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The ontogenic study of early life stages of culture-bred *Nomorhamphus* sp. (Zenarchopteridae) from Lindu, Central Sulawesi

Studi ontogeni stadia awal hidup pada budidaya *Nomorhamphus* sp. (Zenarchopteridae) asal Lindu, Sulawesi Tengah

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ABSTRACT

Nomorhamphus sp. is a freshwater fish that has been traded as an ornamental fish. This fish is unique as an endemic species with a halfbeak-like jaw and orange color on the caudal fin. However, this fish culture information needs a further information. Based on this condition, it is necessary to conduct a study as a basis for ornamental fish breeding and growing-out activities through domestication. A crucial problem in this fish is larval rearing, which can be observed through ontogeny studies. The study was conducted on the newly-born larval behavior, morphological development, andropodium development, growth, and survival rate at the early stages, namely larvae to juvenile. The results showed that the newly-born larvae of *Nomorhamphus* sp. Lindu had a total length of 1.6-1.8 cm. Larvae could swim four hours 22 minutes after birth and feed artemia nauplii with surface feeding type. The initial juvenile stage occurred 25 days of post-birth period with a total length of 2.0-2.2 cm. The water condition of the rearing during the study could support the larval transformation to juvenile. This study is the first report related to the aquaculture success of the early life stage of *Nomorhamphus* sp. Lindu at the domestication stage.

Keywords: andropodium, domestication, endemic halfbeak, larva development, surface feeding

ABSTRAK

Nomorhamphus sp. adalah ikan air tawar yang telah diperdagangkan sebagai ikan hias. Ikan ini memiliki keunikan pada statusnya sebagai spesies endemik, bentuk mulut menyerupai paruh setengah (*halfbeak*), dan warna oranye pada sirip ekor. Namun informasi budidayanya belum diketahui dengan baik. Karena itu perlu dilakukan kajian sebagai dasar dalam kegiatan pengembangbiakan dan pembesaran sebagai ikan hias melalui domestikasi. Salah satu kegiatan penting dalam budidaya yaitu pemeliharaan larva yang dapat diamati melalui studi ontogeni. Kajian pada studi awal ini dilakukan pada stadia awal hidup yaitu larva sampai juvenil. Pengamatan dilakukan pada tingkah laku larva pascalahir, perkembangan morfologi, perkembangan andropodium, pertumbuhan dan sintasan pada lingkungan budidaya. Hasil penelitian menunjukkan bahwa larva *Nomorhamphus* sp. Lindu yang baru dilahirkan memiliki panjang total 1,6-1,8 cm. Larva telah dapat berenang pada umur empat jam 22 menit pascalahir (pcl) dan bisa makan naupli artemia dengan tipe *surface feeding*. Stadia awal juvenil terlihat pada umur 25 hari pcl dengan ukuran panjang total 2,0-2,2 cm. Kondisi media pemeliharaan selama penelitian dapat mendukung kehidupan larva sampai juvenil. Penelitian ini merupakan catatan pertama terkait keberhasilan budidaya stadia awal hidup *Nomorhamphus* sp. Lindu pada tahap domestikasi.

Kata kunci: andropodium, domestikasi, ikan endemik, tipe makan permukaan, perkembangan larva

INTRODUCTION

Nomorhamphus is a tropical freshwater genus from the Zenarchopteridae family. Seventy percent of *Nomorhamphus* species are found as endemic species in Sulawesi Island (Huylebrouck *et al*., 2014; Hadiaty, 2018; Kraemer *et al*., 2019a; Kobayashi *et al*., 2020). The habitats of this fish are freshwater rivers and lakes (Herjayanto *et al*., 2019; Kraemer *et al*., 2019a; Kobayashi *et al*., 2020), as reported in Lindu Lake, Central Sulawesi (Wicaksono *et al*., 2022). Morphologically, this fish has a distinct superior jaw, as the lower jaw is longer than the upper jaw, called a halfbeak (Kraemer *et al*., 2019a; Kobayashi *et al*., 2020).

This fish also has a unique reproduction strategy through internal fertilization (viviparous), thus male fish have an additional sexual organ called andropodium. This organ functions as a spermatozoan distributor to female genital pore during the mating process (Kraemer *et al*., 2019b). The uniqueness of endemic species causes *Nomorhamphus* sp. is collected by hobbyists or foreign ornamental fish collectors. Moreover, the color and shape are also unique as part of the aquascape biota. However, these fish are supplied from natural catch, but limited culture studies information.

Wicaksono *et al*. (2022) have reported the initial characterization of biometrical and morphological approaches of *Nomorhamphus* sp. from Lindu. Yet, information regarding breeding, larval rearing, and controlled seed maintenance remains unknown. In general, studies that have been conducted in *Nomorhamphus* species in Central Sulawesi are species description (Kraemer *et al*., 2019a) and the latest habitat condition (Bandjolu *et al*., 2021; Kraemer *et al*., 2019a). Therefore, a cultural approach for *Nomorhamphus* sp. rearing is necessary to support its sustainable production as an ornamental fish. Early life history observation is highly important to gain basic data for larval rearing management (Dos Santos *et al*., 2016).

This observation is related to morphological development, namely body shape and behavior during the developmental stage, which can be called an ontogenic study (Baras *et al*., 2012; Sørensen *et al*., 2016; Tinguely *et al*., 2019). The ontogenic study in fish is necessary to provide information for further breeding activities in the nursery sector as part of the aquaculture activities (Baras *et al*., 2012). Based on the following condition, a study was performed to describe the ontogenic early life period of *Nomorhamphus* sp. Lindu in the cultural environment. The ontogenic studies are mainly focused on the initial larval development to become juvenile. Observations were performed following the post-birth larval behavior (pbl), morphological development, andropodium development, growth, and survival rate in the culture environment.

MATERIALS AND METHODS

Fish Catching

Fish samples were caught in the inlet flow of Lindu Lake at >1,000 asl (1°22’19.20” S 120°01’40.80” E). using a net at 5.5-mm mesh size. The total female fish caught was nine individuals with a total length of 6.6-9.0 cm. After being caught, the fish were maintained by stocking them in an aerated container. During maintenance, the fish remained unfed.

Maintenance was performed for 24 hours. Fish were packed and transported in a closed system, following Hadiroseyani *et al*. (2016), Herjayanto *et al*. (2018), and Herjayanto *et al*. (2020), which were modified at low stocking density. The initial transportation was performed from Lindu Lake to Palu City through a land route. Then, fish were sent through an air route from Palu City to Soekarno-Hatta Airport, followed by a land route from the airport to Laboratory of Aquaculture, University of Sultan Ageng Tirtayasa, Banten. In this location, fish were reared in a controlled tank.

Fish acclimatization

Acclimatization was performed based on the wild fish maintenance method modified by Herjayanto *et al*. (2018) and Hadiroseyani *et al*. (2016) by stocking the fish in a packing plastic in the rearing tank for 30 minutes for temperature adaptation. Then the fish was removed from the plastic and allowed to escape slowly into the rearing tank for acclimatization usingaquarium of 100×50×40-cm^3.

Handling Pregnant-broodstock

The pregnant female broodstock of *Nomorhamphus* sp. from the wild were separated and reared solitary (Figure 1). The rearing tank for pregnant female fish used an aerated clear plastic container at 35 cm×25 cm×15.5 cm size. The total pregnant female fish was five individuals with a total length of 7-8 cm. During the rearing, the female broodstocks were fed with live feeds, namely *Tubifex* sp. and *Daphnia* sp. three times

a day with *ad libitum* method. Broodstocks were reared until giving birth to the larvae.

Larval rearing

The 40 individuals of *Nomorhamphus* sp. larvae were reared in an aerated clear plastic container at 35 cm×25 cm×15.5 cm size. The rearing tank was filled with water until 5 L volume. Larval rearing used five larvae that were stocked at 1 larvae/L. Larvae were fed with *Artemia* nauplii at one day of post-birth (pbl). Rearing was performed for 30 days after giving birth. Behavior, ontogenic morphology, growth (total length, head length, lower jaw length, and fin length), and survival rate were observed at 0, 15, 25, and 30 days of pbl.

The larval swimming behavior was observed from the 10 post-birth larvae. The ontogenic morphology development was documented in a photo to observe the difference between larval and juvenile stadia, as modified by Baras *et al*. (2012) and Wicaksono *et al*. (2022). The photo was taken in a glass container at 24 cm×7 cm×15 cm, filled with water at 10 cm height. During the rearing, water quality parameters, namely temperature, dissolved oxygen, and pH were measured. Feed residue and fecal waste were siphonized once in three days.

Data analysis

Data from behavior, ontogenic morphology, growth, i.e., total length, head length, lower jaw length, and anal fin length (Figure 2), and survival rate were analyzed descriptively using Microsoft Excel 2010. The analysis results are presented in figures and tables.

RESULTS AND DISCUSSION

Results

The newborn larvae of *Nomorhamphus* sp. had a total length of 1.6-1.8 cm (1.69 ± 0.08 cm) (n=3). Larvae could open their mouth with an orange-colored lower jaw. The pectoral, ventral, anal, dorsal, and caudal fins have become definitive organs (Figure 3). The newborn larvae

Figure 1. The pregnant female broodstock morphology of *Nomorhamphus* sp. Lindu.

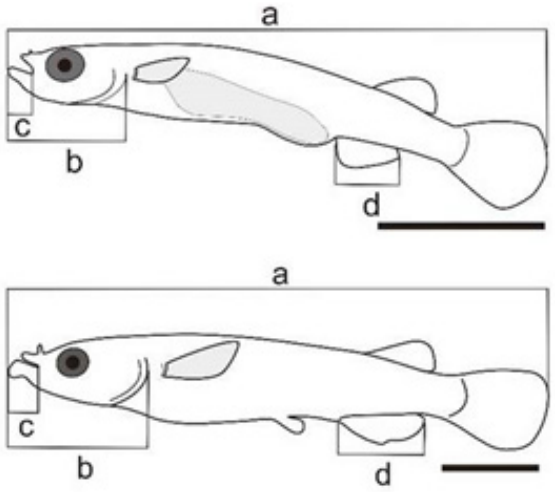

Figure 2. Larval measurement method in 0 days of post-birth (above) and juvenile at 25 days of post-birth (below) in *Nomorhamphus* sp. Lindu. a. Total length; b. Head length; c. Lower jaw length; d. Anal fin length. Line scale: 0.5 cm.

existed on the bottom contained and occasionally swam onto the water surface.

Based on the behavior of 10 larvae, 60% of the larvae could swim on the water column at 20 minutes of post-birth (pbl), then increased by 90% at 3 hours 6 minutes of pbl. All larvae could swim after 4 hours 22 minutes of pbl. This swimming capability has occurred as the swimming bladder has been filled. The swimming position of the larvae at early life stage was only swimming with their head and water surface in perpendicular position.

As the larvae grew older, they could swim onto the water column horizontally. After horizontal swimming, larvae could chase the *Artemia* nauplii. During the developmental stage, the *Nomorhamphus* sp. larvae had a morphological change in their belly shape. Based on the observational results in five days of pbl, larvae had a digestive tract full of *Artemia* nauplii. Also, the body pigmentation started to spread on the transparent body surface (Figure 4).

The digestive tract of *Nomorhamphus* sp. Lindu larvae were still visible until 15 days of pbl as the belly was still transparent. After 20 days of pbl, the fish belly became non-transparent, as the *Artemia* consumed by the larvae was invisible (Figure 5). The anal fin as a secondary sexual character in male fish (andropodium) was yet visible at 15 and 20 days of pbl. The orange color in the ventral fin and caudal fin edge was visible when the fish reached 25 days of pbl.

The anal fin of *Nomorhamphus* sp. Lindu, which was modified as andropodium began to emerge at 25 days of pbl (Figure 6). Therefore, the early stage of juvenile stadia in *Nomorhamphus* sp. Lindu emerges at 25 days of pbl. The total length of *Nomorhamphus* sp. juvenile was 2.11 cm with a head length of 0.47 cm, lower jaw length of 0.11 cm, and anal fin length of 0.30 cm (Table 1).

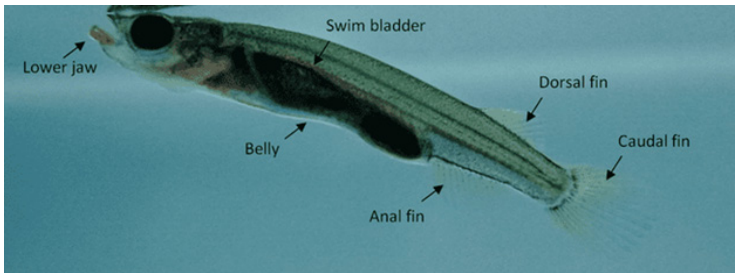

Figure 3. Ontogenic morphology of the newly-born *Nomorhamphus* sp. Lindu larvae. Fish size is presented in Table 1.

Figure 4. Ontogenic morphology of *Nomorhamphus* sp. Lindu larvae. The newborn larvae (above) and five days of pbl larvae (center and below). Fish size is presented in Table 1.

The survival rate of *Nomorhamphus* sp. at the final rearing period (30 days of pbl) was 75-100% (90.60 ± 11%). Fish death has only occurred at three days of pbl and 11 days of pbl. The water quality during the rearing period could support the larval and juvenile life of *Nomorhamphus* sp. Based on the water quality parameters measurement, the temperature was maintained at 22.30-25.50°C (24.00 ± 0.62°C), dissolved oxygen at 4.80-8.90 mg/L (6.85 ± 1.19 mg/L), and pH at 7.19-9.41 (8.08 ± 0.58).

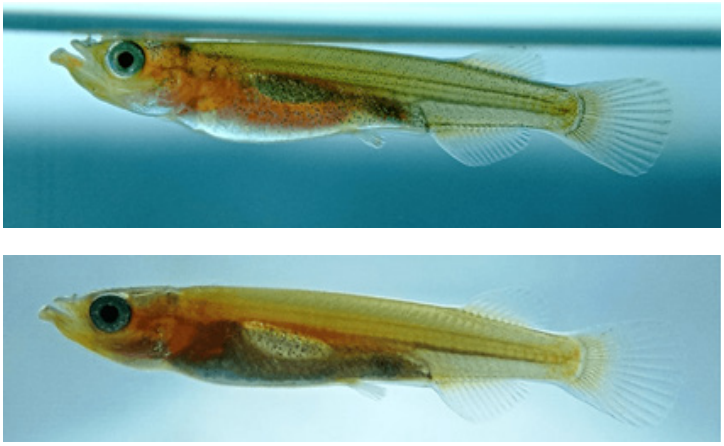

Figure 5. Ontogenic morphology of *Nomorhamphus* sp. Lindu larvae at 15 days of pbl (above) and 20 days of pbl (below). Fish size is presented in Table 1.

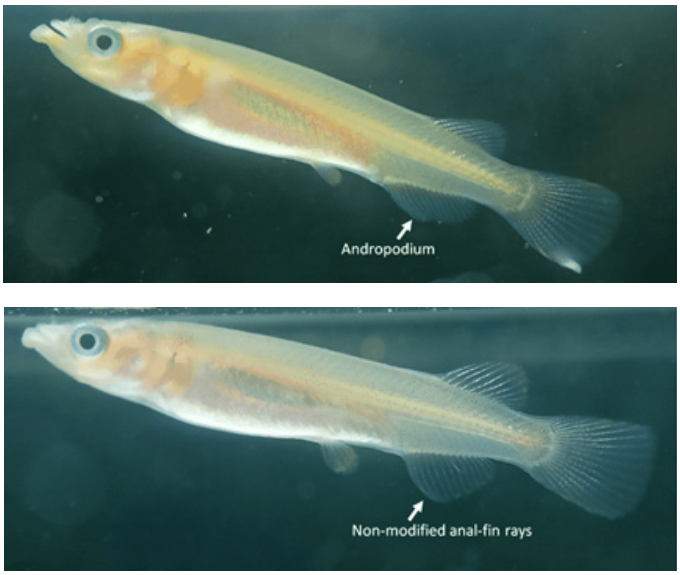

Figure 6. Ontogenic morphology of *Nomorhamphus* sp. Lindu juvenile at 25 days of pbl. Male (above) and female (below). Fish size is presented in Table 1.

Table 1. Survival rate (n=40) and growth (n=12) of *Nomorhamphus* sp. Lindu. early life stages.

Parameter	Age (days of post-birth)			
	0	15	25	30
Developmental stage	Larvae	Larvae	Juvenile	Juvenile
Survival rate (%)	100.00 ± 00	90.60 ± 11	90.60 ± 11	90.60 ± 11
Total length (cm)	1.69 ± 0.08	2.10 ± 0.05	2.11 ± 0.08	2.69 ± 0.19
Head length (cm)	0.32 ± 0.04	0.45 ± 0.03	0.47 ± 0.03	0.53 ± 0.09
Lower jaw length (cm)	0.08 ± 0.02	0.10 ± 0.00	0.11 ± 0.01	0.14 ± 0.02
Anal fin length (cm)	0.24 ± 0.03	0.29 ± 0.01	0.30 ± 0.00	0.39 ± 0.05

Discussion

The early life stage of fish requires more thorough management as larvae are susceptible to environmental changes and feed availability. Therefore, an ontogenic study of early life stages is important to provide basic data for larval rearing management (Baras *et al*., 2012; dos Santos *et al*., 2016). It has been confirmed that *Nomorhamphus* sp. is viviparous fish, as embryonic development occurs in the female gonad and birth larvae (Kraemer *et al*., 2019a; Kraemer *et al*., 2019b; Kobayashi *et al*., 2020). Based on the newborn *Nomorhamphus* sp. Lindu larvae observation, larvae were unable to swim and remained in the bottom container.

This condition has occurred as *Nomorhamphus* sp. Lindu fish are lecithotrophic viviparous fish, as their larvae have yolk as a nutrient source during the developmental stage in the female gonad. This reproductive strategy causes the newborn *Nomorhamphus* sp. larvae to require more time to develop and swim freely by counting on the yolk energy remaining after birth. Another lecithotrophic viviparous fish is *Poecilia vivipara* (Poecillidae), which has yolk as a nutrient source until the final stage of embryonic development (Arcanjo *et al*., 2014). During larval development, transformation emerges in the organ system and body shape (Shahjahan *et al*., 2013; Herjayanto *et al*., 2017). The *Nomorhamphus* sp. larvae gradually performed a jerky motion.

This behavior is an effort for larvae to fill the swimming bladder (Herjayanto *et al*., 2017). Another viviparous species, *P. reticulata*, has newborn larvae that can swim, feed, and move away from danger (Shahjahan *et al*., 2013). In rainbow fish larvae, *Iriatherina werneri*, the newborn larvae spend most of their time resting in the bottom rearing tank, while the fish in the juvenile stage can swim in the water column. Swimming activity elevates as age increases (Herjayanto *et al*., 2017). After the swimming bladder has been filled, the *Nomorhamphus* sp. larvae could swim well to chase *Artemia* nauplii as their feed.

This external feeding becomes an energy source for larvae to conduct metabolism, grow, and develop. The larval body shape causes changes and development to reach a definitive shape along with increasing age. As they were newly born, the *Nomorhamphus* sp. larvae had a transparent body. The morphological change started to emerge at 20 days of pbl, as the belly started to become transparent. At 25 days of pbl (initial juvenile stage), the orange color in the ventral fin and caudal fin edge was visible.

Fish sexual dichromatics and dimorphism can be used as secondary sexual characteristics in fish (Wicaksono *et al*., 2022). In viviparous fish, *Xenotoca eiseni*, orange color in male fish caudal fin initially emerge at four weeks of pbl or 28 days of pbl (Tinguely *et al*., 2019). The secondary sexual character observed in *Nomorhamphus* sp. is andropodium in male fish (Kraemer *et al*., 2019b). In this study, andropodium was confirmed to emerge at 25 days of pbl. Thus, 25 days of pbl is considered as the initial stage of juvenile in *Nomorhamphus* sp. Lindu.

The juvenile fish can grow as a young fish then become a mature broodstock. It has been reported that sexual maturity in another viviparous fish, namely *P. reticulata*, is occurred at 8-10 weeks of pbl or 56-70 days of pbl, then becomes a broodstock at six months of pbl or around 180 days of pbl (Shahjahan *et al*., 2013). The sexual dimorphism in the anal fin, namely gonopodium, is visible at 28 days of pbl (Ahasan *et al*., 2014). The modified anal fin in *Xenotoca eiseni* was initially emerged at four weeks of pbl or 28 days of pbl (Tinguely *et al*., 2019). The secondary sexual character formation in modified anal fin was affected by the androgen hormone, based on the expression of *sonic hedgehog* (Shh) gene (Ogino *et al*., 2004).

The live feed, *Artemia* nauplii, can be used as a feed for *Nomorhamphus* sp. Lindu larvae, as this feed is suitable with larval mouth opening. It has been reported that *Artemia* nauplii had a size of 379-480 μm, commonly used as a live feed for fish culture (Piotrowska *et al*., 2021). The newborn *Nomorhamphus* sp. Lindu larvae were incapable of feeding *Daphnia* due to its size of 0.48-0.81 mm (Ebert, 2022). However, this study did not measure the size of the larval mouth opening, so it is necessary to carry out a more detailed characterization of the ontogeny of the larval mouth opening in future studies. The preliminary observations indicate that *Daphnia* can be applied at 30 days of pcl.

Another halfbeak species, *Zenarchopterus dunckeri*, has been reported to change its diet as it grows in nature. In general, juvenile fish feed more on zooplankton and small gastropods, while larger juveniles feed land insects, such as ants and flies (Kanai *et al*., 2017). In another halfbeak species, *Hemiramphus far*, is known as an omnivorous fish, as it feeds on fish, isopods, ostracods, *Daphnia*, shellfish, algae, and unidentified parts

of plants and animals (Tabassum *et al*., 2017). The superior jaw shape of the *Nomorhamphus*, where the lower jaw is longer than the upper jaw, indicates that this fish is a type of surface feeding, as seen in *Z. dunckeri* (Hirota *et al*., 2015), *H. far* (Tabassum *et al*., 2017), and other *Nomorhamphus* as endemic species in Sulawesi (Kraemer *et al*., 2019a; Kobayashi *et al*., 2020). The success of maintaining the early life stages in this study provides important information for the culture management of *Nomorhamphus* sp. Lindu is an ornamental fish.

CONCLUSION

The *Nomorhamphus* sp. Lindu larvae have a total length of 1.6-1.8 cm and can swim after 4 hours and 22 minutes post-birth (pbl). These larvae feed on *Artemia* nauplii through the surface feeding method. The juvenile stage has occurred at 25 days of pbl with a total length of 2.0-2.2 cm. The rearing media condition can support the early life stages of *Nomorhamphus* sp. Lindu.

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