Vertebral ossification, growth, and survival of nilem carp, *Osteochilus hasselti* larvae using shell flour of local mussel, *Pilsbryocancha exilis*

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Abstract. Nilem carp is one of the local freshwater fish species in Indonesia that has an economical value. The present study was to determine the optimal of water hardness level on the vertebral ossification, growth, and survival of nilem carp larvae. The four treatments of hardness level with 3 replicates applied using A) 80 mg/L (control), B) 100 mg/L, C) 120 mg/L, and D) 140 mg/L on one day old. Larvae were reared in the fiber tanks with the density of 40 larvae/L fed with artemia and artificial diet. The parameters observed were the vertebrae ossification, absolute length, absolute weight, and survival. The result showed that the best vertebrae ossification, absolute length, absolute weight, and survival was found at water hardness with concentration of 120 mg/L compared to the others treatments ($P<0.05$) under optimal range of temperature, pH, and dissolved oxygen during the rearing period. The use of shell flour of local mussel (*P. exilis*) in the rearing water media is very important to support vertebrae ossification, growth, and survival of nilem carp larvae.

1. Introduction

The nilem carp is one of the important species in Southeast Asia. The distribution of nilem carp in Indonesia are in Borneo, Sumatera, and Java islands. Nowadays, nilem carp have been introduced to Celebes and Bali Islands [1]. Restocking is to improve population. Introducing is to bring new fish in the new environment whereas they are not exist in the lake, reservoir, and river [2]. The nilem carp is belong to Cyprinid family has an economical value for "baby fish", chips made from fingerling sized, and the fried egg. This species has potential to be cultured due to high fecundity, easy to culture, tolerate to environmental conditions, and resistance against diseases [3]. The most popular of nilem carp culture is concentrated in West Java, e.g. Bogor, Sukabumi, Tasikmalaya, Garut, and Ciamis regions [4]. Since a herbivorous carp mainly consumes phytoplankton and peryphyton, the existence

of this fish in aquatic ecosystem enables to be use as bio-cleaning agent. Do to this advantages, nilem carp often kept together with other fish in floating net cage culture to clean the net [3].

Some efforts have been carried out to improve production of this species through genetic [4], gonadal development and female seed production [5], improving broodstock via management hormonal, local feed (formulated fed), egg management, and broodstock candidate through ginogenesis [6], polyculture [7], using high oxy of aeration stone [8], grow out technique [9], feeding management [10], and disease [11]. Although the seed production of the nilem carp have been successful but the production is not constant yet due to some of problems in the larval rearing stage. The water hardness (CaCO mg_3/L) plays an important role in terms of fish growth and survival. The control and balance of calcium (Ca^{2+}) ions is essential to bony skeletal development (ossification) or a cartilaginous skeletonof fish [12]. The water hardness have been reported influencing the growth and survival *Labeo rohita* fry [13], *Rutilusfrisii* fingerlings [14], and nile tilapia [15]. Aside of that, the water hardness is also important for ossification, growth and survival but total amount needed depends on species. Therefore, the research of the water hardness for nilem carp larvae culture is needed.

The shell of local mussel, *Pilsbryocancha exilis* consists of three layers. The first layer mainly contains chitin rather than calcium carbonate. The second layer contains crystals of calcium carbonate ($CaCO_3$), and the third layer or pearl layer contains calcium carbonate [16]. The mussel shell can be used as shell flour containing calcium carbonate ranging from 28.97-39.55% [17]. The calcium carbonate in the shell flour is higher than bone flour of fish [18].

The objective of this research is to determine the optimal water hardness supporting vertebral ossification, growth, and survival rate of nilem carp larvae.

2. Materials and methods

2.1. Time and experimental site

The research was conducted from April to May 2018 at Research Station for Freshwater Aquaculture Environment Technology and Toxicology, Cibalagung, Bogor, West Java, Indonesia.

2.2. Test fish

The newly hatched larvae (D1) were used. The stocking density was 40 larvae/L. The larvae were cultivated for 30 days and fed artificial diet. Artemia was fed to the larvae until day 15, thereafter artificial diet.

2.3. The Container

Twelve of fiber tanks with size of 40 × 30 × 25 cm (30 L of water volume) were used. Each fiber tank was aerated. The water exchange rate (30%) was conducted after cleaning its bottom every three days. New water was added according to water hardness concentration.

2.4. The treatment of the experiment

The treatment of water hardness (as mg $CaCO_3$ /L) concentrations were A) 80 mg/l; B) 100 mg/l); C) 120 mg/L; and D) 140 mg/L with three replicates.

To control the water hardness, the formula was calculated below:

Hardness (as mg CaCO3/l) = m titrant x M titrant x 100.1 x 1000/ml sample.....(1)

The procedure to adjust water hardness was done by diluting and homogenizing one gram of shell flour in one liter aquadest, followed by adding HCl with the ration 1:1. The solution was used as standard solution. The water hardness concentration of different level was made using formula:

M mix (mg $CaCO_3$/L) = V1 × M1 + V2 x M2/V mix .....(2)

Where:
M mix (mg $CaCO_3$/L) = Expected concentration
V mix = Expected volume
V1 = Water resource volume
M1 = Water resource concentration
V2 = Standard solution volume
M2 = Standard solution concentration
The percentage of vertebral ossification was counted using formula:

$$VO\ (\%) = \frac{VBF}{VBUF} \times 100 \quad \text{..... (3)}$$

Where:
VO = the percentage of vertebral ossification
VBF = the number of vertebrae bone formed
VBUF = the number of vertebrae bon unformed
The vertebrae bone was check through Alizarin Red staining method [19].

Twenty larvae were sampled every five days (D5, D10, D15, D20, D25, and D30) to measure the weight and length growth bone staining work. The water quality e.g. temperatur, oxygen dissolved, and pH were recorded every five days.

The absolute growth of weight, and length were calculated by reducing final weight or length with initial measurement. For survival was calculated by dividing number of fish at the end experiment with initial stocking.

2.5. Statistical analysis

The design experiment with four treatments and three replicates using complete randomized design were applied. All data such as the absolute weight, absolute length, and survival rate were analyzed using one way ANOVA continued to post hoc multiple comparison using Tukey's test when significant different exist. The data of vertebral ossification and water quality parameters were discussed descriptively.

3. Results

3.1. Vertebral ossification

The percentage of vertebra ossification was presented in Figure 1.

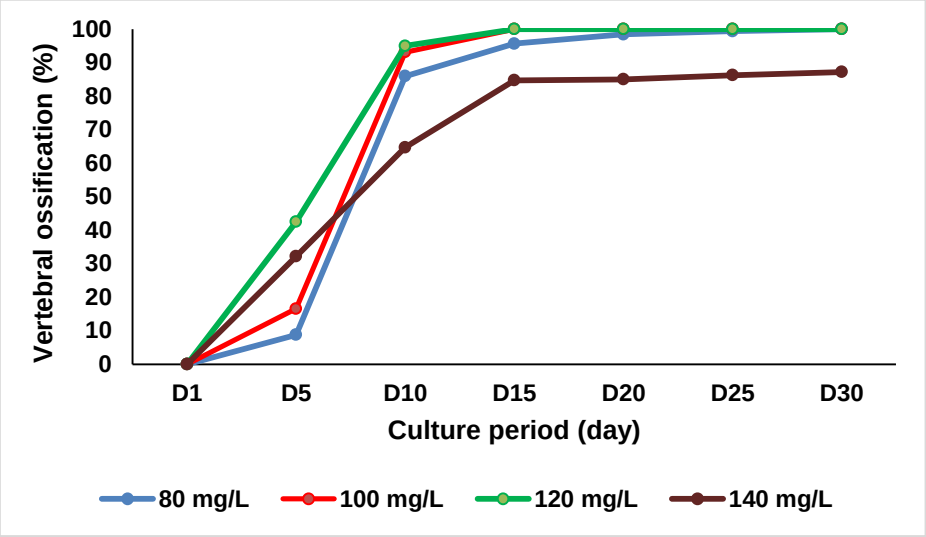
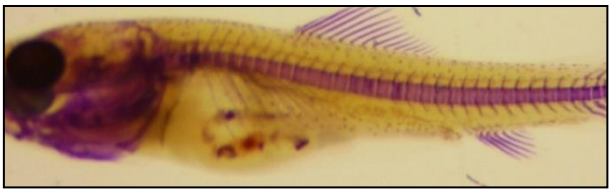


Figure 1. The percentage of vertebral ossification of nilem carp larvae at different water hardness levels

Vertebral ossification of nilem carp (Figure 1) showed that on day 5th, the highest percentage of vertebral-ossification (42.50±6.62%) found in water hardness concentration of 120 mg/L, followed by 140 mg/L (32.19±6.60%), 100 mg/L (16.56±6.26%), and 80 mg/L (8.75±6.72%). The vertebrae formed complete ossification in 100 and 120 $CaCO_3$ mg/L on the day 15th, where in 80 mg/L (95.63±4.93%) and the lowest (84.69±6.82%) at 140 mg/L. The nilem carp cultured in 80 mg/L showed that the vertebral bone formed that reach 100% found on day 30th while at the water hardness with concentration of 140 mg/L did not achieved (87.19±4.53%) still remained incomplete up to 30 day olds (Figure 2 a & b). The results indicated that water hardness of 120 mg/L was the suitable concentration for vertebral bone formation in contrast with 140 mg/L one, it was concluded that the water hardness affect the vertebrae bone formation.



a

b

Figure 2. The vertebral bone formation (Description: a. Complete vertebral formation; and b. Incomplete vertebral formation)

3.2. Growth and survival

The absolute length, absolute weight, and survival rate of nilem carp larvae cultured at different water hardness levels is presented at Figure 3, 4, and 5.

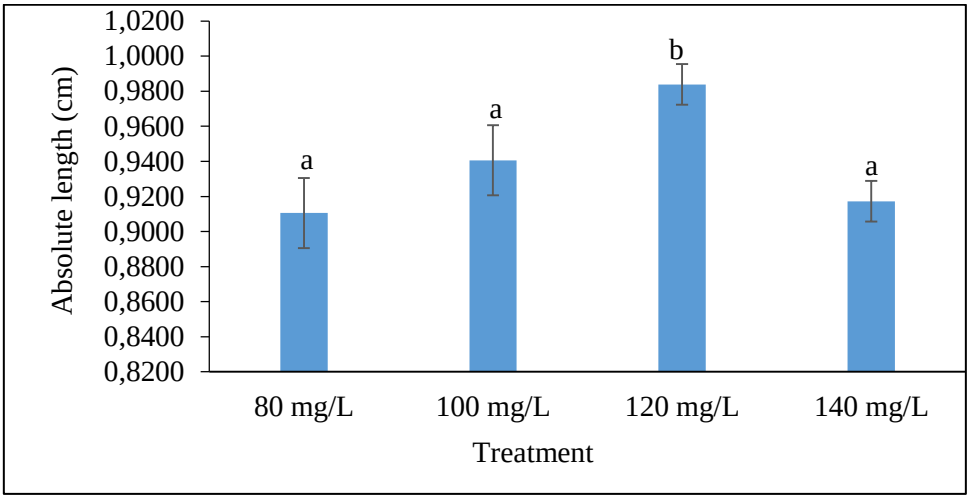


Figure 3. The absolute length of nilem carp, *O. hasselti* larvae cultured at different water hardness levels. The bar followed by the same letter did not significantly different (P>0.05)

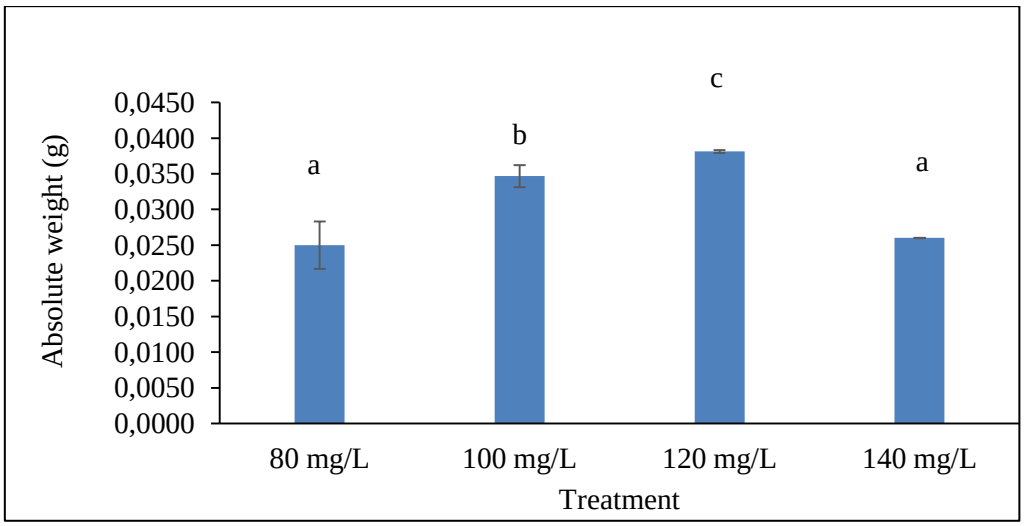


Figure 4. The absolute weight of nilem carp, *O. hasselti* larvae cultured at different water hardness levels. The bar followed by the same letters did not significantly different (P>0.05)

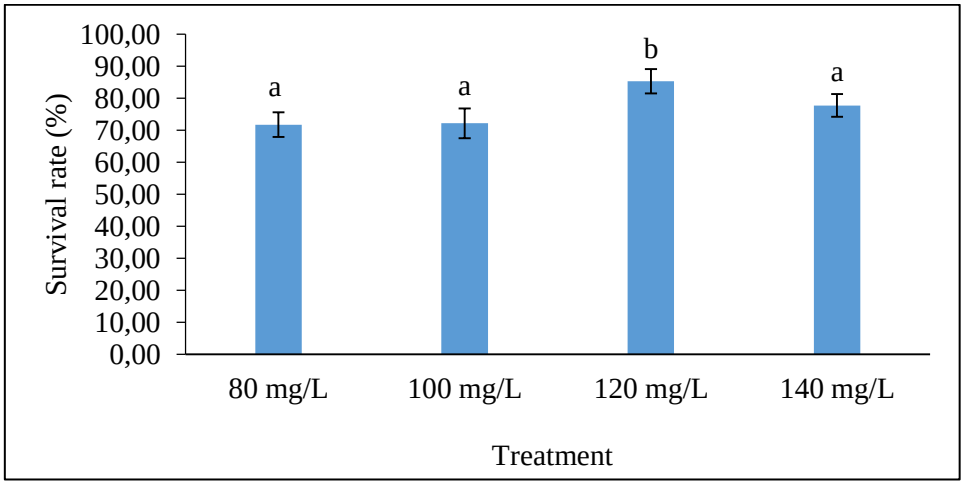


Figure 5. The survival rate of nilem carp, *O. hasselti* larvae cultured at different water hardness levels. The bar followed by the same letters did not significantly different (P>0.05)

The absolute length (Figure 3) showed that nilem carp larvae cultured at water hardness with concentration of 120 mg/L (0.98±0.012 cm) was the highest then followed by 100 mg/L (0.94±0.02 cm), 140 mg/L (0.92±0.01 cm), and 80 mg/L (0.91±0.02 cm). The absolute weight (Figure 4) of nilem carp larvae cultured at water hardness with concentration of 120 mg/L (0.038±0.0002 g) was the highest compared to the others while the lowest of absolute weight found at water hardness with concentration of 80 mg/L (0.025±0.0033 g). The survival rate of nilem carp larvae cultured at water hardness with concentration of 120 mg/L (85.33±3.78%) was the highest then followed by 140 mg/L (77.78±3.56%), 100 mg/L (72.22±4.64%), and 80 mg/L (71.78±3.86%) (Figure 5). Statistical analyzed revealed that water hardness affect to absolute length, absolute weight, and survival rate which the best all of those parameters found at water hardness with concentration of 120 mg/L compared to the others (P<0.05).

3.3. Water quality

The dissolved oxygen, pH, and temperature during the larval rearing period was presented in Table 1.

Table 1. The range of water quality parameters (DO, pH, and Temperature) during the culture period

Parameters	Unit	Treatment				Recommended
		80 mg/L	100 mg/L	120 mg/L	140 mg/L	
DO	mg/L	3.36– 4.38	3.78 – 4.62	3.72 – 5.25	3.82 – 5.38	>3 [20]
pH		6.32 – 6.85	6.63 – 6.86	6.80 – 7.10	7.30- 7.60	6,00-8.00 [20]
Temperature	°C	27.4 – 27.7	27.3 – 27.6	27.3 – 27.7	27.3 – 27.5	27.0-31.0°C [20]

All the data of water quality parameters such as DO, pH, and water temperature (Table 1) during the culture period showed that all of parameters were within in the optimal range but the pH value tended to increase with increasing the water hardness concentration. This indicated that the water hardness affect the pH value.

4. Discussion

Calcium and magnesium ions (Ca^{2+} and Mg^{2+}) are important for ionic regulation of freshwater fish because both ions influence the permeability of biological membranes and preventing diffusive flow through the membrane of cations loss to water. The present experiment shows that the high of hardness concentration has an inhibited the vertebral formation. Certain concentration would not support complete vertebral formation even though culture period was prolonged. This condition occurs due to imbalance of the Ca^{2+} in the water and in the fish body. Thus, it affects the formation of vertebral. An essential difference between terrestrial animals and fish is that the fish can absorb Ca directly from their environment and can fulfil their entire Ca requirement or apart from Ca present in water while terrestrial animals are supplied from food only which contains Ca [21]. The uptake of Ca occurs through gills, fins, gastrointestinal tract and oral epithelia, however, gills are considered the most important organ for Ca regulation [22]. Fish maintain the blood Ca level through mobilization of bone Ca, whenever necessary. Although there is little exchange of bone Ca with body fluids in marine fish [23], in a low-Ca environment, such as freshwater where fish must extract Ca against a steep gradient, mobilization of Ca stores from bones and scales may be necessary under certain conditions [21].

The fish skeleton supports body posture, development and locomotion, functions as attachment point for muscles, protects organs and cells and is a reservoir for ions [24]. The present experiment shows that the larvae culture in the water hardness of 100 and 120 mg/L is the highest for growth and survival. This indicated that the vertebral bone has supported to any activities of larvae for food and locomotion due to the vertebral forming has already achieve up to more than 90% on day 10 and 100% on day 15. Thus, the vertebral forming is correlated to fish in feeding and any locomotion activities such as food competition, of course, the larvae will faster in locomotion to capture the food and any activities compare to the others. Similar results have been reported to such condition in silver catfish larvae which recommended 20 mg/L of water hardness [25], and 15 mg/L for *Labeo rohita* [13], and 100 mg/L for angelfish [26]. The water hardness concentration is vary among fishes while the optimal for fish grow and survive of nilem carp larvae is 120 mg/L.

During the study, temperature, dissolved oxygen, and pH values are within in the optimal ranges. The water hardness is correlated to the pH value, where the high concentration of water hardness will be led to increase pH. Although, the pH value tend to increase at the present experiment but it still in the optimal condition for nilem carp culture. The pH value for nilem carp culture is ranging from 6 – 8 [20].

5. Conclusion

Based on the result of this research can be concluded that the water hardness affect the vertebral ossification, growth, and survival. The increasing concentration of water hardness will be led to increase the pH value. The optimal of water hardness concentration for larvae rearing of nilem carp is 120 mg/L. To improve the nilem carp productivity should be cultivated the aquaculture media with water hardness concentration is 120 mg/L.

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