Effects of different concentrations of bioslurry on the productivity of *Daphnia*

Yuli Andriani, Nidia Puspitasari, Herman Hamdani, Zahidah

Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Sumedang 45363, Indonesia. Corresponding author: Y. Andriani, yuli.andriani@unpad.ac.id

Abstract. *Daphnia* species are commonly used as a live fish feed with high nutritional value .The purpose of this study was to find the bioslurry concentration that resulted in the highest *Daphnia* production. *Daphnia* neonates of the same age were cultivated in jars at a density of 100 individuals per litre of water and were used in an experiment with a completely randomized design involving five feeding treatments, each with three replications. The treatments provided feed in the form of quail droppings at 2.4 g L^{-1} (the control or treatment A) or 2.5, 2.75, 3, or 3.25 g L^{-1} of wind-dried bioslurry (treatment B, C, D or E, respectively). The observed parameters were the growth and reproduction rates of *Daphnia* and water quality measures. Analysis of variance, with *F*–tests at 95% confidence interval, was used to compare treatments. Treatments D and E yielded the highest growth rates at 0.42-0.50 individuals day^{-1} and the highest reproduction rates at 0.34–0.44 individuals day^{-1}. Water quality in the bioslurry treatments was within the normal range for *Daphnia* species. Based on these results, a culture medium with a bioslurry concentration of 3-3.25 g L^{-1} is suitable for obtaining the highest yields of *Daphnia* species.

Key Words: bioslurry, *Daphnia*, death rate, growth rate, reproduction rate.

Introduction. In aquaculture, a suitable diet for the rearing of fish larvae is critical, since it affects their growth, survival, and disease resistance in the early stages of development (Sontakke et al 2019). The egg yolk is the only food source for newborn fish larvae. After the yolk is depleted, larvae seek alternative sources of nutrition, one of which is their natural diet (Budiardi et al 2005). Proteins, lipids, carbohydrates, vitamins, minerals, amino acids, fatty acids, and carotenoids are abundant in natural fish diets (Simhachalam et al 2015).

Daphnia spp. are a food source with high nutritional value which is recommended as food for fish larvae (El-Feky & Abo-Taleb 2020). Exoenzymes such as proteinases, amylases, lipases, and cellulases are abundant in *Daphnia*, and they help in the digestion and development of fish larvae. Exoenzymes are not generally available in other natural diets (Wang et al 2008). *Daphnia* species require nutrients for growth during the cultivation process (Zahidah et al 2012).

Bioslurry is an anaerobic organic material that is a by-product of biogas production from cow dung. It is the fermentation residue produced as a slurry through a series of anaerobic or fermentation processes in a biogas digester (Kabir et al 2017). *Daphnia* species can use bioslurry as a food source since it contains micro- and macronutrients (Dhull et al 2004). According to Singgih & Yusmiati (2018), a single biogas digester unit of 10 m^3 can yield 10 kg of dry bioslurry per day. Bioslurry waste from biogas production is still not properly managed, and has, therefore, become an environmental problem (Jeptoo et al 2012).

Bioslurry has the potential to be an important food option in *Daphnia* culture as it may boost *Daphnia* production, while its use in aquaculture could reduce waste. However, bioslurry has never been studied before as a food source for *Daphnia* species. Therefore, research on its application as a food source for *Daphnia* is necessary to evaluate the optimum concentration that yields the highest *Daphnia* production.

Material and Method. This study was carried out at the Aquaculture Laboratory, Building 4, Faculty of Fisheries and Marine Science, Universitas Padjadjaran from 13 October 2020 to 11 December 2020. *Daphnia* brood stock was obtained from the Ciparanje Hatchery Zone of the Faculty of Fisheries and Marine Science. Quail manure was obtained from Sumedang's Puyuh Nyampak Jaya and farmers in Batu Kuda, Sumedang provided the bioslurry.

The following devices were used in this study: a 2-litre plastic jar, an aerator set, a label sticker, a water heater, a gauze, a camera, a hand counter, a scale with 0.01% accuracy, a measuring glass, test solutions that included O_2, $MnSO_4$, and H_2SO_4 reagents for dissolved oxygen (DO) titration. This study involved three steps: research preparation; the formation of a *Daphnia* cohort; and the main research. Quail droppings and bioslurry were wind dried. They then underwent proximate testing at the Faculty of Animal Husbandry of Universitas Padjadjaran. A two-litre jar was filled with one litre of water. Different amounts of quail droppings or bioslurry were placed on gauze according to each treatment. The gauzes were then placed in the jar and aerated for two days. A *Daphnia* cohort (individuals of the same age group) was then formed. *Daphnia* individuals (F0) were first acclimatised for ±15 min and cultured, and neonates (F1) were removed after a day to be re-cultured in other jars, while the adults were moved to a separate container. *Daphnia* neonates (F1) were then cultured to maturity and reproduced (F2). For each replicate of each treatment, 100 *Daphnia* neonates (F2) were transferred to a separate container, resulting in the use of a total of 1,500 neonates for five treatments with three replications. The number of neonates and the number of adults were recorded every day until they all died.

The experimental method was a completely randomised design that included five treatments with three replications: treatment A (the control with 2.4 g L^{-1} quail manure); treatment B (2.5 g L^{-1} of bioslurry); treatment C (2.75 g L^{-1} of bioslurry); treatment D (3 g L^{-1} of bioslurry); and treatment E (3.25 g L^{-1} of bioslurry). The water quality parameters measured were pH, DO, and temperature. The following formulae (Birch 1948) were used to calculate the growth rate of *Daphnia*:

$$r = \frac{\ln Ro}{T}$$

$$\sum e^{7-rx} l_x m_x = 1097$$

where: Ro was the net reproduction rate; T was average length of life; e was the natural logarithm; r was growth rate; x was maintenance time; l_x was the number of individuals alive at time x; and m_x was the number of individuals at time x.

The reproduction rate was calculated using the following formulae (Birch 1948):

$$b - \frac{r\beta}{e^r - 1}$$

$$1/\beta = \sum L_x e^{-r(x+1)}$$

where: b was the rate of reproduction (births); L_x = (lx + lx+1)/2 was the average number of individuals in the x-age group (KU-x) and the x+1 age group (KU-x+1); e was the natural logarithm; and r was the growth rate.

Statistical analysis*.* Analysis of variance (ANOVA) was used to compare the treatments, with the *F*-statistic at 95% confidence interval. Duncan's multiple range test at 95% confidence interval was used for multiple means comparisons in the case of significant differences between treatments. The data were then analysed using regression to determine the concentration yielding the highest *Daphnia* growth. Water quality data were compared to national standards or other available standards from the literature.

Results

Water quality. The range of water quality parameters measured for each treatment is shown in Table 1. When determining the number of *Daphnia* individuals, water quality parameters, such as temperature, DO, and pH, were taken into account.

Table 1

Water quality parameters

Treatment	*Temperature (^oC)*	*pH*	*DO (mg L^{-1})*
A	26.9-27.9	8.5-9.0	4.1-4.3
B	26.9-28.0	7.3-7.7	4.6-5.1
C	26.9-28.0	7.6-8.0	4.5-5.0
D	26.8-28.0	7.6-8.1	4.5-5.0
E	26.9-28.1	7.8-8.2	4.4-4.8
Optimum value	25-30 (BSNI 2000)	6.5-8.5 (BSNI 2000)	2.82-5.77 (Meilisa et al 2015)

According to Utami et al (2018), the ideal temperature range for *Daphnia* growth is 25.1-28.6^{o}C; thus, this study was conducted within that range. The pH range of the bioslurry treatments B, C, D, and E was 7.3-8.2, whereas it was 8.5-9 in treatment A. According to Ocampo et al (2012), the ideal pH range for the cultivation of *Daphnia* species is 7-8.6. Thus, treatments B, C, D, and E were in the ideal pH range, but treatment A had a pH that was too high for *Daphnia* to survive. Darmawan (2014) suggested that a high pH may affect egg viability and zooplankton health.

Throughout the study, DO levels in all treatments were in the range of 4.1-5.1 mg L^{-1}. According to Rakhman et al (2012), *Daphnia* species can survive at the DO range of 3.00-5.51 mg L^{-1}. In order for *Daphnia* species to thrive and reproduce, all treatments required appropriate DO levels. Mubarak & Triastuti (2009) stated that the ideal DO concentration for *Daphnia* species is > 3 mg L^{-1}.

Adult Daphnia individuals. The number of *Daphnia* adults varied over the 52 days of observation (Figure 1). The number of adults was significantly lower in treatment A (control) with 2.4 g L^{-1} of quail droppings as the food source. In contrast to other treatments which continued for up to 52 days, all adults in treatment A died on the ninth day. This is likely due to the fact that quail droppings did not sustain the *Daphnia* cohort in the water.

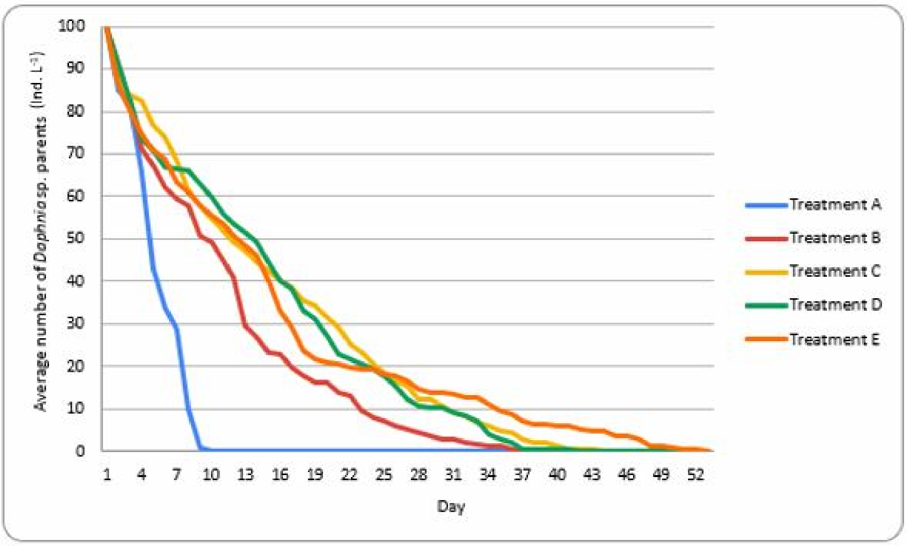

Figure 1. *Daphnia* cohort growth.

In treatments B, C, D, and E, *Daphnia* individuals could live for 36 to 52 days when bioslurry was added as a food source. According to Martínez-Jerónimo et al (1994), the average age of *Daphnia* species can range from 40 to 82 days, with a minimum age of 24 days and a maximum age of up to 119 days. Treatment E enabled the *Daphnia* to live until the age of 52 days. In treatment C, they lasted for 43 days, followed by 41 days in treatment D and 36 days in treatment B. The bioslurry in treatments B, C, D, and E was able to accommodate the needs of the *Daphnia* species. According to Paray & Al-Sadoon (2016), the advantages of cultivating *Daphnia carinata* with animal waste include faster growth and a reasonably longer life cycle. Wibisono et al (2017) stated that the use of fermented organic matter has a greater impact on the growth and reproduction of *Daphnia* species than the use of non-fermented organic matter. Bioslurry is the fermented by-product of biogas which can be used as a diet to promote the growth and reproduction of *Daphnia* species. According to Ghosh et al (1999), bioslurry contains soluble substances and can be used as a food source.

***Daphnia neonates**.* The number of neonates was counted over a period of 52 days. Each treatment had a different number of neonates (Figure 2). We observed that there was no population of *Daphnia* neonates until the fifth day. This was because *Daphnia* individuals had not yet reached the adult stage and could not reproduce. In treatments A and E, the *Daphnia* parents started to give birth to neonates on the sixth day. According to Deken (2005), *Daphnia* species can reach maturity in five to ten days. Darmanto et al (2000) also stated that *Daphnia* species begin reproducing at five days of age and produce offspring every one and a half days.

Treatment A only produced neonates on the sixth and seventh day, and notably, there were no new neonates on the eighth day because the parent population of *Daphnia* in treatment A was only effective in the first eight days. The highest number of neonates was in treatment D, whereas treatment B had the lowest. In all treatments, the neonates were born on the seventh day. Treatment B, with a bioslurry diet for *Daphnia*, enabled the parents to produce neonates from day 7 to day 34, treatment C from day 7 to day 40, and treatment D from day 7 to day 31. Treatment E, on the other hand, produced neonates from day 6 to day 49. On day 13, treatment D produced the most neonates, with 202 in total. In contrast to the other bioslurry treatments, individuals in treatment D died the quickest. According to Enserink et al (1995), *Daphnia* species that reproduce often and produce neonates will die sooner. Pietrzak et al (2010) also stated that high reproduction rates in the early life of *Daphnia* species can reduce their lifespan.

***Daphnia productivity**.* Based on 52 days of observation, the growth and reproduction rates of *Daphnia* differed between treatments (Figure 3). Treatment D showed the highest growth rate of 0.42 individuals day^{-1} and the highest reproduction rate of 0.505 individuals day^{-1}. This is likely due to treatment D having a bioslurry concentration suitable for *Daphnia* cultivation. Treatment A had the lowest growth and reproduction rates, -0.0145 individuals day^{-1} and 0.305 individuals day^{-1}, respectively. The negative growth rate in this treatment was in contrast to the rates in the other treatments. The water quality of treatment A might have been lower due to quail droppings and might have led to the decrease in the growth and reproduction rates of *Daphnia*. According to Mokoginta et al (2003), *Daphnia* abundance is influenced by the availability of food in proportion to the number of individuals in the culture container, as well as suitable environmental conditions.

The bioslurry concentration in treatment D might have been the optimum for *Daphnia* growth; consequently, treatment E, which had a higher bioslurry concentration, showed lower growth and reproduction rates. Pursetyo et al (2011) suggested that the amount of food in the culture medium is affected by the quality and amount of fertiliser in the medium. A suitable diet will fulfil the requirements of *Daphnia* for optimal growth and reproduction. Based on Islama et al's (2018) findings, the 3 g L^{-1} of bioslurry in treatment D was intended to meet the needs of *Daphnia* in this study. The results of the ANOVA showed that the variation in bioslurry concentration had no significant influence on the growth, but significantly different on reproduction rates of *Daphnia* (Figure 3).

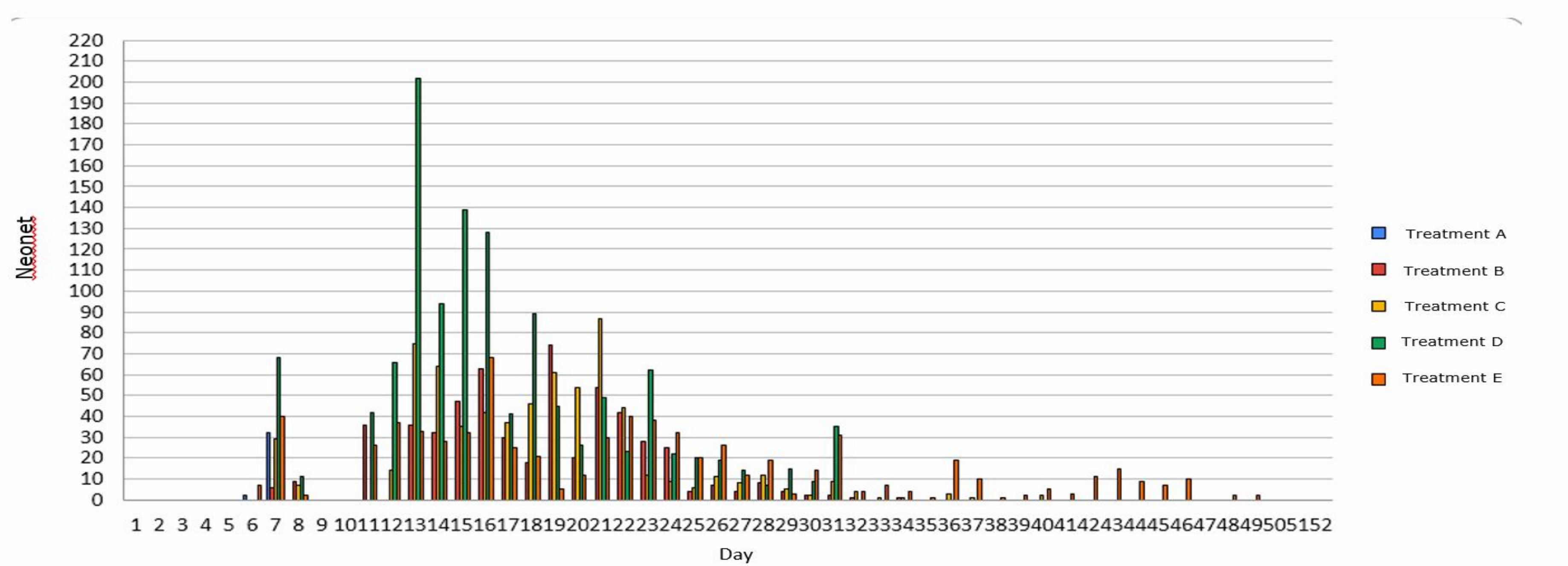

Figure 2. Population growth of *Daphnia* neonates.

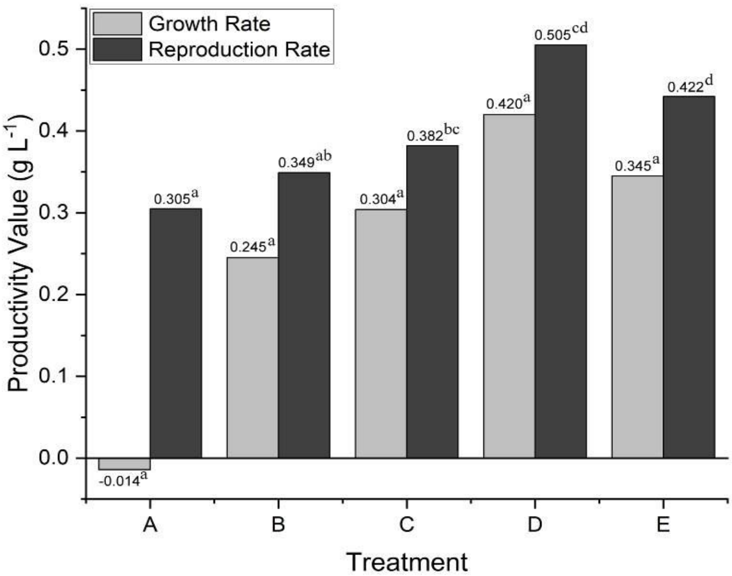

Figure 3. The growth and reproduction rates of *Daphnia*.

The protein level in bioslurry feed was 30.96%, while the protein level in quail droppings was 18.54%. Hence, the difference of protein level resulted in significantly lower both growth and reproduction rate value from treatment A. Fink et al (2011) suggested that the amino acids arginine and histidine should be administered to *Daphnia* to increase the number of neonates and promote a higher growth rate. Nainggolan et al (2017) claimed that protein, which contains nitrogen, can influence *Daphnia* growth.

The relationship between bioslurry concentration and growth rate. According to the results of regression analysis, the concentration of bioslurry in the diet influenced the growth rate. According to the regression curve with the equation $Y = -0.5371x^2 + 3.2546x-4.547$ and the coefficient of determination (R^2) of 0.3658 (Figure 4), 36% of the growth rate was influenced by bioslurry concentration, while the remainder was influenced by other variables. The correlation coefficient of the relationship between bioslurry concentration and growth rate was 0.604, indicating that bioslurry concentration and growth rate were significantly and positively associated. Figure 4 indicates that the optimal bioslurry concentration for growth is 3.06 g L^{-1}.

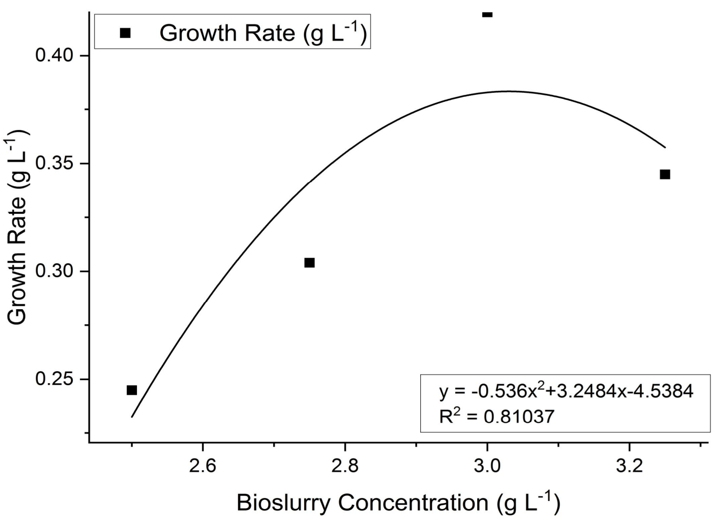

Figure 4. The relationship between bioslurry concentration and growth rate.

The relationship between bioslurry concentration and reproduction rate. According to the results of the regression analysis, the concentration of bioslurry in the diet influenced the reproduction rate. According to the regression curve with the equation $Y = -0.3851x^2 + 2.3746x - 3.1941$ and R^2 of 0.414 (Figure 5), 40% of the reproduction rate was influenced by bioslurry concentration, while the remainder was influenced by other variables. The correlation coefficient (*r*) of the relationship between bioslurry concentration and growth rate was 0.604, indicating that the two factors were significantly and positively correlated. Figure 5 shows that the optimal bioslurry concentration for reproduction is 3.25 g L^{-1} in treatment E.

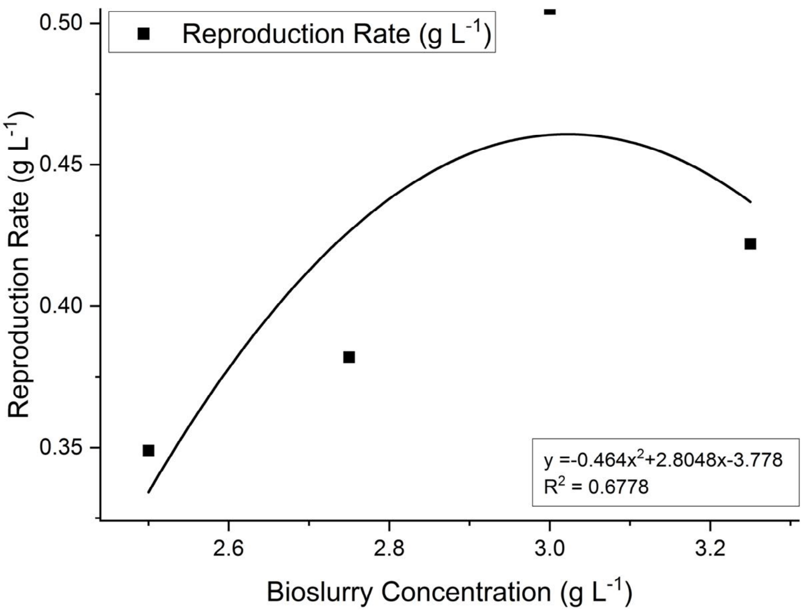

Figure 5. The relationship between bioslurry concentration and reproduction rate.

Conclusions. The growth and reproduction rates of *Daphnia* were considerably influenced by the various concentrations of bioslurry. Compared to other treatments, culture medium with a bioslurry concentration of 3-3.25 g L^{-1} resulted in the highest growth and reproduction rates. Regression analysis indicated that the optimal bioslurry concentration to reach the highest growth rate of *Daphnia* was 3.06 g L^{-1} and to obtain the highest reproduction rate was 3.25 g L^{-1}. In all treatments with bioslurry feed, the range of observed water parameters, such as temperature, DO, pH, and ammonia, was within the tolerance range of *Daphnia* species.

Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Yuli Andriani, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Raya Jatinangor Street, Km 21, Sumedang 45363, Indonesia; e-mail: yuliyusep@yahoo.com
Nidia Puspitasari, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Raya Jatinangor Street, Km 21, Sumedang 45363, Indonesia; e-mail: nidiaps28@gmail.com
Herman Hamdani, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Raya Jatinangor Street, Km 21, Sumedang 45363, Indonesia; e-mail: hamdani_herman@yahoo.com
Zahidah, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Raya Jatinangor Street, Km 21, Sumedang 45363, Indonesia; e-mail: ibuzah@gmail.com

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