

Original article

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Bone formation and growth of climbing perch *Anabas testudineus* larvae fed with Zn enriched *Artemia* nauplii

Pembentukan tulang dan pertumbuhan larva ikan betok *Anabas testudineus* yang diberi pakan naupli *Artemia* yang diperkaya dengan Zn

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ABSTRACT

The aim of this study was to determine the optimum level of Zinc (Zn) enrichment in *Artemia* sp. nauplii as a live feed to improve bone formation and growth of climbing perch *Anabas testudineus* larvae. The study consisted of four different Zn enrichment levels (0.0, 0.05, 0.1, and 0.15 mg/mL) in *Artemia* sp. nauplii. The enrichment was performed for 12 hours with the nauplii density of 1 ind/mL. Climbing perch larvae with an average initial length of 1.65 ± 0.15 mm were fed four times daily with the enriched nauplii. For the first 5 days, all larvae were fed with rotifer followed by feeding with enriched *Artemia* nauplii ad libitum. The results showed that the application of *Artemia* sp. enrichment at 0.1 mg Zn/mL influenced the bone formation, increased the growth, and improved the fish survival of climbing perch larvae. Feeding with 0.1 mg/mL Zn enriched *Artemia* nauplii could be recommended as a strategy to improve the bone formation and growth performance of climbing perch larvae.

Keywords: *Anabas testudineus*, *Artemia* sp., climbing perch, live feed, zinc

ABSTRAK

Penelitian ini bertujuan menentukan dosis optimum pengayaan seng (Zn) pada naupli *Artemia* sp. terhadap pembentukan tulang dan peningkatan pertumbuhan larva ikan betok. Penelitian dilakukan selama 18 hari dengan empat dosis pengayaan Zn yang berbeda (0; 0,05; 0,1; dan 0,15 mg/mL) pada naupli *Artemia*. Pengayaan dilakukan selama 12 jam dengan kepadatan naupli 1 ind/mL. Larva ikan betok dengan ukuran panjang awal rata-rata 1.65 ± 0.15 mm diberi pakan naupli yang diperkaya sebanyak 4 kali sehari. Pada lima hari pertama, semua larva diberi pakan rotifer, diikuti dengan pemberian naupli *Artemia* yang diperkaya secara ad libitum. Hasil yang diperoleh menunjukkan bahwa pengayaan naupli *Artemia* sp. dengan Zn 0,1 mg/mL berpengaruh terhadap pembentukan tulang belakang dan dapat meningkatkan pertumbuhan serta meningkatkan kelangsungan hidup larva ikan betok. Pemberian naupli *Artemia* dengan Zn sebanyak 0.1 mg Zn/mL dapat direkomendasikan untuk perbaikan pembentukan tulang dan pertumbuhan larva ikan betok.

Kata kunci: Naupli *Artemia* sp., ikan betok, pakan alami, seng

INTRODUCTION

Climbing perch *Anabas testudineus* is a freshwater fish that is potentially cultured due to a high economical value, but having a slow growth (Kohinoor *et al.*, 2012; Morioka *et al.*, 2009). One factor influencing the slow growth is feed. Growth is initially begun with a bone formation. Bone formation happens in two ways, namely through the intra-membrane and endochondral ossification (Boglione *et al.*, 1982). Bone formation needs macro and micro mineral elements. One of important micro minerals in bone formation is zinc (Zn). Zn is a mineral element that is mostly accumulated in bones compared to liver as the main organ of micro mineral preservation (Setiawati *et al.*, 2007) and as a cofactor in bone formation (Yamaguchi, 2010). Kaliky *et al.* (2019) reported that Zn also played an important role in the reproduction process of striped catfish.

Commonly, Zn deficiency in feed can lead to low growth and bone demineralization, especially either on juvenile or adult fish (Baeverfjord *et al.*, 2019). On red sea bream larvae, Zn is also functioned to improve growth and backbone formation (Nguyen *et al.*, 2008). In osteology, Zn has anabolic effect on the osteogenesis in osteoblast (Seo *et al.*, 2010). Excessive Zn in feed can decrease the Ca and P content in bones and body of giant gouramy fish and increase the Mn content in bones (Setiawati *et al.*, 2007). The addition of Zn in feed should follow the requirement, thus can balance the other mineral element performances, such as Ca, P, and Mg as also important in bone formation and growth (Holt, 2011). Therefore, sufficient Zn is required for bone formation.

Fish larvae commonly requires Zn content around 20–30 mg/kg (NRC, 1993). Giant gouramy requires Zn supplementation of 25 – 50 mg/kg feed (Setiawati *et al.*, 2007). Fish larvae are commonly and initially fed with live feeds in the form of rotifers and *Artemia* sp. nauplii. The climbing perch larvae given rotifers on 2 days after hatching (DAH) (3.027 mm) until 10 DAH (5.4 mm), while *Artemia* sp. nauplii are given on 7 DAH (4.2 mm) until 20 DAH (10.23 mm) (Yulintine, 2012). The *Artemia* sp. nauplii are small-sized live feed with high nutrient contents (Chahyaningrum *et al.*, 2015), *Artemia* sp. nauplii and rotifer have a character that can be used as initial feed for larvae based on mouth-opening and slow movement (Yulintine, 2012).

Artemia sp. nauplii have 55.38% proteins,

4.42% lipids, 12.15% ash, 3.32% crude fiber, and 24.73% NFE (Chahyaningrum *et al.*, 2015). However, *Artemia* sp. nauplii has lower Zn content than other zooplanktons (Holt, 2011). Zn content increased on rotifer and *Artemia* sp. nauplii after enrichment (Matsumoto *et al.*, 2009; Nguyen *et al.*, 2008). Therefore, Zn needs to be supplemented on rotifer and *Artemia* sp. through enrichment. Red sea bream larvae improves their growth and normal backbone formation through Zn enrichment on *Artemia* sp. nauplii (Nguyen *et al.*, 2008). Thereby, Zn enrichment on *Artemia* sp. nauplii was importantly conducted in this study to improve growth and bone formation of climbing perch larvae.

MATERIALS AND METHODS

Container and materials

The container used was aquarium sized 15×15×20 cm³ as much as 8 pieces for Zn enrichment on *Artemia* sp. nauplii and 20×20×30 cm³ as much as 12 pieces for larval rearing. The climbing perch larvae used were 1.65 ± 0.15 mm and stocked around 40 ekor/L, therefore resulting 1700 larvae in total. *Artemia* sp. nauplii used had 12.18% proteins, 72.24% moisture, 3.22% ash, 0.70% lipids, 2.02% crude fiber, and 9.64% NFE. The Zn used was in the form of ZnSO₄·7H₂O.

Artemia sp. cyst hatching

Artemia sp. cysts were hatched gradually to fulfill the climbing perch larvae live feed availability. *Artemia* sp. cysts were hatched from 4 days after climbing perch egg hatching. *Artemia* sp. nauplii were hatched using water filled container with 30 g/L salinity. Then, the *Artemia* sp. cysts were distributed into the hatching container as much as 1 g and given an aeration for 24 hours. *Artemia* sp. nauplii were harvested and rinsed with freshwater, then moved into the enrichment container (Setiawati *et al.*, 2013) and taken as much as 2 g samples of *Artemia* sp. nauplii for Zn mineral analysis and 10 g for proximate analysis.

Zn enrichment on *Artemia* sp. nauplii

The Zn enrichment on *Artemia* sp. nauplii was performed by following the nutrient enrichment method for live feed (Rahardja *et al.*, 2009). The enrichment container was filled with 200 mL freshwater and 6 g salt. The hatched *Artemia* sp. nauplii were stocked into the available container with 1 nauplius/mL density (Setiawati *et al.*, 2013).

Then, different doses of Zn (0.0, 0.05, 0.1, and 0.15 mg/L) were added and dissolved with water into the enrichment container. The *Artemia* sp. nauplii were harvested after 12 hours (Matsumoto et al., 2009) and rinsed with freshwater, then directly given to the climbing perch larvae. The enriched *Artemia* sp. nauplii were taken 2 g from each treatment for Zn mineral analysis and 10 g from each treatment for proximate analysis.

Climbing perch larval rearing

The newly hatched climbing perch larvae were reared on the prepared aquaria with the stocking density of each aquarium was 120 larvae (40 larvae/L) for 18 days. before stocked, larvae were acclimatized for 15 minutes in the aquarium. Larvae were initially fed on 2 days after hatching (when the egg yolk was fully absorbed) until 5 days after hatching by giving 5 ind/ml rotifers with 4 times daily. Climbing perch larvae were given Zn-enriched *Artemia* sp. nauplii *ad libitum* with 4 times daily. The water quality during the larval rearing period was controlled at 6.5–7.0 mg/L dissolved oxygen, pH 6.9–7.0, and 28–30°C temperature. Water quality maintenance was performed by syphonization every morning

before feeding and 50% water exchange. After the climbing perch larvae were harvested, larvae were taken 20 larvae from each replication as absolute length growth samples and 3 larvae from each treatment for bone staining using Alizarin red and Alcian blue method.

Parameters

Parameters obtained were; *Artemia* sp. nauplii proximate analysis, Zn mineral analysis on *Artemia* sp. nauplii and rotifer, Zn, P, Ca, and Mg mineral analysis on fish larvae, survival rate, absolute length growth, and backbone formation.

RESULT AND DISCUSSION

Hasil

Artemia sp. nauplii proximate

The proximate analysis result showed that Zn enriched *Artemia* sp. nauplii had a different proximate composition (Table 1). Table 2 showed the mineral analysis result of Zn on rotifers and *Artemia* sp. nauplii, besides mineral analysis of Zn, P, Ca, and Mg in fish larvae given nauplii enriched with different Zn. The Zn content on *Artemia* sp. nauplii enriched with Zn was higher

Table 1. The proximate composition of *Artemia* sp. nauplii enriched with different Zn (mg/mL).

Nutrient content (% dry weight)	Zn enrichment concentration (mg/mL)			
	0	0.05	0.1	0.15
Moisture	72.24	71.98	72.21	72.32
Ash	3.22	3.24	3.23	3.19
Protein	12.18	12.2	12.19	12.15
Lipid	0.7	0.75	0.73	0.69
Crude fiber	2.02	2.08	2.03	2
NFE (Nitrogen free extract)	9.64	9.75	9.61	9.65

Table 2. The mineral concentration of Zn on *Artemia* sp. and mineral composition of Zn, P, Ca, and Mg on fish larvae fed *Artemia* nauplii enriched with different Zn concentrations

Mineral content (mg/kg)	Zn enrichment concentration (mg/mL)			
	0	0.05	0.1	0.15
<i>Artemia</i> sp. nauplii				
Zn	20.05 ± 0.11	24 ± 0.10	25.47 ± 0.15	26.19 ± 0.13
Climbing perch larvae				
Zn	35.12 ± 0.29 ^a	46.53 ± 0.19 ^b	47.65 ± 0.05 ^c	48.58 ± 0.14 ^d
P	3160 ± 1 ^a	3286 ± 11 ^b	3513 ± 41 ^c	3728 ± 20 ^d
Ca	5170 ± 1 ^a	5373 ± 7 ^b	5473 ± 4 ^c	5608 ± 6 ^d
Mg	209 ± 1 ^a	214 ± 1 ^b	221 ± 1 ^c	237 ± 0 ^d

Note: Zn = Zinc, P = phosphorous, Ca = calcium, and Mg = magnesium. Different letters behind the average values (± standard deviation) on the same line show a significant difference (P<0.05)

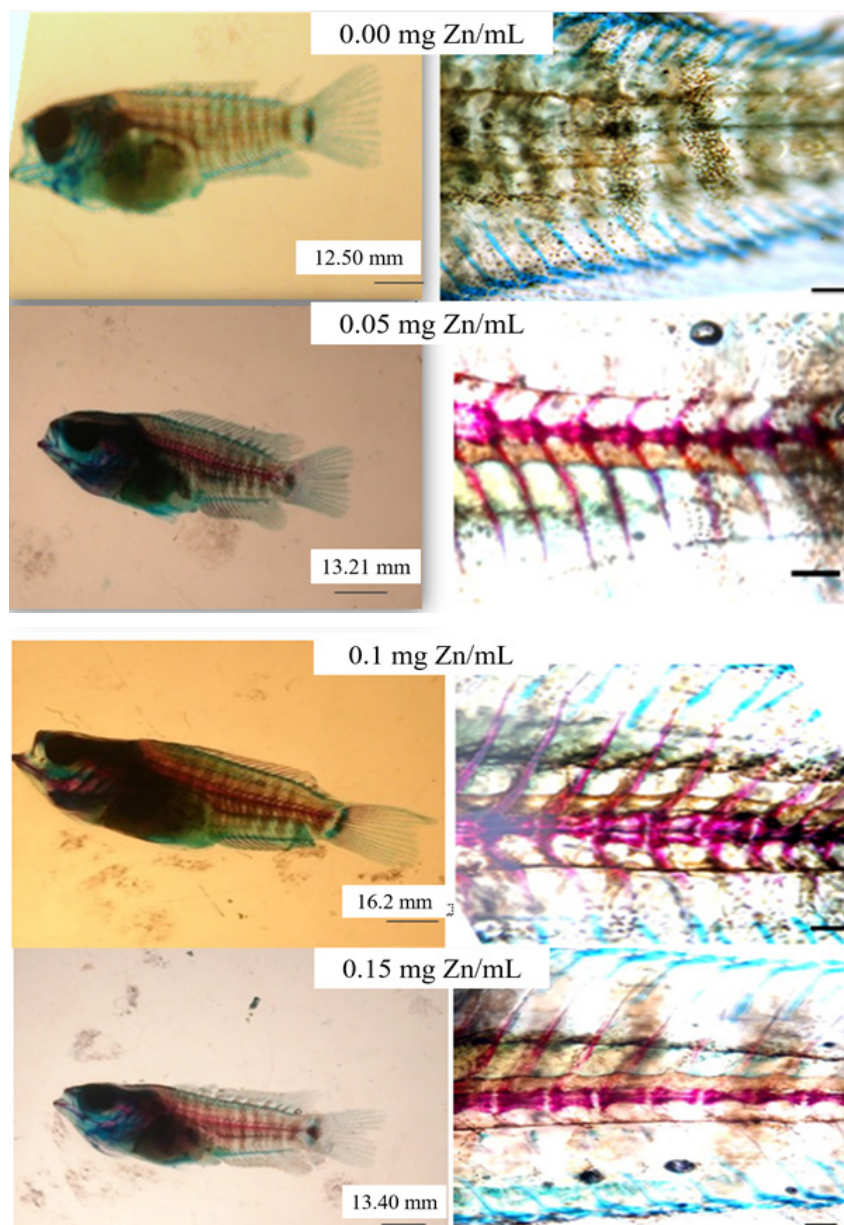


Figure 1. The micrograph of climbing perch larvae backbones after fed *Artemia* sp. nauplii enriched with different Zn concentrations on 18 days after hatching.

Table 3. Final length (mm), absolute length growth (mm), and survival rate (%) of climbing perch larvae fed *Artemia* nauplii enriched with different Zn doses after 18 days of rearing period.

Parameter	Zn enrichment concentration given to <i>Artemia</i> sp. nauplii (mg/mL)			
	0	0.05	0.1	0.15
Lt (mm)	12.62 ± 0.25 ^a	13.47 ± 1.03 ^{ab}	15.03 ± 1.11 ^b	14.07 ± 0.95 ^{ab}
ALG (mm)	10.96 ± 0.246 ^a	11.81 ± 1.026 ^{ab}	13.38 ± 1.10 ^b	12.41 ± 0.95 ^{ab}
SR (%)	45.55 ± 7.47 ^a	48.61 ± 3.46 ^a	64.72 ± 7.51 ^b	70.55 ± 11.70 ^b

Note: SR = Survival rate, PPM= absolute length growth, Pt= final length. Different letters behind the average values (± standard deviation) on the same line show a significant difference ($P < 0.05$).

than without Zn enrichment. Hasil analisis mineral larva ikan menunjukkan bahwa semakin tinggi konsentrasi pengayaan Zn maka semakin tinggi kandungan mineral (Zn, P, Ca, dan Mg) pada larva ikan betok. The mineral analysis on fish larvae indicated that the higher Zn enrichment concentration given, the higher mineral contents (Zn, P, Ca, and Mg) obtained.

Backbone formation on climbing perch larvae

Artemia sp. nauplii enriched with 0.05, 0.1, and 0.15 mg/mL Zn influenced the climbing perch backbone formation (Figure 1). Based on the observation of climbing perch larvae backbone formation, the enriched *Artemia* sp. nauplii given with 0.1 mg/mL Zn obtained a red colored hard bone phase condition. Meanwhile, *Artemia* sp. nauplii without Zn enrichment only obtained a transition phase between cartilaginous to hard bone shown from the transition of transparent blue to red colored bones.

Absolute length growth and survival rate

The climbing perch larva fed *Artemia* sp. nauplii enriched with 0.1 mg/mL Zn had a higher absolute length growth value than control ($P < 0.05$) (Table 3). The highest survival rate of climbing perch larvae was obtained from larvae fed *Artemia* sp. nauplii enriched with 0.1 and 0.15 mg/mL Zn compared to other treatments.

Discussion

The Zn content on *Artemia* sp. nauplii enriched with Zn increased along with the increased concentration of Zn in the enrichment solution. This followed the previous results of Nguyen *et al.* (2008) and Matsumoto *et al.* (2009). The increased Zn content was caused as *Artemia* sp. nauplii could absorb available Zn better in the enrichment container. This study result showed that *Artemia* nauplii enriched with Zn increased the Zn concentration of larval body. This condition followed the result of Eid and Ghonim (1994), who stated that the increased Zn content in *Oreochromis niloticus* fish feed could also increase the Zn content in bones and growth. The increased mineral contents of P, Ca, and Mg was due to the synergetic relationship with Zn mineral. According to Setiawati *et al.* (2007), excessive Zn content in feed could decline P and Ca content in bones as the analysis result on giant gourami indicated that Zn in feed tended to increase the body Zn content, therefore accumulated in the body as one of which was in the bone tissues. The

increased Zn mineral in climbing perch larval body influenced the backbone formation, besides survival rate and growth of climbing perch larvae. The same result was reported on gilthead seabream that showed increased growth, bone mineralization, and decreased abnormality level, when given Se, Mn, and Zn supplemented feed (Izquierdo *et al.*, 2016).

The result indicated that *Artemia* sp. nauplii enriched with 0.1 mg/mL Zn could accelerate the backbone formation marked by the increased length growth of climbing perch larvae. Zn is known to induce the cell differentiation, cell proliferation, and mineralization in osteoblast (bone tissue), as well as improving the insulin-like growth factor I (IGF-I) and transforming growth factor $\beta 1$ (TGF $\beta 1$) production in osteoblastic cells (Yamaguchi, 2010). The larval backbone formation is closely related to feed utilization effectivity and nutrient content completeness in feed consumed, therefore will be expressed on the morphological appearance (Aslianti *et al.*, 2014). Bone formation that is still in the cartilaginous phase affects on the low survival rate and slow growth. Larvae on the cartilaginous phase are at high risk to be dead during the rearing period.

This study result showed that climbing perch larval growth was commonly better than the previous study. This condition happened as only taking 18 days to reach more than 12 mm length, which was faster than the study of Yulintine (2012) that required more than 20 days. The Zn enriched *Artemia* nauplii given produced better growth and survival rate than control. This condition indicated that Zn had a very important role in fish larval growth. A study on carp showed that the supplementation of Zn in feed could improve protein concentration in blood and intestine (Chupani *et al.*, 2017; 2018), while on giant freshwater prawn, the supplementation of Zn was reported to improve growth, survival rate, and immunity (Muralisankar *et al.*, 2014; 2015). Fish feed with 20 mg/kg Zn produced the highest weight and SGR and was significantly different among other treatments ($P < 0.05$) (Mondal *et al.*, 2020).

The survival rate value of climbing perch larvae on *Artemia* sp. nauplii enriched with 0.1 mg/mL Zn treatment was 64.72%, higher than the survival rate of climbing perch reported by Morioka *et al.* (2009) with 20% and Bugar *et al.* (2013) with 18-31%. *Artemia* sp. nauplii enrichment with Zn was suspected to induce the climbing perch larval resistance. Zn also had an

important role on the growth of *rohu* fish *Labeo rohita*. SGR of *rohu* fish was reported to improve along the increased doze of Zn until 42 mg/kg (Akram *et al.*, 2019). The increased survival rate of climbing perch larvae was due to the normal condition occurred to maintain the movement space and utilize the feed maximally for sustaining the survival rate (Bugar *et al.*, 2013). Moreover, the increased survival rate of climbing perch larvae was also due to the sufficient live feed given as based on the larval mouth-opening and number of feed given that could create a normal condition for larval movement space during the rearing period (Suriansyah, 2012).

CONCLUSION

The *Artemia* sp. nauplii given with 0.1 mg/mL Zn enrichment could increase the larval backbone formation and growth rate, as well as survival rate of climbing perch larvae.

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