Optimized Live Feed Regime Significantly Improves Growth Performance and Survival Rate for Early Life History Stages of Pangasius Catfish (*Pangasianodon hypophthalmus*)

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Abstract: This study aimed to determine the optimal live feed regime (i.e., initial feeding moment, density, and frequency) for maximum growth and survival of pangasius catfish (*Pangasianodon hypophthalmus*) early life history stages. The first experiment assessed the optimal initial feeding moment (30, 36, 42, and 48 h post hatching, hph). The second experiment assessed feeding density (3, 5, 8 and 11 individuals per mL, ind/mL) at the optimal initial feeding moment (30 hph) which was the best result from the first experiment. The third experiment assessed optimal feeding frequency (1, 2, 4, and 6 times per day) at the optimal initial feeding moment (30 hph) and density (8 ind/mL) which was drawn upon from the second experiment. All experiments were conducted in 20 L containers containing 20 hph *P. hypophthalmus* larvae at a density of 10 ind/L and fed rotifers (*Brachionus angularis*) for 3 days and then water fleas (*Moina macrocopa*) for 7 days. The first experiment demonstrated that larvae initially fed at 30 hph exhibited a significantly higher survival rate (24%) than larvae initially fed at 36, 42, and 48 hph (19%, 16%, and 16%), respectively. The second experiment demonstrated that larvae fed at 8 and 11 ind/mL densities exhibited significantly higher survival rates (32% and 32%) than larvae fed at 3 and 5 ind/mL densities (13% and 23%), respectively. The third experiment demonstrated that the highest survival rate (66%) was obtained when larvae were fed 6 times per day. These results provide valuable insights regarding the optimal live feed regime for better growth and survival of *P. hypophthalmus* larvae, which are commercially important and numerously cultured throughout the Mekong Delta region.

Keywords: pangasius catfish; feeding rate; feeding frequency; feeding moment; rotifer *Brachionus angularis*; water flea *Moina macrocopa*

1. Introduction

Live feeds play an important role in the aquaculture production of various fish and shellfish species [1–4]. In addition to the provided nutritional value, the small size of live feeds is critical for early life history stages of many fish, especially species with a narrow mouth opening gape at the developmental timepoint when exogenous feeding begins [5–7]. Among the available live feed options, rotifers are considered one of the best and, accordingly, one of the most widely used for small fish species [8–10]. However, in addition to the use of an optimal live feed species, optimal feeding regime also plays a vital role in survival and growth performance during early life history stages.

Higher feeding rates and frequencies can result in higher performance of fish larvae although optimal feeding regimes may vary depending on fish species, life history stage, sizes, or rearing conditions. For example, growth performance of various fish larvae improved when administered live

feed at higher frequencies or rates [11–15]. Administering live feed at the optimal time (i.e., switching on exogenous feeding) is critical, especially for fish species that exhibit cannibalism [16,17]. Hecht and Pienaar [18] revealed that cannibalism in larviculture relates to intra- and inter-cohort sibling cannibalism, which is mainly driven by genetic and behavior factors with the latter usually being driven by environmental factors (e.g., food availability, population density, light intensity, and feeding frequency). Cannibalism can be reduced during larviculture if these environmental factors are effectively controlled [18]. Furthermore, a delay in the timing of first feeding can increase mortality in fish larvae due to starvation and, thus, optimal fish larvae performance depends on availability of prey organism when the yolk sac is resorbed and fish larvae switch on exogenous food [19,20].

Pangasius catfish (*Pangasianodon hypophthalmus*) has been known as one of the most important aquaculture species of Vietnam, especially in the Mekong Delta [21,22]. High production of this species not only supports the economic growth of Vietnam but also contributes to the global fish production [23,24]. Despite high production having been maintained throughout the years, early life history survival rates for this commercially important aquaculture species remain variable and low (per. communication).

Subagja et al. [25] highlighted that although *P. hypophthalmus* cultivation has been practiced for 30 years, published information on optimal rearing methods are scarce. This remained true until the recent investigations into the causes of high mortality during *P. hypophthalmus* larvae rearing, which demonstrated that cannibalism was the major cause during the first week of development [26,27]. Cannibalism was significantly reduced during aquaculture rearing of *P. hypophthalmus* larvae by increasing darkness duration, increasing food availability, and decreasing stocking density [27]. Moreover, the use of increased feeding rate and frequency have also been demonstrated to increase the survival rates of several fish species reared under aquaculture conditions such as large yellow croaker (*Pseudosciaena crocea*) [28], spotted seatrout (*Cynoscion nebulosus*) [29], red-spotted grouper (*Epinephelus akaara*) [11] and Ayu sweetfish (*Plecoglossus altivelis*) [13].

This study aimed to determine suitable feeding environment including (i) initiation of feeding moment; (ii) feeding rate, and (iii) feeding frequency for optimal growth performance and survival of *P. hypophthalmus* larvae. These optimal feeding regimes obtained from the research could be practically applied to increase survival rate of the early life history stages of the commercially important and intensively cultured pangasius catfish.

2. Materials and Methods

2.1. Pangasius Catfish Larvae

All experiments used 20 h post hatch (hph) *P. hypophthalmus* larvae obtained from the Can Tho University fish hatchery.

2.2. Live Feeds Preparation

Freshwater rotifer (*Brachionus angularis*) with a size range of approx. 90–100 μm in length were provided from a mass culture system operated in the Laboratory for Natural Foods, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University, Vietnam. Rotifers were originally collected and isolated from the wild and mass culture was performed under controlled conditions. The initial rotifer stock was maintained in a 2 L glass bottle with continuous aeration. Rotifers were fed once a day with freshly cultured *Chlorella* sp. microalgae at a density of approx. 60,000 individuals per rotifer per day. Up-scaling of the rotifer culture was initiated in 8 L glass bottles at a density of 50 rotifers per milliliter (mL). The same *Chlorella* sp. microalgae diet was provided to this up-scaled rotifer culture. Density of rotifer culture was determined daily and the biomass was partly harvested as live feed for *P. hypophthalmus* larvae experiments when it reached approx. 400–600 rotifers per mL.

Water fleas (*Moina macrocopa*) were originally collected from a commercial culture pond where biomass was regularly harvested for sale. The water fleas were mass cultured in 250 L composite tanks at a density of 200 individuals per L. The water flea culture was provided a 1:1 mixture of rice bran and shrimp feed size 0 three times a day. Water flea culture biomass was harvested as live feed for *P. hypophthalmus* larvae experiments when density reached approx. 8000 individuals per L. The average size of cultured water fleas was 598 ± 250 μm; however, water fleas were size graded to <500 μm before feeding to *P. hypophthalmus* larvae by filtering biomass through a plankton net with 500 μm mesh size.

2.3. Experimental Design and Rearing Management

Three discrete experiments were undertaken. Each experiment was conducted in a 20 L container system located in an open air compartment within a wet lab with water volume maintained at 18 L and continuous aeration. Twenty hours post hatching (hph) *P. hypophthalmus* larvae were allocated in the system at a density of 10 individuals per L (ind/L) [27]. In all experiments larvae were fed *B. angularis* rotifers only for first 3 days and then *M. macrocopa* water fleas only for the remaining 7 days. Feeding densities of each treatment were consistent over the 10 day rearing period. Before each feeding, the number of live feed organisms was counted using a Sedgewick-Rafter counting chamber and additional live feed was supplemented as needed to maintain the standardized feeding densities.

The first experiment was designed to determine the optimal initial feeding moment (i.e., developmental time point at which larvae transition from endogenous to exogenous feeding). As the fish larvae of this species exhibit an intense cannibalistic behavior at an early age, about 36 h after hatching [30,31], early provision of live feed may reduce mortality during this period. The experiment was therefore designed with four treatments with three replicates each in which initial feeding was commenced at 30, 36, 42 and 48 hph. Larval yolk sac exhaustion was observed throughout the first 3 days of development. Each experimental container (*n* = 180) was fed daily at 7 am, 11 am, 3 pm, and 7 pm with rotifers and water fleas at a density of 5 ind/mL for the first 3 and remaining 7 days, respectively.

The second experiment was designed to determine the optimal feeding density (i.e., concentration of live feed organisms). Four treatments with three replicates each were undertaken wherein larvae were fed 3, 5, 8, and 11 ind/mL of live feed at the optimal developmental time point determined by the first experiment. Consistent with the first experiment, larvae were fed daily at 7 am, 11 am, 3 pm and 7 pm.

The third experiment was designed to determine the optimal feeding frequency (i.e., number of feeds per day). Four treatments with three replicates each were undertaken wherein larvae were fed one (at 7 am), two (at 7 and 11 am), four (at 7 am, 11 am, 15 pm, and 19 pm) and six (7 am, 11 am, 15 pm, 19 pm, 23 pm, and 3 am) times per day at the optimal developmental time point and density determined by the first and second experiments, respectively.

All three experiments are summarized in Table 1. All experiments were run across the first 10 days of development as this is the critical period in *P. hypophthalmus* larval development (i.e., transition from endogenous to exogenous feeding and before weaning onto granulated feed) ([31]; unpublished data). Water temperature and pH were monitored daily in the morning (7 am) and afternoon (2 pm), while dissolved oxygen (DO), total ammonium nitrogen (TAN) and nitrite (NO_2^-) were measured every three days.

Table 1. Summary of experimental design of three experiments.

No	Experiments	Treatments			
1	**Experiment 1:** Determination of optimal initiation of feeding moment (hour post hatching—hph)	30	36	42	48
2	**Experiment 2:** Determination of optimal feeding density (ind/L)	3	5	8	11
3	**Experiment 3:** Determination of optimal feeding frequency (times/day)	1	2	4	6

Fish (n = 15 per container or n = 45 per treatment) were sampled every three days to determine growth rate (length and weight) and mouth opening gape. Survival rate of larvae within each container was determined at the termination of each 10 day experiment.

2.4. Sample Analysis and Calculations

Temperature and pH were recorded using a thermometer and pH meter (HI98107, Romania), respectively, while DO, TAN, and NO_2^--N were analyzed following APHA [32]. Mouth opening gape was determined at 90° by measuring the length of the upper jaw using an optical microscope eyepiece and applying by the formula suggested by Shirota [33]:

$$D\,(90^o) = AB \times \sqrt{2}$$

where D is mouth opening gape (μm) and AB is upper jaw length (mm). Growth (length and weight) and survival rates were calculated as follows:

Length-Specific Growth Rate (L-SGR; %) = [(ln final length−ln initial length)]/days of rearing) × 100

Weight-Specific Growth Rate (W-SGR; %) = [ln (final weight) −ln (initial weight)]/days of rearing) × 100

Survival (%) = (final number of fish/initial number of fish) × 100

2.5. Data Analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) was used to identify differences among treatments followed by Tukey's HSD multiple comparisons test to determine differences among groups. All percentage data (survival, L-SGR, and W-SGR) were normalized by arcsine transformation prior to analysis. Alpha was set to 0.05 for all statistical comparisons.

3. Results

3.1. Determination of Optimal Feeding Time

3.1.1. Growth Performance

Monitored water parameters fell within suitable ranges. Temperature ranged from 26.3 to 27.8 °C in the morning and afternoon, while pH was consistent (7.4–7.5). DO concentrations ranged from 4.8 to 5 mg/L (expected given aeration) while both TAN and NO_2^--N concentrations ranged from 0.3 to 0.4 mg/L.

During the first three days of development, the gape of larval fish mouth opening increased from 232 ± 48 to 496 ± 27 μm (Table 2). The mouth opening gape increased steadily to the end of the experiment and no significant difference was found among treatments (p > 0.05). After 10 days of rearing, the larvae mouth opening gape was approximately four times larger than day 0 for all

four treatments (Table 2). More specifically, the mouth opening gape was 825 ± 60 μm, 812 ± 59 μm, 841 ± 45 μm, and 867 ± 48 μm at day 10 for the 30, 36, 42 and 48 hph treatments, respectively.

Table 2. Mouth opening gape (μm) of *P. hypophthalmus* larvae at four developmental time points following different initiation of feeding moment.

Days after Hatching	Initiation of Feeding Moment Treatments			
	30 hph	**36 hph**	**42 hph**	**48 hph**
0	232 ± 48 [a]	232 ± 48 [a]	232 ± 48 [a]	232 ± 48 [a]
3	496 ± 27 [a]	455 ± 37 [a]	485 ± 19 [a]	476 ± 39 [a]
6	666 ± 70 [b]	719 ± 19 [a,b]	730 ± 45 [a,b]	764 ± 46 [a]
10	825 ± 60 [a]	821 ± 59 [a]	841 ± 45 [a]	867 ± 48 [a]

Data (mean ± SD) in the same row with different letters are significantly different (*p* < 0.05). hph: hour post hatching.

After 10 days of development there was no statistically significant difference in growth rate metrics among larvae from the 30, 36, 42, and 46 hph feeding moment treatments (*p* > 0.05; Table 3). The best L-SGR and W-SGR growth rates were observed for larvae from the 36 hph treatment (10 ± 2%/day and 27 ± 2%/day), respectively. The least growth in length (14 ± 4 mm) and L-SGR (9 ± 3%/day) were observed in larvae from the 42 hph treatment while the least growth in weight (26 ± 9 mg) and W-SGR (23 ± 4 %/day) were observed in larvae from the 30 hph treatment (Table 3).

Table 3. Growth performance of *P. hypophthalmus* larvae after 10 days of development following different initial feeding times.

Parameter	Initiation of Feeding Moment Treatments			
	30 hph	**36 hph**	**42 hph**	**48 hph**
Final length (mm)	16 ± 3 [a]	16 ± 4 [a]	14 ± 4 [a]	17 ± 5 [a]
Final weight (mg)	26 ± 9 [a]	40 ± 8 [a]	29 ± 10 [a]	34 ± 7 [a]
L-SGR (%/day)	10 ± 2 [a]	10 ± 2 [a]	9 ± 3 [a]	10 ± 4 [a]
W-SGR (%/day)	23 ± 4 [a]	27 ± 2 [a]	24 ± 4 [a]	25 ± 2 [a]

Data (mean ± SD) in the same row with different letters are significantly different (*p* < 0.05). hph: hours post hatching.

3.1.2. Survival Rate

Larvae from the 30 hph treatment exhibited significantly higher survival (24 ± 3%) than larvae from the 36, 42, and 48 hph treatments (19 ± 2%, 16 ± 1%, and 16 ± 1%, respectively) (*p* > 0.05) while larvae from the 36 hph treatment exhibited significantly higher survival than larvae from the 42 and 48 hph treatments (*p* < 0.05; Figure 1).
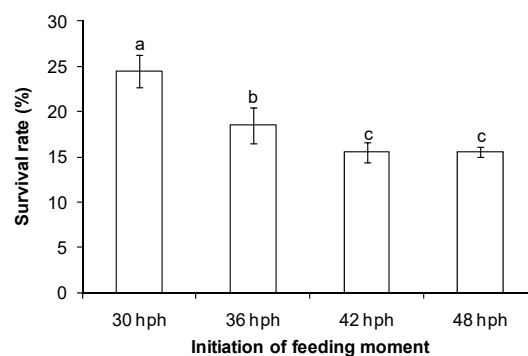
Figure 1. Survival rates of *P. hypophthalmus* larvae following 10 days of rearing under four initiation of feeding moments: 30 h post hatching (hph), 36 hph, 42 hph, and 48 hph. Each bar represents the mean value and standard deviation data for each feeding time treatment. Data with different letters are significantly different (*p* < 0.05).

3.2. Determination of Optimal Feeding Density

3.2.1. Growth Performance

Consistent with the first experiment, monitored water parameters fell within suitable ranges. Temperature and pH were stable (26.3–27.7 °C and 7.4–7.5, respectively), while DO was maintained in a range of 4.8–5.0 mg/L. Both TAN and $NO_2{}^-$-N concentrations were relatively low, ranging from 0.33 to 0.37 mg/L.

There was no significant difference in mouth opening gapes among feeding density treatments ($p > 0.05$; Table 4) when initial feeding commenced at 30 hph. Mouth opening gape did, however, steadily increase from 231 ± 48 μm in larvae from the 3, 5, 8 and 11 ind./L treatments to 864 ± 54 μm, 880 ± 74 μm, 803 ± 60 μm, and 861 ± 45 μm after 10 days of development, respectively (Table 4).

Table 4. Mouth opening gape (μm) of *P. hypophthalmus* larvae at four developmental time points following subjection to different feeding density treatments.

Days after Hatching	Feeding Density Treatments			
	3 ind/mL	**5 ind/mL**	**8 ind/mL**	**11 ind/mL**
0	231 ± 48 [a]	231 ± 48 [a]	231 ± 48 [a]	231 ± 48 [a]
3	505 ± 14 [a]	465 ± 45 [a]	466 ± 35 [a]	485 ± 19 [a]
6	648 ± 52 [b]	717 ± 15 [a,b]	741 ± 37 [a]	790 ± 54 [a]
10	864 ± 54 [a]	880 ± 74 [a]	803 ± 60 [a]	861 ± 45 [a]

Data (mean ± SD) in the same row with different letters are significantly different ($p < 0.05$). ind/mL: individuals per milliliter.

After 10 days of development, there were significant increases in length and L-SGR observed for larvae fed higher densities of live feed starting at 30 hph while no significant differences were observed for weight or W-SGR (Table 5). More specifically, length and L-SGR were significantly higher for larvae from the 8 and 11 ind/mL treatments (12.6 ± 0.4 and 12.5 ± 0.1 %/day, respectively) than for larvae from the 3 ind/mL treatment (10.3 ± 1.5 %/day) but not the 5 ind/mL treatment (Table 5). The lowest growth rate metrics were observed in larvae from the lowest feeding density treatment (3 ind/mL).

Table 5. Growth performance of *P. hypophthalmus* larvae after 10 days of development under different feeding density treatments.

Parameter	Feeding Density Treatments			
	3 ind/mL	**5 ind/mL**	**8 ind/mL**	**11 ind/mL**
Final length (mm)	16.1 ± 2.8 [b]	19.7 ± 1.4 [a,b]	20 ± 0.7 [a]	20.0 ± 0.2 [a]
Final weight (mg)	29.1 ± 3.9 [a]	33.6 ± 3.8 [a]	29.8 ± 5.1 [a]	31.3 ± 1.1 [a]
L-SGR (%/day)	10.3 ± 1.5 [b]	12.4 ± 0.7 [a,b]	12.5 ± 0.4 [a]	12.5 ± 0.1 [a]
W-SGR (%/day)	25.8 ± 1.4 [a]	27.2 ± 1.1 [a]	25.9 ± 1.7 [a]	26.5 ± 0.4 [a]

Data (mean ± SD) in the same row with different letters are significantly different ($p < 0.05$). ind/mL: individuals per milliliter.

3.2.2. Survival Rate

Larvae from the 8 and 11 ind/mL treatments exhibited the highest survival rates (32 ± 1% and 32 ± 2%, respectively), which were significantly higher than the survival rates observed for larvae from the 3 and 5 ind/mL treatments ($p < 0.05$) but not significantly different from one another ($p > 0.05$; Figure 2). Larvae from the 3 ind/mL treatment exhibited the lowest survival rate, which was significantly lower than all three other feeding density treatments ($p < 0.05$). Lastly, larvae from the 5 ind/mL treatment exhibited a significantly higher survival rate ($p < 0.05$) than larvae from the 3 ind/mL treatment but a significantly lower survival rate ($p > 0.05$) than larvae from both 8 and 11 ind/mL treatments (Figure 2).
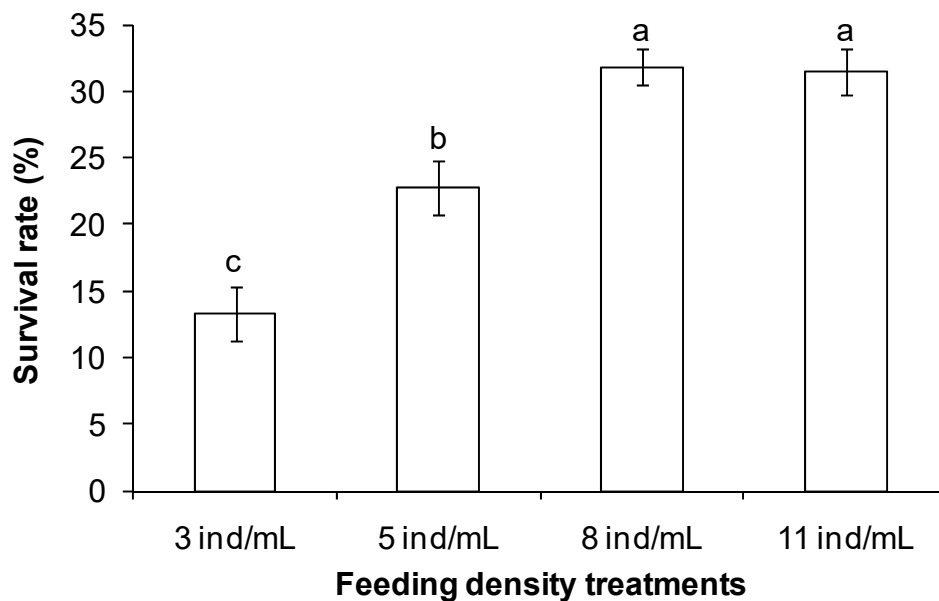
Figure 2. Survival rates of *P. hypophthalmus* larvae following 10 days of development under four feeding density treatments: 3 ind/mL, 5 ind/mL, 8 ind/mL, and 11 ind/mL. Each bar represents the mean and standard deviation data for each feed density treatment. Data with different letters are significantly different ($p < 0.05$).

3.3. Determination of Optimal Feeding Frequency

3.3.1. Growth Performance

Consistent with the first two experiments, monitored water parameters fell within suitable ranges. Temperatures varied from 26.2 to 27.0 °C while pH and DO varied from 7.6 to 8.3 and 4.8 to 4.9 mg/L, respectively. Both TAN and NO_2^--N concentrations were maintained at <0.4 mg/L.

There was no significant difference in mouth opening gapes among feeding frequency treatments ($p > 0.05$; Table 6) when initial feeding commenced at 30 hph and utilized a density of 8 ind/mL. Mouth opening gape did, however, steadily increase from 236 ± 41 μm in larvae from the one, two, four and six times/day treatments to 870 ± 57 μm, 877 ± 65 μm, 879 ± 43 μm, and 889 ± 51 μm after 10 days of development, respectively (Table 6).

Table 6. Mouth opening gape (μm) of *P. hypophthalmus* larvae at four developmental time points following subjection to different feeding frequency treatments.

Days after Hatching	Feeding Frequency Treatments			
	1 Time/Day	**2 Times/Day**	**4 Times/Day**	**6 Times/Day**
0	236 ± 41 [a]	236 ± 41 [a]	236 ± 41 [a]	236 ± 41 [a]
3	484 ± 45 [a]	452 ± 49 [a]	450 ± 51 [a]	444 ± 31 [a]
6	695 ± 46 [a,b]	784 ± 76 [a]	632 ± 60 [b]	699 ± 94 [a,b]
10	870 ± 57 [a]	877 ± 65 [a]	879 ± 43 [a]	889 ± 51 [a]

Data (mean ± SD) in the same row with different letters are significantly different ($p < 0.05$).

After 10 days of development there were significant increases in length, weight, L-SGR, and W-SGR observed for larvae fed more frequently compared to larvae fed less frequently when initial feeding commenced at 30 hph and utilized a density of 8 ind/mL (Table 7). More specifically, all growth rate metrics observed for larvae from the one time/day treatment were significantly lower ($p < 0.05$) than larvae from the four or six times/day treatments while no significant difference in growth rate metrics was observed between larvae from the two and four times/day treatments. Similarly,

no significant difference in growth rate metrics was observed between larvae from the four and six times/day treatments except for weight, which was significantly higher in the six times/day treatment (52.87 ± 7.22 mg) than the four times/day treatment (39.33 ± 6.99 mg).

Table 7. Growth performance of *P. hypophthalmus* larvae after 10 days of development under different feeding frequency treatments.

Parameter	Feeding Frequency Treatments			
	1 Time/Day	2 Times/Day	4 Times/Day	6 Times/Day
Final length (mm)	17 ± 2.2 [b]	19 ± 0.8 [a,b]	21 ± 0.2 [a]	22 ± 1.3 [a]
Final weight (mg)	24 ± 0.4 [c]	30 ± 2.3 [b,c]	39 ± 6.9 [b]	53 ± 7.2 [a]
L-SGR (%/day)	11 ± 1.4 [b]	13 ± 0.42 [a,b]	14 ± 0.10 [a]	14 ± 0.6 [a]
W-SGR (%/day)	20 ± 0.2 [c]	22 ± 0.8 [b,c]	25 ± 1.7 [a,b]	28 ± 1.3 [a]

Data (mean ± SD) in the same row with different letters are significantly different ($p < 0.05$).

3.3.2. Survival Rate

Larvae from the six times/day treatment exhibited the highest survival rate (66 ± 5%), which was significantly higher ($p < 0.05$) than the survival rates observed for larvae from the one, two, and four times/day treatments (9 ± 2%, 11 ± 1%, and 28 ± 1%), respectively (Figure 3). Larvae from the four and six times/day treatments exhibited significantly higher survival rates than larvae from the one and two times/day treatments ($p < 0.05$) while the one and two times/day treatments did not differ significantly from each other ($p > 0.05$).
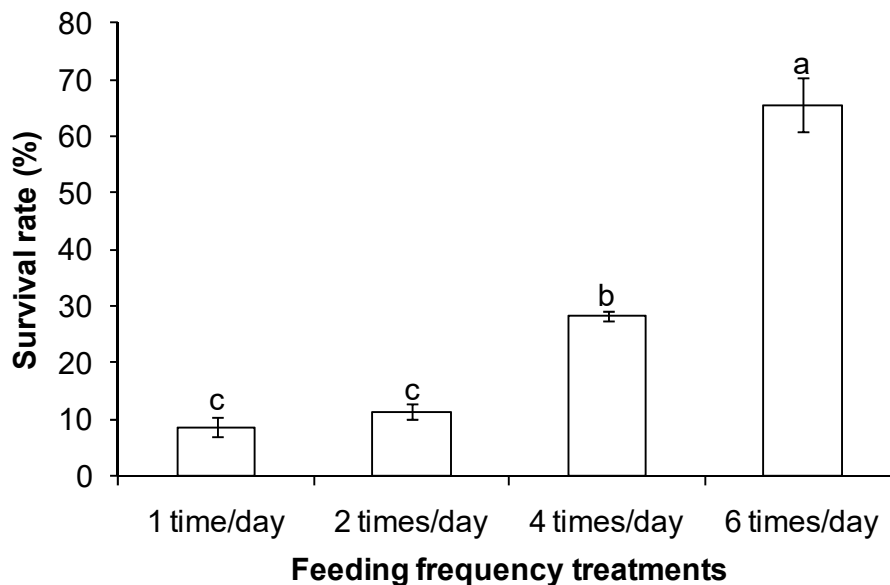
Figure 3. Survival rates of *P. hypophthalmus* larvae following 10 days of development under four feeding frequency treatments: 1 time/day, 2 times/day, 4 times/day, and 6 times/day. Each bar represents the mean and standard deviation data for each feeding frequency treatment. Data with different letters are significantly different ($p < 0.05$).

4. Discussions

In this study *P. hypophthalmus* larvae fed earlier in development (30 and 36 hph) exhibited higher rates of survival compared to larvae fed later in development (42 and 46 hph). Providing *B. angularis* rotifers as live feed earlier in development (i.e., closer to the onset of exogenous feeding) helped minimize starvation as well as cannibalism among *P. hypophthalmus* larvae. Cannibalism in fish is a predatory feeding strategy that involves killing and eating conspecifics (reviewed by [18,34]).

Cannibalism can be caused by various population and environmental stress factors [17] and tends to cause a loss of individuals and, thus, a reduction in fish production. Although pangasius catfish (*P. hypophthalmus*) was not indicated in the list of cannibalistic culture species by Hecht and Pienaar [18] and Naumowicz and Pajdak [17], *P. hypophthalmus* has been demonstrated to exhibit cannibalistic behavior during the first week after hatching [25–27,30]. This temporary but intense cannibalism has been demonstrated to be the cause of high mortality during the early life history stages of *P. hypophthalmus* development. Food or prey availability and feeding frequency have been found to increase possibility of prey encounter of *P. hypophthalmus* larvae and thus reduce cannibalism [18]. Complete yolk sac absorption was reported at day 3 after hatching for *P. hypophthalmus* larvae; however, cannibalism was observed to begin at or before 36 hph [30,31]. As such, when feed is provided earlier in development (e.g., 30 hph) cannibalism and associated mortality during aquaculture rearing can both be minimized. This is supported by Lima et al. [20] who demonstrated that a delay in initial feeding time (i.e., into exogenous feeding stage) increased mortality and retarded growth performance of silver catfish (*Rhamdia voulezi*) larvae and recommended that initial feeding should commence before complete yolk sac absorption. Baolong et al. [35] also confirmed the importance of initial feeding moment on red sea bream (*Pagrosomus major*) and olive flounder (*Paralichthys olivaceus*) larvae in that both growth and survival of these fish larvae decreased as initial feeding time increased beyond complete yolk sac absorption (i.e., into exogenous feeding stage). Shan et al. [36] also demonstrated that growth and survival of rock bream (*Oplegnathus fasciatus*) larvae were significantly reduced when the time of initial exogenous feeding was commenced 7 or 15 days after hatching compared zero day after hatching.

At higher feeding densities fish larvae are more likely to encounter and ingest prey, which, in turn, improves growth performance due to providing sufficient nutrition [37]. In this study, *P. hypophthalmus* larvae provided higher live feed densities (5, 8, and 11 ind/mL) starting at 30 hph and exhibited better growth compared to larvae provided lower live feed density (3 ind/mL). Importantly, survival of *P. hypophthalmus* larvae was significantly improved when provided with higher densities of live feed (Figure 2). Slembrouck et al. [38] also studied the effects of feeding densities on growth and survival of *P. hypophthalmus* larvae and similarly demonstrated that survival increased when live feed density was increased. Wang and Eckmann [39] also demonstrated that higher rotifer feeding densities resulted in improved growth and survival rates of European perch (*Perca fluviatilis*) larvae. One explanation could be that if prey are scarce during each feeding then larvae will likely forage for a long period of time and, thus, become more exposed to the risk of being cannibalized by siblings. As such, it appears that cannibalism due to starvation can be reduced by providing larvae with higher densities of live feed during rearing under aquaculture conditions [17,18,34].

Higher growth (L-SGR and W-SGR) and survival rates were observed in *P. hypophthalmus* larvae treatments that were fed four or six times/day compared to larvae treatments that were fed one or two times/day (Figure 3) starting at 30 hph with a density of 8 ind/mL for both rotifers (days 1–3) and water fleas (days 4–10). These findings are consistent with previous studies on different freshwater and marine fish species. For example, gold fish (*Carassius auratus*) fry exhibited a significant increase in growth and survival rates when fed more frequently (three times per day) than less frequently (one or two times per day) [40]. Higher growth performance was also observed for angel fish (*Pterophyllum scalare*) larvae when fed more frequently (2–4 times per day) than less frequently (once per day and every other day) although, for this species, survival rate did not differ between feed frequency treatments [41]. Increased weight gain and survival rate were also observed for young red-spotted grouper (*Epinephelus akaara*) fed at a higher frequency [11]. The transition from endogenous to exogenous feeding in fish larvae is considered the most critical period during development as this is when high mortality is most likely to occur [42] because the survival and growth of fish larvae depend on food availability [43]. This limitation in availability and accessibility of live feed to fish larvae can be overcome by increasing feeding frequency because an increase in feeding frequency would likely increase the likelihood of live feed ingestion by larvae and, thus, likely increase growth and survival. Kasiri et al. [41] confirmed

that increasing the feeding frequency of live feed to fish larvae resulted in improved food accessibility, growth, and survival. The present study has demonstrated the fact that provision of live food at the right initiation of feeding moment with high availability would reduce significant cannibalism and improve survival of the early history stage of pangasius catfish larvae during the first 10 days of rearing process.

5. Conclusions

Feeding pangasius catfish larvae at right initial feeding moment, density, and frequency has resulted in higher growth performance and survival of *P. hypophthalmus* larvae during the first 10 days of development. Initial exogenous feeding should commence between 30 and 36 hph with *B. angularis* rotifers for the first 3 days and then *M. macrocopa* water fleas for the subsequent 7 days. Both live feed organisms are best fed at a density of 8–11 ind/mL and frequency of six times/day. Routine application of this optimal feeding regime for *P. hypophthalmus* larvae reared under aquaculture conditions can improve survival rates to be quintupled (66%).

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