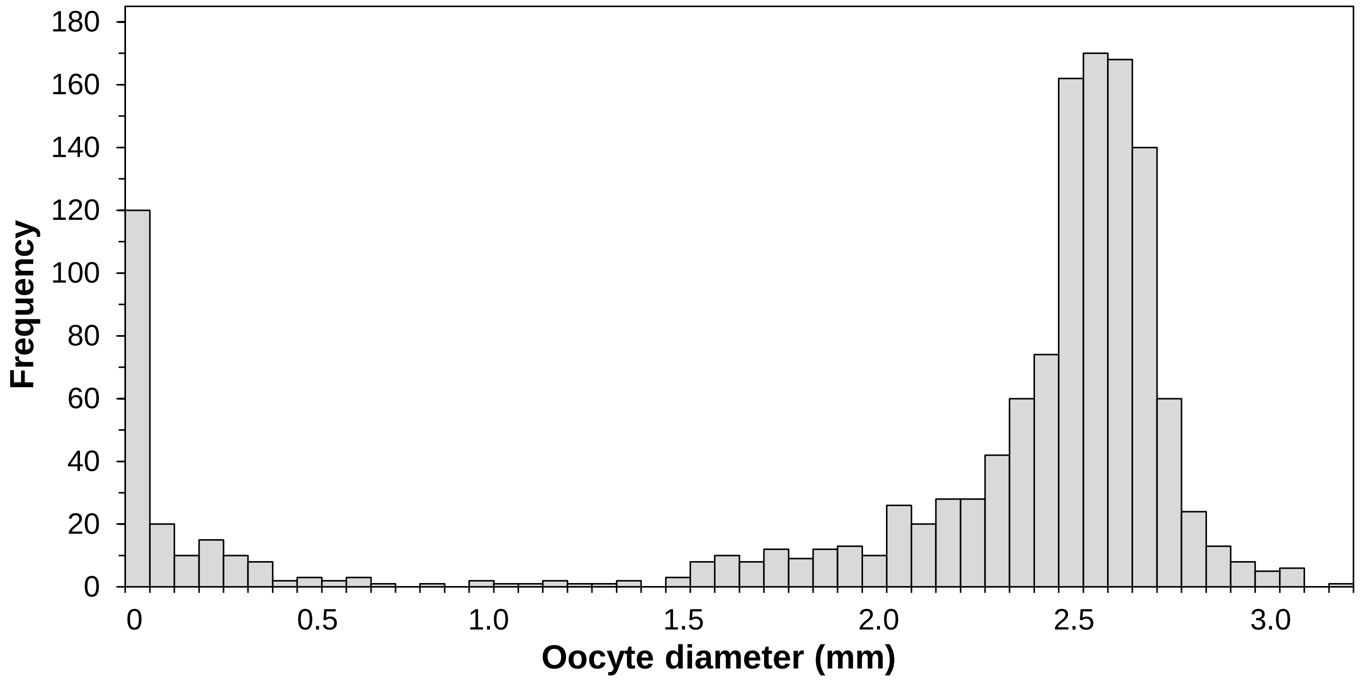

Frequency
180
160
140
120
100
80
60
40
20
0
0
0.5
1.0
1.5
2.0
2.5
3.0
Oocyte diameter (mm)

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 m^2 spawning compartments) or open communal ponds (OP) using a sex ratio of 1 male for 3 females. Data are means ± 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**				
	Pond A	**Pond B**	**Pond C**	**Pond D**	**F or H**	***df***	***p***
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 ± 77^{a}	2,901 ± 60^{a}	2,794 ± 61^{a}	2,921 ± 68^{a}	1.13	309	NS
Male BM (g)	3,954 ± 138^{ab}	4,264 ± 95^{a}	3,771 ± 103^{b}	4,112 ± 84^{ab}	4.69	101	*
Total number of spawns collected	147	166	72	79	-	-	-
Number of spawns female^{-1} month^{-1}	0.16 ± 0.02^{a}	0.16 ± 0.02^{a}	0.07 ± 0.01^{b}	0.11 ± 0.02^{b}	14.75	56	***
Number of eggs spawn^{-1}	4,179 ± 187^{a}	3,791 ± 176^{a}	2,389 ± 258^{b}	2,371 ± 255^{b}	* 62.67	469	***
Egg quality (% viable eggs)	87.7 ± 1.4^{ab}	89.3 ± 1.3^{a}	84.2 ± 2.0^{b}	83.6 ± 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 m^2 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 ± 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)**				
	3	**7**	***F***	***df***	***p***
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 ± 33^{a}	2,972 ± 46^{a}	0.03	469	NS
Male BM (g)	4,133 ± 64^{a}	4,195 ± 88^{a}	0.33	152	NS
Number of spawns compartment^{-1} over experiment	5.6 ± 0.5^{a} (2-11)	6.7 ± 0.5^{a} (2-13)	2.63	49	NS
Number of spawns female^{-1} month^{-1}	0.15 ± 0.01^{a} (0.05-0.29)	0.16 ± 0.01^{a} (0.05-0.30)	0.00	49	NS
Number of eggs in a spawn	3,889 ± 206^{a} (193-14,398)	4,040 ± 184^{a} (109-12,803)	0.36	311	NS
Egg quality (clear eggs %)	87.8 ± 1.4^{a} (12.6-99.8)	89.2 ± 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 ± 157^{a} (716-3,414)	1,887 ± 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

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Asependi Asependi, Anang Hari Kristanto, Marc Legendre

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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.