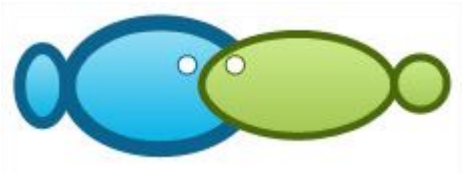

The effect of heat shock on the tetraploidy of catfish, *Pangasius hypophthalmus*

Dwi P. Hartono, Pindo Witoko, Ninik Purbosari

Department of Aquaculture, State Polytechnic of Lampung, Jl. Soekarno-Hatta No. 10 Bandar Lampung, Indonesia. Corresponding author: D. P. Hartono, dwiph@polinela.ac.id

Abstract.This research aims to discover the effect of heat shock and shock duration on the formation of tetraploid catfish. This research was designed using factorial completely randomized design consisting of two main treatments, i.e. shock temperature (39°C, 40°C, and 41°C) and shock duration (90 seconds, 120 seconds, 150 seconds, and 180 seconds). Each treatment was replicated three times. The parameters observed were, inter alia, the percentage of tetraploid individuals, fertilization rate, and hatching rate. The identification of tetraploid individuals was based on observation of the nucleolus in the cell. The result of research indicated that there was a significant effect of shock treatment on the formed tetraploid individuals ($p < 0.01$). Each treatment also significantly affected the formation of tetraploid individuals ($p < 0.01$), but the interaction between factors did not affect the formation of tetraploid individuals ($p > 0.01$). Treatment with shock temperature of 40°C and the duration of 120 seconds produced the highest percentage of tetraploid individuals, i.e. 28.33%. The determination of ploidy level in catfish was done by calculating the number of nucleoli. A tetraploid individual has 5-6 nucleoli at the maximum. Besides, the treatment factor affected the hatching rate of treated catfish. However, based on the statistical test, there was no interaction between shock temperature and shock duration towards the hatching rate ($p > 0.05$).
Key Words: tetraploid, catfish, ploidy, fertilization rate, hatching rate.

Introduction. Efforts to increase the production of cultivated fish can be achieved through improvement in cultivation techniques. Improved techniques of cultivation must always be followed by provision of high quality fingerlings, which can be done by provision of fingerlings that are sterile or triploid. Thorgaard (1983) pointed out that triploid fish are sterile and have the potential to control overpopulation, increase the juvenile growth rate, maintain survival rate, and the growth of adult fish. According to Zohar (1989) and Chao et al (1993) the gonad of triploid fish does not develop, thereby enabling it to overcome the problem or rapid maturity of sexual organs, and the body growth and the increase of meat quality. Beaumont (1994) maintained that sterility can be utilized to increase production because the energy of metabolism, which is usually used for gonadal development, is utilized for growth. Hartono et al (2009) stated that the growth level of triploid catfish can reach 11.13% or an average increase of 3.00% from the growth level of the normal diploid catfish.

The heat shock is one of the common technique in triploidy of fish, but the application of heat shock to induce triploidy is not always 100% effective and can cause a detrimental side-effect and decreased viability. Other forms of ploidy and tetraploidy have been used to facilitate triploid production. The formation of triploid individuals can be done by retaining the polar body II (Thorgaard & Gaal 1979) or generated by crossing a tetraploid (4n) with a diploid (2n) is an alternative approach first developed for fish (Chourrout et al 1986; Myers & Hershberger 1991; Weber et al 2013, 2014). The formation of tetraploid individuals is an interim process in producing fingerlings of triploid fish. Tetraploid broodstocks are used for mass production of triploids (Luo et al 2011; Zhang et al 2014).

Tetraploidization is a method of chromosome engineering for the formation of individuals that have 4n set of chromosomes and is done by giving physical or chemical treatment through prevention of the bound of fission of the first cell (Carman 1992).

Thorgaard (1983) explained that a practical approach for tetraploidy induction through temperature shock is an applicative treatment soon after the first fission at a lethal temperature. Besides being cheap and easy, temperature shock is efficient as it can be done in large numbers (Rustidja 1989). Bidwell et al (1985) reported that the production of tetraploid fish is determined by an optimum condition, the final fertilization time, and the length of shock. Tetraploidy can be induced by suppression of early cell division in the zygote (Chourrout 1984; Zhang et al 2007). Tetraploid induction has been accomplished by pressure, temperature, or chemical shocking of zygotes in many species, including tilapias (*Oreochromis* spp., Myers 1986; El Gamal et al 1999), channel catfish (*Ictalurus punctatus*, Bidwell et al 1985; Goudie et al 1995), yellow perch (*Perca flavescens*, Malison et al 1993; Malison & Garcia-Abiado 1996), and masu salmon (*Oncorhynchus masou*, Sakao et al 2006). Development of a tetraploid broodstock allows production of triploid fish without needing to shock all production fish to induce triploidy (Weber et al 2013, 2014).

The specific objective of the research was to find out the treatment of optimal temperature, the time of shock application and the length of shock in producing the population of tetraploid catfish, *Pangasius hypophthalmus*.

Material and Method. The research was conducted in February to June 2015 in the Fisheries Laboratory at the State Polytechnic of Lampung, Indonesia. The research design was factorial completely randomized design and the treatments given were shock temperature and shock duration. Shock temperature treatment used temperatures at 39°C, 40°C, and 41°C and shock duration comprised 60, 90, 120, and 150 seconds. The zygote that received treatment (initial time) was 40 minutes from the process of fertilization.

Spawning of catfish. Spawning was done by using artificial insemination. The dose used in the insemination was 0.9 mL kg^{-1}. The broodstock was inseminated twice in the ratio of one-third dose in the first insemination and two-thirds dose in the second insemination. The interval between insemination was 10 h. Ovulation was done after 6-8 h from the second insemination by striping. The egg produced by striping was then fertilized by the sperm of the male parent and was given 0.9% NaCl solution to help the fertilization process of the eggs. The fertilized eggs were scattered in an aquarium equipped with a glass plate as a place for sticking catfish eggs. Temperature shock was done 40 minutes after egg fertilization. The temperature and duration of temperature shock were adjusted according to the treatment given. The different shock temperature treatments used temperatures at 39°C, 40°C, and 41°C and shock duration treatments were 60, 90, 120, and 150 seconds. After the temperature shock was done, the eggs were put into a hatching aquarium for the incubation process, done in an aquarium with water at 20 cm in height until hatching.

Rearing of embryo and larva. The development of the embryo was observed in the incubation to determine the fertilization rate. This observation was done by observing the characteristics of a fertilized egg. A catfish egg would hatch after 20-26 h from the fertilization process. The hatched larva was reared in an aquarium. Feeding was done after the larva was three days old. The feed given was artemia with a frequency of five times, i.e. at 07.00, 15.00, 19.00, 22.00, and 02.00 a.m. local time. The feeding was done until the larva was ten days old and continued with feeding with silkworm until it was 21 days old. Replacement of water was done every two days to keep the media clean. Larva rearing was done for 30 days.

Observation of the nucleolus. Observation of the number of nucleoli in each individual from each treatment was done by taking a sample of 50 fish from which the caudal fin was taken. The fin was chopped in a KCL solution of 0.75 M and left for 30 minutes. Then the solution was replaced with Carnoy solution (mixture of acetic acid and absolute ethanol in the ratio of 3:1) and left to soak for 60 minutes. After soaking, preparations were made by taking the organ and it was sprinkled with 50% acetate on the glass object

followed by coloring with silver nitrate. Then the preparations were put into a staining box for 20 minutes at a temperature of 45°C. The preparations were then rinsed with water and left to dry so that it could be observed under a microscope at a magnification of 400 or 1000 times. The tetraploid individuals were characterized according to the number of nucleoli in one cell, i.e. at a maximum of six.

Data analysis. Observation of the treated fish was done in terms of the percentage of tetraploid individuals (4n). The success of tetraploidization was based on the treatment result done using the calculation of the number of nucleoli. The determination of the ploidy level in catfish was done by calculating the number of nucleoli. A tetraploid catfish had a maximum of 5-6 nucleoli.
Kt (% tetraploid parent) = (Σ tetraploid parent / Σ tested fish) x 100%
Observation of the degree of fertilization was done 8-10 h after the shock treatment. The value of the fertization rate (% FR) was calculated with the formula (Effendi 1979):

FR = (number of fertilized eggs / number of total eggs) x 100%

Observation of the degree of hatching was done after the eggs hatched or 30 h after fertilization. The value of the hatching rate (% HR) was calculated with the formula (Effendi 1979):

HR = (number of hatched larvae / number of fertilized eggs) x 100%

The shock heat and duration of temperature shock were subjected to analysis of variance (ANOVA). The result of the statistical analysis would show the optimal temperature and duration of temperature shock which would be used as a basis for application of the formation of tetraploid catfish individuals.

Results and Discussion. Result of the observation of the degree of catfish fertilization can be seen in Figure 1. The statistical test using ANOVA showed there was no significant difference between treatments ($p > 0.05$). Besides, the treatment temperature and shock duration did not show a significant effect on the fertilization rate. Figure 2 shows that the degree of catfish fertilization ranged between 65.63% and 92.31%. This signifies that the quality of eggs and sperms used was good and almost all the eggs were fertilized by the sperms so that there was no difference in the degree of fertilization. The temperature shock after fertilization did not affect the fertilization rate.

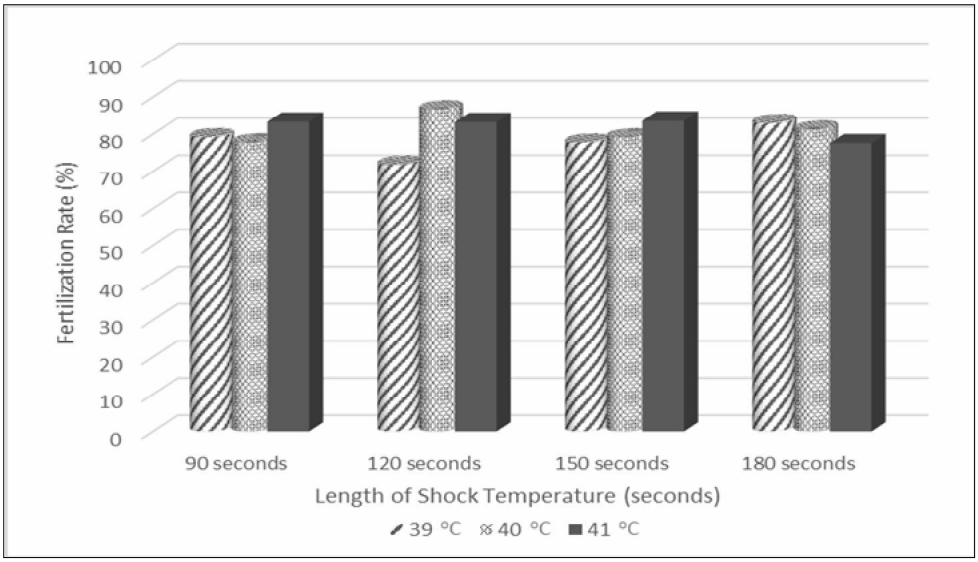

Figure 1. The fertilization rate (%) of catfish eggs resulting from shock treatment.

The result of the observation of the hatching rate of the catfish treated with temperature shock is shown in Figure 2. The statistical test showed that in general treatment of shock temperature or duration of shock had a significant effect on hatching rate ($p < 0.01$). Besides, shock temperature and shock duration affected the hatching rate of the treated catfish. The hatching rates were decreased with increasing of temperature and duration shocks. This shows that both the treatment of shock temperature and shock duration had a direct effect on the hatching rate. The similar result was reported by Lebeda & Flajshans (2015) that the temperature shock causes the decrease hatching rate of fish eggs Siberian sturgeon (*Acipenser baerii*). In addition, Ding et al (2007) reported that increasing the pressure intensity or duration would decrease the hatching rates of sea cucumber, *Apostichopus japonicus*.

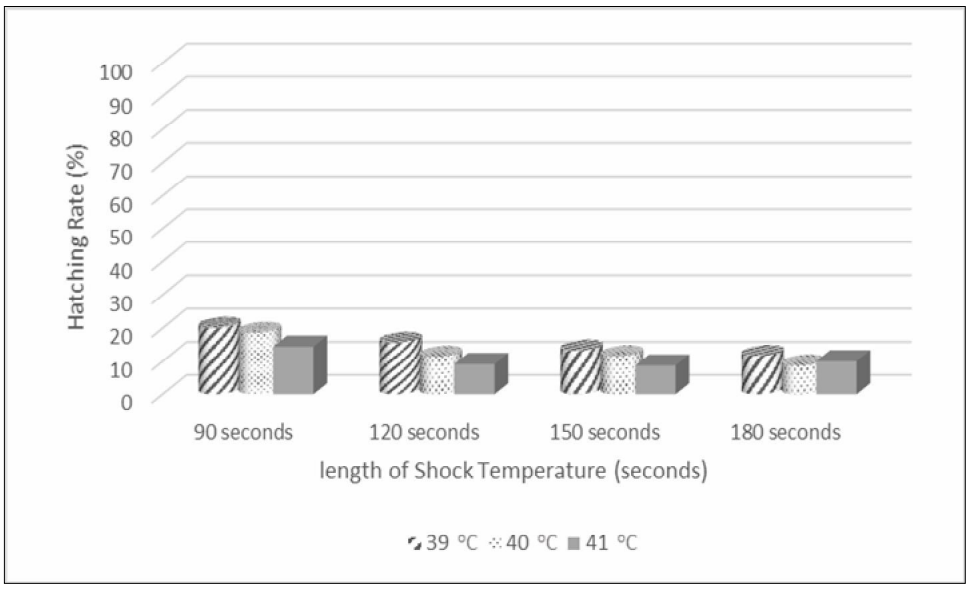

Figure 2. The hatching rate of eggs resulting from temperature shock.

This could be caused by the difference in the survival of the embryo due to the shake that occurred during tetraploidization. This is congruent with a statement by Lagler et al (1977) that shake, shock, and rapid change of temperature are extremely harmful during the early sensitive period and even result in the death of the embryo. It was this condition that affected the low hatching in each treatment of temperature shock. Tave (1993) pointed out that mortality is likely to be caused by a number of detrimental effects of shock treatment on the cytoplasm of eggs. Temperature shock treatment can cause damage to the spindle threads that form during the process of cell fission in the egg (Gill et al 2016). The results of observation of the percentage of catfish tetraploid individuals treated with temperature shock are shown in Figure 3.

The statistical test on the treatment of temperature shock and shock duration showed a significant effect on the formation of tetraploid catfish ($p < 0.01$). In each treatment factor given, the shock temperature had a significant effect on the level of formation of the tetraploid individuals produced ($p < 0.05$) and similarly the shock duration treatment also had a significant effect among the treatments on the formation of tetraploid individuals ($p < 0.01$). However, the statistical analysis showed no interaction between shock temperature treatment and shock duration treatment towards the formation of tetraploid individuals. The highest percentage of tetraploid was obtained in the treatment of shock temperature at 40°C and shock duration of 150 seconds with an average success of 28.33% followed by shock treatment of 41°C and shock duration of 150 seconds with an average success of 16.67%. Temperature shock treatment produced tetraploid individuals although with varying percentages. This shows that shock treatment at a temperature given to eggs was capable of preventing the occurrence of cell fission at the mitosis stage resulting in the formation of tetraploid individuals and did not result in the total death of the zygote. Treatment of temperature shock at 40°C and

the duration of 120 seconds produced the highest percentage of tetraploid individuals. According to Corely-Smith et al (1996), temperature shock for two minutes is an effective time to inhibit the first mitosis fission in the production of androgens and gynogens in zebra fish, *Danio rerio*.

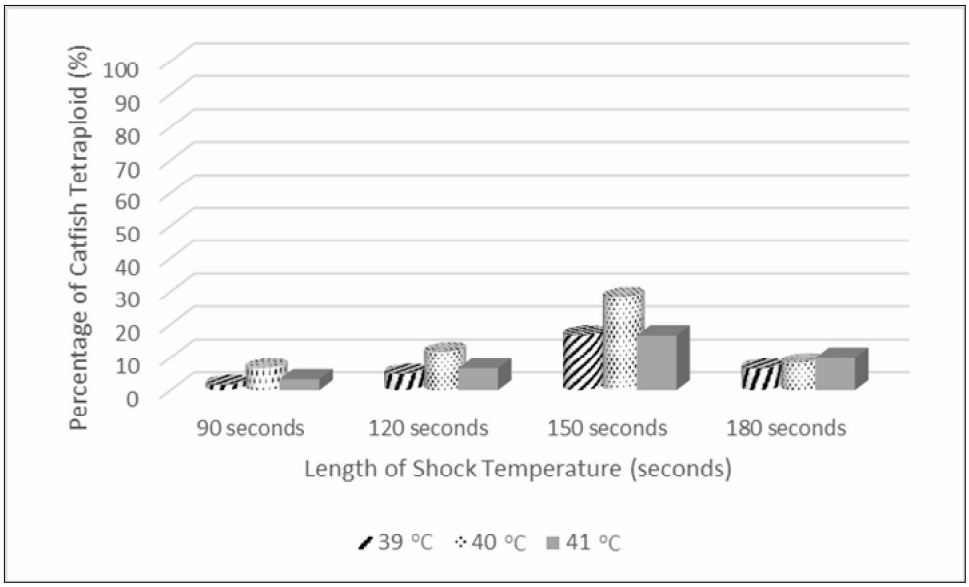

Figure 3. Percentage of catfish tetraploid resulting from shock treatment.

This study showed that the tetraploid catfish can be done with the heat shock on the embryo. The catfish tetraploid can be used as an alternative to produce catfish triploids by mating with diploid individuals. The availability of tetraploids for triploid production would increases availability of high quality of larvae and decreases cost for sterile triploid (Weber et al. 2015). Tetraploid progeny could be used for production of diploid, which could be used to avoid negative consequences of ploidy restoration treatment in production of triploids (Lebeda & Flajshans 2015). This can improve the quality of catfish that can improve the productivity of catfish culture. Hartono et al (2009) suggest that the growth rate of triploid catfish can reach 11.13% or an average increase of 3.00% from the rate of growth of normal diploid catfish.

Conclusions. Tetraploid individuals were found in all the temperature shock treatment given. The highest percentage of tetraploid individuals was found at shock temperature of 40°C and the duration of 150 seconds.

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References

Beaumont A. R., 1994 Genetics and evolution of aquatic organisms. Chapman & Hall, London, pp. 467-485.

Bidwell C. A., Chrisman C. L., Libey G. S., 1985 Polyploidy induced by heat shock in channel catfish. Aquaculture 51:25-32.

Carman O., 1992 Chromosome set manipulation in some warm-water fish. PhD Thesis, Tokyo University of Fisheries, Tokyo, 131 pp.

Chao N. H., Hsu H. W., Hsu H. Y., Liang W. H., Liao I. C., 1993 Studies on methods of triploidy percentage analysis. TML Conference Proceedings 3:203-210.

Chourrout D., 1984 Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout eggs: production of all-triploids, all-tetraploids, and heterozygous and homozygous diploid gynogenetics. Aquaculture 36:111-126.
Chourrout D., Chevassus B., Krieg F., Happe A., Burger G., Renard P., 1986 Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females - potential of tetraploid fish. Theoretical and Applied Genetics 72:193-206.
Corely-Smith G. E., Lim C. J., Brandhorst B. P., 1996 Production of androgenetic zebrafish (*Danio rerio*). Genetics 142:1265-1276.
Ding J., Chang Y., Wang Z., Song J., 2007 Polyploidy induction by hydrostatic pressure shock and embryo development of sea cucumber, *Apostichopus japonicus*. Chinese Journal of Oceanoloy and Limnology 25:184-190.
Effendi M. I., 1979 [Fisheries biological method]. Dewi Sri Foundation, Bogor, 112 pp. [in Indonesian].
El Gamal A. R. A., Davis K. B., Jenkins J. A., Torrans E. L., 1999 Induction of triploidy and tetraploidy in Nile tilapia, *Oreochromis niloticus* (L). Journal of the World Aquaculture Society 30:269-275.
Gil H. W., Kong H. J., An C. M., Kim B. S., Lim S. G., Park I. S., 2016 Cytogenetic study of diploid and induced tetraploid in Korean rose bitterling, *Rhodeus uyekii*. SpringerPlus 5:186. doi:10.1186/s40064-016-1830-4.
Goudie C. A., Simco B. A., Davis K. B., Liu Q., 1995 Production of gynogenetic and polyploid catfish by pressure-induced chromosome set manipulation. Aquaculture 133:185-198.
Hartono D. P., Febriani D., Marlina E., 2009 [The temperature influence on the formation of individual cold shock triploid catfish (*Pangasius* sp.)]. Proceedings of the National Seminar on Appropriate Technology Agroindustri, Lampung State Polytechnic, Lampung, 55 pp. [in Indonesian]
Lagler K. F., Bardach J. E., Miller R. R., Passino D. R. M., 1977 Ichthyology. John Willey and Sons Inc, New York-London, 506 pp.
Lebeda I., Flajshans M., 2015 Technical note: production of tetraploid sturgeons. Journal of Animal Science 93:3759-3764.
Luo K., Xiao J., Liu S., Wang J., He W., Hu J., Qin Q., Zhang C., Tao M., Liu Y., 2011 Massive production of all-female diploids and triploids in the crucian carp. International Journal of Biological Sciences 7:487-495.
Malison J. A., Garcia-Abiado M. A. R., 1996 Sex control and ploidy manipulations in yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*). Journal of Applied Ichthyology 12:189-194.
Malison J. A., Kayes T. B., Held J. A., Barry T. P., Amundson C. H., 1993 Manipulation of ploidy in yellow perch (*Perca flavescens*) by heat shock, hydrostatic pressure shock, and spermatozoa inactivation. Aquaculture 110:229-242.
Myers J. M., 1986 Tetraploid induction in *Oreochromis* spp. Aquaculture 57:281-287.
Myers J. M., Hershberger W. K., 1991 Early growth and survival of heat-shocked and tetraploid-derived triploid rainbow trout (*Oncorhynchus mykiss*). Aquaculture 96:97-107.
Rustidja S., 1989 [Artificial induced breeding and triploidy in the Asian catfish (*Clarias batrachus* Linn.)]. PhD thesis, Faculty of Graduate Studies, Bogor Agricultural Institute, Bogor, 80 pp. [in Indonesian]
Sakao S., Fujimoto T., Kimura S., Yamaha E., Arai K., 2006 Drastic mortality in tetraploid induction results from the elevation of ploidy in masu salmon *Oncorhynchus masou*. Aquaculture 252:147-160.
Tave D., 1993 Genetics for fish hatchery managers. Second edition, Van Nostrand Reinhold, New York, pp. 267-304.
Thorgaard G. H., 1983 Chromosome set manipulation and sex control in fish. In: Fish physiology. Hoar W. S., Randall D. J., Donaldson M. (eds), Volume IX B, Academic Press, Inc. London, pp. 405-434.
Thorgaard G. H., Gall G. A. E., 1979 Adult triploid in rainbow trout family genetics. Genetics 93:961-973.

Weber G. M., Wiens G. D., Welch T. J., Hostuttler M. A., Leeds T. D., 2013 Comparison of disease resistance between diploid, induced-triploid, and intercross-triploid rainbow trout including trout selected for resistance to *Flavobacterium psychrophilum*. Aquaculture 410-411:66-71.
Weber G. M., Hostuttler M. A., Cleveland B. M., Leeds T. D., 2014 Growth performance comparison of intercross-triploid, induced triploid, and diploid rainbow trout. Aquaculture 433:85-93.
Weber G. M., Hostuttler M. A., Semmens K. J., Beers B. A., 2015 Induction and viability of tetraploids in brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 72(10):1443-1449.
Zhang X., Asami T., Onozato H., 2007 Polypolar spindle formation during first cell cycle in rainbow trout *Oncorhynchus mykiss* embryos after heat-shock treatment. Fisheries Science 73:1325-1331.
Zhang Z., Chen J., Li L., Tao M., Zhang C., Qin Q., Xiao J., Liu Y., Liu S., 2014 Research advances in animal distant hybridization. Science China. Life Sciences 57:889-902.
Zohar Y., 1989 Fish reproduction: its physiology and artificial manipulation. In: Fish culture in warm water systems: problems and trends. Shilo M., Sarig S. (eds), CRC Press, Inc. Boca Raton, Florida, pp. 65-120.

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Authors:
Dwi Puji Hartono, Department of Aquaculture, State Polytechnic of Lampung, Jl. Soekarno-Hatta No. 10 Bandar Lampung, Indonesia, e-mail: dwiph@polinela.ac.id
Pindo Witoko, Department of Aquaculture, State Polytechnic of Lampung, Jl. Soekarno-Hatta No. 10 Bandar Lampung, Indonesia, e-mail: pw@polinela.ac.id
Ninik Purbosari, Department of Aquaculture, State Polytechnic of Lampung, Jl. Soekarno-Hatta No. 10 Bandar Lampung, Indonesia, e-mail: purbosari_nnk@polinela.ac.id

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