ORIGINAL ARTICLE

Behavior and reproduction of belida fish (*Notopterus notopterus*, Pallas 1769) in different stocking density and nurtured with different types of feed

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Received: March 27, 2020 ▪ Accepted: May 05, 2020 ▪ Published Online: May 28, 2020

Abstract Belida fish (*Notopterus notopterus*, Pallas 1769) is one of Indonesia's endemic fish that has high economic value so that the catch is so high and has begun to decline in population from nature. The purpose of this study was to analyze the behavior and reproduction of belida fish that nurtured with stocking density and different types of feed. This study was conducted from April to June 2018 in the Fish hatchery and Breeding Laboratory of Fisheries and Marine Science Faculty, Riau University. The design used is a randomized complete design with stocking density treatment consisting of 5, 10, and 15 tail/m3 and type of feed consisting of pellet + vitamin E feed and trash fish feed (dumbo catfish cubs). The results showed that the best treatment was in the stocking density of 5 tail/m3 and trash fish feed (dumbo catfish cubs) showed aggressive fish behavior in chasing the feed given, resulting in reproductive value consisting of the fish number of gonad maturity (TKG IV) as many as 5 fishes, gonado somatic index value of 0.58%, the fecundity of 6,053 eggs, egg diameter of 2.49 mm and semen volume of 0.045 ml.

Keywords: egg diameter, fecundity, fish behavior, gonad maturity, gonadosomatic index, knife fish

Introduction

Belida fish (*Notopterus notopterus*, Pallas 1769) is one of Indonesia's endemic fish that has high economic value. Community consumption of belida fish is so high (Sunarno 2002). In addition to consumption fish, belida fish can also be used as ornamental fish. Community needs for these fish are still obtained from catches in public waters so that the population of belida fish is decreasing (Yulindra et al 2017). Aquaculture activities can be applied to reduce the exploitation of fish from nature for human consumption (Anderson et al 2011; Benkendorff 2009). With the cultivation of belida fish, the community's need for this fish can be met and its preservation from nature will also be maintained.

To carry out fish farming activities, one aspect that needs to be done is the supply of mature gonads and ready for breeding. In gonad maturation activities, fish need to pay attention to stocking density and type of feed given. Both of these aspects will affect the behavior of fish in utilizing the feed given at the same time will determine the success of the fish gonad maturation process known as the process of vitellogenesis in female fish and spermatogenesis in male fish.

Stocking density affects stress levels in fish and continues to physiological activities in fish that affect the welfare status of fish which is divided into primary, secondary, and tertiary responses (Zahedi et al 2019). The primary stress response influences neuro-hormonal stimulation which increases catecholamine and corticosteroid secretion (Foo and Lam 1993). The secondary stress response influences the hydromineral balance, increased heart rate, oxygen absorption, and energy mobilization to meet high energy demand (Wendelaar 1997). The tertiary stress response involves changes in growth, reduction in feed consumption, impaired fish health and immunity, reproduction, and survival of individuals or (Ellis et al 2002; Suarez et al 2015; Yarahmadi et al 2016). Until now it has not been known that the best stocking density is to assess the behavior of belida fish in the utilization of feed used for gonad maturation.

Feed management is an important part of the fish culture (Ullman et al 2019). One of the most important parts of feed management is the type of feed given. Belida fish is a type of carnivorous fish, (Shillewar and Nanware 2009). Carnivorous fish have a lower ability to utilize carbohydrates in the feed compared to herbivorous fish and omnivorous fish (NRC 2011). Based on the results of study on the intestinal content of belida fish, it is known that belida fish in nature eat organic detritus, fish scales, insects, fish, shrimp, sand, and

doi.org/10.31893/jabb.20024

plant litter (Srivastava et.al 2012). Until now not yet known the best type of food for the maturation of belida gonads.

The purpose of this study was to analyze the behavior and reproduction of belida fish that are cultivated with stocking densities and different types of feed.

Materials and Methods

This study was conducted at the Fish hatchery and Breeding Laboratory of Fisheries and Marine science Faculty of Riau University (Figure 1) from April to June 2018. Gonad maturation was carried out by nurturing broodfish prospective of belida fish gonad maturity levels II (TKG) in a concrete tub that has been sealed off with a 1 x 1 x 1 m3 net (Figure 2).

The fish are nurtured in cultivation containers for 60 days with stocking densities of 5, 10, and 15 fish/m3 and the type of feed consisting of pellet + vitamin E feed and trash fish feed (dumbo catfish cubs). Feeding is done 3 times a day, in the morning, afternoon, and evening. Observation of the test fish's behavior in the utilization of the given feed is done after each feeding while observing the reproduction of the test fish is done once in ten days by observing the reproduction of all fish that are nurtured. The belida fish gonad maturity obtained was taken to be weighed using Shimadzu ELB600 type analytic scales and measured in length with graph paper. To find out the reproduction parameters, a fish is operated on the abdomen to retrieve and observe fish gonads. The gonads obtained were weighed and observed for eggs (female fish) and semen (male fish). The behavior of the test fish was analyzed in the description while the reproduction of the test fish was analyzed statistically.

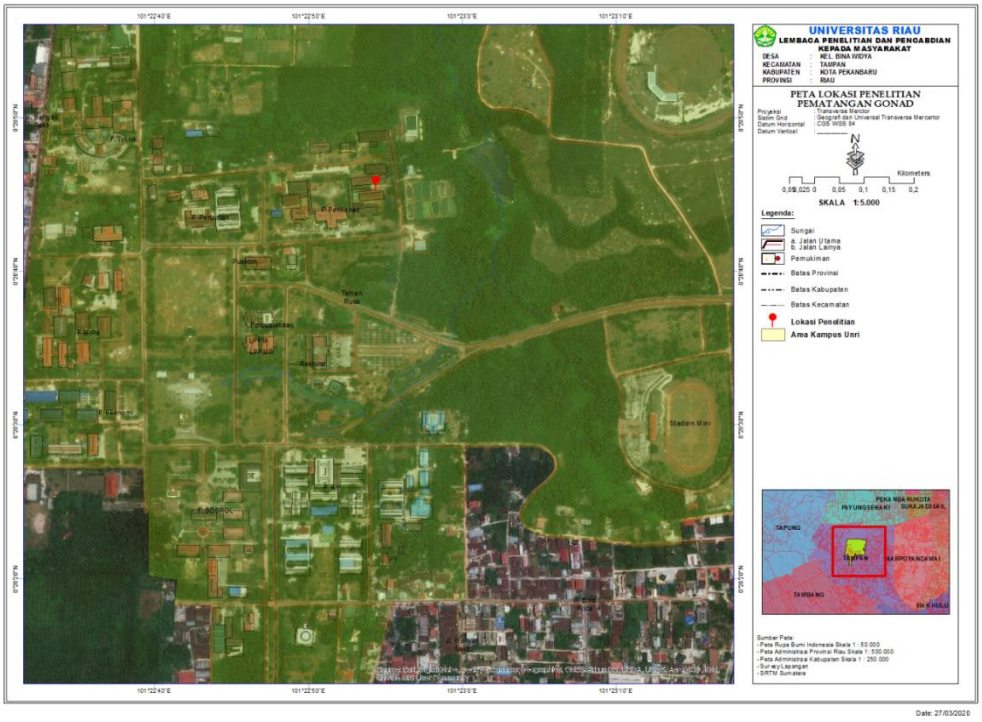

Figure 1 Study location map. Source: Google maps

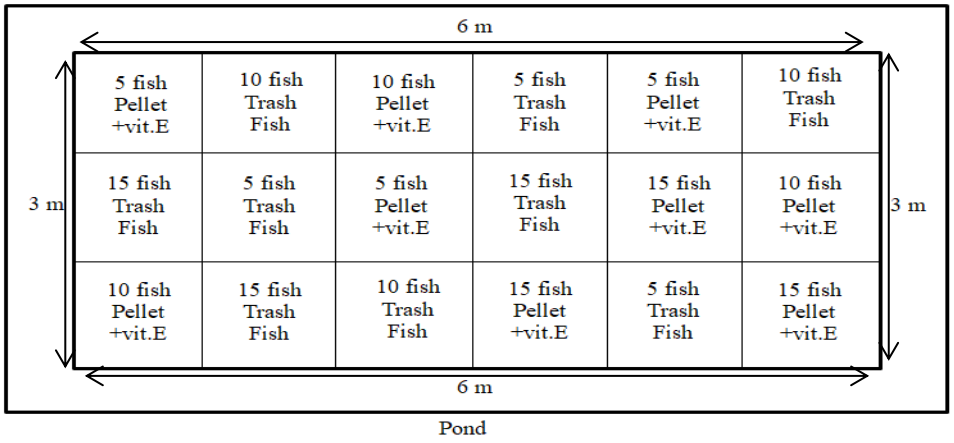

Figure 2 Experiment Design.

The design used was a completely randomized design (CRD) with 6 combination treatments and 3 replications. The design model is as follows: $Yij = \mu + \tau i + \sum ij$
where : Yij = The results of observations of individuals who are treated to i and replications - j, μ = General average, τ i = Effect of treatment to - i and $\sum ij$ = Effect of error treatment to – i, replications to – j.

The test parameters measured to analyze the behavior and reproduction of belida fish kept with stocking densities and different types of feed are:
1. Fish behavior, analyzed in the description that is by directly observing the behavior of belida fish in the utilization of the feed provided, namely feeding in the morning, afternoon, and evening.
2. Gonad Maturity Level (TKG), determined by observing fish that have TKG IV which is done once in ten days
3. Gonad Maturity Index (IKG), determined using the equation:

$$GSI = \frac{\text{Gonad Weight (g)}}{\text{Body Weight (g)}} \times 100\ \%$$

4. Fecundity, determined using the sub-example method with gravimetric (Nikolsky 1963), namely: F: t = B b; where: F = fecundity (grains), t = number of eggs from gonad samples (grains), B = total gonad weight (grams) and b = gonad sample weights (grams).
5. Egg diameter, measured by taking egg samples of 50 eggs from female fish that have had TKG IV, then measured under a microscope with the assistance of an ocular micrometer.
6. Semen volume is determined by dissecting the male fish gonad maturity then the semen is sucked using a syringe without a needle.

The water quality of the belida fish nurturing container is checked once a week in the morning, afternoon, and evening. Temperature and pH are measured using a Milwaukee pH55 type pH meter. Dissolved oxygen was measured using a DO meter with the type Lutron DO-5510. Water quality data are written and described.

Results and Discussion

Belida fish behavior

Based on observations made on test fish, it is known that there are differences in fish behavior when given different feeds. All of the same feed treatments showed the same fish behavior on each stocking density treatment. Belida fish are more aggressive and have a strong response to the trash fish feed given (dumbo catfish cubs). Trash fish that is given alive so that it attracts the attention of belida fish to eat it. When trash fish swim in nurturing containers, belida fish grabbed and ate them immediately. In contrast to the treatment of pellet + vitamin E feed given, the response of the test fish is less interested in the feed given, even rarely eaten by belida fish. Differences in the behavior of fish with the type of feed given are due to the nature of the belida fish which is a predatory fish that is hunting for food. This is consistent with the results of study conducted by Shillewar and Nanware (2009) that belida fish are a type of carnivorous fish. Furthermore, Srivastava et.al. (2012) states based on the results of study on the intestinal content of belida fish it is known that belida fish in nature eat organic detritus, insects, fish, shrimp, sand and plant litter. Burnawi and Pamungka (2016) stated that the percentage of food content in the intestines of belida fish were fish (90.044%), aquatic insects (5.568%), detritus (1.075%), insects (0.669%), worms (0.048%), detritus (0.048%) 1,075%) and unknown (0.670%).

Belida fish reproduction

Giving stocking densities and different types of feed give an effect on the measured parameters of belida fish reproduction, namely gonad maturity level (TKG) IV, gonad maturity index (IKG), fecundity, egg diameter and semen volume (Table 1).

Table 1 Average values of gonad maturity level (TKG), gonad maturity index (IKG), fecundity, egg diameter and volume of belida fish semen from each treatment during the study.

Treatment	TKG IV (Fishes)	IKG (%)	Fecundity (Grains)	Egg Diameter (mm)	Semen volume (ml)
P1	3	4,1	4330	1,91	0,035
P2	5	5,8	6053	2,49	0,045
P3	5	4,0	4316	1,83	0,035
P4	7	5,2	5560	2,37	0,041
P5	8	3,7	3935	1,82	0,034
P6	10	4,8	5162	2,21	0,04

Remarks: P1 = stocking density 5 tail/m^3 and pellet feed + vitamin E; P2 = stocking density 5 tail /m^3 and trash fish feed; P3 = stocking density 10 tail /m^3 and pellet feed + vitamin E; P4 = stocking density 10 tail /m^3 and trash fish feed; P5 = stocking density 15 tail /m^3 and pellet feed + vitamin E; P6 = stocking density 15 tail /m^3 and trash fish feed

doi.org/10.31893/jabb.20024

Gonad maturity level (TKG) IV belida broodfish

The results of observing the level of gonad maturity (TKG) IV of belida fish during nurture ranged from 3-10 fishes. Highest TKG IV of belida fish obtained in treatment P6 (stocking density of 15 fishes/m^3 and trash fish feed) of 10 fish (6 females and 4 males) followed by P5 (stocking density of 15 fish/m^3 and pellet feed + vitamin E) as much as 8 fish (5 females and 3 males), P4 treatment (stocking density 10 fish/m^3 and trash fish feed) of 7 fishes (4 females and 3 males), P3 treatment (10 stocking densities)/m^3 and pellet feed + vitamin E) and P2 treatment (stocking density of 5 fish/m^3 and trash fish feed) of 5 fish (3 females and 2 males) and P1 treatment (stocking density of 5 fish/m^3 and feed pellets + vitamin E) of 3 fish (2 females and 1 male). For more defishes on the attainment of maturity level (TKG IV) belida fish can be seen in Figure 3.

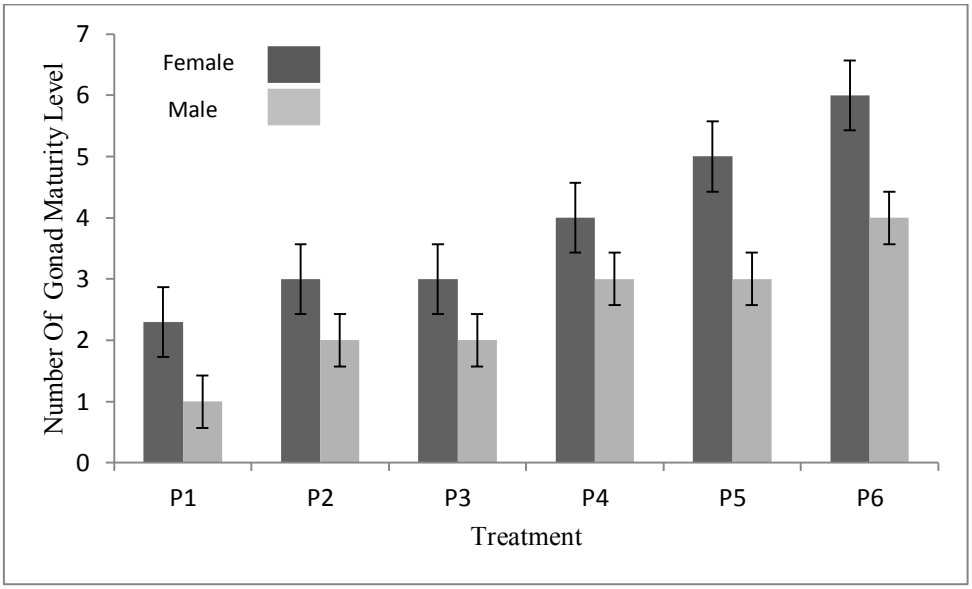

Figure 3 Histogram of the achievement of TKG IV belida fish (*Notopterus notopterus*, Pallas 1769) from each treatment during the study.

Based on the results of statistical tests using analysis of variance (ANOVA) it is known that the treatment given has significant influence ($P < 0.05$) on the value of gonad maturity level (TKG IV) of female fish and the value of gonad maturity achievement (TKG IV) of male fish.

The highest result of gonad maturity level (TKG IV) obtained from this study was 10 fish with 15 fish/m^3 stocking density treatments fed trash fish. This is because belida fish prefer trash fish (dumbo catfish cubs) compared to artificial feed (pellets + vitamin E), according to observations of the behavior of aggressive belida fish in response to the feed given. Belida fish in nature are carnivorous fish and actively foraging at night (Norhidayah et al 2016). Furthermore, Sunarno (2015) states that the development of belida fish eggs depends on the adequacy and balance of feed nutrients received and climatological conditions.

Test fish Gonad maturity level (TKG IV) was always obtained at the nurturing of the fourth week of each treatment, other than that the difference in the number of mature belida gonads in each observation week was not significant. This indicates that the fish do not spawn at certain times. This fact is also consistent with the opinion of Gustomi et al (2016) that based on observations of the maturity of belida gonads in the simpur dam pond, it was found that the discovery of belida fish gonad maturity in each observation month.

Gonad maturity index (IKG) of belida broodfish

The results of observations of the gonad maturity index (IKG) of belida fish during nurturing ranged from 3.7-5.8% (Table 1). The highest average IKG value was obtained in P2 treatment (5 /m^3 stocking density and trash fish feed) by 5.8%, followed by P4 (10 fish/m^3 stocking density and trash fish feed) by 5.2%, P6 (stocking density of 15 fish/m^3 and trash fish feed) at 4.8%, P1 (stocking density of 5 fish/m^3 and feed pellets + vitamin E) by 4.1%, P3 (stocking density of 10 fish/m^3 and feed pellets + vitamin E) by 4.0%, and P5 (stocking density of 10 fish/m^3 and pellet + vitamin E feed) by 3.7%. For more defishes, the average value of belida fish IKG from each treatment during the study can be seen in Figure 4.

Based on the results of statistical tests using analysis of variance (ANOVA) it is known that the treatment given has a very significant effect ($P < 0.01$) on the IKG value of the belida fish. The value of IKG obtained in P2 (5-fish) stoking density treatment with trash fish feed) of 5.8% is due to the 5-fish/m^3 stocking density giving free space to the test fish to be able to catch the trash fish feed (dumbo catfish cubs) is given,

following the results of observing the behavior that has been obtained previously. The more effective the test fish is eating trash fish feed will increase the IKG value of the fish. The IKG value obtained in this study is greater than the IKG value of belida fish from Shankar and Kulkarni's research (2007) where the highest IGS value obtained was 3.67%, but smaller than the value of the belida fish IKG originating from simpur dam pond where the highest value of belida fish IKG was 7.92% (Gustomi et al 2016) Santoso (2009) stated that the value of belida fish IKG was around 0.16-0.89%. Chakrabarti and Chowdhury (2013) stated that the highest belida fish IKG value was obtained in June with an IKG value of 18.571. According to Shankar et al (2007) the condition of fish is influenced by seasonal changes and feed intensity.

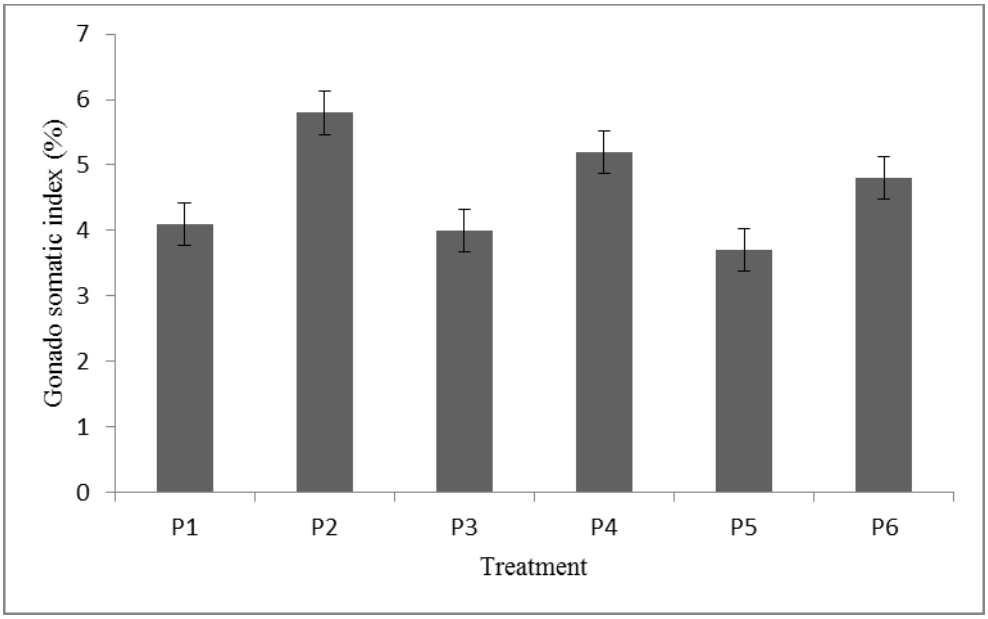

Figure 4 Histogram of belida fish IKG values (*Notopterus notopterus*, Pallas 1769) from each treatment during the study.

Belida fish fecundity

The results of observations of the belida fish fecundity during their nurturing ranged from 3,935 to 6,053 (Table 1). The highest average fecundity was obtained in P2 treatment (5 fish/m^3 stocking density and trash fish) as many as 6,053 eggs, followed by P4 (10 fish/m^3 stocking density and trash fish feed) as much as 5,560 eggs, P6 (15 fish stocking density/15 m^3 and trash fish feed) as much as 5,162 eggs, P1 (stocking density of 5 fish/m^3 and feed pellets + vitamin E) as much as 4,330 items, P3 (stocking density of 10 fish/m^3 and feed pellets + vitamin E) as much as 4,316 items and P5 (stocking density of 15 fish/m^3 and pellet + vitamin E feed) of 3935 grains. For more defishes, the average value of belida fish fecundity from each treatment during the study can be seen in Figure 5.

Based on the results of statistical tests using analysis of variance (ANOVA) it is known that the treatment given has a very significant effect ($P < 0.01$) on the value of belida fecundity. The value of belida fish fecundity obtained from this study shows that it is not much different from belida fish fecundity found in simpur dam ponds, which ranges from 1,052 to 6057 items (Gustomi et al 2016), but higher, when compared to belida fish (*Notopterus notopterus*) in the Bung Lahan Chaiyapum Province, Thailand with fecundity values ranging from 246-989 grains (Jantrachit and Nuangsit 2008). Adjie et al (1999) states that the belida fish fecundity is low when compared to other fish. Notopterus notopterus type of belida fish has a lower fecundity value compared to Chitala chitala fish whose eggs reach 11,972 grains (Wibowo et al 2010). Furthermore, Srivastava et al (2012) stated that the fecundity of belida fish with a body size of 180-189 mm was very low, the fish body size of 260-269 mm ranged from 140-161 items, the fish body size of 320-329 mm ranged from 481 to 535 grains and fish with a size of 340-349 mm have 1,392-grain fecundity. Fecundity is also very dependent on the size of the gonad, while the size of the gonad depends on the value of the IKG, the greater the value of the IKG, the greater the value of fecundity. This fact is evident from the results of study in which the largest IKG value was obtained in P2 treatment (5 stocking densities with trash fish feed) of 5.8%, it turns out that the largest fecundity was also obtained in the treatment of 6,053 grains. The interaction of IKG values with fecundity is presented in Figure 6.

Belida fish egg diameter

The results of observations of the diameter of belida fish eggs during their nurturing ranged from 1.82 mm to 2.49 mm (Table 1). The highest average egg diameter values obtained at P2 treatment (5 fish/m^3 stocking density and trash fish feed) were 2.49 mm, followed by P4 (10 fish/m^3 stocking

density and trash fish feed) of 2.37 mm, P6 (stocking density of 15 heads / m3 and trash fish feed) of 2.21 mm, P1 (stocking density of 5 heads/m^3 and pellet feed + vitamin E) of 1.91 mm, P3 (stocking density of 10 heads/m^3 and pellet feed + vitamin E) of 1.83 mm, and P5 (stocking density of 15 fish/m^3 and pellet feed + vitamin E) of 1.82 mm. For more details, the average value of belida fish egg diameter from each treatment during the study can be seen in Figure 7.

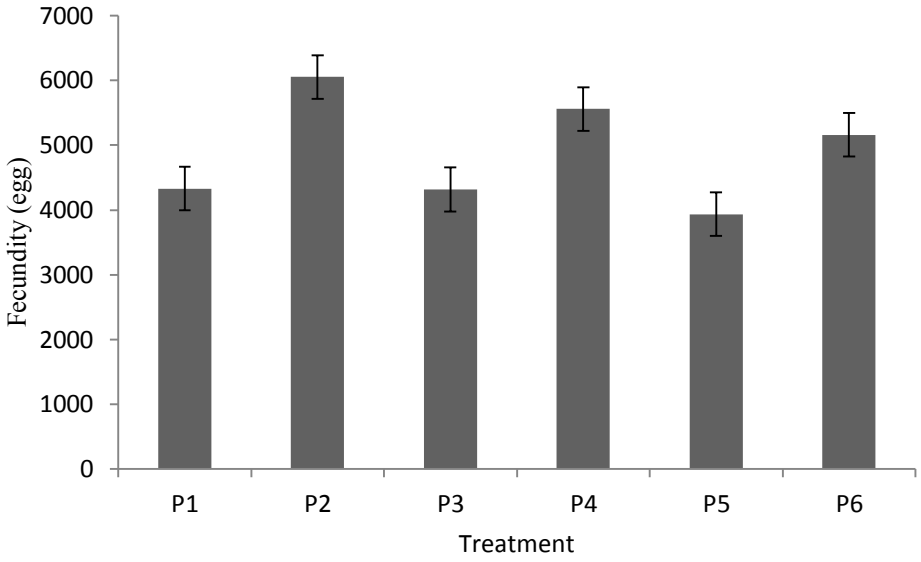

Figure 5 Histogram of belida fecundity (*Notopterus notopterus*, Pallas 1769) from each treatment during the study.

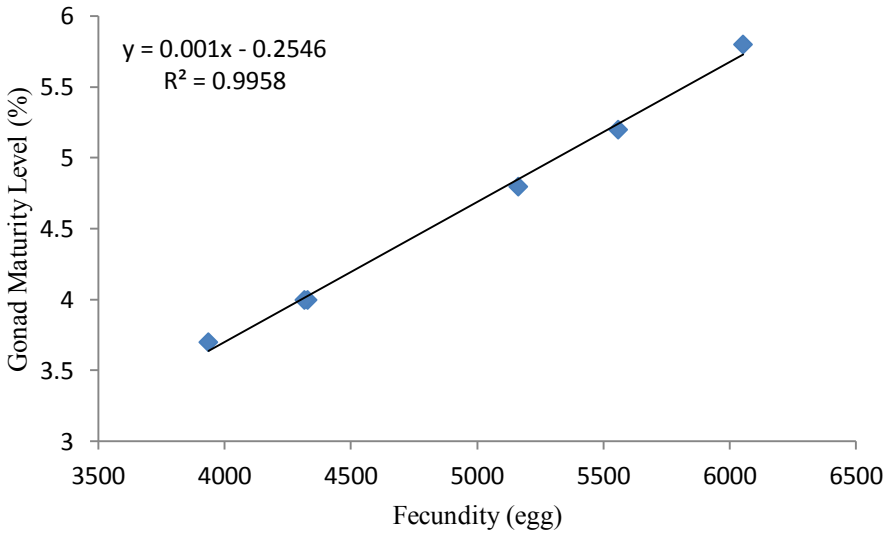

Figure 6 Interaction of gonad maturity index (IKG) with fecundity.

Based on the results of statistical tests using analysis of variance (ANOVA), it was shown that the treatment is given had a very significant effect ($P < 0.01$) on the value of the belida fish egg diameter. Value of belida fish egg diameter obtained from each treatment following the results of study by Gustomi et al (2016) which states that the diameter of belida fish eggs (*Notopterus notopterus*) in TKG IV ranged from 1.21-2.52 mm. The results also showed that the diameter of belida fish eggs obtained depends on the value of fish fecundity and IKG, the greater the IKG value, the greater the fecundity value and the greater the egg diameter value. While the IKG value is highly dependent on the ability of fish to utilize the feed provided. When connected with observations of the behavior of fish in utilizing the feed given, in the P2 treatment (5 stocking density with trash fish) the test fish has an aggressive behavior in chasing the feed given, and it is

evident in the treatment that the IKG value is also obtained, the largest egg fecundity and diameter. The interaction of IKG values with egg diameter can be seen in Figure 8 while the interaction of fecundity values with egg diameter can be seen in Figure 9.

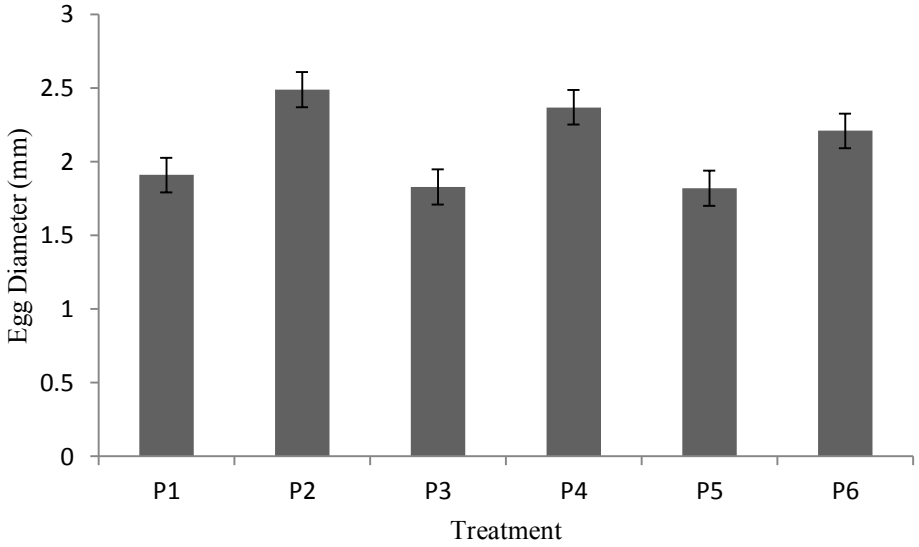

Figure 7 Histogram of belida fish egg diameter (*Notopterus notopterus*, Pallas 1769) from each treatment during the study.

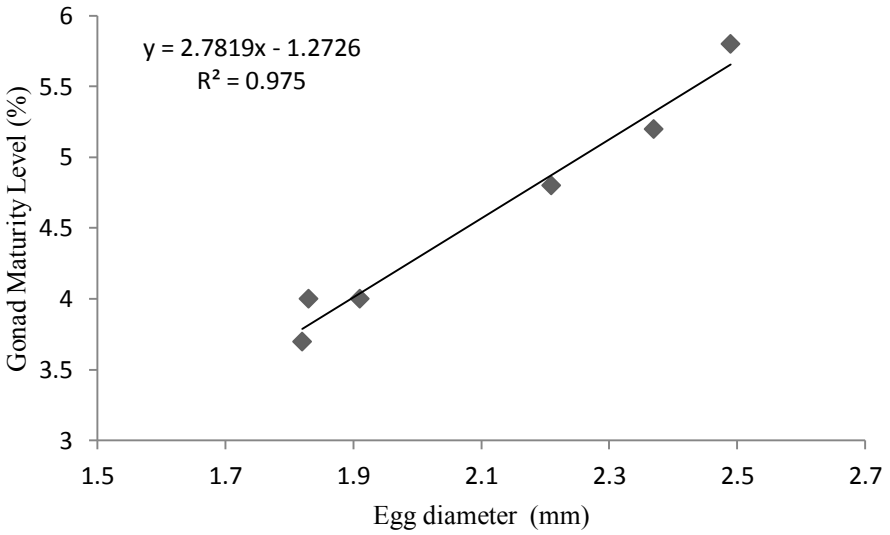

Figure 8 Interaction of gonad maturity index (IKG) with egg diameter.

The volume of belida fish semen

The results of observations of the volume of semen belida fish during nurturing ranged from 0.034 ml to 0.045 ml (Table 1). The highest average volume of semen was obtained in the treatment of P2 (stocking density of 5 fish/m^3 and trash fish feed) of 0.045 ml, followed by P4 (stocking density of 10 fish/m^3 and trash fish feed) of 0.041 ml, P6 (stocking density 15 fish/m^3 and trash fish feed) by 0.040 ml, P1 (stocking density of 5 fish/m^3 and pellet feed + vitamin E) and P3 (stocking densities of 10 fish/m^3 and pellet feed + vitamin E) respectively by 0.035 ml and P5 (stocking density of 15 fish/m^3 and pellet feed + vitamin E) of 0.034 ml. For more details, the average value of belida semen volume from each treatment during the study can be seen in Figure 10.

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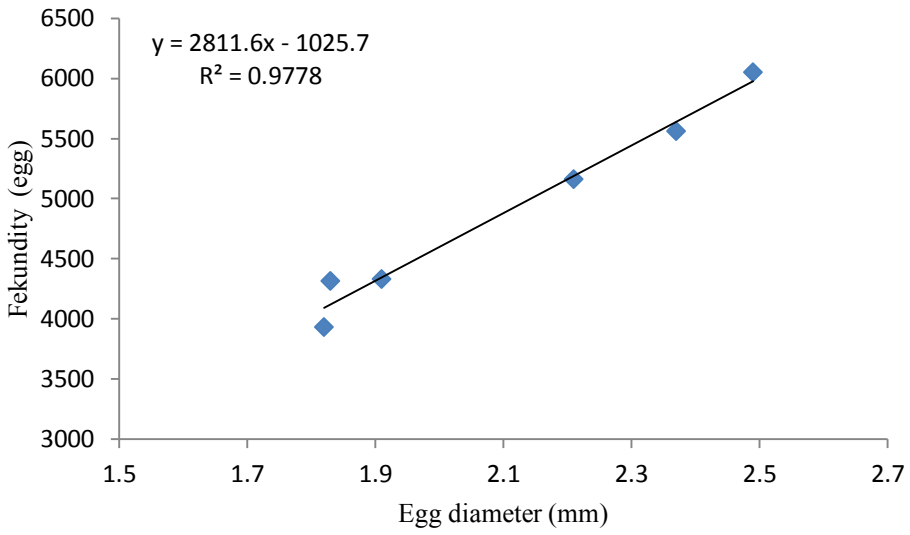

Figure 9 Interaction of fecundity with egg diameter.

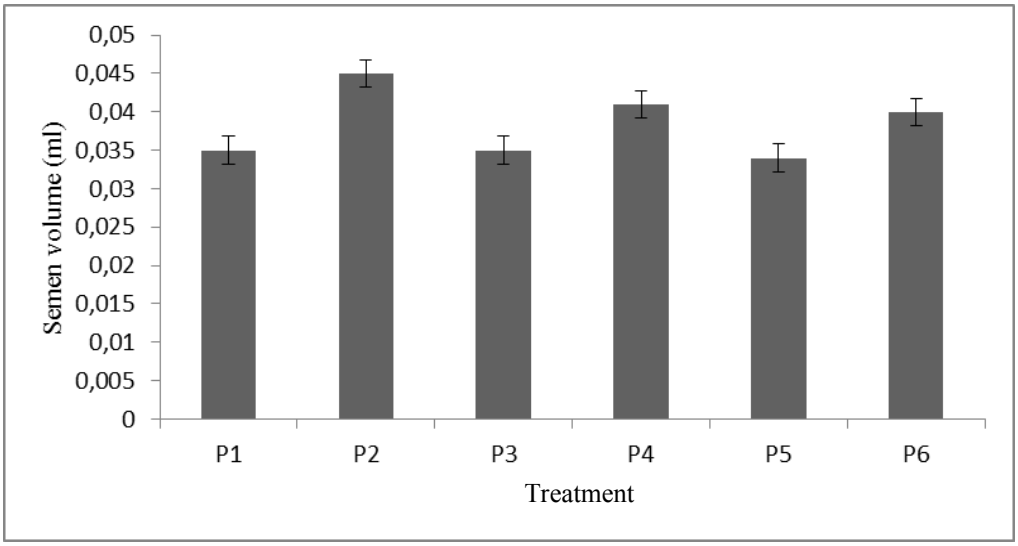

Figure 10 Histogram of belida semen (*Notopterus notopterus*, Pallas 1769) semen volume from each treatment during the study.

Based on the results of statistical tests using the analysis of variance (ANOVA) it shows that the treatment given does not affect ($P > 0.05$) on the volume of semen of belida fish. The highest volume of belida fish semen obtained during maintenance is 0.045 ml. The value of the semen volume of belida fish obtained is relatively small compared to pawas fish with the highest semen volume obtained 0.575 ml (Sukendi et al 2016). The greater the value of the cement volume, the better the quality of fish spermatozoa. According to Sukendi (2012) with the large value of the volume of semen produced is needed for spawning in fish, because this large volume of semen will also increase the value of spermatozoa viability and totality. Sukendi (2001) states that in principle the process of spermatozoa removal in male fish is the same as the maturation of eggs in female fish.

Water quality

The quality of water for the nurturation of belida fish during the study is an average temperature of 29 °C, pH value of 6.5 and Dissolved Oxygen of 5.2 ppm. The value of water quality is still relatively good for the nurturation of freshwater fish.

Conclusions

It can be concluded that the treatment of stocking density and type of feed affects the behavior and reproduction of belida fish. Treatment of stocking 5 fish/m^3 and trash fish feed is the best treatment of fish behavior in response to the feed given at the same time will produce the highest reproductive value, with IKG value of 5.8%, the fecundity of

6,053 eggs, egg diameter of 2.49 mm and semen volume of 0.045 ml.

Acknowledgments

Thanks to the Ministry of Research and Technology, Indonesia for funding this research through the National Strategic research program.

Conflict of Interest

The authors declare no conflict of interest.

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