Webmail Univ. Bung Hatta

**hafrizal syandri <syandri_1960@bunghatta.ac.id>**

# Automated Reminder - 2: F1000Research - article53760

1 message

**Michael** <production.research@f1000.com> Sun, Jul 11, 2021 at 3:00 PM
To: Syandri <syandri_1960@bunghatta.ac.id>
Cc: anithajohny.mariasusai@straive.com, baskaran.elumalai@straive.com, nishikanth.doble@straive.com, production.research@f1000.com

Dear **Syandri,**

You should recently have received the proofs of your F1000Research article **"Reproductive characteristics of the giant gurami sago strain (Osphronemus goramy Lacepède, 1801): basic knowledge for a future hatchery development strategy"** from us.

We'd be grateful if you could let us know if there are any corrections that need to be made to the article, and if so - mark them on the proof using the following link:

https://ops.spi-global.com/eProofingF1000/VerifyTokenandAuthenticate.aspx?token=jYYV5poXzp/j6o09HeJLPg&ChapterOrArticleOrBook=Article

If not, please can you confirm it can be published without any changes.

If you haven't received the proof email, do let us know and we will resend it.

Kind regards,
The Production Team, F1000Research

# Reproductive characteristics of the giant gourami sago strain broodfish (*Osphronemus goramy* Lacepède, 1801): basic knowledge for a future hatchery strategy development

Azrita[1], Hafrijal Syandri*[2], Netti Aryani[3]

[1]Department of Biology Education, Faculty of Education, Universitas Bung Hatta, Padang, West Sumatera 25133, Indonesia

[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang, West Sumatera 25133, Indonesia

[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, Riau 28293, Indonesia

*Corresponding Author: syandri_1960@bunghatta.ac.id

**Abstract**

**Background:** The giant gourami sago strain (*Osphronemus goramy* Lacepède) has been approved in 2018 as a candidate for freshwater aquaculture in Indonesia. However, information on the species' reproduction is minimal. This study analyzed the reproduction characteristics of the gourami sago strain broodfish to provide basic knowledge for a hatchery development strategy in the future.

**Methods:** A total of- 10 female and 10 male gourami sago strain broodfish with mature oocytes were measured for body weight and length, and were evaluated for their reproductive characteristics. Breeding fish were spawned naturally in a 2×1×0.6 m concrete pond with a male-female sex ratio of 1:1. Egg weight and diameter were measured in 25 eggs per female using ACIS AD- 600i scales with 0.01 g accuracy and a microscope (Labo model L-711) using Canon Digital Camera Software 3 . Semen was collected using plastic syringes in 3 mL aliquots, then placed in an insulated ice-cooled container, and analyzed within 2 hours of collection.

**Results:** Average weights of female and male broodfish before spawning were 2180±159.78 g and 3060±134.99 g, respectively. The relative fecundity and egg diameter were 1029±36 eggs $kg^{-1}$ and 2.42±0.05 mm, respectively. The hatching rate and embryo survival to eyed-egg stage were respectively 76.40±2.27% and 94.76±0.42%. Sperm characteristics showed that volume was 0.60±0.12 ml $kg^{-1}$ and percentage motile was 70.04±2.27%. Female broodfish weight after spawning was strongly corelated with the weight before spawned ($r^2$ = 0.999) and absolute fecundity was also strongly corelated with female broodfish weight before spawning ($r^2$= 0.921). Sperm concentration was a moderately corelated with sperm motility ($r^2$ = 0.556) and duration of sperm motility ($r^2$ = 0.502).



Style Definition: FollowedHyperlink
Formatted: Not Highlight
Formatted: Font color: Auto
Formatted: Font color: Black
Formatted: Font color: Auto

**Conclusions:** The gourami sago strain broodfish has suitable reproductive characteristics for the development of future hatcheries. Successful natural spawning should be followed by larval weaning and feeding technology to increase growth and survival.

**Keywords**: Aquaculture, giant gourami, broodfish, egg, sperm, hatchery performance.

**Introduction**

Freshwater aquaculture practiced in inland waters such as lakes, rivers, reservoir, floodplains and oxbow lakes, and freshwater ponds, has expanded during the last decades in Indonesia[1,2,3,4,5]. Approximately 77.57% of fish produced in freshwater aquaculture in Indonesia are sourced from freshwater ponds and inland waters[6]. However, its development depends upon many factors, such as fish species, aquaculture systems, water depletion, fish diseases, farmers' knowledge and aquaculture practices[7,4,8,9,10].

Freshwater aquaculture is one of the fastest growing aquacultures in Indonesia, with more than 3,378,298.92 metric tons produced in 2018[6,11,12]. Nile tilapia (*Oreochromis niloticus)* contributed 37.93% of the total aquaculture production, African catfish (*Clarias gariepinus*) 33.35%, *Pangasius* catfish (*Pangasius hypophthalmus*) 12.38%, common carp (*Cyprinus carpio*) 9.28% and giant gourami (*Osphronemus goramy*) 6.96% [13,14,15].

Indonesian giant gourami strains include the local "tambago", "palapa", "soang", "galunggung" and "blusafir" strains, which have been grown semi-intensively in small-scale farms for decades[8,14,13,16]. However, they have not been able to contribute majorly to freshwater aquaculture production in Indonesia. Therefore, as the gourami sago strain has a limited is widely distributed in the West Sumatra Province of Indonesia[17,18], it is important to develop a hatchery gourami sago strain. The gourami sago strain is considered to support food security along many other freshwater fish species in Indonesia.

The gourami sago strain was approved as a candidate for freshwater aquaculture in 2018 (Decree of the Ministry of Marine and Fisheries, Republic of Indonesia No.56/KEPMEN-KP/2018)[19]. However, data on its reproductive characteristics are still limited. The evaluation of reproductive performance in other fish species has had beneficial impacts in the development of freshwater aquaculture in Asia[20,21,22,23,24]. In contrast, there are still gaps in knowledge of giant gourami sago strain broodfish regarding size at oocyte maturity, age of sexual maturity, sperm characteristics, egg hatchability, survival after eyed-egg stage, larva weaning and growth rate. These factors were identified as key challenges for successful giant gourami sago strain hatchery performance in the future. Therefore,

**Formatted:** Highlight

**Commented [SL1]:** Please rephrase – meaning still unclear

**Commented [S2R1]:** Has been revised

**Commented [SL3R1]:** It is unclear whether you mean short life span, or not often seen in West Sumatra: please see edit and confirm/modify accordingly

**Commented [S4R1]:** Has been revised

**Formatted:** Font color: Black

**Formatted:** Not Highlight

**Formatted:** Highlight

**Formatted:** Not Highlight

the present study was conducted to evaluate the reproductive characteristics in giant gourami sago strain to provide basic knowledge for hatchery development for the future.

## Methods

### *Ethical considerations*

There are no required permits from the government of the Republic of Indonesia to evaluate reproductive characteristics in gourami sago strain broodfish (*Osphronemus goramy*) as a candidate for future aquaculture. The study was funded by Research and Community Service Universitas Bung Hatta under a competitive grants scheme called the research of Professor in 2021 (Contract number: 06.02.1.46.03.2021). This grant included ethical approval and permits to collect fish specimens, rear and spawn giant gourami sago strain in the Aquaculture Laboratory Faculty of Fisheries and Marine Science Universitas Bung Hatta facilities. There was no animal suffering involved in this study and gourami sago strain broodfish were still in good condition when returned to the pond. Ethical approval was granted by the Ethics Commission for Research and Community service at Universitas Bung Hatta (023/LPPM/Hatta/I-2021).

### *Rearing and selection of breeders*

Juvenile fishes were selected about six years ago from a local hatchery in Luhak District, Lima Puluh Kota Regency, West Sumatra Province. The juvenile fish were kept in tanks and transported by truck to the Aquaculture Laboratory, Faculty of Fisheries and Marine Science University of Bung Hatta. A total of 200 individuals giant gourami sago strain juvenile fishes were reared during five years to sexual maturity in a concrete 8.4 $m^3$ (6×2×0.7 m) freshwater pond. During the rearing to sexual maturity, the juvenile fish were given commercial fish feed pellets (781-2 with 30% crude protein content and 4% crude fat; PT Japfa Comfeed Indonesia Tbk).

After the sago strain of giant gourami was reared five years, afterwards a total of 20 mature individuals broodfish were separated according to sex and reared in two concrete 18 $m^3$ (6×2×1.5 m) freshwater ponds during 60 days. The broodfish were fed twice daily (09:00 AM and 16:00 PM), with extruded feed pellets containing 39.50% crude protein and 12.21% fat with a predetermined quantity of 3% of fish weight per day. Besides that, the fish were given sente leaves (*Alocasia macrorriza* L.) at amounts of 1% of fish wet weight per day with the 2.85% protein content and 0.47% crude fat (% wet weight). Each concrete freshwater pond had a 50 mm drain in the middle, which was covered with a net of 0.5 cm mesh size to prevent the fish from escaping and predators from entering. Water was pumped from borehole wells at a rate of 5 litres per minute.

**Formatted:** Highlight

**Commented [SL5]:** How long between rearing and selection of mature fish?

**Commented [S6R5]:** Has been revised

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [SL7]:** As you wrote 20 before (10 M 10 F), please confirm the number and check underlying data

**Commented [S8R7]:** Has been check underlying data and revised

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [SL9]:** Please specify – this is contained in what?

**Commented [S10R9]:** Has been revised

**Formatted:** Highlight

A total of 20 broodfish with mature oocytes were selected, consisting of 10 females and 10 males. Prior to spawning, female and male broodfish were weighed using scales (OHAUS model CT 6000-USA with 0.1 g accuracy), and body length was measured using a meter ruler with 1mm accuracy. Average weight and length of the 10 female broodfish were 2140±159 g and 39.70±1.77 cm, while those of the male broodfish were 3060±135 g and 43.1±1.79 cm.

Reproductive parameters in gourami sago strain broodfish were analyzed using the following formulae:

- Condition factor (CF) = wet weight in gram/length$^3$ × 100
- Ovulated egg weight (g) = fish weight before spawning (g) – fish weight after spawning (g)
- Ova somatic index (%) = egg weight ovulation (g)/ fish weight before spawning (g) x 100

Absolute fecundity was the total number of eggs estimated per nest, and relative fecundity was the total number of eggs per kg body weight.

***Female reproductive performance***

Starting in August 2020 onwards, the broodfish were checked monthly for eggs and semen production. The broodfish were captured with a hand net and anesthetized by oral ingestion of Tricaine methanesulfonate (MS-222, ethyl 4-aminobenzoate methanesulfonate 98%, Sigma Aldrich Co, USA, MO; 50 mg L$^{-1}$), based on the dosage used for *Hemibagrus wyckii*[21]. Oocyte maturation was assessed for each individual. The oocyte maturity in giant gourami females was assessed from oocytes sampled by intraovarian biopsies using a flexible polyethylene catheter[21]. Egg diameter was measured using Labo microscope model L-711 and Canon Digital Camera Software 3.

Formatted: Not Highlight

Natural spawning of broodfish was carried out in 1.2 m$^3$ (2×1×0.6 m) concrete freshwater ponds with a male-female sex ratio of 1:1. Before the broodfish spawned, the ponds were drained, cleaned and all other species removed. Then, palm fibers were placed on top of a bamboo raft in the pond. The pond was then filled with water and the female and male broodfish were released into the spawning pond. The male broodfish made a nest for 5 to 7 days, after which spawning took place and the female broodfish laid eggs. Spawning occurred in the afternoon (between 15.00 to 17.00 PM). Due to the presence of a very large oil globule, giant gourami eggs float[8]. After the broodfish had finished laying eggs, the eggs were kept by the female broodfish in the nest. After the eggs had been kept by the broodfish for four hours in the nest, the eggs were collected and transferred to an incubation tray, which was placed in a ventricular hatching system. A total of 100 eggs for each broodfish were incubated in incubation trays. Meanwhile, the broodfish were returned to their pond once spawned, and no mortality occurred.

Egg weight and diameter were measured for 25 eggs per female using SHIMADZU-model AY 220 scales with 0.1 mg accuracy and a microscope (Labo model L-711) using Canon Digital Camera Software 3. A total of 25 eggs were randomly sampled 16 hours after spawning to determine the fertility rate (FR). The hatching rate (HR) was determined by counting all hatched fry 48 hours after spawned. Then, the endogenous feeding period of the fish larvae was counted until the egg yolks run out (in days), and embryo survival rate (%) to eyed-egg stage was measured.

***Determination of sperm quality***

To stimulate the spermiation process in male broodfish, an LHRH-preparation ( Ovaprim, manufactured for Syndel Laboratories Ltd, 2595 Mccullough Rd Nanaimo B.C 9VS 4m9 Canada) was injected to male fish, with a dosage 0.5 ml per kg of brooder. Semen samples were obtained from 10 gourami sago broodfish randomly selected from the farm. The male broodfish were first anesthetized with 50 mg $L^{-1}$ of MS-222[25], then fish weights (MaW) and total lengths (MaL) were measured. Special care was taken to avoid any contamination of semen with urine, feces, mucus and water. Semen samples were collected using plastic syringes in 3 mL aliquots, and then placed in an insulated ice-cooled container, transported to the laboratory and analyzed within two hours.

The sperm assessment included gross (visual) and microscopic examination as reviewed by Rurangwa *et al.*[26] and Cabrita *et al.*,[27]. The gross examination was based on visual and physical observation of parameters like semen volume, semen pH, sperm concentration, motility and duration motility. Semen volume was determined by collecting the semen in a graduated cylinder. Semen pH was determined with a hand pH meter (HI8424 Hanna Instrumen,USA).Microscopic examination was carried out using the Olympus model CX40, with × 10 and × 25 magnification, to determine parameters such as motility (MO) percentage and duration, by observing water-activated semen placed on a glass slide. Motile sperm were observed and expressed as a percent of non-moving sperm. Motility duration (DMO) was determined as the period between movements of the sperm to cessation of any progress using Neubauer's hemocytometer and calculated as the number of sperm $ml^{-1}$ [28]. Semen pH was determined with a hand pH meter (HI8424 Hanna Instruments, USA).

***Water quality***

Water samples were collected in the spawning pond and incubation trays to determine alkalinity, hardness and pH. The protocol for determining alkalinity by standard methodology is presented by Rice *et al.*, 2012[29]. pH values were determined with a pH meter (digital mini pH meter, 14pH, IQ Scientific, Chemo-science Thailand Co., Ltd, Thailand). An oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA) was used *in situ*, and water temperature in the spawning pond and incubation trays were measured with a thermometer (Celsius scale).

***Statistical analysis***

Results were given as the mean values (± SD). Simple linear regression analyses were performed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL). The standard deviation of each parameter was determined. For linear regression analyses, correlations were considered significant at $p<0.05$, and trends or tendencies were considered significant at $p<0.05$.

**Results**

The reproductive characteristics of female broodfish in sago giant gourami is summarized in Table 1. Total number of eggs per nest (absolute fecundity) varied from 2000 to 2650, while relative fecundity (total number of eggs per kg female brooder) varied between 977 and 1071. The fertility rate ranged from 76 to 84%, and the hatching success rate ranged from 72 to 80%. Endogenous feeding period ranged from 10 to 12 days, and embryo survival rate to eyed-egg stage varied between 94.73 and 95%.

Reproductive characteristics for male broodfish and sperm samples are presented in Table 2. The average live weight of the males was 3340±275.68 g. Sago giant gourami male broodfish were found to be slightly bigger than female broodfish. Gonad weight ranged from 25 to 30 g, whereas gonad somatic index ranged from 0.83 to 0.93%.

The linear correlation ($r^2$) between variables of reproduction characterization parameters in sago strain of giant gourami females broodfish results are shown in Table 3. In this study, the reproductive parameters that showed strong correlations with the absolute fecundity were female fish weight before spawning ($r^2$ = 0.921) and female fish weight after spawning ($r^2$ = 0.864). Similarly, results revealed significant correlations between egg diameter and hardened egg diameter ($r^2$ = 0.833), egg diameter and percentage of the hardened diameter ($r^2$ = 0.699), while egg diameter and fertility rate were moderately correlated ($r^2$ = 0.568). In contrast, the egg diameter was not strongly related to absolute fecundity ($r^2$ = 0.169) and relative fecundity ($r^2$= 0.096). On the other hand, the survival rate of larvae (10 days) also had strong correlations with the hatching rate ($r^2$ = 0.998) and endogenous feeding period ($r^2$ = 0.757).

Linear correlation analysis results ($r^2$) between variables of reproduction characterization parameters in sago giant gourami male broodfish are shown in Table 4. The reproductive parameters that had a strong correlation with gonad weight were somatic index of gonads ($r^2$ = 0.836), while semen volume ($r^2$ = 0.521), semen pH ($r^2$ = 0.521) and sperm concentration ($r^2$ = 0.506) were moderately correlated with gonad weight. In contrast, the gonadal weight negatively

**Commented [SL11]:** Please rephrase as this is still unclear – percentage of what?

**Commented [S12R11]:** Has been revised

**Commented [SL13R11]:** Please note this is still unclear

**Commented [S14R11]:** Has been revised

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

correlated with sperm motility ($r^2$ = 0.017) and duration of motility ($r^2$ = 0.275). In addition, the sperm concentration was also moderately correlated with the sperm motility ($r^2$= 0.556) and duration of motility ($r^2$= 0.502).

The physico-chemical water quality parameters in the spawning ponds and incubation trays for embryo development were as follows: water alkalinity ranged from 50.5 mg $L^{-1}$ to 52.5 mg $L^{-1}$, hardness varied from 65.5 mg $L^{-1}$ to 67.5 mg $L^{-1}$, pH ranged from 6.4 to 6.6, oxygen ranged from 6.1 mg $L^{-1}$ to 6.7 mg $L^{-1}$, and temperature varied from 28°C to 30°C.

**Table 1. Reproduction characterization in sago strain of giant gourami females broodfish (Mean ± SD)**

| | Variables | Range (Min-Max) |
|---|---|---|
| Fish length (cm) | 39.70±1.77 | 38-43 |
| Fish weight before spawning (g) | 2140±159.78 | 1958-2500 |
| Fish weight after spawning (g) | 2108±157.64 | 1930-2465 |
| Condition factor | 3.30±0.42 | 2.54-3.86 |
| Egg weight ovulated (g) | 32.80±2.86 | 28-38 |
| Ova somatic index (%) | 1.55±0.07 | 1.43-1.65 |
| Absolute fecundity (egg/fish) | 2205±201 | 2000-2650 |
| Relative fecundity (egg/kg body weight) | 1029±36 | 977-1071 |
| Egg diameter (mm) | 2.42±0.05 | 2.32-2.46 |
| Hardened egg diameter (mm) | 3.42±0.02 | 3.40-3.45 |
| Egg diameter increase (%) | 29.63±1.43 | 27.8-32.14 |
| Egg weight (mg) | 10.33±1.09 | 9.02-12.20 |
| Hardened egg weight (mg) | 13.36±1.27 | 11.74-15.20 |
| Hardened egg weight increase (%) | 22.69±2.24 | 19.74-24.81 |
| Fertility rate (%) | 81.60±3.37 | 76-84 |
| Hatching rate (%) | 76.40±2.27 | 72-80 |
| Endogenous feeding period (day) | 11.2±0.63 | 10-12 |
| Embryo survival rate to eyed-egg stage (%) | 94.76±0.42 | 94.73-95 |

**Table 2. Reproduction characterization in sago strain of giant gourami males broodfish (Mean ± SD)**

| | Variables | Range (Min-Max) |
|---|---|---|
| Fish weight (g) | 3060±134.99 | 2800-3200 |
| Fish length (cm) | 43.1±1.79 | 40- 5 |
| Condition factor | 3.74±0.43 | 3.08-4.38 |
| Gonads weight (g) | 27.5±1.72 | 25-30 |
| Gonadosomatic index (%) | 0.90±0.03 | 0.83-0.94 |
| Semen volume (mL per kg body weight) | 0.60±0.12 | 0.4-0.7 |
| Semen pH | 8.18±0.15 | 7.9-8.4 |
| Sperm concentration ($10^9$/mL) | 1.44±0.14 | 1.2-1.6 |
| Motility (%) | 70.04±2.27 | 68-75 |
| Duration of motility (sec) | 50.2±7.25 | 43-61 |

**Table 3. Correlations of variables ($r^2$) in sago strain of giant gourami females broodfish**

| | FEL | FWBS | FWAS | CF | OEW | OVI | AF | RF | EW | HEW | EWI | ED | HED | HDI | FR | HR | EFP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FEL | | | | | | | | | | | | | | | | | |
| FWBS | **0.720** | | | | | | | | | | | | | | | | |
| FWAS | **0.717** | **0.999** | | | | | | | | | | | | | | | |
| CF | **0.757** | **0.575** | **0.574** | | | | | | | | | | | | | | |
| OEW | **0.539** | **0.565** | **0.553** | 0.365 | | | | | | | | | | | | | |
| OVI | 0.281 | 0.191 | 0.012 | 0.000 | **0.774** | | | | | | | | | | | | |
| AF | 0.255 | **0.921** | **0.864** | **0.637** | 0.387 | 0.072 | | | | | | | | | | | |
| RF | 0.012 | 0.207 | 0.063 | 0.011 | 0.065 | 0.321 | **0.524** | | | | | | | | | | |
| EW | **0.894** | **0.659** | **0.655** | **0.677** | 0.552 | 0.246 | **0.567** | 0.041 | | | | | | | | | |
| HEW | **0.841** | **0.631** | **0.626** | **0.514** | **0.582** | 0.295 | 0.468 | 0.004 | **0.924** | | | | | | | | |
| EWI | 0.165 | 0.109 | 0.109 | 0.354 | 0.033 | 0.000 | 0.010 | 0.002 | **0.924** | 0.041 | | | | | | | |
| ED | 0.164 | 0.132 | 0.338 | 0.131 | 0.378 | **0.688** | 0.169 | 0.096 | 0.030 | 0.263 | 0.000 | | | | | | |
| HED | 0.064 | 0.029 | 0.237 | 0.126 | 0.025 | 0.207 | 0.022 | 0.064 | 0.468 | 0.184 | 0.030 | **0.833** | | | | | |
| HDI | 0.266 | 0.293 | 0.342 | 0.209 | 0.103 | 0.085 | 0.373 | 0.195 | 0.318 | 0.298 | 0.006 | **0.699** | 0.294 | | | | |
| FR | 0.026 | 0.229 | 0.020 | 0.000 | 0.135 | 0.264 | 0.000 | 0.004 | 0.067 | 0.004 | 0.160 | **0.568** | 0.064 | 0.054 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HR | 0.035 | 0.020 | 0.060 | 0.046 | 0.000 | 0.009 | 0.003 | 0.009 | 0.226 | 0.108 | 0.364 | 0.018 | 0.001 | 0.143 | **0.703** | | |
| EFP | 0.113 | 0.186 | 0.190 | 0.094 | 0.006 | 0.098 | 0.231 | 0.103 | **0.747** | **0.806** | 0.147 | 0.317 | 0.001 | 0.116 | 0.015 | 0.013 | |
| SR | 0.070 | 0.032 | 0.034 | 0.027 | 0.007 | 0.003 | 0.112 | 0.024 | 0.194 | 0.019 | 0.005 | 0.033 | 0.000 | 0.324 | 0.063 | **0.998** | **0.757** |

Statistically important at $r^2 > 0.500$ (underlined)

FeL: female fish length (cm); FWBS: female fish weight before spawning (g); FWAS: female fish weight after spawning (g); CF: condition factor; OEW: egg weight ovulation (g), OVI: Ova somatic index (%), AF: absolute fecundity (eggs), RF: relative fecundity (eggs), EW: egg weight (mg), HEW: Hardened egg weight (mg), EWI: eggs weight increase (%), ED: egg diameter (mm), HED: hardened egg diameter (mm), HDI: hardened egg diameter increase (%), FR: fertility rate (%), HR: hatching rate (%), EFP: endogenous feeding period (days), SR: survival rate (10 days).

**Table 4. Correlations of variables ($r^2$) in sago strain of giant gourami males broodfish**

| | MaL | MaW | CF | GW | GI | SV | pH | SC | MO |
|---|---|---|---|---|---|---|---|---|---|
| MaL | - | | | | | | | | |
| MaW | **0.714** | | | | | | | | |
| CF | **0.807** | 0.347 | | | | | | | |
| GW | 0.399 | **0.550** | 0.187 | | | | | | |
| GI | 0.003 | 0.042 | 0.071 | **0.836** | | | | | |
| SV | 0.025 | **0.576** | 0.042 | **0.521** | 0.000 | | | | |
| pH | **0.516** | **0.772** | 0.353 | **0.521** | 0.127 | 0.296 | | | |
| SC | 0.186 | **0.661** | 0.131 | **0.506** | 0.068 | 0.425 | **0.645** | | |
| MO | 0.068 | 0.453 | 0.061 | 0.017 | 0.130 | 0.393 | 0.280 | **0.556** | |
| DMO | 0.159 | 0.322 | 0.083 | 0.275 | 0.012 | 0.082 | 0.430 | **0.502** | **0.519** |

Statistically important at $r^2 > 0.500$ (underlined)

MaW: male fish weight (g), MaL: male fish length (g), CF: condition factor; GW: gonadal weight (g); GI: gonad somatic index (%), SV: semen volume (ml), SC: sperm concentration ($10^9$/mL), MO: motility (%), DMO: duration of motility (sec).

**Table 5. Summary of the fecundity, gonadal somatic index, egg diameter and hatching rate of giant gourami.**

| Species | Strain | Relative fecundity (egg/kg fish) | GSI (%) | Eggs diameter (mm) | Hatching rate (%) | Reference |
|---|---|---|---|---|---|---|
| *Osphronemus goramy* | Sago | 1037±90 | 1.91±0.35 | 2.42±0.05 | 76.40±6.33 | This study |
| *Osphronemus goramy* | Bastar | 2423±348 | 2.78±1.16 | 2.2 ± 0.2 | 96.36± 2.30 | [16] |
| *Osphronemus goramy* | Galunggung | 4011±287 | 4.15±0.63 | 2.5±0.05 | 89.3±1.30 | [8] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Osphronemus goramy* | - | 5508±1547 | 2.32±0.50 | 2.18±0.19 | 61.60±0.0 | [30] |
| *Osphronemus goramy* | Tambago | 2.896±185 | 3.16±0.11 | 2.47±0.03 | 91.06±4.06 | [31] |

**Discussion**

In our study, body weight in female sago giant gourami broodfish before spawning ranged from 1958 to 2500 g per fish and ova somatic index ranged from 1.43 to 1.65%. Body weight in female sago strain gourami broodfish was smaller than that of giant gourami belonging to the galunggung strain, which ranged from 2500 to 3500 g[8]. Conversely, ova somatic index of galunggung strain are found to be slightly bigger than that of sago giant gourami, which ranged from 3.7 to 4.6%[8]. The differences in reproductive characteristics in broodfish can be explained by strains, brood size, age of broodfish, previous spawning history and the production setting[23].Absolute fecundity in sago giant gourami ranged from 2000 to 2650 eggs fish$^{-1}$ and relative fecundity (RF) ranged from 977 to 1071 eggs kg$^{-1}$. Egg produced in kg fish$^{-1}$ (RF) is thought to be more informative than absolute fecundity. RF in sago strain of giant gourami were smaller compared to those in galunggung strain, palapah strain and blusafir strain[8,31,16]. On the other hand, the difference of relative fecundity can also be related to differences in broodfish size and age used[23]. Environmental factors such as rainfall also influenced the number of eggs per spawn in giant gourami brood, while the water temperature negatively related to number of eggs per spawn[15]. Furthermore, egg diameter in sago strain of giant gourami is found to be almost the same than others strain of giant gourami (Table 5). In this study, egg diameters average was 2.42±0.05 mm, consistent with those reported by other researchers; 2.18±0.19 mm for the giant gourami[30], 2.40±0.05 mm for blusafir strain[16] and 2.5±0.05 mm for galunggung strain[8]. The differences the RF, ova somatic index, egg diameter and hatching rate of giant gourami can be influenced by differences in the strains. Furthermore, egg diameter has been influenced by dietary protein level[32,33,34,35], age of broodfish[36], and spawning season[37,38]. In our study, egg diameter was shown to be positively correlated with egg weight, hardened egg weight, and egg weight increase. Egg weight of rainbow trout also increased after the hardening process and is a positively correlated with the viability of eggs[39]. Other egg quality metrics, such as hatching rate, and survival to first feeding, has been correlated with good egg quality[21].

In this study, the hatching rate of embryo in sago strain of giant gourami were smaller than those of other strains of giant gouramy[8,31,16]. This condition might be affected by the egg and sperm quality in giant gourami sago strain broodfish. In the present study, whether eggs and sperm quality of sago

giant gourami breeders are affected by feed type was poorly understood. Broodfish sex ratio had no influence on egg quality[8]. The reproductive parameters that were strongly correlated with the hatching rate were fertility rate ($r^2$=0.703) and survival rate (10 days) ($r^2$= 0.998). According to Sink *et al*. [40] the biochemical composition of broodfish eggs is strongly correlated with egg quality. In this study, we did not evaluate the biochemical composition of egg, because the relationship between egg quality and biochemical composition is difficult to interpret[41].

The keys regulator hormones of fish reproduction are gonadotropins, follicle-stimulating hormone, luteinizing hormone, and sex steroids[42,43]. In addition, oocyte development and maturation are also regulated by locally acting paracrine and autocrine signalling [44,45]. However, there is no information about the effects of such factors on oocyte development in giant gourami sago. Still, the extrusion feed enriched with vitamin E (d-α-tocopherol) at concentrations of 137.8, 238.05, 338.72 and 439.39 mg per kg feed had an effect on markers of reproductive functions of giant gourami broodfish, such as the sexual maturity cycles, ovum somatic index, relative fecundity and egg diameter[31].

Various efforts have been made by scientists to increase reproductive performance of female broodfish, such as increasing dietary protein level for *Xiphophorus helleri*[33], *Channa marulius*[46] and *Ictalurus punctatus*[40]. Additionally, implantation of 17ß-estradiol has also improved the reproductive performance in *Hemibagrus nemurus*[47]. Currently, whether the increase in protein level of feed and use of hormones can increase the reproductive potential in sago giant gourami is poorly understood. Therefore, we recommend the -use of proteins in feed (at levels of 25%, 30% or 35%) and the addition of 17β-estradiol (for example 200, 400 or 600 µg/kg body weight) to increase the reproductive potential of giant gourami sago the future.

Average semen volume in sago giant gourami were lower (0.4 to 0.6 ml) than those of *Hemibagrus wyckii* (0.60 to 1.20 ml)[21], but higher than those of *Pterygoplichthys gibbiceps*[48]. It appears that the semen volume depend on fish species[21,49,50]. Many factors influenced sperm quality and quantity such as genetic, physiological, spawning season and environmental factors.[26,49,51,52]. On the other hand, improvements in feed nutrition of broodfish can increase gamete quality and semen volume [46] .Commercial honey combined with 10% Dimethyl Sulfoxide (DMSO) was also shown to increase sperm motility[53]. Synthetic hormones such as salmon gonadotropin-releasing hormone analogues (GnRHa), with or without dopamine antagonist; domperidone (Dom) has effective on sperm quality[54,55]. Nevertheless, the duration of motility of sperm was strongly positively correlated with the water quality in ponds. In this study, the water quality parameters in spawning ponds included an alkalinity of 50.5 to 52.5 mg/L and hardness 65.5 to 67.5 mg/L, a pH between 6.4 and 6.6 and

**Commented [SL15]:** Time of what?
**Commented [S16R15]:** Has revised
**Formatted:** Highlight
**Formatted:** Highlight
**Formatted:** Not Highlight
**Formatted:** Not Highlight
**Formatted:** Not Highlight
**Formatted:** Not Highlight
**Commented [SL17]:** Please rephrase/clarify
**Commented [S18R17]:** Has been revised
**Formatted:** Highlight
**Formatted:** Highlight
**Formatted:** Highlight
**Formatted:** Highlight
**Commented [SL19]:** Please specify: mg of what?
**Commented [S20R19]:** Has been added

water temperature between 28 and 30 °C. These water quality parameters were able to support the ability of the sperm to fertilized egg[21].

The sperm motility in sago giant gourami ranged from 68 to 75%, and duration of motility ranged from 43 to 61 sec. These results are consistent with *Genypterus blacodes* and *Esox lucius*[56,50]. Sperm motility includes the percentage of motile sperm, straight line velocity, curvi linear velocity, average path velocity and linearity[50]. In this study, we did not investigate those parameters. In addition, the percentage of motile sperm is influenced by addition of extenders and cryoprotectants[57,58,59]. However, sperm motility from fresh semen was slightly greater compared to cryopreserved semen from *Esox lucius*[50]. The fertility rate of eggs ranged from 76 and 84%; however, no significant correlation was detected between fertility rate and sperm parameters, such as semen volume, semen pH, motility and duration of motility. Conversely, the sperm concentration was moderately correlated with sperm motility and duration of motility. The parameters commonly measured to assess sperm quality in brood were volume, density and motility (such as the percentage of motile sperm, straight line velocity curvilinear velocity, average path velocity, linearity and amplitude of lateral head displacement), including fertilizing capacity [49,50,52,60]. In this study, we did not investigate the ionic composition of the semen, but this phenomenon could be related to the ionic composition of semen which might has a significant influence on sperm motility and duration of motility.

**Conclusions**

This research analyzed the reproductive characteristics in giant gourami sago broodfish reared in concrete freshwater ponds, in the Aquaculture Laboratory Faculty of Fisheries and Marine Science, Universitas Bung Hatta. Relative fecundities of the giant gourami sago strain broodfish ranged from 977 to 1071 eggs, and egg diameter ranged from 2.32 to 2.46 mm. Semen volume ranged from 0.4 to 0.7 ml per kg body weight and sperm motility was comprised between 68 to 75%. A strong linear relationship was observed between absolute fecundity and female fish weight before and after spawning. A similar, strong positive correlation was observed between survival rate (10 days) and hatching rate. The sperm concentration was also moderately positive correlated with the motility and duration of sperm motility. Keys to increasing reproduction performance in gourami sago -strain depends on broodfish weight, relative fecundity and hatching rates. Although data on the reproductive characteristics of gourami sago strain broodfish have been obtained., there are still knowledge gaps in larval weaning, and feeding technologies during rearing. Therefore, for successful

**Commented [SL21]:** Do you have a reference to support this?

**Commented [S22R21]:** Has been added

**Formatted:** Font color: Red, Highlight

**Commented [SL23]:** Please note this is the syntax seen in the literature

**Commented [S24R23]:** Has been revised

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Not Highlight

**Formatted:** Not Highlight

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [SL25]:** Please clarify: which are correlated together? Are they all correlated to each other (concentration with motility, concentration with duration of motility, motility and duration of motility)?

**Commented [S26R25]:** Has been revised

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [SL27]:** Please note edit here is for correct grammar and syntax

**Commented [S28R27]:** Ok

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

practices in hatcheries, further research is recommended to determine a proper feed formulation and the development of appropriate aquaculture systems.

**Data availability**

*Underlying data*

Figshare: Reproduction characterization of the gurami sago (*Osphronemus goramy* Lacepède, 1801): basic knowledge for a hatchery development strategy for the future.

https://doi.org/10.6084/m9.figshare.14661189.v3 [61].

This project contains the following underlying data:

- Table 1. Raw data of fish length, weight, absolute fecundity and relative fecundity of gurami sago broodfish
- Table 2. Raw data of egg diameter (mm) in sago strain of giant gourami broodfish
- Table 3. Raw data of hardened egg diameter (mm) in sago strain of giant gourami broodfish
- Table 4. Raw data of egg diameter increase (%) in sago strain of giant gourami broodfish
- Table 5. Raw data of egg weight (mg) in sago strain of giant gourami broodfish
- Table 6. Raw data of hardened egg weight (mg) in sago strain of giant gourami broodfish
- Table 7. The data of egg weight increase (%) in sago strain of giant gourami broodfish
- Table 8. The data of fertilization rate (%) in sago strain of giant gourami broodfish
- Table 9. The data of hatching rate (%), endogenous feeding period (day) and embryo survival rate to eyed-egg stage (%) and in sago strain gourami
- Table 10. Male size, gonadal weight and semen in sago strain of giant gourami broodfish
- Table 11. Sperm concentration ($10^9$/mL) in sago strain of giant gourami broodfish
- Table 12. Sperm Motility (%) in sago strain of giant gourami broodfish
- Table 13. Duration motility (sec) in sago strain of giant gourami broodfish

Formatted: Font: 10 pt, Highlight

Formatted: Font: 10 pt, Highlight



**Competing interests**

No competing interests were disclosed.

**Grant information**

This study was funded by a competitive grants scheme called the Professor 2021. Contract number: 06.02.1.46.03.2021

**Acknowledgements**

The authors thank for Professor. Dr. Tafdil Husni the Rector of Universitas Bung Hatta for supporting this study through the competitive grant's schema called Research Professor, 2021. The appreciation goes to all of the students (Puji Kurniawan and Ranji Rinaldi and Muhammad Vajri Djauhari) who helped the author during data collection in the field.

**Commented [SL29]:** Please ensure you have obtained consent from these people to be named in your manuscript

**Commented [S30R29]:** they have agreed to have their names written on the Acknowledgements

**References**

1. Syandri H, Azrita, Mardiah A.: Nitrogen and phosphorus waste production from different fish species cultured at floating net cages in Lake Maninjau, Indonesia. *Asian J. Sci. Res*., 2018;11 (2): 287-294.
2. Aryani N, Suharman I, Azrita, *et al*.: Diversity and distribution of fish fauna of upstream and downstream areas at Koto Panjang Reservoir, Riau Province, Indonesia. F1000Research, 2020; 8:1435. Publisher Full Text
3. Mungkung R, Aubin J, Prihadi TH, *et al*.: Life Cycle Assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. *J. Clean. Prod*, 2013; 47: 249-256. Publisher Full Text
4. Pouil S, Samsudin R, Slembrouck J, *et al*.: Nutrient budgets in a small-scale freshwater fish pond system in Indonesia. *Aquaculture*, 2019; 504:267–274. Publisher Full Text
5. FAO, The State of World Fisheries and Aquaculture: Meeting the Sustainable Development Goals. *Food and Agriculture Organization of the United Nations*, Rome.2018.
6. CDSI (Central Data System Information). Ministry of Marine and Fisheries Republic of Indonesia, 2018 (In Indonesian). Reference Source
7. Azrita, Aryani N, Mardiah A, *et al*.: Growth, production and feed conversion performance of the gurami sago (*Osphronemus goramy* Lacepède, 1801) strain in different aquaculture systems. *F1000Research* 2020, 9:161. Publisher Full Text
8. Arifin OZ, Slembrouck J, Subagja J, *et al*.: New insights into giant gourami (*Osphronemus goramy*) reproductive biology and egg production control. *Aquaculture*, 2019; 519:734743
9. Ahmad N, Thompson S.: The blue dimensions of aquaculture: A global synthesis. *Sci. Total Environ*. 2019; 652: 851–861. Publisher Full Text

10. Ranjan R, Megarajan S, Xavier B, *et al*.: Broodstock development, induced breeding and larval rearing of Indian pompano, *Trachinotus mookalee* (Cuvier, 1832) – A new candidate species for aquaculture. *Aquaculture*, 2018; 495: 550–557. Publisher Full Text

11. Henriksson, PJG, Nhuong T, Chadag VM, *et al*.: Indonesian aquaculture futures-evaluating environmental and socioeconomic potentials and limitations.2017; J. Cleaner Prod., 162: 1482-1490. Publisher Full Text

12. Tran N, Rodriguez UP, Chan CY, *et al*.: Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. Marine Policy, 2017; 79, 25–32. Publisher Full Text

13. Aryani N, Azrita, Mardiah A, *et al*.: Influence of feeding rate on the growth, feed efficiency and carcass composition of the Giant gourami (*Osphronemus goramy*). *Pakistan J. Zool*. 2017; 49(5): 1775-1781. Reference Source

14. Nugroho E, Azrita, Syandri H, *et al*.: DNA barcoding of giant gourami (*Osphronemus goramy*) from West Sumatra, Indonesia. *AACL Bioflux*, 2019, 12, (4): 1074-1079. Reference Source

15. Slembrouck J, Arifin OZ, Pouil S, *et al*.: Seasonal variation of giant gourami (*Osphronemus goramy*) spawning activity and egg production in aquaculture ponds. *Aquaculture*,2020; 735450. PubMed Abstract Publisher Full Text

16. Radona D, Nafiqoh N.: Reproductive Characteristics and Heterosis value of Bastar and Bluesafir population of giant gourami crosses. *Berita Biologi*, 2014: 13(2):153-159 (in Indonesian). Reference Source

17. Azrita, Syandri H.: Effects of salinity on survival and growth of gurami sago (*Osphronemus goramy*, Lacepède, 1801) Juveniles. *Pak. J. Biol. Sci*., 2018; 21 (4): 171-178. Reference Source

18. Nugroho E, Azrita, Syandri H, *et al*.: Evaluation of genetic divergence of kalui fish (*Osphronemus goramy*) strains from West Sumatra revealed by random amplified polymorphism DNA (RAPD) marker. *Jurnal Riset Akuakultur*, 2016; 11 (4), 313-319 (in Indonesian). Reference Source

19. CDSI (Central Data System Information). Ministry of Marine and Fisheries, Republic of Indonesia, 2018 (In Indonesian). Reference Source.

20. Kanyilaz M.: Reproductive performance of a newly described Salmonid fish, Alakir Trout (*Salmo Kottelati*), a candidate species for aquaculture. *Pak. J. Zool*. 2016; 48(1): 83–89. Reference Source

21. Aryani A, Suharman I, Syandri H.: Reproductive performance of asian catfish (*Hemibagrus wyckii* Bleeker, 1858), a candidate species for aquaculture. *F1000Research* 2018, 7:683 Publisher Full Text

22. Aruho C, Walakira JK, Rutaisire J.: An overview of domestication potential of *Barbus altianalis* (Boulenger, 1900) in Uganda. *Aquac. Rep.*, 2018; 11, 31–37. Publisher Full Text

23. Osure GO, Phelps RP.: Evaluation of reproductive performance and early growth of four strains of Nile tilapia (*Oreochromis niloticus*, L) with different histories of domestication. *Aquaculture*, 2006; 253(1-4): 485–494. Publisher Full Text

24. Krejszeff S, Katarzyna T, Daniel Z, *et al.*: Domestication affect spawning of the ide (*Leuciscus idus*)-preliminary study. *Aquaculture*. 2009; 295(1–2): 145–147. Publisher Full Text

25. Weber RA, Peleteiro JB, García Martín LO, *et al*.: The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). *Aquaculture*. 2009; 288(1–2): 147–150. Publisher Full Text

26. Rurangwa E, Kime D, Ollevier F, *et al*.: The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 2004; 234(1-4): 1-28. Publisher Full Text

27. Cabrita E, Martínez-Páramo S, Gavaia PJ, *et al*.: Factors enhancing fish sperm quality and emerging tools for sperm analysis. *Aquaculture*, 2014; 432, 389–401. Publisher Full Text.

28. Mylonas CC, Duncan NJ, Asturiano JF.: Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. *Aquaculture*, 2017: 472, 21–44. Publisher Full Text.

29. Rice EW, Baird RB, Eaton AD, *et al.*: Standard methods for the examination of water and wastewater, 22nd ed**.** American Public Health Association, American Water Works Association, Water Environment Federation. 2012. Reference Source

30. Amornsakun T, Kullai S, Hassan A.: Some aspects in early life stage of giant gourami, *Osphronemus goramy* (Lacepede) larvae. *Songklanakarin J. Sci. Technol*, 2014; 36 (5): 493 – 498. Reference Source

31. Bari Y.: Addition of vitamin E to artificial feed in an effort to increase the reproductive potential of giant gourami (*Osphronemus goramy* Lacepede) broodstock. Thesis of

Postgraduate, Bogor Agricultural University (unpublish, in Indonesian), 1997. Reference Source

32. Izquierdo M, Fernández-Palacios H, Tacon AG.: Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 2001; 197(1-4): 25–42. Publisher Full Text

33. Chong ASC, Ishak SD, Osman Z, *et al*.: Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture*, 2004; 234: 381–392. Publisher Full Text.

34. Hafeez-ur-Rehman M, Abbas F, Ashraf M, *et al*.: Effect of different dietary protein levels on egg development and its response to inducing agents during induced spawning of *Channa marulius*. *Pakistan J. Zool*., 2017: 49 (1):337 – 343. Reference Source

35. Sarih S, Djellata A, Roo J, *et al*.: Effects of increased protein, histidine and taurine dietary levels on egg quality of greater amberjack (*Seriola dumerili*, Risso, 1810). *Aquaculture*, 2018; 499:72-79. Publisher Full Text

36. Jeuthe H, Brännäs E, Nilsson J.: Effects of egg size, maternal age and temperature on egg, viability of farmed Arctic charr. *Aquaculture*, 2013; 408-409, 70–77. Publisher Full Text

37. Stuart KR, Armbruster L, Johnson R, *et al*.: Egg diameter as a predictor for egg quality of California yellowtail (*Seriola dorsalis*). *Aquaculture*, 2020; 522: 735154. PubMed Abstract | Publisher Full Text.

38. Grant B, Davie A, Taggart JB, *et al*.: Seasonal changes in broodstock spawning performance and egg quality in ballan wrasse (*Labrus bergylta*). *Aquaculture*, 2016; 464: 505–514. Publisher Full Text.

39. Lahnsteiner F, Patzner RA.: Rainbow trout egg quality determination by the relative weight increase during hardening: a practical standardization. *J. Appl. Ichthyol*. 2002; 8(1):24-26. Reference Source

40. Sink TD, Lochmann RT, Pohlenz C, *et al*.: Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. *Aquaculture*, 2010; 298(3-4): 251– 259. Publisher Full Text.

41. Morehead D, Hart P, Dunstan G, *et al*.: Differences in egg quality between wild striped trumpeter (*Latris lineata*) and captive striped trumpeter that were fed different diets. *Aquaculture*, 2001; 192 (1): 39–53. Publisher Full Text.

42. Mateos J, Mañanos E, Carrillo M, *et al*.: Regulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) gene expression by gonadotropin-releasing hormone (GnRH) and sexual steroids in the Mediterranean Sea bass. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2002; 132(1): 75–86. Publisher Full Text

43. Paullada-Salmerón JA, Cowan ME, Loentgen GH, *et al*.: The gonadotropin-inhibitory hormone system of fish: The case of sea bass (*Dicentrarchus labrax*). *Gen. Comp. Endocrinol.,* 2019; 279: 184-195. Publisher Full Text.

44. Lubzens E, Young G, Bobe J, *et al*.: Oogenesis in teleosts: How fish eggs are formed. *Gen. Comp. Endocrinol.*, 2010; 165 (3): 367–389. Publisher Full Text.

45. Lubzens E, Bobe J, Young G, *et al*.: Maternal investment in fish oocytes and eggs: The molecular cargo and its contributions to fertility and early development. *Aquaculture*, 2017; 472: 107–143. Publisher Full Text.

46. Hafeez-ur-Rehman M, Abbas F, Ashraf M *et al*.: Effect of Different Dietary Protein Levels on Egg Development and its Response to Inducing Agents during Induced Spawning of *Channa marulis*, *Pakistan J. Zool.*, 2017; 49 (1):337-343. Reference Source.

47. Aryani N, Suharman I.: Effect of dietary protein level on the reproductive performance of female of green catfish (*Hemibagrus nemurus* Bagridae). *J Aquac Res Dev*., 2015; 6:11. Reference Source

48. Collazos-Lasso LF, Mariana CGE, Elisabeth AB.: Induced reproduction of the sailfin pleco, *Pterygoplichthys gibbiceps* (Kner, 1854) (Pisces: Loricariidae). *AACL Bioflux*, 2018, 11 (3): 724-729. Reference Source

49. Caldas JS, Godoy L.: Sperm characterization of the endangered Amazonian fish *Hypancistrus zebra*: basic knowledge for reproduction and conservation strategies. *Anim. Reprod. Sci.*, 2019; 204: 117-124. Publisher Full Text.

50. Dietrich GJ, Nynca J, Szczepkowski M, *et al*.: The effect of cryopreservation of semen from whitefish (*Coregonus lavaretus*) and northern pike (*Esox lucius*) using a glucose-methanol extender on sperm motility parameters and fertilizing ability. *Aquaculture*, 2016; 464: 60–64. Publisher Full Text.

51. Butt IAE, Litvak MK, Trippel EA.: Seasonal variations in seminal plasma and sperm characteristics of wild-caught and cultivated Atlantic cod, *Gadus morhua*. *Theriogenology*, 2010; 73(7): 873–885. Publisher Full Text.

52. Abinawanto, Intan AP, Retno L.: Sperm motility of giant gourami (*Osphronemus goramy*, Lacepede, 1801) at several concentrations of honey combined with DMSO after short-term storage. *AACL Bioflux*, 2017, 10 (2): 156-163. Reference Source

53. Valdebenito II, Gallegos PC, Effer BR.: Gamete quality in fish: evaluation parameters and determining factors. *Zygote*, 2013; 23(02): 177–197. Publisher Full Text.

54. Cejko BI, Krejszeff S, Żarski D, *et al*.: Effect of carp pituitary homogenate (CPH) and sGnRHa (Ovaprim) on northern pike (*Esox lucius*) spermiation stimulation and its effect on quantity and quality of sperm. *Anim. Reprod. Sci.*, 2018; 193: 217–225. Publisher Full Text.

55. Zadmajid V.: Comparative effects of human chorionic gonadotropin (hCG) and Ovaprim™ (sGnRHa+domperidone) on the reproductive characteristics of wild-caught male Longspine scraper, *Capoeta trutta* (Heckel, 1843). *Aquaculture*,2016; 463: 7–15. Publisher Full Text

56. Dumorné K, Valdebenito I, Contreras P, *et al*.: Effect of pH, osmolality and temperature on sperm motility of pink cusk-eel (*Genypterus blacodes*, (Forster, 1801)). *Aquac. Rep*., 2018; 11: 42-46. Publisher Full Text.

57. Golshahi K, Aramli MS, Nazari RM, *et al*.: Disaccharide supplementation of extenders is an effective means of improving the cryopreservation of semen in sturgeon. *Aquaculture*, 2018; 486: 261–265. Publisher Full Text.

58. Yusoff M, Hassan BN, Ikhwanuddin M, *et al*.: Successful sperm cryopreservation of the brown-marbled grouper, Epinephelus fuscoguttatus using propylene glycol as cryoprotectant. *Cryobiology*, 2018; 81: 168–173. Publisher Full Text

59. Kommisrud E, Myromslien FD, Stenseth EB, *et al*.: Viability, motility, ATP content and fertilizing potential of sperm from Atlantic salmon (*Salmo salar* L.) in milt stored before cryopreservation. *Theriogenology*. 2020; 151: 58-65. Publisher Full Text

60. Cejko BI, Żarski D, Palińska-Żarska K, *et al*.: Artificial seminal plasma improves motility and fertilization capacity of common carp *Cyprinus carpio* L. sperm during one hour of storage. *Aquaculture*, 2019; 506: 224-228. Publisher Full Text.

61. Syandri, Hafrijal; Azrita, Azrita; Aryani, Netti (2021): Untitled ItemReproduction characterization of the gurami sago (Osphronemus goramy Lacepède, 1801): basic knowledge for a hatchery development strategy for the future. figshare. Journal contribution. https://doi.org/10.6084/m9.figshare.14661189.v3

**Reproductive characteristics of the giant gourami sago strain broodfish (*Osphronemus goramy* Lacepède, 1801): basic knowledge for a future hatchery strategy development**

Azrita[1], Hafrijal Syandri*[2], Netti Aryani[3]

[1]Department of Biology Education, Faculty of Education, Universitas Bung Hatta, Padang, West Sumatera 25133, Indonesia

[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang, West Sumatera 25133, Indonesia

[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, Riau 28293, Indonesia

*Corresponding Author: syandri_1960@bunghatta.ac.id

**Abstract**

**Background:** The giant gourami sago strain (*Osphronemus goramy* Lacepède) has been approved in 2018 as a candidate for freshwater aquaculture in Indonesia. However, information on the species' reproduction is minimal. This study analyzed the reproduction characteristics of the gourami sago strain broodfish to provide basic knowledge for a hatchery development strategy in the future.

**Methods:** A total of 10 female and 10 male gourami sago strain broodfish with mature oocytes were measured for body weight and length, and were evaluated for their reproductive characteristics. Breeding fish were spawned naturally in a 2×1×0.6 m concrete pond with a male-female sex ratio of 1:1. Egg weight and diameter were measured in 25 eggs per female using ACIS AD- 600i scales with 0.01 g accuracy and a microscope (Labo model L-711) using Canon Digital Camera Software 3 . Semen was collected using plastic syringes in 3 mL aliquots, then placed in an insulated ice-cooled container, and analyzed within 2 hours of collection.

**Results:** Average weights of female and male broodfish before spawning were 2180±159.78 g and 3060±134.99 g, respectively. The relative fecundity and egg diameter were 1029±36 eggs $kg^{-1}$ and 2.42±0.05 mm, respectively. The hatching rate and embryo survival to eyed-egg stage were respectively 76.40±2.27% and 94.76±0.42%. Sperm characteristics showed that volume was 0.60±0.12 ml $kg^{-1}$ and percentage motile was 70.04±2.27%. Female broodfish weight after spawning was strongly corelated with the weight before spawned ($r^2$ = 0.999) and absolute fecundity was also strongly corelated with female broodfish weight before spawning ($r^2$= 0.921). Sperm concentration was a moderately corelated with sperm motility ($r^2$ = 0.556) and duration of sperm motility ($r^2$ = 0.502).

**Conclusions:** The gourami sago strain broodfish has suitable reproductive characteristics for the development of future hatcheries. Successful natural spawning should be followed by larval weaning and feeding technology to increase growth and survival.

**Keywords**: Aquaculture, giant gourami, broodfish, egg, sperm, hatchery performance.

**Introduction**

Freshwater aquaculture practiced in inland waters such as lakes, rivers, reservoir, floodplains and oxbow lakes, and freshwater ponds, has expanded during the last decades in Indonesia[1,2,3,4,5]. Approximately 77.57% of fish produced in freshwater aquaculture in Indonesia are sourced from freshwater ponds and inland waters[6]. However, its development depends upon many factors, such as fish species, aquaculture systems, water depletion, fish diseases, farmers' knowledge and aquaculture practices[7,4,8,9,10].

Freshwater aquaculture is one of the fastest growing aquacultures in Indonesia, with more than 3,378,298.92 metric tons produced in 2018[6,11,12]. Nile tilapia (*Oreochromis niloticus)* contributed 37.93% of the total aquaculture production, African catfish (*Clarias gariepinus*) 33.35%, *Pangasius* catfish (*Pangasius hypophthalmus*) 12.38%, common carp (*Cyprinus carpio*) 9.28% and giant gourami (*Osphronemus goramy*) 6.96% [13,14,15].

Indonesian giant gourami strains include the local "tambago", "palapa", "soang", "galunggung" and "blusafir" strains, which have been grown semi-intensively in small-scale farms for decades[8,14,13,16]. However, they have not been able to contribute majorly to freshwater aquaculture production in Indonesia. Sago gourami strain that have been approved as aquaculture candidates is limited in distribution in the West Sumatra Province of Indonesia[17,18]. Therefore their hatchery is very importantly developed in order to be able to contribute to the production of freshwater aquaculture. The gourami sago strain is considered to support food security along many other freshwater fish species in Indonesia.

The gourami sago strain was approved as a candidate for freshwater aquaculture in 2018 (Decree of the Ministry of Marine and Fisheries, Republic of Indonesia No.56/KEPMEN-KP/2018)[19]. However, data on its reproductive characteristics are still limited. The evaluation of reproductive performance in other fish species has had beneficial impacts in the development of freshwater aquaculture in Asia[20,21,22,23,24]. In contrast, there are still gaps in knowledge of giant gourami sago strain broodfish regarding size at oocyte maturity, age of sexual maturity, sperm characteristics, egg hatchability, survival after eyed-egg stage, larva weaning and growth rate. These factors were identified as key

**Commented [SL1]:** Please rephrase – meaning still unclear

**Commented [S2R1]:** Has been revised

**Commented [SL3R1]:** It is unclear whether you mean short life span, or not often seen in West Sumatra: please see edit and confirm/modify accordingly

**Commented [S4R1]:** Has been revised

**Formatted:** Highlight

challenges for successful giant gourami sago strain hatchery performance in the future. Therefore, the present study was conducted to evaluate the reproductive characteristics in giant gourami sago strain to provide basic knowledge for hatchery development for the future.

## Methods

### *Ethical considerations*

There are no required permits from the government of the Republic of Indonesia to evaluate reproductive characteristics in gourami sago strain broodfish (*Osphronemus goramy*) as a candidate for future aquaculture. The study was funded by Research and Community Service Universitas Bung Hatta under a competitive grants scheme called the research of Professor in 2021 (Contract number: 06.02.1.46.03.2021). This grant included ethical approval and permits to collect fish specimens, rear and spawn giant gourami sago strain in the Aquaculture Laboratory Faculty of Fisheries and Marine Science Universitas Bung Hatta facilities. There was no animal suffering involved in this study and gourami sago strain broodfish were still in good condition when returned to the pond. Ethical approval was granted by the Ethics Commission for Research and Community service at Universitas Bung Hatta (023/LPPM/Hatta/I-2021).

### *Rearing and selection of breeders*

Juvenile fishes were selected about six years ago from a local hatchery in Luhak District, Lima Puluh Kota Regency, West Sumatra Province. The juvenile fish were kept in tanks and transported by truck to the Aquaculture Laboratory, Faculty of Fisheries and Marine Science University of Bung Hatta. A total of 200 individuals giant gourami sago strain juvenile fishes were reared for five years to increase sexual maturity, which were reared in 8.4 m3 (6×2×0.7 m) concrete freshwater pond. During the rearing of the fishes to reach sexual maturity, the juvenile fish were fed with commercial fish pellets (781-2 with 30% crude protein content and 4% crude fat; PT Japfa Comfeed Indonesia Tbk).

After the giant gourami sago was reared for five years, 20 mature individuals broodfish were separated according to sex and reared in 18 $m^3$ (6×2×1.5 m) two concrete freshwater ponds for 60 days. The broodfish were fed twice daily (09:00 AM and 16:00 PM), with extruded feed pellets containing 39.50% crude protein and 12.21% fat with a predetermined quantity of 3% of fish weight per day. Besides that, the fish were given sente leaves (*Alocasia macrorriza* L.) at amounts of 1% of fish wet weight per day. The leaves contain 2.85% protein and 0.47% crude fat in wet weight). Each concrete freshwater pond had a 50 mm drain in the middle, which was covered with a net of 0.5 cm

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [SL5]:** How long between rearing and selection of mature fish?

**Commented [S6R5]:** Has been revised

**Commented [SL7]:** As you wrote 20 before (10 M 10 F), please confirm the number and check underlying data

**Commented [S8R7]:** Has been check underlying data and revised

**Formatted:** Highlight

**Commented [SL9]:** Please specify – this is contained in what?

**Commented [S10R9]:** Has been revised

**Formatted:** Highlight

mesh size to prevent the fish from escaping and predators from entering. Water was pumped from borehole wells at a rate of 5 litres per minute.

A total of 20 broodfish with mature oocytes were selected, consisting of 10 females and 10 males. Prior to spawning, female and male broodfish were weighed using scales (OHAUS model CT 6000-USA with 0.1 g accuracy), and body length was measured using a meter ruler with 1mm accuracy. Average weight and length of the 10 female broodfish were 2140±159 g and 39.70±1.77 cm, while those of the male broodfish were 3060±135 g and 43.1±1.79 cm.

Reproductive parameters in gourami sago strain broodfish were analyzed using the following formulae:

- Condition factor (CF) = wet weight in gram/length$^3$ × 100
- Ovulated egg weight (g) = fish weight before spawning (g) – fish weight after spawning (g)
- Ova somatic index (%) = egg weight ovulation (g)/ fish weight before spawning (g) x 100

Absolute fecundity was the total number of eggs estimated per nest, and relative fecundity was the total number of eggs per kg body weight.

***Female reproductive performance***

Starting in August 2020 onwards, the broodfish were checked monthly for eggs and semen production. The broodfish were captured with a hand net and anesthetized by oral ingestion of Tricaine methanesulfonate (MS-222, ethyl 4-aminobenzoate methanesulfonate 98%, Sigma Aldrich Co, USA, MO; 50 mg L$^{-1}$), based on the dosage used for *Hemibagrus wyckii*[21]. Oocyte maturation was assessed for each individual. The oocyte maturity in giant gourami females was assessed from oocytes sampled by intraovarian biopsies using a flexible polyethylene catheter[21]. Egg diameter was measured using Labo microscope model L-711 and Canon Digital Camera Software 3.

Natural spawning of broodfish was carried out in 1.2 m$^3$ (2×1×0.6 m) concrete freshwater ponds with a male-female sex ratio of 1:1. Before the broodfish spawned, the ponds were drained, cleaned and all other species removed. Then, palm fibers were placed on top of a bamboo raft in the pond. The pond was then filled with water and the female and male broodfish were released into the spawning pond. The male broodfish made a nest for 5 to 7 days, after which spawning took place and the female broodfish laid eggs. Spawning occurred in the afternoon (between 15.00 to 17.00 PM). Due to the presence of a very large oil globule, giant gourami eggs float[8]. After the broodfish had finished laying eggs, the eggs were kept by the female broodfish in the nest. After the eggs had been kept by the broodfish for four hours in the nest, the eggs were collected and transferred to an incubation tray, which was placed in a ventricular hatching system. A total of 100 eggs for each broodfish were

incubated in incubation trays. Meanwhile, the broodfish were returned to their pond once spawned, and no mortality occurred.

Egg weight and diameter were measured for 25 eggs per female using SHIMADZU-model AY 220 scales with 0.1 mg accuracy and a microscope (Labo model L-711) using Canon Digital Camera Software 3. A total of 25 eggs were randomly sampled 16 hours after spawning to determine the fertility rate (FR). The hatching rate (HR) was determined by counting all hatched fry 48 hours after spawned. Then, the endogenous feeding period of the fish larvae was counted until the egg yolks run out (in days), and embryo survival rate (%) to eyed-egg stage was measured.

***Determination of sperm quality***

To stimulate the spermiation process in male broodfish, an LHRH-preparation ( Ovaprim, manufactured for Syndel Laboratories Ltd, 2595 Mccullough Rd Nanaimo B.C 9VS 4m9 Canada) was injected to male fish, with a dosage 0.5 ml per kg of brooder. Semen samples were obtained from 10 gourami sago broodfish randomly selected from the farm. The male broodfish were first anesthetized with 50 mg $L^{-1}$ of MS-222[25], then fish weights (MaW) and total lengths (MaL) were measured. Special care was taken to avoid any contamination of semen with urine, feces, mucus and water. Semen samples were collected using plastic syringes in 3 mL aliquots, and then placed in an insulated ice-cooled container, transported to the laboratory and analyzed within two hours.

The sperm assessment included gross (visual) and microscopic examination as reviewed by Rurangwa *et al.*[26] and Cabrita *et al.*,[27]. The gross examination was based on visual and physical observation of parameters like semen volume, semen pH, sperm concentration, motility and duration motility. Semen volume was determined by collecting the semen in a graduated cylinder. Semen pH was determined with a hand pH meter (HI8424 Hanna Instrumen,USA).Microscopic examination was carried out using the Olympus model CX40, with × 10 and × 25 magnification, to determine parameters such as motility (MO) percentage and duration, by observing water-activated semen placed on a glass slide. Motile sperm were observed and expressed as a percent of non-moving sperm. Motility duration (DMO) was determined as the period between movements of the sperm to cessation of any progress using Neubauer's hemocytometer and calculated as the number of sperm $ml^{-1}$ [28]. Semen pH was determined with a hand pH meter (HI8424 Hanna Instruments, USA).

***Water quality***

Water samples were collected in the spawning pond and incubation trays to determine alkalinity, hardness and pH. The protocol for determining alkalinity by standard methodology is presented by Rice *et al.*, 2012[29]. pH values were determined with a pH meter (digital mini pH meter, 14pH, IQ Scientific, Chemo-science Thailand Co., Ltd, Thailand). An oxygen meter (YSI model 52, Yellow Spring

Instrument Co., Yellow Springs, OH, USA) was used *in situ*, and water temperature in the spawning pond and incubation trays were measured with a thermometer (Celsius scale).

***Statistical analysis***

Results were given as the mean values (± SD). Simple linear regression analyses were performed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL). The standard deviation of each parameter was determined. For linear regression analyses, correlations were considered significant at $p<0.05$, and trends or tendencies were considered significant at $p<0.05$.

**Results**

The reproductive characteristics of female broodfish in sago giant gourami is summarized in Table 1. Total number of eggs per nest (absolute fecundity) varied from 2000 to 2650, while relative fecundity (total number of eggs per kg female brooder) varied between 977 and 1071. The fertility rate ranged from 76 to 84%, and the hatching success rate ranged from 72 to 80%. Endogenous feeding period ranged from 10 to 12 days, and embryo survival rate to eyed-egg stage varied between 94.73 and 95%.

Reproductive characteristics for male broodfish and sperm samples are presented in Table 2. The average live weight of the males was 3340±275.68 g. Sago giant gourami male broodfish were found to be slightly bigger than female broodfish. Gonad weight ranged from 25 to 30 g, whereas gonad somatic index ranged from 0.83 to 0.93%.

The linear correlation ($r^2$) between variables of reproduction characterization parameters in sago strain of giant gourami females broodfish results are shown in Table 3. In this study, the reproductive parameters that showed strong correlations with the absolute fecundity were female fish weight before spawning ($r^2 = 0.921$) and female fish weight after spawning ($r^2 = 0.864$). Similarly, results revealed significant correlations between egg diameter and hardened egg diameter ($r^2 = 0.833$), and between egg diameter and percentage of the hardened diameter ($r^2 = 0.699$). Meanwhile egg diameter and fertility rate were moderately correlated ($r^2 = 0.568$). In contrast, the egg diameter was not strongly related to absolute fecundity ($r^2 = 0.169$) and relative fecundity ($r^2 = 0.096$). On the other hand, the survival rate of larvae (10 days) also had strong correlations with the hatching rate ($r^2 = 0.998$) and endogenous feeding period ($r^2 = 0.757$).

Linear correlation analysis results ($r^2$) between variables of reproduction characterization parameters in sago giant gourami male broodfish are shown in Table 4. The reproductive parameters that had a strong correlation with gonad weight were somatic index of gonads ($r^2$ =

**Commented [SL11]:** Please rephrase as this is still unclear – percentage of what?

**Commented [S12R11]:** Has been revised

**Commented [SL13R11]:** Please note this is still unclear

**Commented [S14R11]:** Has been revised

**Formatted:** Highlight

**Formatted:** Highlight

0.836), while semen volume ($r^2$ = 0.521), semen pH ($r^2$ = 0.521) and sperm concentration ($r^2$ = 0.506) were moderately correlated with gonad weight. In contrast, the gonadal weight negatively correlated with sperm motility ($r^2$ = 0.017) and duration of motility ($r^2$ = 0.275). In addition, the sperm concentration was also moderately correlated with the sperm motility ($r^2$= 0.556) and duration of motility ($r^2$= 0.502).

The physico-chemical water quality parameters in the spawning ponds and incubation trays for embryo development were as follows: water alkalinity ranged from 50.5 mg $L^{-1}$ to 52.5 mg $L^{-1}$, hardness varied from 65.5 mg $L^{-1}$ to 67.5 mg $L^{-1}$, pH ranged from 6.4 to 6.6, oxygen ranged from 6.1 mg $L^{-1}$ to 6.7 mg $L^{-1}$, and temperature varied from 28°C to 30°C.

**Table 1. Reproduction characterization in sago strain of giant gourami females broodfish (Mean ± SD)**

| | Variables | Range (Min-Max) |
|---|---|---|
| Fish length (cm) | 39.70±1.77 | 38-43 |
| Fish weight before spawning (g) | 2140±159.78 | 1958-2500 |
| Fish weight after spawning (g) | 2108±157.64 | 1930-2465 |
| Condition factor | 3.30±0.42 | 2.54-3.86 |
| Egg weight ovulated (g) | 32.80±2.86 | 28-38 |
| Ova somatic index (%) | 1.55±0.07 | 1.43-1.65 |
| Absolute fecundity (egg/fish) | 2205±201 | 2000-2650 |
| Relative fecundity (egg/kg body weight) | 1029±36 | 977-1071 |
| Egg diameter (mm) | 2.42±0.05 | 2.32-2.46 |
| Hardened egg diameter (mm) | 3.42±0.02 | 3.40-3.45 |
| Egg diameter increase (%) | 29.63±1.43 | 27.8-32.14 |
| Egg weight (mg) | 10.33±1.09 | 9.02-12.20 |
| Hardened egg weight (mg) | 13.36±1.27 | 11.74-15.20 |
| Hardened egg weight increase (%) | 22.69±2.24 | 19.74-24.81 |
| Fertility rate (%) | 81.60±3.37 | 76-84 |
| Hatching rate (%) | 76.40±2.27 | 72-80 |

| | | |
|---|---|---|
| Endogenous feeding period *(*day) | 11.2±0.63 | 10-12 |
| Embryo survival rate to eyed-egg stage (%) | 94.76±0.42 | 94.73-95 |

**Table 2. Reproduction characterization in sago strain of giant gourami males broodfish (Mean ± SD)**

| | Variables | Range (Min-Max) |
|---|---|---|
| Fish weight (g) | 3060±134.99 | 2800-3200 |
| Fish length (cm) | 43.1±1.79 | 40- 5 |
| Condition factor | 3.74±0.43 | 3.08-4.38 |
| Gonads weight (g) | 27.5±1.72 | 25-30 |
| Gonadosomatic index (%) | 0.90±0.03 | 0.83-0.94 |
| Semen volume (mL per kg body weight) | 0.60±0.12 | 0.4-0.7 |
| Semen pH | 8.18±0.15 | 7.9-8.4 |
| Sperm concentration ($10^9$/mL) | 1.44±0.14 | 1.2-1.6 |
| Motility (%) | 70.04±2.27 | 68-75 |
| Duration of motility (sec) | 50.2±7.25 | 43-61 |

**Table 3. Correlations of variables ($r^2$) in sago strain of giant gourami females broodfish**

| | FEL | FWBS | FWAS | CF | OEW | OVI | AF | RF | EW | HEW | EWI | ED | HED | HDI | FR | HR | EFP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FEL | | | | | | | | | | | | | | | | | |
| FWBS | **0.720** | | | | | | | | | | | | | | | | |
| FWAS | **0.717** | **0.999** | | | | | | | | | | | | | | | |
| CF | **0.757** | **0.575** | **0.574** | | | | | | | | | | | | | | |
| OEW | **0.539** | **0.565** | **0.553** | 0.365 | | | | | | | | | | | | | |
| OVI | 0.281 | 0.191 | 0.012 | 0.000 | **0.774** | | | | | | | | | | | | |
| AF | 0.255 | **0.921** | **0.864** | **0.637** | 0.387 | 0.072 | | | | | | | | | | | |
| RF | 0.012 | 0.207 | 0.063 | 0.011 | 0.065 | 0.321 | **0.524** | | | | | | | | | | |
| EW | **0.894** | **0.659** | **0.655** | **0.677** | 0.552 | 0.246 | **0.567** | 0.041 | | | | | | | | | |
| HEW | **0.841** | **0.631** | **0.626** | **0.514** | **0.582** | 0.295 | 0.468 | 0.004 | **0.924** | | | | | | | | |
| EWI | 0.165 | 0.109 | 0.109 | 0.354 | 0.033 | 0.000 | 0.010 | 0.002 | **0.924** | 0.041 | | | | | | | |
| ED | 0.164 | 0.132 | 0.338 | 0.131 | 0.378 | **0.688** | 0.169 | 0.096 | 0.030 | 0.263 | 0.000 | | | | | | |
| HED | 0.064 | 0.029 | 0.237 | 0.126 | 0.025 | 0.207 | 0.022 | 0.064 | 0.468 | 0.184 | 0.030 | **0.833** | | | | | |
| HDI | 0.266 | 0.293 | 0.342 | 0.209 | 0.103 | 0.085 | 0.373 | 0.195 | 0.318 | 0.298 | 0.006 | **0.699** | 0.294 | | | | |
| FR | 0.026 | 0.229 | 0.020 | 0.000 | 0.135 | 0.264 | 0.000 | 0.004 | 0.067 | 0.004 | 0.160 | **0.568** | 0.064 | 0.054 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HR | 0.035 | 0.020 | 0.060 | 0.046 | 0.000 | 0.009 | 0.003 | 0.009 | 0.226 | 0.108 | 0.364 | 0.018 | 0.001 | 0.143 | **0.703** | | |
| EFP | 0.113 | 0.186 | 0.190 | 0.094 | 0.006 | 0.098 | 0.231 | 0.103 | **0.747** | **0.806** | 0.147 | 0.317 | 0.001 | 0.116 | 0.015 | 0.013 | |
| SR | 0.070 | 0.032 | 0.034 | 0.027 | 0.007 | 0.003 | 0.112 | 0.024 | 0.194 | 0.019 | 0.005 | 0.033 | 0.000 | 0.324 | 0.063 | **0.998** | **0.757** |

Statistically important at $r^2 > 0.500$ (underlined)

FeL: female fish length (cm); FWBS: female fish weight before spawning (g); FWAS: female fish weight after spawning (g); CF: condition factor; OEW: egg weight ovulation (g), OVI: Ova somatic index (%), AF: absolute fecundity (eggs), RF: relative fecundity (eggs), EW: egg weight (mg), HEW: Hardened egg weight (mg), EWI: eggs weight increase (%), ED: egg diameter (mm), HED: hardened egg diameter (mm), HDI: hardened egg diameter increase (%), FR: fertility rate (%), HR: hatching rate (%), EFP: endogenous feeding period (days), SR: survival rate (10 days).

**Table 4. Correlations of variables ($r^2$) in sago strain of giant gourami males broodfish**

| | MaL | MaW | CF | GW | GI | SV | pH | SC | MO |
|---|---|---|---|---|---|---|---|---|---|
| MaL | - | | | | | | | | |
| MaW | **0.714** | | | | | | | | |
| CF | **0.807** | 0.347 | | | | | | | |
| GW | 0.399 | **0.550** | 0.187 | | | | | | |
| GI | 0.003 | 0.042 | 0.071 | **0.836** | | | | | |
| SV | 0.025 | **0.576** | 0.042 | **0.521** | 0.000 | | | | |
| pH | **0.516** | **0.772** | 0.353 | **0.521** | 0.127 | 0.296 | | | |
| SC | 0.186 | **0.661** | 0.131 | **0.506** | 0.068 | 0.425 | **0.645** | | |
| MO | 0.068 | 0.453 | 0.061 | 0.017 | 0.130 | 0.393 | 0.280 | **0.556** | |
| DMO | 0.159 | 0.322 | 0.083 | 0.275 | 0.012 | 0.082 | 0.430 | **0.502** | **0.519** |

Statistically important at $r^2 > 0.500$ (underlined)

MaW: male fish weight (g), MaL: male fish length (g), CF: condition factor; GW: gonadal weight (g); GI: gonad somatic index (%), SV: semen volume (ml), SC: sperm concentration ($10^9$/mL), MO: motility (%), DMO: duration of motility (sec).

**Table 5. Summary of the fecundity, gonadal somatic index, egg diameter and hatching rate of giant gourami.**

| Species | Strain | Relative fecundity (egg/kg fish) | GSI (%) | Eggs diameter (mm) | Hatching rate (%) | Reference |
|---|---|---|---|---|---|---|
| *Osphronemus goramy* | Sago | 1037±90 | 1.91±0.35 | 2.42±0.05 | 76.40±6.33 | This study |
| *Osphronemus goramy* | Bastar | 2423±348 | 2.78±1.16 | 2.2 ± 0.2 | 96.36± 2.30 | [16] |
| *Osphronemus goramy* | Galunggung | 4011±287 | 4.15±0.63 | 2.5±0.05 | 89.3±1.30 | [8] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Osphronemus goramy* | - | 5508±1547 | 2.32±0.50 | 2.18±0.19 | 61.60±0.0 | [30] |
| *Osphronemus goramy* | Tambago | 2.896±185 | 3.16±0.11 | 2.47±0.03 | 91.06±4.06 | [31] |

**Discussion**

In our study, body weight in female sago giant gourami broodfish before spawning ranged from 1958 to 2500 g per fish and ova somatic index ranged from 1.43 to 1.65%. Body weight in female sago strain gourami broodfish was smaller than that of giant gourami belonging to the galunggung strain, which ranged from 2500 to 3500 g[8]. Conversely, ova somatic index of galunggung strain are found to be slightly bigger than that of sago giant gourami, which ranged from 3.7 to 4.6%[8]. The differences in reproductive characteristics in broodfish can be explained by strains, brood size, age of broodfish, previous spawning history and the production setting[23].Absolute fecundity in sago giant gourami ranged from 2000 to 2650 eggs fish$^{-1}$ and relative fecundity (RF) ranged from 977 to 1071 eggs kg$^{-1}$. Egg produced in kg fish$^{-1}$ (RF) is thought to be more informative than absolute fecundity. RF in sago strain of giant gourami were smaller compared to those in galunggung strain, palapah strain and blusafir strain[8,31,16]. On the other hand, the difference of relative fecundity can also be related to differences in broodfish size and age used[23]. Environmental factors such as rainfall also influenced the number of eggs per spawn in giant gourami brood, while the water temperature negatively related to number of eggs per spawn[15]. Furthermore, egg diameter in sago strain of giant gourami is found to be almost the same than others strain of giant gourami (Table 5). In this study, egg diameters average was 2.42±0.05 mm, consistent with those reported by other researchers; 2.18±0.19 mm for the giant gourami[30], 2.40±0.05 mm for blusafir strain[16] and 2.5±0.05 mm for galunggung strain[8]. The differences the RF, ova somatic index, egg diameter and hatching rate of giant gourami can be influenced by differences in the strains. Furthermore, egg diameter has been influenced by dietary protein level[32,33,34,35], age of broodfish[36], and spawning season[37,38]. In our study, egg diameter was shown to be positively correlated with egg weight, hardened egg weight, and egg weight increase. Egg weight of rainbow trout also increased after the hardening process and is a positively correlated with the viability of eggs[39]. Other egg quality metrics, such as hatching rate, and survival to first feeding, has been correlated with good egg quality[21].

In this study, the hatching rate of embryo in sago strain of giant gourami were smaller than those of other strains of giant gouramy[8,31,16]. This condition might be affected by the egg and sperm quality in giant gourami sago strain broodfish. In the present study, whether eggs and sperm quality of sago

giant gourami breeders are affected by feed type was poorly understood. Broodfish sex ratio had no influence on egg quality[8]. The reproductive parameters that were strongly correlated with the hatching rate were fertility rate ($r^2$=0.703) and survival rate (10 days) ($r^2$= 0.998). According to Sink *et al*. [40] the biochemical composition of broodfish eggs is strongly correlated with egg quality. In this study, we did not evaluate the biochemical composition of egg, because the relationship between egg quality and biochemical composition is difficult to interpret[41].

The keys regulator hormones of fish reproduction are gonadotropins, follicle-stimulating hormone, luteinizing hormone, and sex steroids[42,43]. In addition, oocyte development and maturation are also regulated by locally acting paracrine and autocrine signalling [44,45]. However, there is no information about the effects of such factors on oocyte development in giant gourami sago. Still, the extrusion feed enriched with vitamin E (d-α-tocopherol) at concentrations of 137.8, 238.05, 338.72 and 439.39 mg per kg feed had an effect on markers of reproductive functions of giant gourami broodfish, such as the sexual maturity cycles, ovum somatic index, relative fecundity and egg diameter[31].

Various efforts have been made by scientists to increase reproductive performance of female broodfish, such as increasing dietary protein level for *Xiphophorus helleri*[33], *Channa marulius*[46] and *Ictalurus punctatus*[40]. Additionally, implantation of 17ß-estradiol has also improved the reproductive performance in *Hemibagrus nemurus*[47]. Currently, whether the increase in protein level of feed and use of hormones can increase the reproductive potential in sago giant gourami is poorly understood. Therefore, we recommend the use of proteins in feed (at levels of 25%, 30% or 35%) and the addition of 17β-estradiol (for example 200, 400 or 600 µg/kg body weight) to increase the reproductive potential of giant gourami sago the future.

Average semen volume in sago giant gourami were lower (0.4 to 0.6 ml) than those of *Hemibagrus wyckii* (0.60 to 1.20 ml)[21], but higher than those of *Pterygoplichthys gibbiceps*[48]. It appears that the semen volume depend on fish species[21,49,50]. Many factors influenced sperm quality and quantity such as genetic, physiological, spawning season and environmental factors.[26,49,51,52]. On the other hand, improvements in feed nutrition of broodfish can increase gamete quality and semen volume [46] .Commercial honey combined with 10% Dimethyl Sulfoxide (DMSO) was also shown to increase sperm motility[53]. Synthetic hormones such as gonadotropin-releasing hormone analogues (GnRHa), with or without dopamine antagonist; domperidone (Dom) effectively improve sperm quality[54,55]. Nevertheless, the duration of motility of sperm was strongly correlated with the water quality in ponds. In this study, the water quality parameters in spawning ponds included an alkalinity of 50.5 to 52.5 mg/L and hardness 65.5 to 67.5 mg/L, a pH between 6.4 and 6.6 and water temperature

**Commented [SL15]:** Time of what?

**Commented [S16R15]:** Has been revised

**Commented [SL17]:** Please rephrase/clarify

**Commented [S18R17]:** Has been revised

**Formatted:** Highlight

**Commented [SL19]:** Please specify: mg of what?

**Commented [S20R19]:** Has been added

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

between 28 and 30 °C. These water quality parameters were able to support the ability of the sperm to fertilized egg[21].

The sperm motility in sago giant gourami ranged from 68 to 75%, and duration of motility ranged from 43 to 61 sec. These results are consistent with *Genypterus blacodes* and *Esox lucius*[56,50]. Sperm motility includes the percentage of motile sperm, straight line velocity, curvi linear velocity, average path velocity and linearity[50]. In this study, we did not investigate those parameters. In addition, the percentage of motile sperm is influenced by addition of extenders and cryoprotectants[57,58,59]. However, sperm motility from fresh semen was slightly greater compared to cryopreserved semen from *Esox lucius*[50]. The fertility rate of eggs ranged from 76 and 84%; however, no significant correlation was detected between fertility rate and sperm parameters, such as semen volume, semen pH, motility and duration of motility. Conversely, the sperm concentration was moderately correlated with sperm motility and duration of motility. The parameters commonly measured to assess sperm quality in brood were volume, density and motility (such as the percentage of motile sperm, straight line velocity curvilinear velocity, average path velocity, linearity and amplitude of lateral head displacement), including fertilizing capacity [49,50,52,60]. In this study, we did not investigate the ionic composition of the semen, but this phenomenon could be related to the ionic composition of semen which might has a significant influence on sperm motility and duration of motility.

**Conclusions**

This research analyzed the reproductive characteristics of giant gourami sago strain broodfish reared in concrete freshwater ponds, in the Aquaculture Laboratory Faculty of Fisheries and Marine Science, Universitas Bung Hatta. Relative fecundities of the giant gourami sago strain broodfish ranged from 977 to 1071 eggs, and egg diameter ranged from 2.32 to 2.46 mm. Semen volume ranged from 0.4 to 0.7 ml per kg body weight and sperm motility was comprised between 68 to 75%. A strong linear relationship was observed between absolute fecundity and female fish weight before and after spawning. Similarly, strongly positive correlation was observed between survival rate (10 days) and hatching rate. The sperm concentration was also moderately positive correlated with the motility and duration of sperm motility. Keys to increase reproduction performance in gourami sago strain- depends on broodfish weight, relative fecundity and hatching rates. Although data on the reproductive characteristics of gourami sago strain broodfish have been obtained, there are still knowledge gaps in feeding technologies and larval weaning during rearing. Therefore, for successful

**Commented [SL21]:** Do you have a reference to support this?

**Commented [S22R21]:** Has been added

**Commented [SL23]:** Please note this is the syntax seen in the literature

**Commented [S24R23]:** Has been revised

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [SL25]:** Please clarify: which are correlated together? Are they all correlated to each other (concentration with motility, concentration with duration of motility, motility and duration of motility)?

**Commented [S26R25]:** Has been revised

**Formatted:** Highlight

**Commented [SL27]:** Please note edit here is for correct grammar and syntax

**Commented [S28R27]:** Ok

**Formatted:** Highlight

practices in hatcheries, further research is recommended to determine a proper feed formulation and the development of appropriate aquaculture systems.

**Data availability**

*Underlying data*

Figshare: Reproduction characterization of the gurami sago (*Osphronemus goramy* Lacepède, 1801): basic knowledge for a hatchery development strategy for the future.

https://doi.org/10.6084/m9.figshare.14661189.v3 [61].

This project contains the following underlying data:

- Table 1. Raw data of fish length, weight, absolute fecundity and relative fecundity of gurami sago broodfish
- Table 2. Raw data of egg diameter (mm) in sago strain of giant gourami broodfish
- Table 3. Raw data of hardened egg diameter (mm) in sago strain of giant gourami broodfish
- Table 4. Raw data of egg diameter increase (%) in sago strain of giant gourami broodfish
- Table 5. Raw data of egg weight (mg) in sago strain of giant gourami broodfish
- Table 6. Raw data of hardened egg weight (mg) in sago strain of giant gourami broodfish
- Table 7. The data of egg weight increase (%) in sago strain of giant gourami broodfish
- Table 8. The data of fertilization rate (%) in sago strain of giant gourami broodfish
- Table 9. The data of hatching rate (%), endogenous feeding period (day) and embryo survival rate to eyed-egg stage (%) and in sago strain gourami
- Table 10. Male size, gonadal weight and semen in sago strain of giant gourami broodfish
- Table 11. Sperm concentration ($10^9$/mL) in sago strain of giant gourami broodfish
- Table 12. Sperm Motility (%) in sago strain of giant gourami broodfish
- Table 13. Duration motility (sec) in sago strain of giant gourami broodfish

Formatted: Font: 10 pt, Highlight

Formatted: Font: 10 pt, Highlight



**Competing interests**

No competing interests were disclosed.

**Grant information**

This study was funded by a competitive grants scheme called the Professor 2021. Contract number: 06.02.1.46.03.2021

**Acknowledgements**

The authors thank for Professor. Dr. Tafdil Husni the Rector of Universitas Bung Hatta for supporting this study through the competitive grant's schema called Research Professor, 2021. The appreciation goes to all of the students (Puji Kurniawan and Ranji Rinaldi and Muhammad Vajri Djauhari) who helped the author during data collection in the field.

**Commented [SL29]:** Please ensure you have obtained consent from these people to be named in your manuscript

**Commented [S30R29]:** they have agreed to have their names written on the Acknowledgements

**References**

1. Syandri H, Azrita, Mardiah A.: Nitrogen and phosphorus waste production from different fish species cultured at floating net cages in Lake Maninjau, Indonesia. *Asian J. Sci. Res*., 2018;11 (2): 287-294.
2. Aryani N, Suharman I, Azrita, *et al*.: Diversity and distribution of fish fauna of upstream and downstream areas at Koto Panjang Reservoir, Riau Province, Indonesia. F1000Research, 2020; 8:1435. Publisher Full Text
3. Mungkung R, Aubin J, Prihadi TH, *et al*.: Life Cycle Assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. *J. Clean. Prod*, 2013; 47: 249-256. Publisher Full Text
4. Pouil S, Samsudin R, Slembrouck J, *et al*.: Nutrient budgets in a small-scale freshwater fish pond system in Indonesia. *Aquaculture*, 2019; 504:267–274. Publisher Full Text
5. FAO, The State of World Fisheries and Aquaculture: Meeting the Sustainable Development Goals. *Food and Agriculture Organization of the United Nations*, Rome.2018.
6. CDSI (Central Data System Information). Ministry of Marine and Fisheries Republic of Indonesia, 2018 (In Indonesian). Reference Source
7. Azrita, Aryani N, Mardiah A, *et al*.: Growth, production and feed conversion performance of the gurami sago (*Osphronemus goramy* Lacepède, 1801) strain in different aquaculture systems. *F1000Research* 2020, 9:161. Publisher Full Text
8. Arifin OZ, Slembrouck J, Subagja J, *et al*.: New insights into giant gourami (*Osphronemus goramy*) reproductive biology and egg production control. *Aquaculture*, 2019; 519:734743
9. Ahmad N, Thompson S.: The blue dimensions of aquaculture: A global synthesis. *Sci. Total Environ*. 2019; 652: 851–861. Publisher Full Text

10. Ranjan R, Megarajan S, Xavier B, *et al*.: Broodstock development, induced breeding and larval rearing of Indian pompano, *Trachinotus mookalee* (Cuvier, 1832) – A new candidate species for aquaculture. *Aquaculture*, 2018; 495: 550–557. Publisher Full Text

11. Henriksson, PJG, Nhuong T, Chadag VM, *et al*.: Indonesian aquaculture futures-evaluating environmental and socioeconomic potentials and limitations.2017; J. Cleaner Prod., 162: 1482-1490. Publisher Full Text

12. Tran N, Rodriguez UP, Chan CY, *et al*.: Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. Marine Policy, 2017; 79, 25–32. Publisher Full Text

13. Aryani N, Azrita, Mardiah A, *et al*.: Influence of feeding rate on the growth, feed efficiency and carcass composition of the Giant gourami (*Osphronemus goramy*). *Pakistan J. Zool*. 2017; 49(5): 1775-1781. Reference Source

14. Nugroho E, Azrita, Syandri H, *et al*.: DNA barcoding of giant gourami (*Osphronemus goramy*) from West Sumatra, Indonesia. *AACL Bioflux*, 2019, 12, (4): 1074-1079. Reference Source

15. Slembrouck J, Arifin OZ, Pouil S, *et al*.: Seasonal variation of giant gourami (*Osphronemus goramy*) spawning activity and egg production in aquaculture ponds. *Aquaculture*,2020; 735450. PubMed Abstract Publisher Full Text

16. Radona D, Nafiqoh N.: Reproductive Characteristics and Heterosis value of Bastar and Bluesafir population of giant gourami crosses. *Berita Biologi*, 2014: 13(2):153-159 (in Indonesian). Reference Source

17. Azrita, Syandri H.: Effects of salinity on survival and growth of gurami sago (*Osphronemus goramy*, Lacepède, 1801) Juveniles. *Pak. J. Biol. Sci*., 2018; 21 (4): 171-178. Reference Source

18. Nugroho E, Azrita, Syandri H, *et al*.: Evaluation of genetic divergence of kalui fish (*Osphronemus goramy*) strains from West Sumatra revealed by random amplified polymorphism DNA (RAPD) marker. *Jurnal Riset Akuakultur*, 2016; 11 (4), 313-319 (in Indonesian). Reference Source

19. CDSI (Central Data System Information). Ministry of Marine and Fisheries, Republic of Indonesia, 2018 (In Indonesian). Reference Source.

20. Kanyilaz M.: Reproductive performance of a newly described Salmonid fish, Alakir Trout (*Salmo Kottelati*), a candidate species for aquaculture. *Pak. J. Zool*. 2016; 48(1): 83–89. Reference Source

21. Aryani A, Suharman I, Syandri H.: Reproductive performance of asian catfish (*Hemibagrus wyckii* Bleeker, 1858), a candidate species for aquaculture. *F1000Research* 2018, 7:683 Publisher Full Text

22. Aruho C, Walakira JK, Rutaisire J.: An overview of domestication potential of *Barbus altianalis* (Boulenger, 1900) in Uganda. *Aquac. Rep.*, 2018; 11, 31–37. Publisher Full Text

23. Osure GO, Phelps RP.: Evaluation of reproductive performance and early growth of four strains of Nile tilapia (*Oreochromis niloticus*, L) with different histories of domestication. *Aquaculture*, 2006; 253(1-4): 485–494. Publisher Full Text

24. Krejszeff S, Katarzyna T, Daniel Z, *et al.*: Domestication affect spawning of the ide (*Leuciscus idus*)-preliminary study. *Aquaculture*. 2009; 295(1–2): 145–147. Publisher Full Text

25. Weber RA, Peleteiro JB, García Martín LO, *et al*.: The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). *Aquaculture*. 2009; 288(1–2): 147–150. Publisher Full Text

26. Rurangwa E, Kime D, Ollevier F, *et al*.: The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 2004; 234(1-4): 1-28. Publisher Full Text

27. Cabrita E, Martínez-Páramo S, Gavaia PJ, *et al*.: Factors enhancing fish sperm quality and emerging tools for sperm analysis. *Aquaculture*, 2014; 432, 389–401. Publisher Full Text.

28. Mylonas CC, Duncan NJ, Asturiano JF.: Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. *Aquaculture*, 2017: 472, 21–44. Publisher Full Text.

29. Rice EW, Baird RB, Eaton AD, *et al.*: Standard methods for the examination of water and wastewater, 22nd ed**.** American Public Health Association, American Water Works Association, Water Environment Federation. 2012. Reference Source

30. Amornsakun T, Kullai S, Hassan A.: Some aspects in early life stage of giant gourami, *Osphronemus goramy* (Lacepede) larvae. *Songklanakarin J. Sci. Technol*, 2014; 36 (5): 493 – 498. Reference Source

31. Bari Y.: Addition of vitamin E to artificial feed in an effort to increase the reproductive potential of giant gourami (*Osphronemus goramy* Lacepede) broodstock. Thesis of

Postgraduate, Bogor Agricultural University (unpublish, in Indonesian), 1997. Reference Source

32. Izquierdo M, Fernández-Palacios H, Tacon AG.: Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 2001; 197(1-4): 25–42. Publisher Full Text

33. Chong ASC, Ishak SD, Osman Z, *et al*.: Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture*, 2004; 234: 381–392. Publisher Full Text.

34. Hafeez-ur-Rehman M, Abbas F, Ashraf M, *et al*.: Effect of different dietary protein levels on egg development and its response to inducing agents during induced spawning of *Channa marulius*. *Pakistan J. Zool*., 2017: 49 (1):337 – 343. Reference Source

35. Sarih S, Djellata A, Roo J, *et al*.: Effects of increased protein, histidine and taurine dietary levels on egg quality of greater amberjack (*Seriola dumerili*, Risso, 1810). *Aquaculture*, 2018; 499:72-79. Publisher Full Text

36. Jeuthe H, Brännäs E, Nilsson J.: Effects of egg size, maternal age and temperature on egg, viability of farmed Arctic charr. *Aquaculture*, 2013; 408-409, 70–77. Publisher Full Text

37. Stuart KR, Armbruster L, Johnson R, *et al*.: Egg diameter as a predictor for egg quality of California yellowtail (*Seriola dorsalis*). *Aquaculture*, 2020; 522: 735154. PubMed Abstract | Publisher Full Text.

38. Grant B, Davie A, Taggart JB, *et al*.: Seasonal changes in broodstock spawning performance and egg quality in ballan wrasse (*Labrus bergylta*). *Aquaculture*, 2016; 464: 505–514. Publisher Full Text.

39. Lahnsteiner F, Patzner RA.: Rainbow trout egg quality determination by the relative weight increase during hardening: a practical standardization. *J. Appl. Ichthyol*. 2002; 8(1):24-26. Reference Source

40. Sink TD, Lochmann RT, Pohlenz C, *et al*.: Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. *Aquaculture*, 2010; 298(3-4): 251– 259. Publisher Full Text.

41. Morehead D, Hart P, Dunstan G, *et al*.: Differences in egg quality between wild striped trumpeter (*Latris lineata*) and captive striped trumpeter that were fed different diets. *Aquaculture*, 2001; 192 (1): 39–53. Publisher Full Text.

42. Mateos J, Mañanos E, Carrillo M, *et al*.: Regulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) gene expression by gonadotropin-releasing hormone (GnRH) and sexual steroids in the Mediterranean Sea bass. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2002; 132(1): 75–86. Publisher Full Text

43. Paullada-Salmerón JA, Cowan ME, Loentgen GH, *et al*.: The gonadotropin-inhibitory hormone system of fish: The case of sea bass (*Dicentrarchus labrax*). *Gen. Comp. Endocrinol.,* 2019; 279: 184-195. Publisher Full Text.

44. Lubzens E, Young G, Bobe J, *et al*.: Oogenesis in teleosts: How fish eggs are formed. *Gen. Comp. Endocrinol.*, 2010; 165 (3): 367–389. Publisher Full Text.

45. Lubzens E, Bobe J, Young G, *et al*.: Maternal investment in fish oocytes and eggs: The molecular cargo and its contributions to fertility and early development. *Aquaculture*, 2017; 472: 107–143. Publisher Full Text.

46. Hafeez-ur-Rehman M, Abbas F, Ashraf M *et al*.: Effect of Different Dietary Protein Levels on Egg Development and its Response to Inducing Agents during Induced Spawning of *Channa marulis*, *Pakistan J. Zool.*, 2017; 49 (1):337-343. Reference Source.

47. Aryani N, Suharman I.: Effect of dietary protein level on the reproductive performance of female of green catfish (*Hemibagrus nemurus* Bagridae). *J Aquac Res Dev*., 2015; 6:11. Reference Source

48. Collazos-Lasso LF, Mariana CGE, Elisabeth AB.: Induced reproduction of the sailfin pleco, *Pterygoplichthys gibbiceps* (Kner, 1854) (Pisces: Loricariidae). *AACL Bioflux*, 2018, 11 (3): 724-729. Reference Source

49. Caldas JS, Godoy L.: Sperm characterization of the endangered Amazonian fish *Hypancistrus zebra*: basic knowledge for reproduction and conservation strategies. *Anim. Reprod. Sci.*, 2019; 204: 117-124. Publisher Full Text.

50. Dietrich GJ, Nynca J, Szczepkowski M, *et al*.: The effect of cryopreservation of semen from whitefish (*Coregonus lavaretus*) and northern pike (*Esox lucius*) using a glucose-methanol extender on sperm motility parameters and fertilizing ability. *Aquaculture*, 2016; 464: 60–64. Publisher Full Text.

51. Butt IAE, Litvak MK, Trippel EA.: Seasonal variations in seminal plasma and sperm characteristics of wild-caught and cultivated Atlantic cod, *Gadus morhua*. *Theriogenology*, 2010; 73(7): 873–885. Publisher Full Text.

52. Abinawanto, Intan AP, Retno L.: Sperm motility of giant gourami (*Osphronemus goramy*, Lacepede, 1801) at several concentrations of honey combined with DMSO after short-term storage. *AACL Bioflux*, 2017, 10 (2): 156-163. Reference Source

53. Valdebenito II, Gallegos PC, Effer BR.: Gamete quality in fish: evaluation parameters and determining factors. *Zygote*, 2013; 23(02): 177–197. Publisher Full Text.

54. Cejko BI, Krejszeff S, Żarski D, *et al*.: Effect of carp pituitary homogenate (CPH) and sGnRHa (Ovaprim) on northern pike (*Esox lucius*) spermiation stimulation and its effect on quantity and quality of sperm. *Anim. Reprod. Sci.*, 2018; 193: 217–225. Publisher Full Text.

55. Zadmajid V.: Comparative effects of human chorionic gonadotropin (hCG) and Ovaprim™ (sGnRHa+domperidone) on the reproductive characteristics of wild-caught male Longspine scraper, *Capoeta trutta* (Heckel, 1843). *Aquaculture*,2016; 463: 7–15. Publisher Full Text

56. Dumorné K, Valdebenito I, Contreras P, *et al*.: Effect of pH, osmolality and temperature on sperm motility of pink cusk-eel (*Genypterus blacodes*, (Forster, 1801)). *Aquac. Rep*., 2018; 11: 42-46. Publisher Full Text.

57. Golshahi K, Aramli MS, Nazari RM, *et al*.: Disaccharide supplementation of extenders is an effective means of improving the cryopreservation of semen in sturgeon. *Aquaculture*, 2018; 486: 261–265. Publisher Full Text.

58. Yusoff M, Hassan BN, Ikhwanuddin M, *et al*.: Successful sperm cryopreservation of the brown-marbled grouper, Epinephelus fuscoguttatus using propylene glycol as cryoprotectant. *Cryobiology*, 2018; 81: 168–173. Publisher Full Text

59. Kommisrud E, Myromslien FD, Stenseth EB, *et al*.: Viability, motility, ATP content and fertilizing potential of sperm from Atlantic salmon (*Salmo salar* L.) in milt stored before cryopreservation. *Theriogenology*. 2020; 151: 58-65. Publisher Full Text

60. Cejko BI, Żarski D, Palińska-Żarska K, *et al*.: Artificial seminal plasma improves motility and fertilization capacity of common carp *Cyprinus carpio* L. sperm during one hour of storage. *Aquaculture*, 2019; 506: 224-228. Publisher Full Text.

61. Syandri, Hafrijal; Azrita, Azrita; Aryani, Netti (2021): Untitled ItemReproduction characterization of the gurami sago (Osphronemus goramy Lacepède, 1801): basic knowledge for a hatchery development strategy for the future. figshare. Journal contribution. https://doi.org/10.6084/m9.figshare.14661189.v3

**Reproductive characteristics of the giant gourami sago strain broodfish (*Osphronemus goramy* Lacepède, 1801): basic knowledge for a future hatchery strategy development**

Azrita[1*], Hafrijal Syandri[2], Netti Aryani[3]

[1]Department of Biology Education, Faculty of Education, Universitas Bung Hatta, Padang, West Sumatera 25133, Indonesia

[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang, West Sumatera 25133, Indonesia

[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, Riau 28293, Indonesia

*Corresponding Author: azrita31@bunghatta.ac.id

**Commented [S1]:** To changes Corresponding Author azrita31@bunghatta.ac.id

**Abstract**

**Background:** The giant gourami sago strain (*Osphronemus goramy* Lacepède) ~~has been~~was approved in 2018 as a candidate for freshwater aquaculture in Indonesia. However, information on the species' reproduction is minimal. This study analyzed the reproduction characteristics of the gourami sago strain broodfish to provide basic knowledge for a hatchery development strategy in the future.

**Methods:** A total of 10 female and ten male gourami sago strain broodfish with mature oocytes were measured for body weight and length~~,~~ and were evaluated for their reproductive characteristics. Breeding fish were spawned naturally in a 2×1×0.6 m concrete pond with a male-female sex ratio of 1:1. Egg weight and diameter were measured in 25 eggs per female using ACIS AD- 600i scales with 0.01 g accuracy and a microscope (Labo model L-711) using Canon Digital Camera Software 3 . Semen was collected using plastic syringes in 3 mL aliquots, then placed in an insulated ice-cooled container, and analyzed within 2 hours of collection.

**Results:** Average weights of female and male broodfish before spawning were 2180±159.78 g and 3060±134.99 g, respectively. The relative fecundity and egg diameter were 1029±36 eggs $kg^{-1}$ and 2.42±0.05 mm, respectively. The hatching rate and embryo survival to an eyed-egg stage were respectively 76.40±2.27% and 94.76±0.42%. Sperm characteristics showed that volume was 0.60±0.12 ml $kg^{-1}$ and percentage motile was 70.04±2.27%. Female broodfish weight after spawning was strongly correlated with the weight before spawned ($r^2$ = 0.999) and absolute fecundity was also strongly correlated with female broodfish weight before spawning ($r^2$= 0.921). Sperm concentration was a moderately correlated with sperm motility ($r^2$ = 0.556) and duration of sperm motility ($r^2$ = 0.502).

**Conclusions:** The gourami sago strain broodfish has suitable reproductive characteristics for the development of future hatcheries. Successful natural spawning should be followed by larval weaning and feeding technology to increase growth and survival.

**Keywords**: Aquaculture, giant gourami, broodfish, egg, sperm, hatchery performance.

**Introduction**

Freshwater aquaculture practiced in inland waters such as lakes, rivers, reservoirs, floodplains, and oxbow lakes, and freshwater ponds have expanded during the last decades in Indonesia[1,2,3,4,5]. Approximately 77.57% of fish produced in freshwater aquaculture in Indonesia are sourced from freshwater ponds and inland waters[6]. However, their development depends upon many factors, such as fish species, aquaculture systems, water depletion, fish diseases, farmers' knowledge, and aquaculture practices[7,4,8,9,10].

Freshwater aquaculture is one of the fastest-growing aquacultures in Indonesia, with more than 3,378,298.92 metric tons produced in 2018[6,11,12]. Nile tilapia (*Oreochromis niloticus)* contributed 37.93% of the total aquaculture production, African catfish (*Clarias gariepinus*) 33.35%, *Pangasius* catfish (*Pangasius hypophthalmus*) 12.38%, common carp (*Cyprinus carpio*) 9.28%, and giant gourami (*Osphronemus goramy*) 6.96% [13,14,15].

Indonesian giant gourami strains include the local "tambago", "palapa", "soang", "galunggung" and "blusafir" strains, which have been grown semi-intensively in small-scale farms for decades[8,14,13,16]. However, they have not been able to contribute majorly to freshwater aquaculture production in Indonesia. Sago gourami strain that has been approved as aquaculture candidate is limited in distribution in the West Sumatra Province of Indonesia[17,18]. Therefore their hatchery is very importantly developed to be able to contribute to the production of freshwater aquaculture. The gourami sago strain is considered to support food security along with many other freshwater fish species in Indonesia.

The gourami sago strain was approved as a candidate for freshwater aquaculture in 2018 (Decree of the Ministry of Marine and Fisheries, Republic of Indonesia No.56/KEPMEN-KP/2018)[19]. However, data on its reproductive characteristics are still limited. The evaluation of reproductive performance in other fish species has had beneficial impacts in the development of freshwater aquaculture in Asia[20,21,22,23,24]. In contrast, there are still gaps in knowledge of giant gourami sago strain broodfish regarding size at oocyte maturity, age of sexual maturity, sperm characteristics, egg hatchability, survival after eyed-egg stage, larva weaning, and growth rate. These factors were identified as key

**Commented [SL2]:** Please rephrase – meaning still unclear

**Commented [S3]:** Has been revised

**Commented [SL4]:** It is unclear whether you mean short life span, or not often seen in West Sumatra: please see edit and confirm/modify accordingly

**Commented [S5]:** Has been revised

challenges for successful giant gourami sago strain hatchery performance in the future. Therefore, the present study was conducted to evaluate the reproductive characteristics in giant gourami sago strains to provide basic knowledge for hatchery development in the future.

## Methods

### *Ethical considerations*

There are no required permits from the government of the Republic of Indonesia to evaluate reproductive characteristics in gourami sago strain broodfish (*Osphronemus goramy*) as a candidate for future aquaculture. The study was funded by Research, and Community Service Universitas Bung Hatta under a competitive grants scheme called the research of Professor in 2021 (Contract number: 06.02.1.46.03.2021). This grant included ethical approval and permits to collect fish specimens, rear and spawn giant gourami sago strains in the Aquaculture Laboratory Faculty of Fisheries and Marine Science Universitas Bung Hatta facilities. There was no animal suffering involved in this study and gourami sago strain broodfish were still in good condition when returned to the pond. Ethical approval was granted by the Ethics Commission for Research and Community service at Universitas Bung Hatta (023/LPPM/Hatta/I-2021).

### *Rearing and selection of breeders*

Juvenile fishes were selected about six years ago from a local hatchery in Luhak District, Lima Puluh Kota Regency, West Sumatra Province. The juvenile fish were kept in tanks and transported by truck to the Aquaculture Laboratory, Faculty of Fisheries, and the Marine Science University of Bung Hatta. A total of 200 individuals giant gourami sago strain juvenile fishes were reared for five years to increase sexual maturity, which was reared in an 8.4 m3 (6×2×0.7 m) concrete freshwater pond. During the rearing of the fishes to reach sexual maturity, the juvenile fish were fed with commercial fish pellets (781-2 with 30% crude protein content and 4% crude fat; PT Japfa Comfeed Indonesia Tbk).

After the giant gourami sago was reared for five years, 20 mature individuals broodfish were separated according to sex and reared in 18 $m^3$ (6×2×1.5 m) two concrete freshwater ponds for 60 days. The broodfish were fed twice daily (09:00 AM and 16:00), with extruded feed pellets containing 39.50% crude protein and 12.21% fat with a predetermined quantity of 3% of fish weight per day. Besides that, the fish were given sente leaves (*Alocasia macrorriza* L.) at amounts of 1% of fish wet weight per day. The leaves contain 2.85% protein and 0.47% crude fat in wet weight). Each concrete freshwater pond had a 50 mm drain in the middle, which was covered with a net of 0.5 cm

**Commented [SL6]:** How long between rearing and selection of mature fish?

**Commented [S7]:** Has been revised

**Commented [SL8]:** As you wrote 20 before (10 M 10 F), please confirm the number and check underlying data

**Commented [S9]:** Has been check underlying data and revised

**Commented [SL10]:** Please specify – this is contained in what?

**Commented [S11]:** Has been revised

mesh size to prevent the fish from escaping and predators from entering. Water was pumped from borehole wells at a rate of 5 liters per minute.

A total of 20 broodfish with mature oocytes were selected, consisting of 10 females and 10 males. Before spawning, female and male broodfish were weighed using scales (OHAUS model CT 6000-USA with 0.1 g accuracy), and body length was measured using a meter ruler with 1mm accuracy. The average weight and length of the 10 female broodfish were 2140±159 g and 39.70±1.77 cm, while those of the male broodfish were 3060±135 g and 43.1±1.79 cm.

Reproductive parameters in gourami sago strain broodfish were analyzed using the following formulae:

- Condition factor (CF) = wet weight in gram/length$^3$ × 100
- Ovulated egg weight (g) = fish weight before spawning (g) – fish weight after spawning (g)
- Ova somatic index (%) = egg weight ovulation (g)/ fish weight before spawning (g) x 100

Absolute fecundity was the total number of eggs estimated per nest, and relative fecundity was the total number of eggs per kg body weight.

***Female reproductive performance***

Starting in August 2020 onwards, the broodfish were checked monthly for eggs and semen production. The broodfish were captured with a hand net and anesthetized by oral ingestion of Tricaine methanesulfonate (MS-222, ethyl 4-aminobenzoate methanesulfonate 98%, Sigma Aldrich Co, USA, MO; 50 mg L$^{-1}$), based on the dosage used for *Hemibagrus wyckii*[21]. Oocyte maturation was assessed for each individual. The oocyte maturity in giant gourami females was assessed from oocytes sampled by intraovarian biopsies using a flexible polyethylene catheter[21]. Egg diameter was measured using Labo microscope model L-711 and Canon Digital Camera Software 3.

Natural spawning of broodfish was carried out in 1.2 m$^3$ (2×1×0.6 m) concrete freshwater ponds with a male-female sex ratio of 1:1. Before the broodfish spawned, the ponds were drained, cleaned, and all other species removed. Then, palm fibers were placed on top of a bamboo raft in the pond. The pond was then filled with water, and the female and male broodfish were released into the spawning pond. The male broodfish made a nest for 5 to 7 days, after which spawning took place and the female broodfish laid eggs. Spawning occurred in the afternoon (between 3.00 to 5.00 PM). Due to the presence of a very large oil globule, giant gourami eggs float[8]. After the broodfish had finished laying eggs, the eggs were kept by the female broodfish in the nest. After the eggs had been kept by the broodfish for four hours in the nest, the eggs were collected and transferred to an incubation tray, which was placed in a ventricular hatching system. A total of 100 eggs for each

broodfish were incubated in incubation trays. Meanwhile, the broodfish were returned to their pond once spawned, and no mortality occurred.

Egg weight and diameter were measured for 25 eggs per female using SHIMADZU-model AY 220 scales with 0.1 mg accuracy and a microscope (Labo model L-711) using Canon Digital Camera Software 3. A total of 25 eggs were randomly sampled 16 hours after spawning to determine the fertility rate (FR). The hatching rate (HR) was determined by counting all hatched fry 48 hours after spawning. Then, the endogenous feeding period of the fish larvae was counted until the egg yolks run out (in days), and embryo survival rate (%) to the eyed-egg stage was measured.

***Determination of sperm quality***

To stimulate the spermiation process in male broodfish, an LHRH-preparation ( Ovaprim, manufactured for Syndel Laboratories Ltd, 2595 Mccullough Rd Nanaimo B.C 9VS 4m9 Canada) was injected into male fish, with a dosage of 0.5 ml per kg of the brooder. Semen samples were obtained from 10 gourami sago broodfish randomly selected from the farm. The male broodfish were first anesthetized with 50 mg $L^{-1}$ of MS-222[25], then fish weights (MaW) and total lengths (MaL) were measured. Special care was taken to avoid any contamination of semen with urine, feces, mucus, and water. Semen samples were collected using plastic syringes in 3 mL aliquots and then placed in an insulated ice-cooled container, transported to the laboratory, and analyzed within two hours.

The sperm assessment included gross (visual) and microscopic examination as reviewed by Rurangwa *et al.*[26] and Cabrita *et al.*,[27]. The gross examination was based on visual and physical observation of parameters like semen volume, semen pH, sperm concentration, motility, and duration motility. Semen volume was determined by collecting the semen in a graduated cylinder. Semen pH was determined with a hand pH meter (HI8424 Hanna Instrumen, USA). Microscopic examination was carried out using the Olympus model CX40, with × 10 and × 25 magnification, to determine parameters such as motility (MO) percentage and duration, by observing water-activated semen placed on a glass slide. Motile sperm were observed and expressed as a percent of non-moving sperm. Motility duration (DMO) was determined as the period between movements of the sperm to a cessation of any progress using Neubauer's hemocytometer and calculated as the number of sperm $ml^{-1}$ [28]. Semen pH was determined with a hand pH meter (HI8424 Hanna Instruments, USA).

***Water quality***

Water samples were collected in the spawning pond and incubation trays to determine alkalinity, hardness, and pH. The protocol for determining alkalinity by standard methodology is presented by Rice *et al.*, 2012[29]. pH values were determined with a pH meter (digital mini pH meter, 14pH, IQ

Scientific, Chemo-science Thailand Co., Ltd, Thailand). An oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA) was used *in situ*, and water temperature in the spawning pond and incubation trays were measured with a thermometer (Celsius scale).

***Statistical analysis***

Results were given as the mean values (± SD). Simple linear regression analyses were performed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL). The standard deviation of each parameter was determined. For linear regression analyses, correlations were considered significant at $p<0.05$, and trends or tendencies were considered significant at $p<0.05$.

**Results**

The reproductive characteristics of female broodfish in sago giant gourami are summarized in Table 1. The total number of eggs per nest (absolute fecundity) varied from 2000 to 2650, while relative fecundity (total number of eggs per kg female brooder) varied between 977 and 1071. The fertility rate ranged from 76 to 84%, and the hatching success rate ranged from 72 to 80%. Endogenous feeding period ranged from 10 to 12 days, and embryo survival rate to eyed-egg stage varied between 94.73 and 95%.

Reproductive characteristics for male broodfish and sperm samples are presented in Table 2. The average live weight of the males was 3340±275.68 g. Sago giant gourami male broodfish were found to be slightly bigger than female broodfish. Gonad weight ranged from 25 to 30 g, whereas gonad somatic index ranged from 0.83 to 0.93%.

The linear correlation ($r^2$) between variables of reproduction characterization parameters in sago strain of giant gourami females broodfish results are shown in Table 3. In this study, the reproductive parameters that showed strong correlations with the absolute fecundity were female fish weight before spawning ($r^2$ = 0.921) and female fish weight after spawning ($r^2$ = 0.864). Similarly, results revealed significant correlations between egg diameter and hardened egg diameter ($r^2$ = 0.833), and between egg diameter and percentage of the hardened diameter ($r^2$ = 0.699). Meanwhile, egg diameter and fertility rate were moderately correlated ($r^2$ = 0.568). In contrast, the egg diameter was not strongly related to absolute fecundity ($r^2$ = 0.169) and relative fecundity ($r^2$= 0.096). On the other hand, the survival rate of larvae (10 days) also had strong correlations with the hatching rate ($r^2$ = 0.998) and endogenous feeding period ($r^2$ = 0.757).

Linear correlation analysis results ($r^2$) between variables of reproduction characterization parameters in sago giant gourami male broodfish are shown in Table 4. The reproductive

**Commented [SL12]:** Please rephrase as this is still unclear – percentage of what?

**Commented [S13]:** Has been revised

**Commented [SL14]:** Please note this is still unclear

**Commented [S15]:** Has been revised

parameters that had a strong correlation with gonad weight were somatic index of gonads ($r^2$ = 0.836), while semen volume ($r^2$ = 0.521), semen pH ($r^2$ = 0.521) and sperm concentration ($r^2$ = 0.506) were moderately correlated with gonad weight. In contrast, the gonadal weight negatively correlated with sperm motility ($r^2$ = 0.017) and duration of motility ($r^2$ = 0.275). In addition, the sperm concentration was also moderately correlated with the sperm motility ($r^2$= 0.556) and duration of motility ($r^2$= 0.502).

The Physico-chemical water quality parameters in the spawning ponds and incubation trays for embryo development were as follows: water alkalinity ranged from 50.5 mg $L^{-1}$ to 52.5 mg $L^{-1}$, hardness varied from 65.5 mg $L^{-1}$ to 67.5 mg $L^{-1}$, pH ranged from 6.4 to 6.6, oxygen ranged from 6.1 mg $L^{-1}$ to 6.7 mg $L^{-1}$, and temperature varied from 28°C to 30°C.

**Table 1. Reproduction characterization in sago strain of giant gourami females broodfish (Mean ± SD)**

| | Variables | Range (Min-Max) |
|---|---|---|
| Fish length (cm) | 39.70±1.77 | 38-43 |
| Fish weight before spawning (g) | 2140±159.78 | 1958-2500 |
| Fish weight after spawning (g) | 2108±157.64 | 1930-2465 |
| Condition factor | 3.30±0.42 | 2.54-3.86 |
| Egg weight ovulated (g) | 32.80±2.86 | 28-38 |
| Ova somatic index (%) | 1.55±0.07 | 1.43-1.65 |
| Absolute fecundity (egg/fish) | 2205±201 | 2000-2650 |
| Relative fecundity (egg/kg body weight) | 1029±36 | 977-1071 |
| Egg diameter (mm) | 2.42±0.05 | 2.32-2.46 |
| Hardened egg diameter (mm) | 3.42±0.02 | 3.40-3.45 |
| Egg diameter increase (%) | 29.63±1.43 | 27.8-32.14 |
| Egg weight (mg) | 10.33±1.09 | 9.02-12.20 |
| Hardened egg weight (mg) | 13.36±1.27 | 11.74-15.20 |
| Hardened egg weight increase (%) | 22.69±2.24 | 19.74-24.81 |
| Fertility rate (%) | 81.60±3.37 | 76-84 |

| | | |
|---|---|---|
| Hatching rate (%) | 76.40±2.27 | 72-80 |
| Endogenous feeding period *(*day) | 11.2±0.63 | 10-12 |
| Embryo survival rate to the eyed-egg stage (%) | 94.76±0.42 | 94.73-95 |

**Table 2. Reproduction characterization in sago strain of giant gourami males broodfish (Mean ± SD)**

| | Variables | Range (Min-Max) |
|---|---|---|
| Fish weight (g) | 3060±134.99 | 2800-3200 |
| Fish length (cm) | 43.1±1.79 | 40- 5 |
| Condition factor | 3.74±0.43 | 3.08-4.38 |
| Gonads weight (g) | 27.5±1.72 | 25-30 |
| Gonadosomatic index (%) | 0.90±0.03 | 0.83-0.94 |
| Semen volume (mL per kg body weight) | 0.60±0.12 | 0.4-0.7 |
| Semen pH | 8.18±0.15 | 7.9-8.4 |
| Sperm concentration ($10^9$/mL) | 1.44±0.14 | 1.2-1.6 |
| Motility (%) | 70.04±2.27 | 68-75 |
| Duration of motility (sec) | 50.2±7.25 | 43-61 |

**Table 3. Correlations of variables ($r^2$) in sago strain of giant gourami females broodfish**

| | FEL | FWBS | FWAS | CF | OEW | OVI | AF | RF | EW | HEW | EWI | ED | HED | HDI | FR | HR | EFP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FEL | | | | | | | | | | | | | | | | | |
| FWBS | **0.720** | | | | | | | | | | | | | | | | |
| FWAS | **0.717** | **0.999** | | | | | | | | | | | | | | | |
| CF | **0.757** | **0.575** | **0.574** | | | | | | | | | | | | | | |
| OEW | **0.539** | **0.565** | **0.553** | 0.365 | | | | | | | | | | | | | |
| OVI | 0.281 | 0.191 | 0.012 | 0.000 | **0.774** | | | | | | | | | | | | |
| AF | 0.255 | **0.921** | **0.864** | **0.637** | 0.387 | 0.072 | | | | | | | | | | | |
| RF | 0.012 | 0.207 | 0.063 | 0.011 | 0.065 | 0.321 | **0.524** | | | | | | | | | | |
| EW | **0.894** | **0.659** | **0.655** | **0.677** | 0.552 | 0.246 | **0.567** | 0.041 | | | | | | | | | |
| HEW | **0.841** | **0.631** | **0.626** | **0.514** | **0.582** | 0.295 | 0.468 | 0.004 | **0.924** | | | | | | | | |
| EWI | 0.165 | 0.109 | 0.109 | 0.354 | 0.033 | 0.000 | 0.010 | 0.002 | **0.924** | 0.041 | | | | | | | |
| ED | 0.164 | 0.132 | 0.338 | 0.131 | 0.378 | **0.688** | 0.169 | 0.096 | 0.030 | 0.263 | 0.000 | | | | | | |
| HED | 0.064 | 0.029 | 0.237 | 0.126 | 0.025 | 0.207 | 0.022 | 0.064 | 0.468 | 0.184 | 0.030 | **0.833** | | | | | |
| HDI | 0.266 | 0.293 | 0.342 | 0.209 | 0.103 | 0.085 | 0.373 | 0.195 | 0.318 | 0.298 | 0.006 | **0.699** | 0.294 | | | | |
| FR | 0.026 | 0.229 | 0.020 | 0.000 | 0.135 | 0.264 | 0.000 | 0.004 | 0.067 | 0.004 | 0.160 | **0.568** | 0.064 | 0.054 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HR | 0.035 | 0.020 | 0.060 | 0.046 | 0.000 | 0.009 | 0.003 | 0.009 | 0.226 | 0.108 | 0.364 | 0.018 | 0.001 | 0.143 | **0.703** | | |
| EFP | 0.113 | 0.186 | 0.190 | 0.094 | 0.006 | 0.098 | 0.231 | 0.103 | **0.747** | **0.806** | 0.147 | 0.317 | 0.001 | 0.116 | 0.015 | 0.013 | |
| SR | 0.070 | 0.032 | 0.034 | 0.027 | 0.007 | 0.003 | 0.112 | 0.024 | 0.194 | 0.019 | 0.005 | 0.033 | 0.000 | 0.324 | 0.063 | **0.998** | **0.757** |

Statistically important at $r^2 > 0.500$ (underlined)

FeL: female fish length (cm); FWBS: female fish weight before spawning (g); FWAS: female fish weight after spawning (g); CF: condition factor; OEW: egg weight ovulation (g), OVI: Ova somatic index (%), AF: absolute fecundity (eggs), RF: relative fecundity (eggs), EW: egg weight (mg), HEW: Hardened egg weight (mg), EWI: eggs weight increase (%), ED: egg diameter (mm), HED: hardened egg diameter (mm), HDI: hardened egg diameter increase (%), FR: fertility rate (%), HR: hatching rate (%), EFP: endogenous feeding period (days), SR: survival rate (10 days).

**Table 4. Correlations of variables ($r^2$) in sago strain of giant gourami males broodfish**

| | MaL | MaW | CF | GW | GI | SV | pH | SC | MO |
|---|---|---|---|---|---|---|---|---|---|
| MaL | - | | | | | | | | |
| MaW | **0.714** | | | | | | | | |
| CF | **0.807** | 0.347 | | | | | | | |
| GW | 0.399 | **0.550** | 0.187 | | | | | | |
| GI | 0.003 | 0.042 | 0.071 | **0.836** | | | | | |
| SV | 0.025 | **0.576** | 0.042 | **0.521** | 0.000 | | | | |
| pH | **0.516** | **0.772** | 0.353 | **0.521** | 0.127 | 0.296 | | | |
| SC | 0.186 | **0.661** | 0.131 | **0.506** | 0.068 | 0.425 | **0.645** | | |
| MO | 0.068 | 0.453 | 0.061 | 0.017 | 0.130 | 0.393 | 0.280 | **0.556** | |
| DMO | 0.159 | 0.322 | 0.083 | 0.275 | 0.012 | 0.082 | 0.430 | **0.502** | **0.519** |

Statistically important at $r^2 > 0.500$ (underlined)

MaW: male fish weight (g), MaL: male fish length (g), CF: condition factor; GW: gonadal weight (g); GI: gonad somatic index (%), SV: semen volume (ml), SC: sperm concentration ($10^9$/mL), MO: motility (%), DMO: duration of motility (sec).

**Table 5. Summary of the fecundity, gonadal somatic index, egg diameter, and hatching rate of giant gourami.**

| Species | Strain | Relative fecundity (egg/kg fish) | GSI (%) | Eggs diameter (mm) | Hatching rate (%) | Reference |
|---|---|---|---|---|---|---|
| *Osphronemus goramy* | Sago | 1037±90 | 1.91±0.35 | 2.42±0.05 | 76.40±6.33 | This study |
| *Osphronemus goramy* | Bastar | 2423±348 | 2.78±1.16 | 2.2 ± 0.2 | 96.36± 2.30 | [16] |
| *Osphronemus goramy* | Galunggung | 4011±287 | 4.15±0.63 | 2.5±0.05 | 89.3±1.30 | [8] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Osphronemus goramy* | - | 5508±1547 | 2.32±0.50 | 2.18±0.19 | 61.60±0.0 | [30] |
| *Osphronemus goramy* | Tambago | 2.896±185 | 3.16±0.11 | 2.47±0.03 | 91.06±4.06 | [31] |

**Discussion**

In our study, body weight in female sago giant gourami broodfish before spawning ranged from 1958 to 2500 g per fish, and ova somatic index ranged from 1.43 to 1.65%. Bodyweight in female sago strain gourami broodfish was smaller than that of giant gourami belonging to the galunggung strain, which ranged from 2500 to 3500 g[8]. Conversely, the ova somatic index of galunggung strain is found to be slightly bigger than that of sago giant gourami, which ranged from 3.7 to 4.6%[8]. The differences in reproductive characteristics in broodfish can be explained by strains, brood size, age of broodfish, previous spawning history, and the production setting[23]. Absolute fecundity in sago giant gourami ranged from 2000 to 2650 eggs $fish^{-1}$ and relative fecundity (RF) ranged from 977 to 1071 eggs $kg^{-1}$. Egg produced in kg $fish^{-1}$ (RF) is thought to be more informative than absolute fecundity. RF in sago strain of giant gourami was smaller compared to those in galunggung strain, palapah strain, and blusafir strain[8,31,16]. On the other hand, the difference in relative fecundity can also be related to differences in broodfish size and age used[23]. Environmental factors such as rainfall also influenced the number of eggs per spawn in giant gourami brood, while the water temperature negatively related to the number of eggs per spawn[15]. Furthermore, egg diameter in sago strain of giant gourami is found to be almost the same as other strains of giant gourami (Table 5). In this study, egg diameters average was 2.42±0.05 mm, consistent with those reported by other researchers; 2.18±0.19 mm for the giant gourami[30], 2.40±0.05 mm for blusafir strain[16,] and 2.5±0.05 mm for galunggung strain[8]. The differences in the RF, ova somatic index, egg diameter, and hatching rate of giant gourami can be influenced by differences in the strains. Furthermore, egg diameter has been influenced by dietary protein level[32,33,34,35], age of broodfish[36], and spawning season[37,38]. In our study, egg diameter was shown to be positively correlated with egg weight, hardened egg weight, and egg weight increase. The egg weight of rainbow trout also increased after the hardening process and is positively correlated with the viability of eggs[39]. Other egg quality metrics, such as hatching rate, and survival to first feeding, have been correlated with good egg quality[21].

In this study, the hatching rate of the embryo in sago strain of giant gourami was smaller than those of other strains of giant gouramy[8,31,16]. This condition might be affected by the egg and sperm quality in giant gourami sago strain broodfish. In the present study, whether eggs and sperm quality

of sago giant gourami breeders are affected by feed type was poorly understood. Broodfish sex ratio did not influence egg quality[8]. The reproductive parameters that were strongly correlated with the hatching rate were fertility rate ($r^2$=0.703) and survival rate (10 days) ($r^2$= 0.998). According to Sink *et al*. [40,] the biochemical composition of broodfish eggs is strongly correlated with egg quality. In this study, we did not evaluate the biochemical composition of an egg, because the relationship between egg quality and biochemical composition is difficult to interpret[41].

The keys regulator hormones of fish reproduction are gonadotropins, follicle-stimulating hormone, luteinizing hormone, and sex steroids[42,43]. In addition, oocyte development and maturation are also regulated by locally acting paracrine and autocrine signaling [44,45]. However, there is no information about the effects of such factors on oocyte development in giant gourami sago. Still, the extrusion feed enriched with vitamin E (d-α-tocopherol) at concentrations of 137.8, 238.05, 338.72 and 439.39 mg per kg feed affected markers of reproductive functions of giant gourami broodfish, such as the sexual maturity cycles, ovum somatic index, relative fecundity, and egg diameter[31].

Various efforts have been made by scientists to increase the reproductive performance of female broodfish, such as increasing dietary protein levels for *Xiphophorus helleri*[33], *Channa marulius*[46,] and *Ictalurus punctatus*[40]. Additionally, implantation of 17ß-estradiol has also improved the reproductive performance in *Hemibagrus nemurus*[47]. Currently, whether the increase in the protein level of feed and use of hormones can increase the reproductive potential in sago giant gourami is poorly understood. Therefore, we recommend the use of proteins in feed (at levels of 25%, 30%, or 35%) and the addition of 17β-estradiol (for example 200, 400, or 600 µg/kg body weight) to increase the reproductive potential of giant gourami sago the future.

Average semen volume in sago giant gourami was lower (0.4 to 0.6 ml) than those of *Hemibagrus wyckii* (0.60 to 1.20 ml)[21], but higher than those of *Pterygoplichthys gibbiceps*[48]. It appears that the semen volume depends on fish species[21,49,50]. Many factors influenced sperm quality and quantity such as genetic, physiological, spawning season, and environmental factors.[26,49,51,52]. On the other hand, improvements in feed nutrition of broodfish can increase gamete quality and semen volume [46]. Commercial honey combined with 10% Dimethyl Sulfoxide (DMSO) was also shown to increase sperm motility[53]. Synthetic hormones such as gonadotropin-releasing hormone analogs (GnRHa), with or without dopamine antagonist; domperidone (Dom) effectively improve sperm quality[54,55]. Nevertheless, the duration of motility of sperm was strongly correlated with the water quality in ponds. In this study, the water quality parameters in spawning ponds included alkalinity of 50.5 to 52.5 mg/L and hardness 65.5 to 67.5 mg/L, a pH between 6.4 and 6.6, and a water temperature

**Commented [SL16]:** Time of what?

**Commented [S17]:** Has been revised

**Commented [SL18]:** Please rephrase/clarify

**Commented [S19]:** Has been revised

**Commented [SL20]:** Please specify: mg of what?

**Commented [S21]:** Has been added

between 28 and 30 °C. These water quality parameters were able to support the ability of the sperm to fertilize the egg[21].

The sperm motility in sago giant gourami ranged from 68 to 75%, and the duration of motility ranged from 43 to 61 sec. These results are consistent with *Genypterus blacodes* and *Esox lucius*[56,50]. Sperm motility includes the percentage of motile sperm, straight-line velocity, curvilinear velocity, average path velocity, and linearity[50]. In this study, we did not investigate those parameters. In addition, the percentage of motile sperm is influenced by the addition of extenders and cryoprotectants[57,58,59]. However, sperm motility from fresh semen was slightly greater compared to cryopreserved semen from *Esox lucius*[50]. The fertility rate of eggs ranged from 76 and 84%; however, no significant correlation was detected between fertility rate and sperm parameters, such as semen volume, semen pH, motility, and duration of motility. Conversely, the sperm concentration was moderately correlated with sperm motility and duration of motility. The parameters commonly measured to assess sperm quality in brood were volume, density, and motility (such as the percentage of motile sperm, straight-line velocity curvilinear velocity, average path velocity, linearity, and amplitude of lateral head displacement), including fertilizing capacity [49,50,52,60]. In this study, we did not investigate the ionic composition of the semen, but this phenomenon could be related to the ionic composition of semen which might have a significant influence on sperm motility and duration of motility.

**Conclusions**

This research analyzed the reproductive characteristics of giant gourami sago strain broodfish reared in concrete freshwater ponds, in the Aquaculture Laboratory Faculty of Fisheries and Marine Science, Universitas Bung Hatta. Relative fecundities of the giant gourami sago strain broodfish ranged from 977 to 1071 eggs, and egg diameter ranged from 2.32 to 2.46 mm. Semen volume ranged from 0.4 to 0.7 ml per kg body weight and sperm motility was comprised between 68 to 75%. A strong linear relationship was observed between absolute fecundity and female fish weight before and after spawning. Similarly, a strongly positive correlation was observed between survival rate (10 days) and hatching rate. The sperm concentration was also moderately positively correlated with the motility and duration of sperm motility. Keys to increase reproduction performance in gourami sagostrain depend on broodfish weight, relative fecundity, and hatching rates. Although data on the reproductive characteristics of gourami sago strain broodfish have been obtained, there are still knowledge gaps in feeding technologies and larval weaning during rearing. Therefore, for successful practices in hatcheries, further research is recommended to determine a proper feed formulation and the development of appropriate aquaculture systems.

**Commented [SL22]:** Do you have a reference to support this?

**Commented [S23]:** Has been added

**Commented [SL24]:** Please note this is the syntax seen in the literature

**Commented [S25]:** Has been revised

**Commented [S26]:** Has been revised

**Commented [SL27]:** Please note edit here is for correct grammar and syntax

**Commented [S28]:** Ok

**Data availability**

*Underlying data*

Figshare: Reproduction characterization of the gurami sago (*Osphronemus goramy* Lacepède, 1801): basic knowledge for a hatchery development strategy for the future.

https://doi.org/10.6084/m9.figshare.14661189.v3 [61].

This project contains the following underlying data:

- Table 1. Raw data of fish length, weight, absolute fecundity, and relative fecundity of gurami sago broodfish
- Table 2. Raw data of egg diameter (mm) in sago strain of giant gourami broodfish
- Table 3. Raw data of hardened egg diameter (mm) in sago strain of giant gourami broodfish
- Table 4. Raw data of egg diameter increase (%) in sago strain of giant gourami broodfish
- Table 5. Raw data of egg weight (mg) in sago strain of giant gourami broodfish
- Table 6. Raw data of hardened egg weight (mg) in sago strain of giant gourami broodfish
- Table 7. The data of egg weight increase (%) in sago strain of giant gourami broodfish
- Table 8. The data of fertilization rate (%) in sago strain of giant gourami broodfish
- Table 9. The data of hatching rate (%), endogenous feeding period (day), and embryo survival rate to the eyed-egg stage (%) and in sago strain gourami
- Table 10. Male size, gonadal weight, and semen in sago strain of giant gourami broodfish
- Table 11. Sperm concentration ($10^9$/mL) in sago strain of giant gourami broodfish
- Table 12. Sperm Motility (%) in sago strain of giant gourami broodfish
- Table 13. Duration motility (sec) in sago strain of giant gourami broodfish



**Competing interests**

No competing interests were disclosed.

**Grant information**

This study was funded by a competitive grants scheme called the Professor 2021. Contract number: 06.02.1.46.03.2021

**Acknowledgments**

The authors thank Professor. Dr. Tafdil Husni the Rector of Universitas Bung Hatta for supporting this study through the competitive grant's schema called Research Professor, 2021. The appreciation goes to all of the students (Puji Kurniawan and Ranji Rinaldi and Muhammad Vajri Djauhari) who helped the author during data collection in the field.

**Commented [SL29]:** Please ensure you have obtained consent from these people to be named in your manuscript

**Commented [S30]:** they have agreed to have their names written on the Acknowledgements

**References**

1. Syandri H, Azrita, Mardiah A.: Nitrogen and phosphorus waste production from different fish species cultured at floating net cages in Lake Maninjau, Indonesia. *Asian J. Sci. Res*., 2018;11 (2): 287-294.

2. Aryani N, Suharman I, Azrita, *et al*.: Diversity and distribution of fish fauna of upstream and downstream areas at Koto Panjang Reservoir, Riau Province, Indonesia. F1000Research, 2020; 8:1435. Publisher Full Text

3. Mungkung R, Aubin J, Prihadi TH, *et al*.: Life Cycle Assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. *J. Clean. Prod*, 2013; 47: 249-256. Publisher Full Text

4. Pouil S, Samsudin R, Slembrouck J, *et al*.: Nutrient budgets in a small-scale freshwater fish pond system in Indonesia. *Aquaculture*, 2019; 504:267–274. Publisher Full Text

5. FAO, The State of World Fisheries and Aquaculture: Meeting the Sustainable Development Goals. *Food and Agriculture Organization of the United Nations*, Rome.2018.

6. CDSI (Central Data System Information). Ministry of Marine and Fisheries Republic of Indonesia, 2018 (In Indonesian). Reference Source

7. Azrita, Aryani N, Mardiah A, *et al*.: Growth, production and feed conversion performance of the gurami sago (*Osphronemus goramy* Lacepède, 1801) strain in different aquaculture systems. *F1000Research* 2020, 9:161. Publisher Full Text

8. Arifin OZ, Slembrouck J, Subagja J, *et al*.: New insights into giant gourami (*Osphronemus goramy*) reproductive biology and egg production control. *Aquaculture*, 2019; 519:734743

9. Ahmad N, Thompson S.: The blue dimensions of aquaculture: A global synthesis. *Sci. Total Environ*. 2019; 652: 851–861. Publisher Full Text

10. Ranjan R, Megarajan S, Xavier B, *et al*.: Broodstock development, induced breeding and larval rearing of Indian pompano, *Trachinotus mookalee* (Cuvier, 1832) – A new candidate species for aquaculture. *Aquaculture*, 2018; 495: 550–557. Publisher Full Text

11. Henriksson, PJG, Nhuong T, Chadag VM, *et al*.: Indonesian aquaculture futures-evaluating environmental and socioeconomic potentials and limitations.2017; J. Cleaner Prod., 162: 1482-1490. Publisher Full Text

12. Tran N, Rodriguez UP, Chan CY, *et al*.: Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. Marine Policy, 2017; 79, 25–32. Publisher Full Text

13. Aryani N, Azrita, Mardiah A, *et al*.: Influence of feeding rate on the growth, feed efficiency and carcass composition of the Giant gourami (*Osphronemus goramy*). *Pakistan J. Zool*. 2017; 49(5): 1775-1781. Reference Source

14. Nugroho E, Azrita, Syandri H, *et al*.: DNA barcoding of giant gourami (*Osphronemus goramy*) from West Sumatra, Indonesia. *AACL Bioflux*, 2019, 12, (4): 1074-1079. Reference Source

15. Slembrouck J, Arifin OZ, Pouil S, *et al*.: Seasonal variation of giant gourami (*Osphronemus goramy*) spawning activity and egg production in aquaculture ponds. *Aquaculture*,2020; 735450. PubMed Abstract Publisher Full Text

16. Radona D, Nafiqoh N.: Reproductive Characteristics and Heterosis value of Bastar and Bluesafir population of giant gourami crosses. *Berita Biologi*, 2014: 13(2):153-159 (in Indonesian). Reference Source

17. Azrita, Syandri H.: Effects of salinity on survival and growth of gurami sago (*Osphronemus goramy*, Lacepède, 1801) Juveniles. *Pak. J. Biol. Sci*., 2018; 21 (4): 171-178. Reference Source

18. Nugroho E, Azrita, Syandri H, *et al*.: Evaluation of genetic divergence of kalui fish (*Osphronemus goramy*) strains from West Sumatra revealed by random amplified polymorphism DNA (RAPD) marker. *Jurnal Riset Akuakultur*, 2016; 11 (4), 313-319 (in Indonesian). Reference Source

19. CDSI (Central Data System Information). Ministry of Marine and Fisheries, Republic of Indonesia, 2018 (In Indonesian). Reference Source.

20. Kanyilaz M.: Reproductive performance of a newly described Salmonid fish, Alakir Trout (*Salmo Kottelati*), a candidate species for aquaculture. *Pak. J. Zool*. 2016; 48(1): 83–89. Reference Source

21. Aryani A, Suharman I, Syandri H.: Reproductive performance of asian catfish (*Hemibagrus wyckii* Bleeker, 1858), a candidate species for aquaculture. *F1000Research* 2018, 7:683 Publisher Full Text

22. Aruho C, Walakira JK, Rutaisire J.: An overview of domestication potential of *Barbus altianalis* (Boulenger, 1900) in Uganda. *Aquac. Rep.*, 2018; 11, 31–37. Publisher Full Text

23. Osure GO, Phelps RP.: Evaluation of reproductive performance and early growth of four strains of Nile tilapia (*Oreochromis niloticus*, L) with different histories of domestication. *Aquaculture*, 2006; 253(1-4): 485–494. Publisher Full Text

24. Krejszeff S, Katarzyna T, Daniel Z, *et al.*: Domestication affect spawning of the ide (*Leuciscus idus*)-preliminary study. *Aquaculture*. 2009; 295(1–2): 145–147. Publisher Full Text

25. Weber RA, Peleteiro JB, García Martín LO, *et al*.: The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). *Aquaculture*. 2009; 288(1–2): 147–150. Publisher Full Text

26. Rurangwa E, Kime D, Ollevier F, *et al*.: The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 2004; 234(1-4): 1-28. Publisher Full Text

27. Cabrita E, Martínez-Páramo S, Gavaia PJ, *et al*.: Factors enhancing fish sperm quality and emerging tools for sperm analysis. *Aquaculture*, 2014; 432, 389–401. Publisher Full Text.

28. Mylonas CC, Duncan NJ, Asturiano JF.: Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. *Aquaculture*, 2017: 472, 21–44. Publisher Full Text.

29. Rice EW, Baird RB, Eaton AD, *et al.*: Standard methods for the examination of water and wastewater, 22nd ed**.** American Public Health Association, American Water Works Association, Water Environment Federation. 2012. Reference Source

30. Amornsakun T, Kullai S, Hassan A.: Some aspects in early life stage of giant gourami, *Osphronemus goramy* (Lacepede) larvae. *Songklanakarin J. Sci. Technol*, 2014; 36 (5): 493 – 498. Reference Source

31. Bari Y.: Addition of vitamin E to artificial feed to increase the reproductive potential of giant gourami (*Osphronemus goramy* Lacepede) broodstock. Thesis of Postgraduate, Bogor Agricultural University (unpublish, in Indonesian), 1997. Reference Source

32. Izquierdo M, Fernández-Palacios H, Tacon AG.: Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 2001; 197(1-4): 25–42. Publisher Full Text

33. Chong ASC, Ishak SD, Osman Z, *et al*.: Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture*, 2004; 234: 381–392. Publisher Full Text.

34. Hafeez-ur-Rehman M, Abbas F, Ashraf M, *et al*.: Effect of different dietary protein levels on egg development and its response to inducing agents during induced spawning of *Channa marulius*. *Pakistan J. Zool*., 2017: 49 (1):337 – 343. Reference Source

35. Sarih S, Djellata A, Roo J, *et al*.: Effects of increased protein, histidine, and taurine dietary levels on egg quality of greater amberjack (*Seriola dumerili*, Risso, 1810). *Aquaculture*, 2018; 499:72-79. Publisher Full Text

36. Jeuthe H, Brännäs E, Nilsson J.: Effects of egg size, maternal age, and temperature on egg, viability of farmed Arctic charr. *Aquaculture*, 2013; 408-409, 70–77. Publisher Full Text

37. Stuart KR, Armbruster L, Johnson R, *et al*.: Egg diameter as a predictor for egg quality of California yellowtail (*Seriola dorsalis*). *Aquaculture*, 2020; 522: 735154. PubMed Abstract | Publisher Full Text.

38. Grant B, Davie A, Taggart JB, *et al*.: Seasonal changes in broodstock spawning performance and egg quality in Ballan wrasse (*Labrus bergylta*). *Aquaculture*, 2016; 464: 505–514. Publisher Full Text.

39. Lahnsteiner F, Patzner RA.: Rainbow trout egg quality determination by the relative weight increase during hardening: a practical standardization. *J. Appl. Ichthyol*. 2002; 8(1):24-26. Reference Source

40. Sink TD, Lochmann RT, Pohlenz C, *et al*.: Effects of dietary protein source and protein-lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production, and quality, and fry hatching percentage and performance. *Aquaculture*, 2010; 298(3-4): 251– 259. Publisher Full Text.

41. Morehead D, Hart P, Dunstan G, *et al*.: Differences in egg quality between wild striped trumpeter (*Latris lineata*) and captive striped trumpeter that were fed different diets. *Aquaculture*, 2001; 192 (1): 39–53. Publisher Full Text.

42. Mateos J, Mañanos E, Carrillo M, *et al*.: Regulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) gene expression by gonadotropin-releasing hormone (GnRH) and

sex steroids in the Mediterranean Sea bass. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2002; 132(1): 75–86. Publisher Full Text

43. Paullada-Salmerón JA, Cowan ME, Loentgen GH, *et al*.: The gonadotropin-inhibitory hormone system of fish: The case of sea bass (*Dicentrarchus labrax*). *Gen. Comp. Endocrinol.,* 2019; 279: 184-195. Publisher Full Text.

44. Lubzens E, Young G, Bobe J, *et al*.: Oogenesis in teleosts: How fish eggs are formed. *Gen. Comp. Endocrinol.*, 2010; 165 (3): 367–389. Publisher Full Text.

45. Lubzens E, Bobe J, Young G, *et al*.: Maternal investment in fish oocytes and eggs: The molecular cargo and its contributions to fertility and early development. *Aquaculture*, 2017; 472: 107–143. Publisher Full Text.

46. Hafeez-ur-Rehman M, Abbas F, Ashraf M *et al*.: Effect of Different Dietary Protein Levels on Egg Development and its Response to Inducing Agents during Induced Spawning of *Channa marulis*, *Pakistan J. Zool.*, 2017; 49 (1):337-343. Reference Source.

47. Aryani N, Suharman I.: Effect of dietary protein level on the reproductive performance of female of green catfish (*Hemibagrus nemurus* Bagridae). *J Aquac Res Dev*., 2015; 6:11. Reference Source

48. Collazos-Lasso LF, Mariana CGE, Elisabeth AB.: Induced reproduction of the sailfin pleco, *Pterygoplichthys gibbiceps* (Kner, 1854) (Pisces: Loricariidae). *AACL Bioflux*, 2018, 11 (3): 724-729. Reference Source

49. Caldas JS, Godoy L.: Sperm characterization of the endangered Amazonian fish *Hypancistrus zebra*: basic knowledge for reproduction and conservation strategies. *Anim. Reprod. Sci.*, 2019; 204: 117-124. Publisher Full Text.

50. Dietrich GJ, Nynca J, Szczepkowski M, *et al*.: The effect of cryopreservation of semen from whitefish (*Coregonus lavaretus*) and northern pike (*Esox lucius*) using a glucose-methanol extender on sperm motility parameters and fertilizing ability. *Aquaculture*, 2016; 464: 60–64. Publisher Full Text.

51. Butt IAE, Litvak MK, Trippel EA.: Seasonal variations in seminal plasma and sperm characteristics of wild-caught and cultivated Atlantic cod, *Gadus morhua*. *Theriogenology*, 2010; 73(7): 873–885. Publisher Full Text.

52. Abinawanto, Intan AP, Retno L.: Sperm motility of giant gourami (*Osphronemus goramy*, Lacepede, 1801) at several concentrations of honey combined with DMSO after short-term storage. *AACL Bioflux*, 2017, 10 (2): 156-163. Reference Source

53. Valdebenito II, Gallegos PC, Effer BR.: Gamete quality in fish: evaluation parameters and determining factors. *Zygote*, 2013; 23(02): 177–197. Publisher Full Text.

54. Cejko BI, Krejszeff S, Żarski D, *et al*.: Effect of carp pituitary homogenate (CPH) and sGnRHa (Ovaprim) on a northern pike (*Esox lucius*) spermiation stimulation and its effect on quantity and quality of sperm. *Anim. Reprod. Sci.*, 2018; 193: 217–225. Publisher Full Text.

55. Zadmajid V.: Comparative effects of human chorionic gonadotropin (hCG) and Ovaprim™ (sGnRHa+domperidone) on the reproductive characteristics of wild-caught male Longspine scraper, *Capoeta trutta* (Heckel, 1843). *Aquaculture*,2016; 463: 7–15. Publisher Full Text

56. Dumorné K, Valdebenito I, Contreras P, *et al*.: Effect of pH, osmolality and temperature on sperm motility of pink cusk-eel (*Genypterus blacodes*, (Forster, 1801)). *Aquac. Rep*., 2018; 11: 42-46. Publisher Full Text.

57. Golshahi K, Aramli MS, Nazari RM, *et al*.: Disaccharide supplementation of extenders is an effective means of improving the cryopreservation of semen in sturgeon. *Aquaculture*, 2018; 486: 261–265. Publisher Full Text.

58. Yusoff M, Hassan BN, Ikhwanuddin M, *et al*.: Successful sperm cryopreservation of the brown-marbled grouper, Epinephelus fuscoguttatus using propylene glycol as cryoprotectant. *Cryobiology*, 2018; 81: 168–173. Publisher Full Text

59. Kommisrud E, Myromslien FD, Stenseth EB, *et al*.: Viability, motility, ATP content and fertilizing potential of sperm from Atlantic salmon (*Salmo salar* L.) in milt stored before cryopreservation. *Theriogenology*. 2020; 151: 58-65. Publisher Full Text

60. Cejko BI, Żarski D, Palińska-Żarska K, *et al*.: Artificial seminal plasma improves motility and fertilization capacity of common carp *Cyprinus carpio* L. sperm during one hour of storage. *Aquaculture*, 2019; 506: 224-228. Publisher Full Text.

61. Syandri, Hafrijal; Azrita, Azrita; Aryani, Netti (2021): Untitled ItemReproduction characterization of the gurami sago (Osphronemus goramy Lacepède, 1801): basic knowledge for a hatchery development strategy for the future. figshare. Journal contribution. https://doi.org/10.6084/m9.figshare.14661189.v3