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hafrizal syandri <syandri_1960@bunghatta.ac.id>

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Reproductive characteristics of the sago strain gourami 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 sago strain gourami (*Osphronemus goramy*) 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 sago strain gourami broodfish to provide basic knowledge for a hatchery development strategy in the future.

Methods: A total of 20 female and male broodfish sago strain gourami had matured 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 software camera 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. The relative fecundity and egg diameter were 1029±36 eggs kg^{-1}, 2.42±0.05 mm. The hatching rate and embryo survival to an eyed-egg stage were 76.40±2.27% and 94.76±0.42%, respectively. 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 parameters had strongly correlated with the female broodfish weight after spawned (r^2 = 0.999), and absolute fecundity had also strongly correlated with female broodfish before spawning (r^2= 0.921). At the same time, the parameter of sperm concentration has moderately correlated with sperm motility (r^2 = 0.556) and duration of sperm motility (r^2 = 0.502).

Conclusions: The sago strain gourami 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 cultured 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, worthy this a concern to be development of sago strain gourami was living a limited in West Sumatera Province of Indonesia[17,18]. The sago strain gourami is considered a source for nutritional and food security among many freshwater fishes' communities in Indonesia.

The sago strain gourami 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 sago strain gourami 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 sago strain giant gourami hatchery performance in the future. Therefore,

the present study was conducted to evaluate the reproductive characteristics in sago strain gourami 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 giant 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 sago strain gourami in the Aquaculture Laboratory Faculty of Fisheries and Marine Science Universitas Bung Hatta facilities. There was no animal suffering involved in this study and sago strain gourami 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 sago strain gourami juvenile fishes were reared 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 that, a total of 30 mature individuals broodfish were separated according to sex and reared in two concrete 18 m^3 (6×2×1.5 m) freshwater ponds. 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, it is also were given sente leaves (*Alocasia macrorriza* L.) as much as 1% (wet weight) of fish weight per day were contained 2.85% protein and 0.47% 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 liters 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 sago strain gourami 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 sago strain 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 software camera 3, https://id.canon/id/support/0200351909.

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 each broodfish are

incubated in the 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 software camera 3 https://id.canon/id/support/0200351909. 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, endogenous feeding period counted until the egg yolks run out (day) and embryo survival rate (%) counted to eyed-egg stage i.e. day 12.

Determination of sperm quality

To stimulate the spermiation process in male broodfish used LHRH-a (Ovaprim, manufactured for Syndel Laboratories Ltd, 2595 Mccullough Rd Nanaimo B.C 9VS 4m9 Canada) with dosage 0.5 ml per kg of brooder. Semen samples were obtained from 10 broodfish in sago giant gourami 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.* (2004) and Cabrita *et al.* (2017)[26,27]. 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 by collecting the semen in a graduated cylinder and determining the level in millimeters. Semen pH was determined with a hand pH meter (HI8424 Hanna Instruments, 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].

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[29]. The traditional procedure for alkalinity is to measure how much H+ is required to titrate a sample to the methyl orange endpoint (about pH 4.5). The pH at the titration endpoint corresponds

approximately to the point where an amount of H+ has been added to react with all the OH^-, $CO3^{2-}$, and $HCO3^-$ in the sample to produce CO2 and H2O. The milliequivalents of H^+ used in the titration multiplied by 50.04 mg CaCO3/ Meq is the alkalinity. Hardness is determined by titrating a sample with 0.01 M ethylenediaminetetraacetic acid (EDTA) to form complexes with divalent cations. 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 characteristics parameters in sago strain of giant gourami females broodfish results are showing 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 in the diameter of the hardened egg (r^2 =0.699), while egg diameter and fertility rate 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 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 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- 45
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 strain 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 strain 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 strain 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 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 sago strain 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] that biochemical composition of egg strong correlated with egg quality of broodfish. 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 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 sago giant gourami. Conversely, the extruded feed enriched with vitamin E (d-α-tocopherol) as much as 137.8 mg, 238.05 mg, 338.72 mg and 439.39 mg per kg of feed ingredients had an effect on markers of brood reproduction function of giant gourami, such as sexual maturity time, ovum somatic index, relative fecundity and egg diameter31.[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 strain gourami is poorly understood. Therefore, we recommend the use of feed protein levels and hormonal dosage to increase the reproductive potential in sago strain gourami broodfish is very important to be applied in the future.

Average semen volume in sago strain 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 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 nutrition feed of broodfish can be improved gamete quality and semen volume[46]. Commercial honey combined with 10% Dimethyl Sulfoxide (DMSO) it is also had increased motility rate of sperm[53]. While, synthetic hormones such as salmon gonadotropin releasing hormone analogue and domperidone (GnRH + Domperidone) effective on sperm quality[54,55]. Nevertheless, sperm fertility competition occurs in the aquatic environment, and was strongly correlated with the water quality in ponds. In this study, the water quality parameters in spawning ponds of sago strain gourami broodfish in terms of alkalinity (50.5 to 52.5 mg L^{-1}), hardness (65.5 to 67.5 mg L^{-1}), pH (6.4

to 6.6) and water temperature (28 to 30 °C). All of these water quality parameters have been able to support the ability of sperm to fertilize an egg.

The sperm motility in sago strain 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, 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 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 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 sago strain gourami broodfish reared in concrete freshwater ponds, in the Aquaculture Laboratory Faculty of Fisheries and Marine Science, Universitas Bung Hatta. Relative fecundities of the sago strain gourami 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 correlated between survival rate (10 days) with hatching rate. Sperm parameters which had moderately correlated was between sperm concentration with sperm motility and sperm duration of motility. Keys to increasing reproduction performance in sago strain gourami depends on broodfish weight, relative fecundity and hatching rates. Despite data on reproductive characteristic has been obtained, there is still an observed limitations of quality seed supply for aquaculture attributed to knowledge gaps in larval weaning, grow-up feeding technologies. Therefore, for successful 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.v1 [61].

This project contains the following underlying data:

- Table 1. Raw data of fish length, weight, absolute fecundity and relative fecundity in sago strain gurami broodfish
- Table 2. Raw data of egg diameter (mm) in sago strain gourami broodfish
- Table 3. Raw data of hardened egg diameter (mm) in sago strain gourami broodfish
- Table 4. Raw data of egg diameter increase (%) in sago strain gourami broodfish
- Table 5. Raw data of egg weight (mg) in sago strain gourami broodfish
- Table 6. Raw data of hardened egg weight (mg) in sago strain gourami broodfish
- Table 7. The data of egg weight increase (%) in sago strain gourami broodfish
- Table 8. The data of fertilization rate (%) in sago strain gourami broodfish
- Table 9. 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 gourami broodfish
- Table 11. Sperm concentration (10^9/mL) in sago strain gourami broodfish
- Table 12. Sperm Motility (%) in sago strain gourami broodfish
- Table 13. Duration motility (sec) in sago strain gourami broodfish

Competing interests

No competing interests were disclosed.

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Reproductive characteristics of the the sago strain gourami 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 sago strain gourami (*Osphronemus goramy*) 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 sago strain gourami broodfish to provide basic knowledge for a hatchery development strategy in the future.

Methods: A total of 20 female and male broodfish sago strain gourami had matured 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 software camera 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. The relative fecundity and egg diameter were 1029±36 eggs kg^{-1} and 2.42±0.05 mm. The hatching rate and embryo survival to eyed-egg stage were 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 had strongly corelated with the female broodfish weight before spawned (r^2 = 0.999) and absolute fecundity had also strongly corelated with female broodfish before spawning (r^2= 0.921). While, the parameter of sperm concentration has a moderately corelated with the sperm motility (r^2 = 0.556) and duration of sperm motility (r^2 = 0.502).

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Conclusions: The sago strain gourami 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, worthy this concern to be development of sago strain gourami was living a limited in West Sumatera Province of Indonesia[17,18]. The sago strain gourami is considered a source for nutritional and food security among many freshwater fishes' communities in Indonesia.

The sago strain gourami 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,

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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 sago strain gourami 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 sago strain gourami 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 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 that, a total of 30 mature individuals broodfish were separated according to sex and reared in two concrete 18 m^3 (6×2×1.5 m) freshwater ponds. 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, it is also were given sente leaves (*Alocasia macrorriza* L.) as much as 1% (wet weight) of fish weight per day were contained 2.85% protein and 0.47% 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.

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A total of 20 broodfish with mature oocytes were selected, consisting of 10 females and 10 males. Prior to spawned, 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.

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Reproductive parameters in sago strain gourami 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 software camera 3 , https://id.canon/id/support/0200351909.

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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 each broodfish are

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incubated in the 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 software camera 3, https://id.canon/id/support/0200351909. 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, endogenous feeding period counted until the egg yolks run out (day) and embryo survival rate (%) counted to eyed-egg stage i.e. day 12.

Determination of sperm quality

To stimulate the spermiation process in male broodfish used LHRH-a (Ovaprim, manufactured for Syndel Laboratories Ltd, 2595 Mccullough Rd Nanaimo B.C 9VS 4m9 Canada) with dosage 0.5 ml per kg of brooder. Semen samples were obtained from 10 broodfish in sago giant gourami 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.* (2004) and Cabrita *et al.* (2017)[26,27]. 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 by collecting the semen in a graduated cylinder and determining the level in millimeters. 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[29]. The traditional procedure for alkalinity is to measure how much H+ is required to titrate a

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sample to the methyl orange endpoint (about pH 4.5). The pH at the titration endpoint corresponds approximately to the point where an amount of H+ has been added to react with all the OH^-, $CO3^{2-}$, and $HCO3^-$ in the sample to produce CO2 and H2O. The milliequivalents of H^+ used in the titration multiplied by 50.04 mg CaCO3/ Meq is the alkalinity. is determined by titrating a sample with 0.01 M ethylenediaminetetraacetic acid (EDTA) to form complexes with divalent cations[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).

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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 in the diameter of the hardened egg ($r^2 = 0.699$), while egg

diameter and fertility rate 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 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

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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

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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 giant gourami broodfish was smaller than that of giant gourami belonging to the galunggung strain, which ranged from 2500 to 3500 g. 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

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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] that biochemical composition of egg strong correlated with egg quality of broodfish. 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 sago giant gourami. Conversely, the extrusion feed enriched with vitamin E (d-α-tocopherol) as much as 137.8 mg, 238.05 mg, 338.72 and 439.39 mg per kg feed ingredients had an effect on markers of brood reproduction functions of giant gourami, such as sexual maturity time, ovum somatic index, relative fecundity and egg diameter31)[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 feed protein levels and hormonal dosage to increase the reproductive potential in sago strain gourami broodfish is very important to be applied in 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 nutrition feed of broodfish can be improved gamete quality and semen volume [46] .Commercial honey combined with 10% Dimethyl Sulfoxide (DMSO) it is also had increased motility rate of sperm [53]. While, synthetic hormones such as salmon gonadotropin releasing hormone analogue and domperidone (GnRH + Domperidone) effective on sperm quality[54,55]. Nevertheless, sperm fertility competition occurs in the aquatic environment, and was strongly correlated with the water quality in ponds. In this study, the water quality parameters in spawning ponds of sago strain gourami broodfish in terms of alkalinity (50.5 to 52.5 mg L^{-1}) and hardness (65.5

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to 67.5 mg L^{-1}), pH (6.4 to 6.6) and water temperature (28 to 30 °C). The all of these water quality parameters have been able to support the ability of sperm to fertilized an egg.

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, 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 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 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 sago strain gourami 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 correlated between survival rate (10 days) with hatching rate. Sperm parameters which had moderately correlated was between sperm concentration with sperm motility and sperm duration of motility . Keys to increasing reproduction performance in sago strain gourami depends on broodfish weight, relative fecundity and hatching rates. Despite data on reproductive characteristic has been obtained, there is still an observed limitations of quality seed supply for aquaculture attributed to knowledge gaps in larval weaning, grow-up feeding technologies. Therefore, for successful practices in hatcheries, further research is recommended to determine a proper feed formulation and the development of appropriate aquaculture systems.

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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.v1 [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. 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

Competing interests

No competing interests were disclosed.

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