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Reproductive characteristicszation of the gurami sago-sago giant gourami strain (Osphronemus goramy Lacepède, 1801): basic knowledge for a future hatchery strategy development strategy for the future

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 Preparation, Writing Review & Editing

Hafrijal Syandri (team member)

- Data Curation, Formal Analysis, Methodology, Writing-Original Draft Preparation, Writing-Review & Editing.
- Netti Aryani (team member)
- Data Curation, Formal Analysis, Resources, Validation,
   Writing Review & Editing

Abstract

## Background:

The sSago strain of giant gourami (Osphronemus qurami Lacepède) has been released approved on in 2018 as a candidate aquaculture freshwaterfor freshwater aquaculture in Indonesia. -However, information on the reproduction characterizationspecies' reproduction is are minimal. This study analyzed the reproduction characterization characteristics in of the sago strain of the giant gourami broodfish to provide basic knowledge for a hatchery development strategy in the future.

# Methods:

A total of 10 female broodfish and 10 males had matured oocytes were measured for body weight and length, and were evaluated for their reproductive charactericharacteristicszation. Broodfish

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Breeding fish are were spawned naturally using in a-1.2-m³ (2×1×0.6 m) concrete pond with a male-female sex ratio of (1:1). Egg weight and diameter were measured for in 25 eggs per female using balances (ACIS AD- 600i scales with 0.01 g accuracy) and a Labe-microscope (Labo model L-711) using software camera 3. Semen was collected using plastic syringes in 3 mL aliquots, and then placed in an insulated ice-cooled container, and analyzed within 2 hours of collection.

Results:

Average weights of female and male broodfish females and males before spawning ed-were 2180±159.78 g and 3060±134.99 g, respectively. The relative fecundity, and egg diameter and egg weight were 1029±36 eggs kg $^{-1}$ , 2.42±0.05 mm and 10.33±1.09 mg, respectively. The hatching rate, endogenous feeding period and embryo survival to eyed-egg stage were 76.40±2.27%, 11.2±0.63 day and 94.76±0.42% respectively. Sperm characteristics showed that, such as volume (was 0.60±0.12 ml kg $^{-1}$ ) and percentage motile motility was( 70.04±2.27%) were measured. The female fish weight before spawninged parameters that had strong relationships with the female fish weight after spawned ( $r^2$  = 0.999) and absolute fecundity ( $r^2$  = 0.921). While, the parameter of sperm concentration has a strong relationship with the sperm motility ( $r^2$  = 0.556) and duration of sperm motility ( $r^2$  = 0.502).

Conclusions:

Sage-The sage strain of the giant gourami broodfish has a good-suitable reproductive characterization-characteristics for hatchery development for the future the development of future hatcheries. The natural spawning that has been success should be followed up with the larval weaning technology and feeding to increase the growth and survival. Successful natural spawning should be followed by larval weaning and feeding technology to increase the growth and survival.

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

Introduction

Aquaculture freshwater Freshwater aquaculture is a practiced in inland waters such as lakes, rivers, reservoir, floodplains and oxbow lakes, including 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 production of Indonesia are sourced sources from freshwater ponds and inland waters<sup>6</sup>. 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.

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Freshwater aquaculture is one of the fastest growing aquacultures in Indonesia, with more than 3,378,298.92 metric tons produced in 2018<sup>6,11,12</sup>. Nile tilapia (*Oreochromis niloticus*) has contributed (37.93% of the total aquaculture production), African catfish (*Clarias galepinus*) -{33.35%}, 

Pangasius catfish (*Pangasius hypophtalamushypophthalmus*) -{12.38%}, common carp (*Cyprinus carpio*) (9.28%) and Gaiant gourami (*Osphronemus goramy*) (6.96%) - 13,14,15.

Indonesian\_has many strains for giant gourami strains\_belonging to include the local "tambago", "palapa", "soang", "galunggung" and "blusafir" strains, which has have been produced grown semi-intensively in small-scale farms for decades8,14,13,16. But However, it is they have not been able to contributed contribute maximally majorly to the production on freshwater aquaculture production of in Indonesia. This a concern to be developing of newly strain of giant gourami, namely gurami sago was living a limited in West Sumatera Province of Indonesia. The giant gourami sago strainstrain of giant gourami is one considered a source for nutritional and food security, including as an ornamental fish among many freshwater fishes' communities in Indonesia.

Sago strain of giant gouramiThe giant gourami sago strain was approved has been released as a candidate for freshwater aquaculture in 2018 (Decree of the Ministry of Marine and Fisheries, Republic of Indonesia No.56/KEPMEN-KP/2018)<sup>19</sup>. However, data on its reproduction reproductive characteristicsization in sago strain of giant gourami still a are still—limited. Ton the other hand, the evaluationed to reproduction of reproductive performance in on the others fish species has been had beneficial impacts to in the developmented of freshwater aquaculture in the Asia regionAsia<sup>20,21,22,23,24</sup>. Whereas In contrast, in sago strain of giant gourami broodfish there are still gaps in-knowledge of giant gourami sago strain broodfish in terms of regarding size at oocyte maturity, time-age of sexual maturity, sperm characteristics, egg hatchability, survival after eyedeges stage, larva weaning and growth rate. These factors were identified as key challenges for successfully a giant gourami sago strain hatchery performance in sago strain of giant gourami for the futures in the future. Therefore, the present study was conducted to evaluate the reproduction reproductive characterization characteristics in sago strain of giant gurami broodfishgiant gourami sago strain as-to provide basic knowledge about for hatchery development for the future.

## Methods

#### Ethical considerations

There are no required permits from the government of the Republic of Indonesia to evaluation

<u>evaluate reproduction reproductive characterization characteristics</u> in <u>sago strain of giant</u>

<u>gourami</u>giant gourami sago strain broodfish (*Osphronemus qoramy*) for as a candidate for future

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aquaculture for the future. The study was founded 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 collected fish samplesspecimens, reared and spawned in sage strain of giant gouramigiant gourami sage strain in the Aquaculture Laboratory Faculty of Fisheries and Marine Science Universitas Bung Hatta facilities. There no suffering animal activitywas no animal suffering involved in this study and sage strain of giant gouramigiant gourami sage strain broodfish were still in good condition until-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 puvenile fishes 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 in sage strain of giant gouramigiant gourami sage strain juvenile fishes were reared to sexual maturity adult sex under thein a concrete 8.4 m³ (6×2×0.7 m) freshwater pond 8.4 m³ (6×2×0.7 m). During were the reared rearing in sage strain of giant gourami juvenile fish until adult sex to sexual maturity, the juvenile fishes were given commercial feed pellets which contained 30% crude protein and 4% crude fat.

After that, a total of 30 mature individualse adult sex broodfish were separated according by to sex and reared in two concrete 18 m³ (6×2×1.5 m) freshwater ponds 18 m³ (6×2×1.5 m). Sago strain of giant gourami broodfish the broodfish were feed twice daily (09:00 AM and 16:00 PM), were with extruded pellet feed pellets containing was 39.50% crude protein and 12.21% fat with a predetermined ratio quantity as much asof 3% of biomass fish weight per /day. Besides that, it is also were given sente leaves sufficiently were contained 2.85% protein and 0.47% fat (% wet weight). Each concrete freshwater pond hads a 50 mm of middle drain in the middleage, which is was covered with a net of 0.5 cm mesh size to prevent the fish gurami sago broodfish from escaping and predators from entering. WThe water was pumped from the borehole wells at a velocity rate of 5 literes per minute.

A total of 20 broodfish for sage strain of giant gourami has oocyte matured with mature oocytes wereas selected, consistinged of 10 females and 10 males. Prior to stocking, of females and males broodfish were weighed using balance scales (OHAUS model CT 6000-USA with 0.1 g accuracy), and their lengths body length were was measured using a meter ruler with 1mm accuracy. Average

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weight and length of the 10 female broodfish were 2140 $\pm$ 159 g and 39.70 $\pm$ 1.77 cm, while, 10 of those of the male broodfish were 3060 $\pm$ 135 g and 43.1 $\pm$ 1.79 cm.

Reproduction<u>ve</u> characterization parameters in sage strain of giant gourami broodfishgiant gourami sage strain broodfish were analyzed using the following formula<u>e</u>s:

- \_\_Condition factor (CF) = wet weight in gram/length<sup>3</sup> × 100
- ;•• Ovulated egg weight (g) = fish weight before spawning (g) − fish weight after spawning (g)
- ;••• va somatic index (%) = egg weight ovulation (g)/ fish weight before spawning (g) x 100

<u>:\_ aA</u>bsolute fecundity <u>is-was</u> the total number of eggs <del>was</del> estimated per nest, and relative fecundity <u>is-was the</u> total number of eggs per kg body weight.

#### Female reproductive performance

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

Natural spawning of broodfish is-was carried out using in 1.2-m³ (2×1×0.6 m) concrete freshwater ponds with a male-female sex ratio of (1:1). Before the broodfish is-spawned, the ponds are-were drained, cleaned and all other species are-removed. Then, palm fibers are-were provided which are placed on top of a bamboo raft in the pond. Furthermore, tThe pond is-was then filled with water and the female and male broodfish are-were released into the spawning pond. The male broodfish made a nest for 5 to 7 days, after which spawning takes took place and the female broodfish laiyeds eggs. Spawning ed-occurred in the afternoon (ranged from between 15.00 to 17.00 PM). Due to the presence of a very large oil globule, sago strain of giant gourami giant gourami sago strain eggs float. After the broodfish hads finished laying eggs, the eggs are-were kept by the female broodfish in to the nest. Furthermore, Aafter the eggs-are were had been kept by the broodfish for four hours in the nest, the eggs are-were collected and transferred to an incubation tray, which is-was placed in a ventricular hatching system. A total of 100 eggs each incubation trays. Meanwhile, the broodfish were returned to their pond after-once spawned, and no mortality occurred.

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

#### Determination of sperm quality

Hormonal stimulation for spermination efficiency of males of sage strain of giant gourami broodfish giant gourami sage strain broodfish used LHRH-a preparations (Ovaprim) with dosage 0.5 ml per kg of brooder. Semen samples were obtained from 10 broodfish in sage strain of giant gourami randomly selected from the farm. The male broodfish were first anesthetized with 50 mg L<sup>-1</sup> of MS-222<sup>25</sup>, and 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 2-two hours.

The sperm evaluate assessment included gross (visual) and microscopic examination as reviewed by Rurangwa et al. (2004) and Cabrita et al. (2017)<sup>26,27</sup>. The gross examination was based on visual and physical observation of parameters like the semen volume by collecting the semen in a graduated cylinder and determining the level in millimeters. The mMicroscopic examination was carried out using the Olympus model CX40, with magnification between × 10 and × 25 magnification, to determine other parameters such as motility (MO) percentage and duration, were determined by observing water—activated semen placed on a glass slidge under a microscope. 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 progressive using Neubauer's hemocytometer and calculated as the number of sperm ml<sup>-128</sup>. Semen pH was determined with a hand pH meter (HI8424 Hanna Instruments, USA).

## Water **qQ**uality

Water samples were collected in the spawning pond and incubation trays were collected to determine the alkalinity, hardness and pH. Alkalinity and hardness were measured according to standard procedure presudure PH values were determined with a pH meter (digital mini pH meter phylaph, 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 water

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temperature of in the spawning pond and incubation trays as were measured with a thermometer (Celsius scale).

#### Statistical analysis

Results were given as the means\_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, significant\_correlations were considered significant at p<0.05, and whereas significant trends or tendencies were considered significant at p<0.05.

#### Results

The reproduction reproductive characteristics attempted of female broodfish in sage strain of giantsage giant gourami is summarized in Table 1. Total number of eggs per nest was estimated per nest (absolute fecundity) varied from 2000 to 2650, while relative fecundity (total number of eggs per kg female brooder) varied were between 977 and 1071. The fertility rate ranged from 76 to 84%, and the hatching rate 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 were between 94.73 and 95%.

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

The analyzed of the linear correlation  $(r^2)$  between variables of reproduction characterization parameters in sago strain of giant gourami females broodfish <u>results are</u> shown in Table 3. <u>In t</u>This study, the <u>reproduction reproductive characterization</u>-parameters that <u>had-showed</u> 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, <u>results revealed significant correlations</u> between egg diameter <u>with and the hardened egg diameter</u>  $(r^2 = 0.833)$ , hardened egg diameter increased  $(r^2 = 0.699)$  and fertility rate  $(r^2 = 0.568)$ . 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 larvael (10 days) is also had strong correlations with the hatching rate  $(r^2 = 0.998)$  and endogenous feeding period  $(r^2 = 0.757)$ .

The analyzed of the  $I_L$  inear correlation analysis results ( $r^2$ ) between variables of reproduction characterization parameters in sago strain of giant gourami males broodfish <u>are</u> shown in Table 4.

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The reproductive characterization 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. WhereasIn contrast, the gonadal weight negatively related correlated to with sperm motility ( $r^2$  = 0.017) and duration of motility ( $r^2$  = 0.275). Besides thatIn addition, the sperm concentration is was also had moderately correlatedions 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 in sage strain of giant gourami-were as follows: water alkalinity ranged from 50.5 mg L<sup>-1</sup> to 52.5 mg L<sup>-1</sup>, hardness varied from 65.5 mg L<sup>-1</sup> to 67.5 mg L<sup>-1</sup>, pH ranged from 6.4 to 6.6, oxygen ranged from 6.1 mg L<sup>-1</sup> to 6.7 mg L<sup>-1</sup>. and-temperature varied from 28-°C to 30-°C.

Table 1. Reproduction characterization in sago strain of giant gourami females broodfish (Mean  $\pm$  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

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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  $\pm$  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 <sup>9</sup> /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

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Table 3. Correlations of variables (r²) in sago strain of giant gourami females broodfish

	FEL	FWBS	FWAS	CF	OEW	OVI	AF	RF	EW	HEW	EWI	ED	HED	HDI	FR	HR	Е
FEL																	
FWBS	<u>0.720</u>																
FWAS	0.717	<u>0.999</u>															
CF	<u>0.757</u>	<u>0.575</u>	<u>0.574</u>														
OEW	<u>0.539</u>	<u>0.565</u>	<u>0.553</u>	0.365													
OVI	0.281	0.191	0.012	0.000	<u>0.774</u>												
AF	0.255	<u>0.921</u>	<u>0.864</u>	<u>0.637</u>	0.387	0.072											
RF	0.012	0.207	0.063	0.011	0.065	0.321	<u>0.524</u>										
EW	0.894	<u>0.659</u>	<u>0.655</u>	<u>0.677</u>	<u>0.552</u>	0.246	<u>0.567</u>	0.041									
HEW	<u>0.841</u>	<u>0.631</u>	<u>0.626</u>	<u>0.514</u>	<u>0.582</u>	0.295	0.468	0.004	<u>0.924</u>								
EWI	0.165	0.109	0.109	0.354	0.033	0.000	0.010	0.002	<u>0.924</u>	0.041							
ED	0.164	0.132	0.338	0.131	0.378	<u>0.688</u>	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	<u>0.833</u>					
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			

Information Classification: General

HR	0.035 0.	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	<u>0.703</u>		
EFP	0.113 0	0.186	0.190	0.094	0.006	0.098	0.231	0.103	<u>0.747</u>	<u>0.806</u>	0.147	0.317	0.001	0.116	0.015	0.013	
SR	0.070 0	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	<u>0.757</u>

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 (r2) in sago strain of giant gourami males broodfish

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

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 (mI), SC: sperm concentration (10<sup>9</sup>/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	GSI (%)	Eggs	Hatching	Reference
		fecundity		diameter	rate (%)	
		(egg/kg fish)		(mm)		
0.1	•	4027.00	1.04.0.05	2.42.0.05	76 40 (6.22	TI:
Osphronemus	Sago	1037±90	1.91±0.35	2.42±0.05	76.40±6.33	This study
goramy						
Osphronemus	Bastar	2423±348	2.78±1.16	2.2 ± 0.2	96.36± 2.30	16
goramy						
Osphronemus	Galunggung	4011±287	4.15±0.63	2.5±0.05	89.3±1.30	8
•	Galuliggulig	40111207	4.13±0.03	2.3±0.05	03.3±1.3U	
goramy						

 Osphronemus
 5508±1547
 2.32±0.50
 2.18±0.19
 61.60±0.0
 30

 goramy
 Osphronemus
 Tambago
 2.896±185
 3.16±0.11
 2.47±0.03
 91.06±4.06
 31

 goramy
 31
 31
 31
 31
 31
 31
 31

#### Discussion

In our study, body weight in <u>female</u> sago <u>strain of</u> giant gourami <u>female</u> broodfish before spawn<u>ing</u> ranged from 1958 to 2500 g per fish and ova somatic index ranged from 1.43 to 1.65%. Body weight in <u>female</u> sago <u>strain of</u> giant gourami <u>female</u> broodfish <u>was is the smaller</u> than <u>as that of giant gourami belonging to the galunggung strain, <u>which</u> ranged from 2500 to 3500 g. Conversely, ova somatic index of galunggung strain are found to be slightly bigger than <u>with that of</u> sago <u>strain of</u> giant gourami, <u>which</u> ranged from 3.7 to 4.6%. The differences <u>of in</u> reproductive on <u>characterization characteristics</u> in broodfish can be <u>affected explained</u> by strains, brood size, age used, previous spawning history and the production setting<sup>23</sup>.</u>

Absolute fecundity (AFs) in sago strain of giant gourami ranged between from 2000 to 2650 eggs fish<sup>-1</sup> and relative fecundity (RF)s were ranged from 977 to 1071 eggs kg<sup>-1</sup>. Egg produced in kg fish<sup>-1</sup> (RF) is thought to be more informative than absolute fecundity. Relative fecundity (RF) in sago strain of giant gourami were smaller compared to those in galunggung strain, palapah strain and blusafir strain<sup>8,31,16</sup>. On the other hand, the difference of relative fecundity can also be related to differences in broodfish size and age used<sup>23</sup>. Environmental factors such as rainfall is also influenced the number of eggs per spawn in giant gourami brood, while the water temperature negatively related to number of eggs per spawn<sup>15</sup>. 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, EDs egg diameters obtained average was 2.42±0.05 mm, consistent with those reported by another researcher sylfer example 2.18±0.19 mm for the giant gourami<sup>30</sup>, 2.40±0.05 mm for blusafir strain<sup>16</sup> and 2.5±0.05 mm for galunggung strain<sup>8</sup>. Apparently, tThe differences the relative fecundityRF, ova somatic index, egg diameter and hatching rate of giant gourami can be influenced by differences in the strains. Furthermore, the egg diameter has been influenced by the dietary protein level 32,33,34,35, age of broodfish<sup>36</sup>, and spawning season<sup>37,38</sup>. In our study, egg diameter was shown to be positively correlated with egg weight, hardened egg weight, and eggs weight increased. Egg weight of rainbow trout also increased after the hardening process and is a positively correlated with the viability of

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eggs<sup>39</sup>. Other egg quality metrics, such as hatching rate, and survival to first feeding, has been correlated with good egg quality.

In this study, the hatching rate of embryo (HRs) in sago strain of giant gourami were smaller than those of other strains of giant gouramy<sup>8,31,16</sup>. This condition might be affected by the egg and sperm quality in sago strain of giant gourami broodfishgiant gourami sago strain broodfish. In the present study, whether eggs and sperm quality of sago giant gourami breeders are affected by feed nutrition type for sago strain of giant gourami brooder wasis poorly understood. Meanwhile, the bB roodfish sex ratio has nothad no influence d-on egg quality<sup>8</sup>. The reproduction reproductive characterization parameters that had strong correlations 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. (2010)<sup>40</sup> that indicator of egg quality the strong correlation with biochemical composition of eggs. In this study, we did not evaluate the biochemical composition of egg, because due to the relationshiped between egg quality and biochemical composition is difficult to interpret<sup>41</sup>.

The keys of regulator hormoness of fish reproductioned were are gonadotropins (GTHs), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex steroids  $^{42,43}$ . Besides that In addition, oocyte developmented and maturationed are also regulated by locally acting paracrine and autocrine signalling  $^{144,45}$ . However, there is not information about the effects of such kinds of factors on the oocyte development in sago strain of giant gourami. Nevertheless, extrusion feed enriched with the vitamin E (d- $\alpha$ -tocopherol) as much as 137.8, 238.05, 338.72 and 439.39 mg per kg feed ingredients affected to markers of reproductive functions of giant gourami brooder (e.g., time sexual maturity, ova somatic index, relative fecundity and egg diameter)  $^{31}$ .

Various efforts have been made by scientists to increased reproductive performance of female broodfish, such as to-increasing e-about dietary protein level for *Xiphophorus helleri*<sup>33</sup>, *Channa marulius*<sup>46</sup> and *Ictalurus punctatus*<sup>40</sup>. Additionally, implantation of 17ß-estradiol has also managed to improved the reproductive performance in *Hemibagrus nemurus*<sup>47</sup>. Currently In this context, whether the increase in protein level of feed and use of hormonesal can increase the reproductiveed potential in sago strain of giant gourami are is poorly understood. Therefore, the experiment use of feed protein levels and hormonal dosage it is very important to applied for sago strain of giant gourami broodfishgiant gourami sago strain broodfish in the future.

Average SVs semen volume was found in sago strain of giant gourami are were lower (0.4 to 0.6 ml) than those of *Hemibagrus wyckii* (0.60 to 1.20 ml)<sup>21</sup>, but higher than those of *Pterygoplichthys gibbiceps*<sup>48</sup>. It appears that the semen volume depend on fish species<sup>21,49,50</sup>. So mMany factors influenced ensperm quality and quantity of sperm-such as genetic, physiological, spawning season

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and environmental factors.<sup>26,49,51,52</sup>. On the other hand, improvements nutrition feed of broodfish and proofer feeding greatly improve gamete quality and larval production<sup>46,53</sup>. While, synthetic hormones such as salmon gonadotropin releasing hormone analogue and domperidone (GnRH + Domperidone) effective on sperm quality<sup>54,55</sup>. Nevertheless, <u>for sperm fertility competition occurs in the aquatic environment, and that had strong correlationand was strongly</u> with the water quality in ponds. In this study, the water quality parameters in spawning ponds in terms of alkalinity (50.5 to 52.5 mg L<sup>-1</sup>) and hardness (65.5 to 67.5 mg L<sup>-1</sup>), pH (6.4 to 6.6) and water temperature (28 to 30 °C). The all water quality parameters can be supported the ability for sperm to fertilized an egg.

The sperm motility in sago strain of giant gourami ranged from 68 to 75%, and duration of motility ranged from 43 to 61 sec. Theseis results that are consistent with Genypterus blacodes and Esox lucius<sup>56,50</sup>. The Seperm motility includesd the percentage of motile sperm, straight line velocity, curvilinear velocity, average path velocity and linearity<sup>50</sup>. In this study, we did not investigate of those parameters. Meanwhile In addition, the percentage of motileity sperm is influenced by addition of extenders and cryoprotectants<sup>57,58,59</sup>. However, sperm motility on from fresh semen was slightly better greater compared to cryopreser vedvation semen on from the Esox lucius 50. The fertility rate of eggs ranged from 76 and 84% is nevertheless however, it is does not detected any no significanttly correlation was detecteds between fertility rate and sperm parameters. Conversely, the sperm concentration had was moderately correlated on with the 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), includinged fertilizing capacity  $^{49,50,52,60}$ . In  $\mp$ 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 reproduction-reproductive characterization characteristics in the sage strain of giant gourami broodfishgiant gourami sage strain broodfish reared in concrete freshwater ponds, in the Aquaculture Laboratory Faculty of Fisheries and Marine Science, Universitas Bung Hatta. Relative fecundities of the Sage strain of giant gourami broodfishgiant gourami sage strain broodfish had the relative fecundities ranged from 977 to 1071 eggs, and egg diameter between ranged from 2.32 to 2.46 mm. The semen Semen volume ranged from 0.4 to 0.7 ml per kg body weight and sperm motility were was comprised between 68 to 75%. There was aA strong linear

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relationship was observed between absolute fecundity with and the female fish weight before and after spawning. Similarly, the similar relationship was observed between absolute fecundity, and male sperm concentration and sperm motility for the reared sago strain. Keys to increasing reproduction performance in sago strain of giant gourami depends upon on broodfish weight size of broodfish, relative fecundity and hatching rates. Despite initial has been success for spawning, there are observed limitations of quality seed supply for aquaculture attributed to knowledge gaps in larval weaning, grow-up feeding technologies. Therefore, for the success successful practiced practices in hatcheryhatcheries, where further research is recommended for to determine a proper feed formulation and the development of appropriate aquaculture systems.

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#### **Data availability**

Underlying data

RepositoryFigshare: Title. 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].

# DOI: 10.6084/m9.figshare.14661189

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 (%) in sago strain of giant gourami broodfish

- Table 10. Male size, gonadal weight and semen in sago strain of giant gourami broodfish
- Table 11. Sperm concentration (10<sup>9</sup>/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

<u>Data are available under the terms of the Creative Commons Attribution 4.0 International</u> license (CC-BY 4.0). <u>License: CC BY 4.0</u>

#### **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.

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