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Larval rearing of Knife fish (*Notopterus notopterus*) in different feeding regimes

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Abstract
The present study was conducted to assess the effects of different feed regimes for nursing knife fish (*Notopterus notopterus*) larvae up to 35 days. Larvae of knife fish were reared in 50 L tank with Moina, Tubifex worm, and commercial feed powder substituted in different culture days. Feeding larvae with Tubifex worms during nursing found to best for growth rate and survival rate of knife fish. The best result was obtained when larvae were fed 0-5 days Moina, 6-20 days tubifex worm, and 21–35-day feed powder with weight gain 0.31 g, length gain 2.36 cm, and survival rate 59%. It is concluded that feeding larvae with Tubifex worm from day 6 to day 20 resulted in a significant difference (p<0.05) on the growth rate and survival rate of knife fish.

Keywords: Knife fish, nursing larvae, Moina, Tubifex worm, feed powder, survival rate, growth rate

1. Introduction
The knife fish *N. notopterus* (Pallas, 1769) [13], which is distributed throughout the Indian subcontinent and into Southeast Asia, is a unique species in the genus (Nelson *et al*., 2006) [8]. It occurs mainly in clear streams, sluggish lakes, floodplains, canals, and ponds (Jhingram & Talwar, 1991; Rainboth, 1996) [4, 9]. Feeding management plays a critical role in the success of larval rearing. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well-balanced nutritionally complete, and cost-effective compounded feeds. Microalgae and zooplankton play a vital role in the hatchery phase of many aquacultures as feed for larval and juvenile crustaceans and fish. The food value of live food organisms for a particular fish species was primarily determined by its size and form. The nutritional quality of live food in aquaculture is important for the survival and growth of larvae. The Knife fish *N. notopterus* is carnivorous inhabits and feeds on various Annelids, Arthropods, Molluscs, small fishes, etc. (Shillewar and Nanware, 2009) [11]. Despite the temperature is constant, the hatching rate is seen to be varying among different breeding events within the same egg cluster. So, larval rearing needs crucial care to gain a higher survival rate.
Larval rearing of the captive-bred population of *Chitala chitala* which is an endangered freshwater species was reported successful in India (Sarkar *et al*., 2006) [10]. For the stock enhancement development of controlled larval rearing techniques is very crucial. The methods to improve the larval rearing have to be improved further for commercial farming of the species. Sarkar *et al*., (2006) [10] reared the larvae of *Chitala chitala* by feeding different diets such as live food (tubifex worms, Chironomus larvae, zooplankton), dry feed (dry tubifex, spirulina, daphnia), and other feeds like boiled egg yolk. Recirculating systems can also be applied for the larval rearing to attain higher growth and survival during early life stages (88%) by feeding spirulina and daphnia (Sarkar *et al*., 2006) [10]. Larval can be reared in different systems like concrete tanks, fiberglass tanks, recirculating systems. Starting from day 17 the larvae (16.2 mm) of the feather back initiates the exogenous feeding concurrent with endogenous nutrient utilization from the yolk sac. Feeding habit of fishes in natural water bodies is different among the species but all the fishes require protein-rich live food for their better growth, efficient breeding, and survival (Mandal *et al*., 2009) [7]. Advances in live food enrichment techniques have helped to boost the importance and potential of live food organisms in the raising of larval aquatic species.

The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish larvae, fry, and fingerlings (Lim *et al.*, 2003) [6]. This study aims to assess the effect of different feeding regimes on the growth and survival rate of knife fish larvae.

2. Materials and Methods

The experiments were carried out at the wet-laboratory of Department of Freshwater Aquaculture, College of Aquaculture and Fisheries, Can Tho University, Vietnam.

Larval rearing of Knife fish

Larvae were reared for 35 days in a 50L tank with a stocking density of 2 larvae/L, with 3 treatments and 3 replicates to know best feed for larval rearing. Furthermore, water quality parameters were monitored and recorded daily i.e., temperature, D.O, and pH. This experiment was conducted with 3 treatments and 3 replicates as follow:

Treatment 1: 0-5 days Moina, 6-10 days Tubifex worm, 11-35 days feed powder

Treatment 2: 0-5 days Moina, 6-15 days Tubifex worm, 16-35 days feed powder

Treatment 3: 0-5 days Moina, 6-20 days Tubifex worm, 21-35 days feed powder

Feed

The larvae were fed with live food and commercial feed-containing 42% crude protein. The live food consists of Moina (*Moina sp)*, and *Tubifex* worm (*Tubifex tubifex)*. Larvae were fed twice a day with live food and feed powder 4 times a day depending on treatments and culture days. While changing the diet from live food to commercial feed, the fish were provided with a mixture of tubifex 60% and feed powder 40% in the first day and tubifex 30% + feed powder 70% on the second day to train the fish for feed powder. The larvae were fed with live food early morning 7 am and evening at 8 pm. After the substitution of live food with commercial feed larvae were fed 4 times a day (7 am, 12 pm, 5 pm and 8 pm) respectively.

Monitoring of Water quality parameters

The experiment was set up in a freshwater hatchery with a continuous aeration system. A centigrade thermometer within the range of 0°C to 120°C was used to record the water temperature, also for Dissolved Oxygen and pH, hand-held Oxy-Guard digital meter and Handy pH meter was used. Regular siphoning was done to remove remaining feed and water was exchange 50% daily. Water temperature, pH, and Dissolved Oxygen were measured twice a day during the larval rearing period.

Larval Quality

Therefore, after eggs hatching larval quality was evaluated by using the following growth parameters, (El-Hawarry *et al.*, 2016).

- **Weight gain (WG, g):** Final weight – Initial weight
- **Length gain (LG, cm)):** Final length – Initial length
- **Average Daily weight gain (ADG, g)): (**Final weight – Initial weight) / Days
- **Daily length gain (ADL, cm)): (**Final length – Initial length) / Days
- **Specific growth rate (SGR, %):** 100 × **(**ln Final weight-ln Initial weight) / Days
- **Survival Rate (SR, %):** 100 × (No of fish harvested/No of fish stocked)

Sampling

During the larval rearing process, the larvae were sampled 2 times. The first sampling was done, before stocking into the larval rearing tank and second sampling in the 35th day, the fish was sampled for larval quality analysis such as Length, Weight, Survival Rate, and Specific Growth Rate.

Statistical Analysis

Statistical analysis, one-way analysis of variance, and Duncan's multiple range tests were used to evaluate the difference of feeding efficacy between the experimental groups. Data were analyzed for mean, standard deviation, standard error mean, and one way ANOVA to evaluate larval feeding strategy analysis using statistical software SPSS 25.0 and Excel 2016 software. Differences were considered a p-value of 0.05 using computer software SPSS.

3. Results

The effects of feed regimes on the growth and survival rate of *N. notopterus* larvae

Water quality parameters of larval rearing tanks

The different water quality parameters such as temperature (ºC), pH, and dissolved oxygen were monitored between 7:30-8:00 a.m. and between 2-3 pm every day. The temperature was fluctuated 26±1 ºC in the morning and 28±2 ºC in the afternoon in all treatments shown in Table 1.

Table 1: Fluctuation of water quality parameters during larval rearing of Knife fish

Treatments	Temperature ºC		pH		DO mg/l	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
T1	26.53±0.01	28.5±0.02	7.5±0.04	7.59±0.05	5.17±0.09	5.27±0.03
T2	26.67±0.04	28.59±0.07	7.52±0.06	7.63±0.01	5.23±0.03	5.43±0.03
T3	26.55±0.01	28.51±0.01	7.51±0.02	7.63±0.02	5.23±0.03	5.43±0.03

Note: Values shown are means of 3 replicates group's ± SEM

Fish growth and specific growth rates

Variation in the mean values of growth parameters (initial weight, weight gain, final weight, SGR, ADG, survival rate under different treatments is presented in Table 2.

Table 2: Weight gain, ADG, SGR and survival rate of Knife fish larvae

Parameters	Treatments		
	T1	T2	T3
Initial weight (g)	0.018±0.0003[a]	0.019±0.0003[a]	0.018±0.0003[a]
Final weight (g)	0.160±0.0080[a]	0.180±0.0190[a]	0.330±0.0150[b]
Weight gain(g)	0.150±0.0080[a]	0.160±0.0190[a]	0.310±0.0140[b]
Average Daily Weight Gain (g)	0.005±0.0003[a]	0.005±0.0006[a]	0.010±0.0005[b]
Specific Growth Rate (%)	7.30±0.18[a]	7.60±0.38[a]	9.6±0.11[b]
Survival Rate (%)	19.67±1.67[a]	28.33±5.81[a]	59±9.87[b]

Note: The values shown on the table are the mean ± SEM. The different small letters a, b represents significant differences ($p<0.05$)

Table 3: Final length, length gain and ADL of Knife fish larvae

Treatments	Initial length (cm)	Final length (cm)	Length gain (cm)	Average Daily Length Gain (cm)
T1	1.34 ±0.003[a]	2.68±0.073[a]	1.34±0.076[a]	0.045±0.003[a]
T2	1.33±0.003[a]	2.87±0.078[a]	1.53±0.075[a]	0.051±0.003[a]
T3	1.35±0.007[a]	3.71±0.110[b]	2.36±0.104[b]	0.079±0.003[b]

Note: The values shown on the table are the mean ± SEM. The different small letters a, b represents significant differences ($p<0.05$)

Weight of larvae fed with live food and commercial feed

From Table 2 the stocking weight of larvae in different treatment ranges from 0.018 to 0.019 g. The weight of larvae in different treatments during stocking was not significantly different ($p>0.05$). Based on the statistical test of variance (one-way ANOVA), larvae fed with Tubifex up to day 20 show significant difference ($p<0.05$) with other treatments. The highest growth was obtained when larvae fed with 5 days Moina, 15 days Tubifex worm and 15 days feed powder. The highest final weight of larvae within 35 days of culture was 0.31±0.014 g and the lowest was 0.16±0.008 g. Weight gain was also significantly higher when the larvae were fed with live food up to day 20. Statistical results showed that feeding with live food and commercial feed had a significant difference in the growth ($p<0.05$) of *Notopterus notopterus* larvae.

Length of larvae of fed with live food and commercial feed powder

From table 3, it was seen that the initial length was not significantly different ($p<0.05$) during the time of stocking. The highest length of 3.71±0.11 cm was obtained in treatment fed with Tubifex up to 20 days. Based on the analysis of variance and the Duncan multiple range test, feeding live food for more culture days had a significant difference ($p<0.05$).

Specific growth rate (SGR)

The specific growth rates (SGR) of Knife fish reared in various stocking feed types are shown in Table 2. The highest daily growth rate of the fish was 9.6±0.11% obtained in fish fed with Tubifex worm up to day 20. On the other hand, the lowest SGR was obtained in fish fed with Tubifex up to day 10 and commercial feed powder 7.3±0.18%. ANOVA test showed that feed types and their interactions had significant effects ($P < 0.05$) on the specific growth rate (SGR) of the fish.

Average Daily Weight Gain and Average Daily Length Gain

The Average Daily Weight Gain and Average Daily Length Gain was highest in treatment 3 when larvae were fed with live food for a long duration. The highest ADG was 0.01±0.0005 g and ADL was 0.079±0.003 cm obtained in treatment 3. Based on the statistical test, feeding larvae with live food fed in the early life stage had a significant difference ($p<0.05$) on growth rate.

Survival rate of *N. notopterus* larvae in different feeding regimes

Figure 1 shows the survival rate of Knife fish larvae in different feeding regimes. The highest survival rate was 59±9.87% obtained when larvae fed live food (Moina + tubifex) up to 20 days and the lowest was 19.67±1.67% when larvae fed with live food up to 10 days. Statistical test of variance (one way ANOVA) results showed that type of feed and duration of live food had a significant difference ($p<0.05$) on the survival rate of larvae.

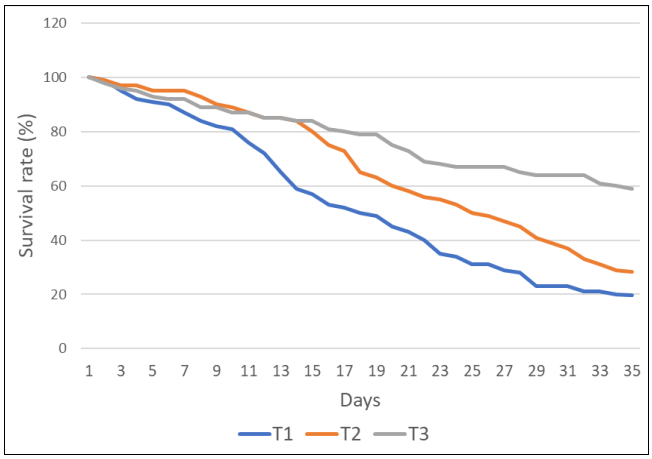

Fig 1: Survival rate of N. notopterus larvae

4. Discussion

Larval rearing of knife fish

The knife fish is a highly demanded freshwater fish species and hence has an excellent culture potential. To expand knife fish culture, knowledge of early larval development and feeding is imperative. But the feeding and larval development of this fish is poorly understood and only a few studies have ever been made. Therefore, the present study was conducted to develop a larval feeding strategy in different feeding regimes.

The fish is carnivorous inhabits and feeds on various Annelids, Arthropods, Mollusks, small fishes, etc. (Shillewar and Nanware, 2009) [11]. During this experiment, live food was replaced by commercial feed in different cultural days. According to Sukendi *et al*., (2020) [13] it was very hard for the knife fish to accept the commercial feed. While changing the diet from live food to commercial feed, the fish were provided with a mixture of tubifex 60% and feed powder 40% in the first day and tubifex 30% + feed powder 70% on the second day to train the fish for feed powder. The weight growth of knife fish larvae during the first 10 days of culture was similar when fed live food. After day 10, the live food was replaced

by commercial feed powder and the growth of larvae was slow. This is due to Knife fish larvae not having a good response to the feed powder and only small portion was eaten, so much of the feed was leftover. During the early days, the digestive system of larvae was not completed. Hossain *et al*., (2009) [3] found high growth and survivability of fry in clown knife fish *Notopterus chitala* when fed with Tubifex worm. The length of the knife fish looks very significant when live food was fed up to day 20. However, switching live food to commercial feed in the early days resulted in slow growth and increases mortality. The highest growth can be obtained when larvae were fed with live food (Srivastava *et al*., 2012) [12] because the digestibility of live food is higher than commercial feed. "The length-weight relationship has an eminent role in fishery biology involving the various trends with the life history of fishes. In *N. notopterus* LWR indicated significantly positive correlation, so weight can be forecasted by length which can be used for assessment of biomass" (Kaur and Rawal, 2017) [5].

Larvae mortality increases with the change in the diet from live food to feed powder. Feed powder was well ingested at the early stage but larvae died with a gut full of food, suggesting that larvae were unable to digest the formulated diet. The hypothesis was that younger larvae have insufficient digestive enzymes to thrive on feed powder, and that exogenous enzymes, provided from live food, are necessary for early stages (Cahu and Zambonino Infante, 2001) [2]. The survival rate was high when larvae fed with Tubifex worms as it contains a high amount of nutrients which is essential during growth and development (Akhtar and Singh, 2012) [1]. Sarkar *et al*., (2007) [10] found a high survival rate in clown Knife Fish *Chitala chitala* up to 80% when feeding with Tubifex worm and zooplankton. In this experiment Specific growth rate was high as compared to Sukendi *et al*., (2020) [13] as fish grows faster in the early life stage.

5. Conclusion

Fish larvae have insufficient digestive enzymes to thrive on feed powder and exogenous enzymes. Thus, younger larvae need live food in early life stages for better development and growth. Feeding larvae from day 6 to 20 with Tubifex worm resulted in a high growth rate and survival rate of knife fish. Thus, Tubifex worm is a vital food source capable for sustaining and hovering the larval stages of fish. Further study needs to be conducted for the standardized mass production system of zooplankton (Moina, Tubifex worms, Rotifers etc.) to accelerate the survival rate of fish in larval stages.

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