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Effect of Overlapping Feeding on the Growth and Survival of Nilem (*Osteochilus vittatus*) Larvae

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Abstract

The nilem (*Osteochilus vittatus*) is an endemic freshwater species native to Indonesia, valued for its therapeutic properties and potential as a raw material for value-added products such as baby fish chips. However, its relatively slow growth limits aquaculture development. This study evaluated the effect of overlapping feeding on the growth performance and survival of nilem larvae. Spawning was conducted over three periods using hormone-induced broodstock, and fertilized eggs were incubated under controlled conditions. Larvae were reared at the Instalasi Riset Plasma Nutfah Perikanan Air Tawar under two feeding regimes with two replicates each: a non-overlapping regime, in which infusoria was administered from day 4 to 7 followed by artificial feed, and an overlapping regime, in which infusoria was provided from day 3 to 10 alongside artificial feed from day 8 to 10 before transfer to outdoor concrete tanks. Overlapping feeding resulted in higher absolute weight gain (0.022 ± 0.001 g), absolute length gain (0.78 ± 0.03 cm), specific growth rate (3.30 ± 0.135%), and survival rate (90.0 ± 0.89%) compared to the non-overlapping treatment (0.018 ± 0.001 g; 0.67 ± 0.01 cm; 2.76 ± 0.111%; and 86.1 ± 0.62%, respectively). Significant differences ($p < 0.05$) were observed in absolute length gain and survival, whereas absolute weight gain and specific growth rate were not significantly affected. Overlapping feeding improved larval performance and is recommended as a strategy to enhance hatchery productivity in nilem aquaculture.

Keywords: nilem fish, overlapping feeding, survival, growth performance

I. INTRODUCTION

Indonesia is home to 12 species of native freshwater fish commonly cultured in rivers and swamps, including the nilem (*Osteochilus vittatus*), an indigenous species (Latuconsina, 2021). Nilem fish offers several advantages, it is easy to cultivate, and individuals weighing up to 5 g can be processed into ready-to-eat products such as baby fish (Subagja *et al.,* 2006). According to aquaculture statistics, nilem production decreased from 33,771 tons in 2019 to 32,854 tons in 2021 (KKP, 2021). A primary limitation in nilem aquaculture is the relatively slow growth rate, highlighting the need for strategies that accelerate growth. Effective feed management is a key determinant of aquaculture success, as it optimizes nutrient utilization and supports optimal growth performance (Kurniati, 2019).

The larval stage is a critical in fish hatchery operations, playing a central role in determining the success of juvenile fish production. At this stage, larval organs are generally not fully developed, resulting in suboptimal physiological function. This condition increases the vulnerability of the larval phase, making it a critical period for fish survival. The transition from endogenous feeding (yolk reserves) to exogenous feeding (natural and artificial diets) poses a major challenge during this stage. A proper understanding of this transitional adaptation is required to minimize mortality and maximize larval growth (Bhagawati *et al.,* 2021).

Nilem fish have higher energy requirements compared to species that inhabit still waters or ponds, as they naturally inhabit rivers with strong currents. Fish in lotic environments allocate part of their assimilated energy from feed toward the energetic costs of swimming. For instance, in Amur grayling fish, increased flow velocity resulted in depletion of glycogen reserves and elevation of lactate levels, indicating greater energy use for locomotion (Zhai *et al.,* 2024). Excess energy in nilem is allocated for growth, whereas insufficient energy inhibits growth processes. Fish expended on swimming activities in flowing water than on growth, reflecting an adaptive mechanism to high-current environments.

Nilem fish also possess a relatively long digestive tract and a slow digestive process due to their herbivorous feeding habits. These characteristics affect both energy-use efficiency and growth rate. The primary constraint in nilem culture is their relatively slow growth; therefore, appropriate feed management is essential, particularly during the transition between feed types. The application of overlapping feeding has been proposed as a strategy to enhance growth performance in nilem (Kurniati, 2019). This study aimed to evaluate the effect of overlapping feeding of infusoria and pellet feed on the growth performance and survival of nilem (*Osteochilus vittatus*) larvae.

II. MATERIALS AND METHODS

The equipment used in this study consisted of four rearing aquariums (80 × 60 × 41 cm), two concrete tanks (7.5 × 5.7 × 1.5 m), five infusoria culture aquariums (50 × 48.5 × 30 cm), a digital scale, and standard water quality measuring devices, consisting of a thermometer, pH meter, dissolved oxygen meter, and ammonia test kit. Materials used for spawning included Ovaprim hormone (0.5 mL kg^{-1} of broodstock), NaCl solution (5 mL), fish anesthetic/stabilizer (5 mL), dried banana leaves, and commercial feed (Fengli).

2.1 Experimental Design

The experiment consisted of two treatments with two replications, resulting in a total of four experimental units. The treatments compared larval rearing without overlapping feeding and with overlapping feeding, as described below.

2.1.1 Non-Overlapping feeding

In the non-overlapping feeding, nilem larvae were fed according to the following schedule (Table 1). Based on the feeding schedule, larvae aged D1–D3 relied on endogenous reserves (yolk sac), those aged D4–D7 were provided with natural feed in the form of infusoria, and larvae aged D8–D30 received commercial artificial feed (Fengli).

2.1.2 Overlapping feeding

In this treatment, natural and artificial feeds were applied in an overlapping manner (Table 2). Larvae aged D1–D3 relied on endogenous reserves (yolk sac). From D3 to D10, larvae were fed infusoria. Artificial feed (Fengli) was introduced from D8 to D10 alongside infusoria. From D11 to D30, the larvae were transferred to outdoor concrete tanks for further rearing.

2.2 Research Procedures

2.2.1 Spawning of Nilem Fish

Spawning was conducted over three spawning periods. Broodstock were placed in aquariums measuring 1 m × 0.5 m × 0.4 m. The spawning aquariums were cleaned by scrubbing all surfaces with a sponge, dried, then filled with water to a height of

Table 1. Feeding management of *Osteochilus vittatus* larvae under the non-overlapping feeding regime.

Diet type	Age (day)								
	1	2	3	4	5-6	7	8	9-29	30
Yolk sac	■	■	■						
Infusoria				■	■	■			
Pf 0 (fengli							■	■	■

Table 2. Feeding management of *Osteochilus vittatus* larvae under the overlapping feeding method.

Diet type	Age (day)								
	1	2	3	4	5-6	7	8	9-29	30
Yolk sac	■	■	■						
Infusoria			■	■	■	■			
Pf 0 (fengli						■	■	■	■

Table 3. Fecundity data of *Osteochilus vittatus* across three spawning periods.

Period	Initial broodstock weight (g)	Final broodstock weight (g)	Total number of eggs (egg)
Spawning 1	261.5	169.9	143,812
Spawning 2	234.8	182.3	82,425
Spawning 3	158	118	62,800
Mean	218.1 ± 53.7	156.7 ± 34.1	96,345.7 ± 42,262

21 cm and aerated. Spawning was carried out semi-artificially through hormone induction using Ovaprim hormone, with a broodstock sex ratio of 1:3 (female : males). Females with mature gonads were identified by a distended abdomen and reddish ovaries. Mature males were identified by the smooth texture of the abdomen and verified by gentle stripping to confirm the release of milt. Gonadal maturity in females was further confirmed by cannulation of sampled broodfish to check egg readiness.

Hormone induction was performed by injecting ovaprim intramuscularly using a syringe at a 45° angle, avoiding contact with the bones. After injection, the syringe was carefully withdrawn, and the injection site was gently massaged. Male and female broodfish were then placed together in the same aquarium. Spawning behavior was indicated by males chasing the female and rubbing their abdomen against the genital area of the female. Ovulation occurred approximately 12–18 hours after hormone injection.

2.2.2 Egg Hatching

Egg hatching was carried out in aquarium measuring 80 cm × 60 cm × 41 cm. The aquariums were cleaned by scrubbing all internal surfaces with a sponge, rinsed, and air-dried before use. Water sourced from a natural spring and stored in a holding reservoir was added to a depth of 20 cm, followed by aeration. Eggs collected from the spawning tanks were filtered using a fine-mesh net and rinsed under running water to remove debris. The eggs were then evenly distributed into the hatching aquaria with continuous aeration. Hatching occurred within 24 hours.

2.2.3 Larval Rearing

Larval rearing was conducted in concrete tanks measuring 7.5 m × 5.7 m × 1.5 m. The tanks were cleaned by scrubbing all walls, rinsed, and dried for 1–2 days. Water from a natural spring was added to a depth of 50 cm by opening the inlet valve and closing the outlet valve. Larvae aged 8 days were transferred from the hatching aquarium to outdoor concrete tanks. Transfer was performed by gently scooping larvae with a fine-mesh net, placing them in water-filled buckets, and evenly stocking them into the concrete tanks.

Larvae were fed infusoria and Fengli commercial feed. Feeding was conducted three times daily at 07:00, 12:00, and 15:00. Sampling was carried out weekly to measure larval length and weight, using a sample size of 50 individuals from each pond. Length measurements were performed by placing larvae individually in a petri dish and observing them over a millimeter block. Weight was measured using a digital scale by weighing groups of 50 larvae and calculating the average weight.

2.2.4 Natural Feed Culture

Natural feed culture was conducted using aquarium measuring 50 cm × 48.5 cm × 30 cm, filled with water to a depth of 20 cm. The culture aquaria were cleaned by scrubbing all surfaces with a sponge and dried before use. Each aquarium was filled with 4.85 L of pond water. Infusoria were cultured using four pieces of dried banana leaves, which were soaked for 5 minutes before being placed into the culture tanks. The culture was ready for harvest after four days. Harvesting was conducted by opening the tank cover and directing a flashlight toward the bottom of the tank to attract infusoria to the surface. They were then collected using a plastic bowl, transferred into a small basin, and evenly distributed to the larvae using a small plastic container.

III. RESULT

3.1 Spawning

Spawning was conducted over three periods

Period	Initial broodstock weight (g)	Final broodstock weight (g)	Total number of eggs (egg)	Total number of eggs (egg)
Spawning 1	261.5	169.9	143,812	143,812
Spawning 2	234.8	182.3	82,425	82,425
Spawning 3	158	118	62,800	62,800
Mean	218.1 ± 53.7	156.7 ± 34.1	96,345.7 ± 42,262	96,345.7 ± 42,262

(Table 3). The lowest total egg production was recorded during spawning period 3, yielding 62,800 eggs from broodstock with an initial body weight of 158 g and a final body weight of 118 g. In contrast, the highest total egg production occured in spawning period 1, with 143,812 eggs produced by broodstock with an initial weight of 261.5 g and a final weight of 169.9 g.

3.2 Egg Hatching Rate

Egg hatching results indicated that the lowest number of fertilized eggs and hatched larvae occurred in spawning period 3, with 59,660 fertilized eggs and 55,364 hatched larvae, respectively. The highest values were recorded in spawning period 1, with 133,742 fertilized eggs and 118,440 hatched larvae. The lowest fertilization rate (FR) was observed in spawning period 1 at 93%, whereas the highest FR was obtained in spawning period 2 at 98%. Similarly, the lowest hatching rate (HR) was found in spawning period 3 at 92.8%, while the highest was observed in spawning period 2 at 93.4%. These data are presented in Table 4.

3.3 Larval Rearing

The highest growth performance was observed in the overlapping feeding treatment, with an absolute weight gain of 0.022 ± 0.001 g, an absolute length gain of 0.78 ± 0.028 cm, and a specific growth rate of 3.30 ± 0.135%. The growth performance values are presented in Table 5.

T-test analysis showed that absolute length gain had homogeneous variance and a p-value of 0.036 (< 0.05), indicating a significant difference between treatments and leading to the rejection of H_0. In contrast, absolute weight gain exhibited homogeneous variance with a p-value of 0.056 (> 0.05), indicating no significant difference between treatments. Similarly, the specific growth rate showed homogeneous variance and a p-value of 0.053 (> 0.05), demonstrating that the treatments did not significantly affect this parameter.

Larval survival after 30 days exhibited homogeneous variance, and statistical analysis using the t-test yielded a p-value of 0.036 (<0.05), indicating a significant difference between treatments. Survival was higher in the overlapping feeding treatment (90.0 ± 0.89%) compared to the non-overlapping feeding treatment (86.1 ± 0.62%), based on an initial stocking density of 25,000 larvae (Table 6).

3.4 Water Quality

Water quality played an important role in supporting nilem larval rearing. Measurements taken over 30 days (Table 7) indicated that water temperature ranged from 22–30 °C, with the lowest temperature recorded in the overlapping feeding treatment and the highest in the non-overlapping feeding treatment. Dissolved oxygen levels ranged from 5.06 to 5.75 mg/L in the non-overlapping treatment and from

Tabel 5 Growth performance of *Osteochilus vittatus* larvae based on absolute length gain, absolute weight gain, and specif-

Rearing	Absolute length gain (cm)	Absolute weight gain (g)	Spesific growth rate (%)
Non-overlapping feeding	0.68 ± 0.01[a]	0.018 ± 0.001[a]	2.84 ± 0.077[a]
Overlapping feeding	0.78 ± 0,028[b]	0.022 ± 0,001[a]	3.30 ± 0.135[a]

*Length: P < 0.05; weight: P > 0.05; SGR: P>0.05

Tabel 6. Survival of *Osteochilus vittatus* larvae over a 30-day rearing period under non-overlapping and overlapping feed-

Treatment	Survival rate (%)
Non-overlapping feeding	86.1 ± 0.62[a]
Overlapping feeding	90.0 ± 0.89[b]

* SR: P < 0,05

Tabel 7. Water quality parameters during the rearing of *Osteochilus vittatus* larvae.

Parameter	Non-overlapping feeding		Overlapping feeding		Bhatnagar dan Devi (2013)
	Morning	Evening	Morning	Evening	
Suhu (˚C)	23–30	22–30	22–27	23,9–27	25–30 °C
pH	7.85–9.61	7.9–9.6	8.35–9.65	8.54–9.6	6.5–9
DO (mg/L)	5.06 –5.75	5.06–5.67	5.4–5.65	5.45–5.76	5 mg/l
Amonia (mg/L)	0.25	0.25	0.25–1.5	0.25–1.5	<0.025

5.4 to 5.76 mg/L in the overlapping treatment. Water pH values ranged from 7.85 to 9.61 in the non-overlapping group and from 8.35 to 9.65 in the overlapping group. Ammonia levels remained between 0.25 mg/L in the non-overlapping treatment to 0.25–1.5 mg/L in the overlapping treatment over the 30-day period.

IV. DISCUSSION

The spawning performance observed in this study indicates that *Osteochilus vittatus* broodstock exhibited consistent reproductive output across the three spawning periods, with fecundity ranging from 62,800 to 143,812 eggs. These values were comparable to previous reports on *O. vittatus* and related cyprinid species. Hastuti *et al.,* (2024) reported that broodstock weighing 220 g could produce an average of 25,350 eggs. The higher fecundity observed in this study may be associated with broodstock condition factor and hormonal responsiveness, suggesting that Ovaprim administration at 0.5 mL/kg was effective in stimulating ovulation.

Growth performance of nilem larvae differed between feeding strategies. The overlapping feeding treatment yielded higher absolute length gain (0.78 ± 0.028 cm), absolute weight gain (0.022 ± 0.001 g), and specific growth rate (3.30 ± 0.135%) compared to the non-overlapping treatment. Survival rate (SR) was significantly influenced by feeding strategy, with the overlapping feeding treatment achieving a survival rate of 90%, compared to 86% in the non-overlapping treatment. This improvement aligns with reports from Wauyai (2021), who reported substatially lower survival (46.6–66.6%) in nilem larvae reared without overlapping feed transition, highlighting the critical role of feeding strategy in mitigating starvation stress and feed rejection during dietary shifts.

Hamre *et al.,* (2013) emphasized that the transition from endogenous to exogenous feeding represents the most vulnerable stage in larval development, during which mortality commonly peaks. Overlapping feeding therefore serves not only as a feeding technique but as an adaptive management approach that aligns with larval physiological readiness. An optimal first feeding strategy that considers timing, feed type, and size enhances larval adaptation, growth, and survival, thereby improving aquaculture efficiency (Malzahn *et al.,* 2022).

Infusoria has been demonstrated to be an effective first feed for fish larvae due to its high nutritional value, digestibility, and ability to support erly growth and survival (Amit 2023). Lahnsteiner *et al.,* (2023) reported that infusoria can serve as a first-feed alternative comparable to rotifers and artemia for freshwater larvae, while Mukai *et al.,* (2022) highlighted efficient infusoria culture methods for marine larvae. Therefore, the provision of infusoria during the early larval stage enhances adaptation to subsequent formulated feeds and reduces mortality, making it a crucial component of larval aquaculture management.

The success of this approach can be attributed to the nutritional and functional characteristics of infusoria as the initial live feed. Cultures based on dried banana leaves have been shown to contain three major genera, namely *Paramecium* sp., *Chlorococcum* sp., and *Trichocerca tenuior* with protein content ranging from 40% to 60% and lipid content between 10% and 20% (Insanni *et al.,* 2022). These microorganisms contain digestive enzymes and probiotic-associated bacteria that stimulate buccal-motor coordination and accelerate intestinal maturation during the weaning phase. Pillay and Kutty (2005) emphasized that live feed not only provides nutrients but also triggers feeding reflexes and enzymatic secretion, making it indispensable in the endogenous–exogenous transition period.

Water quality is a key determinant of larval performance in *Osteochilus vittatus* culture. In this study, temperature, pH, and dissolved oxygen remained within acceptable ranges for cyprinids (Maniagasi *et al.,* 2013), although slightly lower temperatures during the rainy season may have reduced metabolic activity (Bhatnagar & Devi 2013). Ammonia levels were generally low in the non-overlapping treatment but increased slightly in the overlapping treatment due to uneaten feed accumulation. Ramadhan and Yusanti (2020) reported that elevated ammonia concentrations can negatively affect aquatic organisms by disrupting growth and survival. Therefore, maintaining stable temperature and controlling organic waste are critical to optimizing larval growth and survival.

Overall, the findings of this study demonstrate that overlapping feeding significantly enhances larval survival and supports improved growth performance in nilem. Although weight-related parameters showed modest differences, the consistent improvement in survival rate underscores the practical value of overlapping feeding as an adaptive management approach for cyprinid larval rearing. Given that larval viability is a primary determinant of hatchery productivity, the adoption of overlapping feeding may serve as a standardized protocol for nilem and related species in freshwater aquaculture.

V. CONCLUSION

Overlapping feeding significantly improved larval performance in *Osteochilus vittatus*. Although growth differences between treatments were modest, larvae provided with overlapping live and formulated feed showed a higher survival rate (90.0%) compared to non-overlapping feeding (86.1%). Spawning performance was consistent across broodstock, confirming the effectiveness of Ovaprim induction. These results indicate that overlapping feeding is a practical strategy to enhance larvae production efficiency in nilem aquaculture.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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