EFFECTS OF TYPE OF PREY, FEEDING LEVEL, PREY ACCESSIBILITY AND WATER AERATION ON GROWTH AND SURVIVAL OF *PANGASIUS HYPOPHTHALMUS* LARVAE (SILUROIDEI, PANGASIIDAE)

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Abstract

Two feeding experiments were carried out on *Pangasius hypophthalmus* larvae reared in a recirculating water system during 8 days. The first experiment was done at the Can Tho University (Vietnam) and the second at the RIFF Sukamandi station (Indonesia).

The main of the first experiment was to compare survival and growth performance of *P. hypophthalmus* larvae fed with either *Moina* sp. or *Artemia* nauplii and with either strong or moderate aeration. Strong aeration was tested in order to homogenise prey repartition in the water column, since *Artemia* and *Moina* sp. tended normally to be concentrated in corners or at the bottom of the tanks. Two feeding rates (150 and 4000% of fish biomass) were tested.

The second experiment aimed at an evaluation of optimal feeding level with *Artemia* nauplii, by considering growth, number of ingested preys and survival rates of larvae. Each of three feeding levels (R1, R2 and R3) were tested at three different fish stocking densities (10, 30 and 90 larvae.L^{-1}). The feeding level R1 was adjusted daily according to a pre-established model of the number of *Artemia* ingested as a function of age in *P. hypophthalmus* larvae. The rations R2 and R3 were respectively 3 and 9 times greater than R1. The prey accessibility (number of prey per litre), which varied as a function of the different combinations of feeding level x fish stocking density, was also considered as a parameter that could influence larval growth and survival.

The results of the first experiment indicated that both *Artemia* and *Moina* sp. could be used as a first feed for *P. hypophthalmus* larvae. However larvae fed *Artemia* nauplii presented a faster growth rate than those fed *Moina*. Perturbation of the rearing media by strong water aeration resulted systematically in reduced larval survival rates, whatever the type of prey and feeding rate used. The results of the second experiment showed that: 1) at a same feeding level, the stocking density did not affect the growth or survival of larvae; 2) at a same stocking density, larval growth and survival increased significantly with an increase of the feeding level, except for the highest feeding level at the highest stocking density (90 larvae.L^{-1}) which resulted in excessive feed quantity in the tanks; 3) at a same feed accessibility, growth and survival were decreased with a decrease in feeding level associated to an increase in stocking density. Therefore, in the range tested, the feeding level had a predominant effect on larval growth and survival in comparison to stocking density or prey accessibility. In the most favourable conditions, the larvae showed a very high growth rate, reaching up to 50 mg mean body weight at 8-days of age. The optimal feeding levels of *P. hypophthalmus* are discussed in regards to the observed number of *Artemia* nauplii ingested as a function of age of larvae in the different situations tested.

INTRODUCTION

Originating from the Mekong River, *Pangasius hypophthalmus* is the most widely cultured fish species in ponds in the Mekong Delta. Its production reaches several ten thousands tons per year in Vietnam. The species was introduced from Thailand to Indonesia in 1972 (Hardjamulia *et al.*,

1981) where it has been well adapted to local conditions and is appreciated by consumers.

Induced spawning of *P. hypophthalmus* was initially reported in Thailand in 1976 (Charoen Panil, 1977), in Indonesia in 1981 (Hardjamulia *et al.*, 1981) and in Vietnam in 1981 (My Anh *et al.*, 1981). Although *P. hypophthalmus* has been cultured for about 30 years, larval rearing of this species remains problematic (Subagja *et al.*, 1999). It is generally recognised that the first 8 days of life represents the most critical period, during which a marked cannibalistic behaviour was considered as the main cause of mortality. However, Subagja *et al.* (1999) showed that bacterial disease and female parents had more influence on survival rates of *P. hypophthalmus* larvae than direct effect of cannibalism.

Investigations carried out in Indonesia (Yuniardi, 1987) showed that *P. hypophthalmus* larvae fed on *Artemia* nauplii gave higher survival and growth rates than those fed on *Daphnia carinata*. However, *Artemia* is still the most expensive live food in South East Asia and some others natural feed should be found as substitutes.

Feeding level and prey accessibility play also an important role in growth and survival during the larval rearing.

In the present study, two different experiments were conducted:

- The first one with *Artemia* and *Moina* sp. as first feed distributed at two different rations. As these preys tend to be concentrated in corners or at the bottom of the tanks after being distributed to the fish, strong water aeration was tested as a possible mean of increasing prey accessibility by homogenising their distribution in the water column. It has been observed that young *P. hypophthalmus* larvae have a predominantly pelagic behaviour.
- The second one to determine the optimal feeding rates with *Artemia* nauplii, and the effects of prey accessibility for *P. hypophthalmus* larvae by considering growth, ingested number of prey and survival rates at different fish stocking densities.

MATERIAL AND METHODS

Experiment 1

The first experiment was carried out at the Can Tho university (Vietnam) and was designed to compare the effects of a low (150% of fish biomass) and high (4000% of fish biomass) feeding ration on growth and survival of *P. hypophthalmus* larvae fed either with *Artemia* nauplii or *Moina sp.* It aimed also to determine the effects of a strong water aeration on the prey accessibility by homogenising live food in water column. Therefore, the larvae were placed in the following rearing conditions:

- larvae fed with *Artemia* at 150% of fish biomass with strong aeration
- larvae fed with *Artemia* at 150% of fish biomass with slight aeration
- larvae fed with *Artemia* at 4000% of fish biomass with strong aeration
- larvae fed with *Artemia* at 4000% of fish biomass with slight aeration
- larvae fed with *Moina* at 150% of fish biomass with strong aeration
- larvae fed with *Moina* at 150% of fish biomass with slight aeration
- larvae fed with *Moina* at 4000% of fish biomass with strong aeration
- larvae fed with *Moina* at 4000% of fish biomass with slight aeration.

The larvae were obtained from broodfish held in earthen ponds at the Can Tho University (Vietnam). Oocyte maturation and ovulation were induced after hormonal treatment with hCG (human chorionic gonadotropin; Campet, 1997). Twenty four hours after hatching, larvae were individually counted and transferred to the larval rearing structures.

All treatments were tested with three replications. The larvae were reared in 50 litre aquarium at a stocking density of 10 larvae.L^{-1} with a water flow of 0.4-0.5 L.min^{-1}. The experiment was monitored for 8 days and feeding started at 48 hours post-hatching when yolk sac was not completely absorbed. The feeding frequency was 6 meals per day at 08:00, 12:00, 16:00, 20:00, 24:00 and 4:00. Each three day, in each treatment the larvae were weighed in batch in order to readjust the quantity of *Artemia* and *Moina* distributed according to feeding rates.

During the experiment, the water temperature was measured daily with a minimal-maximal thermometer and varied between 28 and 30°C. Water quality was monitored twice a week during the period of larval rearing, pH varied between 7.0 to 7.5 and dissolved oxygen was in all cases higher than 5.0 mg.L^{-1}. Ammonia and nitrite concentrations were determined at the same time using Aquaquant© kits (Merck 14423, 14424) and ranged between 0.007 and 0.02 mg.L^{-1}, and between 0.01 and 0.04 mg.L^{-1}, respectively. Each aquarium was cleaned every day by siphoning off faeces and uneaten food.

On the last day of rearing period (day 8), thirty larvae from each tank were sampled and weighed in batch at an accuracy of 0.1 mg, the fishes being previously placed on paper towels in order to absorb adhering water. Survival rates were determined by counting all the remaining larvae in each aquarium.

Experiment 2

The second feeding experiment was carried out at the Sukamandi station of the Research Institute for Freshwater Fisheries, (West Java, Indonesia). It was designed to evaluate the optimal level of feeding with *Artemia* nauplii for larval rearing of *P. hypophthalmus.* Each of three feeding rations (R1, R2 and R3) were tested at three stocking densities (10, 30 and 90 larvae.L^{-1}).

Feeding levels and fish densities were chosen to ensure that several combinations of these two factors resulted in a same prey accessibility (the level of prey accessibility, which is the product of the feeding level by the fish stocking density, represents the density of *Artemia* available per litre of water, see Table 2). This was done in order to be able to separate, in the effects of feeding level on larval growth and survival, those related directly to the amount of feed distributed (number of *Artemia* per larvae) from those that may result from changes in feed accessibility (number of *Artemia* per litre).

A supplementary treatment (R'3) consisted in larvae stocked at the lowest density (10 larvae.L^{-1}) and fed with the highest quantity of *Artemia* nauplii used in this experiment (ration R3 given to larvae stocked at 90 fish.L^{-1}). These two treatments resulted in the same high prey accessibility (A1 x 81, see Table 2).

The feeding level was basically defined from observations of the number of *Artemia* nauplii ingested as a function of age in *P. hypophthalmus* larvae fed in excess. These observations, were done at the AGIFISFH hatchery, Vietnam (Slembrouck, 1997). The relationship between age and observed-number of *Artemia* nauplii ingested was modelised (Fig. 1) and served as a reference to defined the lowest feeding level used in this experiment. Medium ration (R2) was three times greater than this low ration (R1) and high ration (R3) was three times greater than the medium ration (R2). Feeding level was adjusted daily as a function of this model (Fig. 1) and feeding rate (% of fish biomass) was calculated from an estimated *Artemia* mean body weight of 15 µg (Sorgeloos *et al.*, 1986).

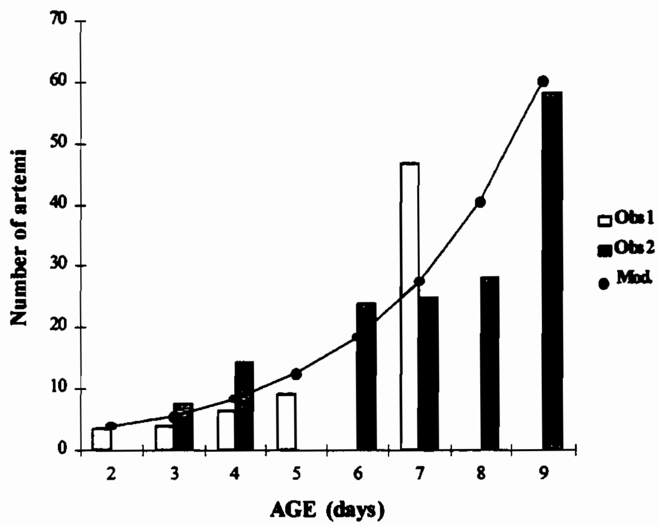


Figure 1: Number of *Artemia* ingested by *Pangasius hypophthalmus* as a function of age of larvae observed in AGIFISH hatchery in 1996 (Slembrouck, 1997). The modelised relationship between number of *Artemia* ingested and age of larvae is of the form: Y=e^(a+bX), with a=0,563541 and b=0,392637(r2 = 0,873).

The larvae used in this experiment were obtained from 3-5 years old *P. hypophthalmus* brooders held in ponds at the Sukamandi station. Induced breeding, artificial fertilisation and incubation of eggs were made following the procedure described by Legendre *et al.* (1999). Twelve hours after hatching, the larvae were individually counted and transferred to the experimental facilities.

All treatments were tested in duplicate and larvae were placed in 30 L tanks of a recirculating water system with mechanical and biological filters. Water flow through the tanks were of 0.25 L.min^{-1} up to 3-day then 0.5 L.mn^{-1}. Oxytetracycline at dose of 10 mg.L^{-1} was applied as a permanent bath to the larvae from the first day up to 8-days of age (Subagja *et al.,* 1999).

Larvae were fed with *Artemia* nauplii starting from 40 hours after hatching. Up to 8-days of age, the feeding frequency was of 8 meals per day at 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00 and 06:00. At each feeding time, the water flow was stopped during 30 minutes in order to maintain the living preys in the tanks.

During the experiment, the water temperature was measured continuously and varied between 28.4 and 30.9°C. Water quality was monitored daily, dissolved oxygen and pH varied in the range of 4.4-7.8 $mg.L^{-1}$ and 8.4–8.5, respectively. Ammonia and nitrite concentrations were determined at the same time using Aquaquant© kits (Merck 14423, 14424) and ranged between 0.00 and 0.22 $mg.L^{-1}$, and between 0.005 and 0.012 $mg.L^{-1}$, respectively. Each aquarium was cleaned daily by siphoning off dead larvae and uneaten *Artemia* nauplii.

Every two days from the 2nd day until the 8th day of age, ten larvae from each tank were randomly sampled 30 minutes after the 6:00 PM meal and weighed in batch at an accuracy of 0.1mg according to the procedure of the first experiment. Then, the larvae were fixed in 5% formalin, individually dissected, and the total number of *Artemia* nauplii present in the first part of their digestive tract was counted. On the last day, survival rates were determined by counting all the remaining larvae in each tank.

Statistical analysis

Final mean body weights and survival rates of larvae were subjected to three way ANOVA (kind of prey x ration x water aeration) in the first experiment and two way ANOVA (ration x density) in the second one. Data from the supplementary treatment (10 $larvae.L^{-1}$; R'3) in the second experiment were compared to those from the treatment with R3 at stocking density of 90 $larvae.L^{-1}$ by one way ANOVA. When necessary, angular transformation of data expressed as percentage was carried out in order to stabilised the residual variance.

RESULTS

Experiment 1

Mean body weight and survival rates of larvae obtained after 8 days of age as a function of feed type (*Artemia* or *Moina)*, feeding level and conditions of water aeration are given in Table 1.

Survival rate

Results of analysis of variance indicated that aeration ($p<0.0001$) and ration ($p<0.001$) had a great influence on survival rates of *P. hypophthalmus* larvae. However, no effect of the type of prey (*Artemia* or *Moina*) were found ($p>0.05$).

When larvae were fed with the low ration (150%), the survival rates obtained with a strong water aeration (21% and 18%) were lower than those obtained with a slight aeration (40 and 27%). The survival rates were higher when larvae were fed with *Artemia* nauplii rather than *Moina* at low ration with strong or slight water aeration and at high ration with strong aeration. However, the highest survival rate (62%) was obtained when larvae were fed with *Moina* at a high ration (4000%) with slight aeration.

Growth

Analysis of variance showed no significant effect of the aeration conditions on the larval growth ($p>0.05$). Mean body weights were significantly higher ($p<0.0001$) for larvae fed *Artemia* nauplii than for those fed *Moina*. The growth rate of larvae was significantly increased with higher ration ($p<0.001$), except for larvae fed *Moina* in the treatment with strong aeration.

Experiment 2

Survival rate

The survival rates ranged between 20% and 60.5% (Table 2). The highest values were observed in treatments where larvae were fed with a high ration (R3) at density of 10 $larvae.L^{-1}$ (60.5%) or 30 $larvae.L^{-1}$ (52.0%). The analysis of variance indicated that the survival rates of *P. hypophthalmus* larvae increased significantly according to the ration ($p<0.001$). However, the treatment at ration R3 and high density (90 $larvae.L^{-1}$) showed a lower survival rate (33.0%), as was also observed in the supplementary treatment (10 $larvae.L^{-1}$; R'3). This indicated a negative effect of too high concentration of *Artemia* nauplii on survival rates.

For a same feeding rate (except for R3 with high stocking density, see upper), there was no effect of the larval stocking density on the survival rates, despite prey accessibility increased with increasing fish stocking densities. For a same larval stocking density, survival rates increased according to the ration, therefore according to the prey accessibility. However, considering a same prey accessibility we observed that the survival rates had a tendency to decrease with higher stocking density, i.e. with a lower feeding rate.

The result obtained (37.5%) with the supplementary treatment (10 $larvae.L^{-1}$; R'3) was close to the one at density of 90 $larvae.L^{-1}$ and

Feeding	Water aeration	Feeding rate (% biomass)	Final body weight (mg)	Survival rate (%)
Artemia	**Strong**	150	13.9	20.7
Artemia	Slight	150	11.2	40.4
Artemia	**Strong**	4000	19.9	28.1
Artemia	Slight	4000	20.6	48.9
Moina	**Strong**	150	5.0	18.4
Moina	Slight	150	7.3	27.3
Moina	**Strong**	4000	6.1	22.3
Moina	Slight	4000	10.9	62.3

Table 1: Body weight and survival rate of *P. hypophthalmus* larvae reared at a stocking density of 10 larvae.L^{-1} at the age of 8 days as a function of prey (*Artemia, Moina)*, feeding level and water aeration (mean for three replications per treatment).

Fish stocking density		Corresponding feeding rate on the first day of feeding	Feeding level at the first feeding	Accessibility at the first feeding	Level of prey accessibility	Final body weight	SGR	Survival rate
(larvae.L^{-1})		(% biomass .day^{-1}) (*)	(Nb *Artemia* .$larvae^{-1}$.$feeding^{-1}$)	(Nb *Artemia* .$litre^{-1}$.$feeding^{-1}$)		(mg)	(%.d^{-1})	(%)
10	R1	40	3	30	A1	20.3	44.5	23.5
10	R2	120	9	90	A1 x 3	29.1	50.1	38.0
10	R3	360	27	270	A1 x 9	43.9	56.1	60.5
30	R1	40	3	90	A1 x 3	23.8	47.1	20.0
30	R2	120	9	270	A1 x 9	33.3	52.0	31.5
30	R3	360	27	810	A1 x 27	49.8	57.9	52.0
90	R1	40	3	270	A1 x 9	22.5	46.3	21.5
90	R2	120	9	810	A1 x 27	33.0	51.9	39.5
90	R3	360	27	2430	A1 x 81	46.9	57.1	33.0
10	R'3	3240	81	2430	A1 x 81	51.3	58.9	37.5

(*) Calculated considering an individual *Artemia* weight of 15µg (Sorgeloos *et al.*, 1986).

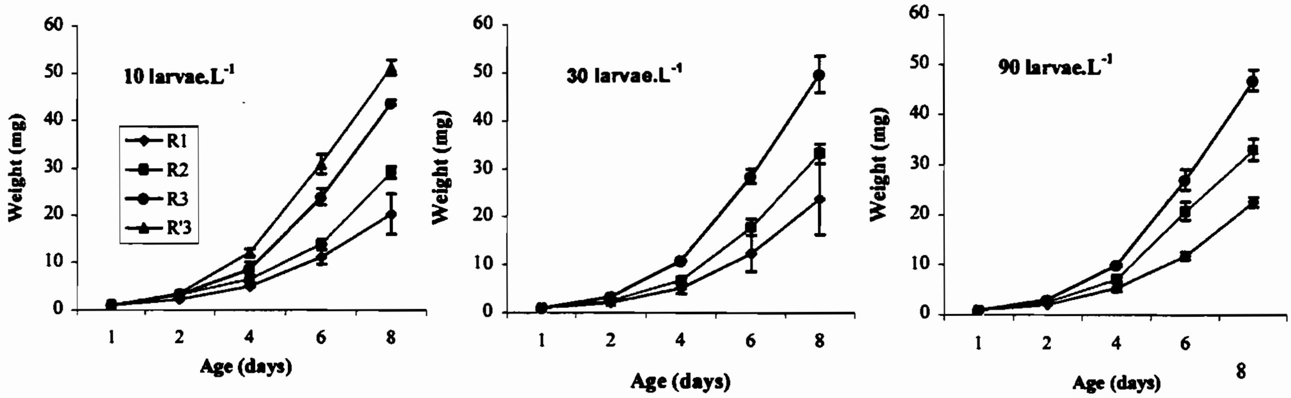
Table 2: Ration, accessibility, mean body weight, specific growth rate (SGR) and survival rate of *P. hypophthalmus* larvae fed with *Artemia nauplii* after 8 days of rearing at different stocking densities (mean for two replications).

ration R3 (33.0%). Then, larvae fed with the highest *Artemia* accessibility (A1 x 81) presented equivalent and rather low survival rates whatever the stocking density (10 or 90 larvae.L^{-1}).

Growth

Growth performances were positively correlated with feeding ration ($p<0.0001$; Table 2 and Fig. 2). By contrast, there was no effect of the stocking density on the growth performances. Considering the same accessibility of prey (A1 x 9 and A1 x 27), the final body weight tended to decrease according to stocking density and to increase according the ration (Table 2).

Growth data were examined per period of two days (Fig. 3), in terms of specific growth rate [SGR=100(LnW2-LnW1)/t]. During a first period from 60 hours to 5 days after hatching, the Figure 3 indicated that the highest SGR were obtained with the highest rations (R3 and R'3). Afterwards, SGR for the high rations generally decreased faster than for the low rations until the end of the experiment. From the 4th day of larval rearing the difference of SGR between high and low rations were reduced and SGR values tended to be very close. It appeared (Fig. 3) that the growth differential observed between larvae fed with ration R1, R2, R3 and R'3 (Fig.2) concerned only the first four days of larval rearing.



Vertical bars indicate range between replicates.

Figure 2: Growth of *Pangasius hypophthalmus* larvae at the age of 8 days from the first feeding as a function of feeding level with *Artemia* nauplii and fish stocking densities.

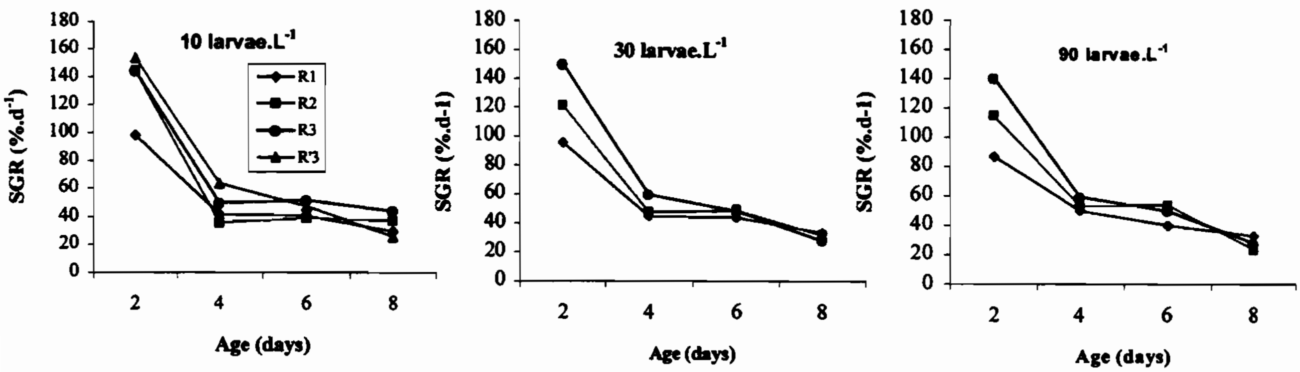


Figure 3: Specific growth rate (SGR) of *P. hypophthalmus* as a function of age of larvae at each two days sampling periods for the different feeding level and densities.

Number of ingested preys

The evolution of the number *Artemia* nauplii ingested by *P. hypophthalmus* larvae as a function of their age, at each two days of sampling, is presented in Figure 4. The number of ingested nauplii increased with the ration given. However, the number of ingested preys decreased at the 8th day of rearing whatever the treatment.

For the stocking density of 10 larvae.L^{-1}, the numbers of ingested preys at the 2nd, 4th and 6th day of larval rearing at the high rations were 13.7, 27.0 and 64.4 *Artemia* nauplii (R3) and 14.5, 29.1 and 70.3 *Artemia* nauplii, (R'3). This showed that increasing the feeding level by a 9 factor (from R3 to R'3) did not led to a strong increase in the number of ingested preys. Therefore, the ration R3 could be considered as a maximal level of rationing.

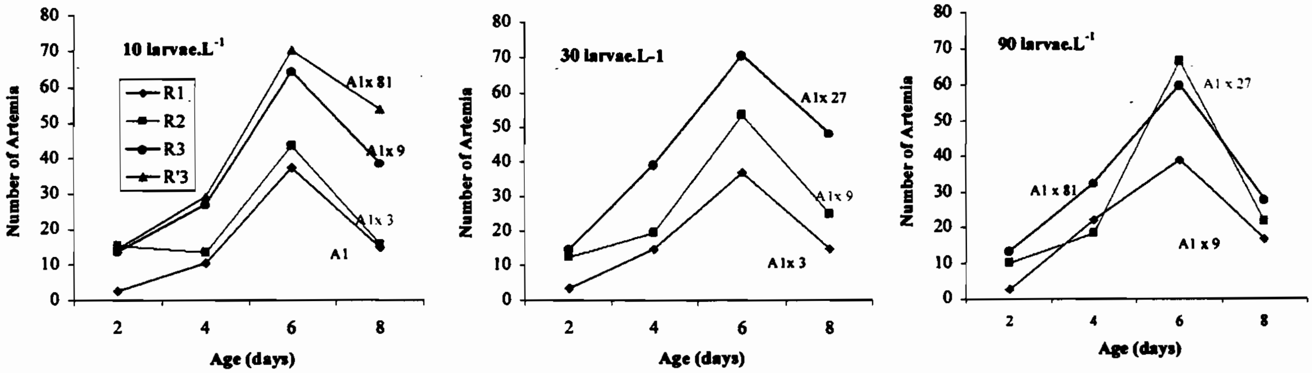
Independently from the stocking density, the mean number of *Artemia* nauplii ingested by larvae fed with the ration of reference (R1) at the 2nd and 4th days were respectively 2.8 and 16 and corresponded approximately to the model curve (Fig. 1). However, after the 4th day and whatever the stocking density, the observed numbers of *Artemia* nauplii ingested were higher (35 to 70) than predicted by the reference curve (23).

When the ration was multiplied by 9 at a same stocking density (from R1 to R3 at 10 or 30 larvae.L^{-1}), the number of ingested *Artemia* was multiplied by 5 at day 2, by 3 at day 4 and by 2 at day 6. For larvae reared at a stocking density of 90 per litre, increasing the ration from R1 to R3 resulted in a lower increase of the number of ingested preys, which was multiplied by 1.5 only from the 4th day of rearing (Fig. 4).

The number of *Artemia* nauplii ingested by larvae fed with equal conditions of prey accessibility decreased when feeding rate decreased, which was also accompanied by an increase in stocking density.

DISCUSSION

The first experiment showed that *P. hypophthalmus* larvae were fragile and sensitive to mechanical chocks and perturbations of the media. In addition, the homogenisation of the preys by strong aeration did not seemed to improve



(A1, A1 x 3, ... : corresponding levels of prey accessibility)

Figure 4: Number of *Artemia* ingested by *P. hypophthalmus larvae* as a function of age at each two days sampling periods for the different feeding level and densities.

the ingestion, as no difference in the final mean body weight of larvae were found between the two levels of water aeration tested. In this experiment the low survival rates were probably not related to feeding (ration and type of food) or distribution of the preys in the water column. Some other factors such as feeding frequency, rearing conditions, water quality or diseases may have resulted in low growth or survival rates. As a matter of fact, *Aeromonas hydrophila* was identified as a pathogenic agent for *P. hypophthalmus* larvae (Hambali *et al.*, 1999). Occurrence of bacterial infection and its negative effect on the survival rate of *P. hypophthalmus* larvae was already demonstrated by Subagja *et al.* (1999). However, these authors did not find any effects of bacterial infection on the growth rate of larvae in their study, as final body weights were equivalent when larvae were reared with or without antibacterial treatment.

In this first experiment, no difference of survival rates was found between larvae fed *Artemia* and *Moina* sp. However, mean body weight were higher for larvae fed *Artemia* nauplii, indicating that this prey was more adapted for first feeding of *P. hypophthalmus* larvae. Yunardi (1987) also demonstrated a better growth and survival for *P. hypophthalmus* larvae fed *Artemia* (21mg) than for those fed *Daphnia carinata* (11mg) at a feeding rate of 200-250% of fish biomass. *Artemia* nauplii was also considered as the best live feed for larval rearing of *Heterobranchus longifilis* (Legendre *et al.*, 1991), *Clarias macrocephalus* (Fermin & Bolivar, 1991) and *Pangasius bocourti* (Hung *et al.*, 1999).

When larvae were reared at a stocking density of 10 larvae.L^{-1} and fed with *Artemia* nauplii in the first experiment, the results did not show difference of survival rates between low (150%) and high feeding rates (4000%). By contrast, the second experiment indicated a decreasing of survival rates of *P. hypophthalmus* larvae when they were fed *Artemia* at a high feeding level (10 fish.L^{-1}; R'3, A1x 81; corresponding to 3240% of initial fish biomass). Similar observations were also made for the treatment with a stocking density of 90 larvae.L^{-1} and ration R3, which resulted in the same high quantity of *Artemia* nauplii (A1 x 81) in the tanks. These results indicated a negative effect of too high quantities of *Artemia* nauplii on larval survival rate. By comparison, the high survival rate of larvae (62%) fed *Moina* distributed at 4000 % of fish biomass in the first experiment could result from the capacity of *Moina* to survive in fresh water, while *Artemia* nauplii die after 3-4 hours in this media and decayed. Thus it can be assumed that larvae fed very high rations of *Artemia* were reared in inadequate hygiene as the tanks were cleaned only once a day.

The highest mean final body weight of larvae obtained in the first experiment was 21 mg only. Similar final weights (20-24 mg) were also observed in the second experiment with the lowest feeding rate. These values are in accordance with those previously reported (20-25 mg) for *P. hypophthalmus* larvae at the age of 10 days (Prihastowo, 1987; Yuniardi, 1987) or 8 days (Subagja *et al.*, 1999). However, observations carried out previously at the Sukamandi station (unpubl. Data) and in Vietnam (see Fig. 1) for larvae fed *Artemia* nauplii in excess, but without antibacterial treatment, showed lower levels of prey ingestion (7-14 *Artemia* nauplii at day 4-5) than for larvae fed high rations in the second experiment reported here (32 *Artemia* nauplii at day 4). Therefore, the lower final weights of larvae (20-25 mg at day 8-10) in these different trials could have been related to a low rate of prey ingestion whatever the feeding level used. The present results showed that, with appropriate rearing conditions, a mean body weight as high as 50 mg can be expected at the age of 8 days.

The results of the second experiment showed that, in the range tested, fish stocking density did not influence growth and survival of larvae. By contrast, both were positively related to the feeding level up to a limit corresponding to an excessive quantity of feed in the tanks.

Until the age of 4 days *P. hypophthalmus* larvae swim with the mouth open and close their jaws only when meeting a prey. Therefore the search for food seems to be passive phenomenon during this early period, and it is only afterwards that larvae search actively for their preys in all the water column (Hardjamulia *et al.*, 1981; Legendre, unpubl. data). These observations suggested that the prey concentration (or accessibility) could be an important parameter to consider for larval rearing, particularly for young stages. However, at a same feeding ration, the larval growth and survival remained roughly the same despite prey accessibility was increased by 3 or 9 times when fish stocking density was increased in the same ratio. As an example, the mean body weight, survival and numbers of ingested preys remained unchanged in fish fed ration R1 and reared at stocking density of 10, 30 or 90 per litre, corresponding to prey accessibility of 30, 90 and 270 *Artemia* per litre (see Table 2 and Fig. 4). Conversely, for the same prey accessibility, the growth and survival of larvae, and the number of ingested *Artemia* nauplii, increased as a function of the feeding ration given. Therefore, the lowest level of prey accessibility (A1 = 30 *Artemia* nauplii.L^{-1}.feeding^{-1}) tested in this experiment was probably above the threshold at which this parameter could have been a limiting one for the larvae.

Hung *et al.* (unpublished data) showed a progressive and important increase of ingested preys as a function of age in *Heterobranchus longifilis* when larvae were fed *Artemia* nauplii (a mean of 221 preys per fish was observed at 9 days of age). By contrast, the present investigation showed a diminution of the number of preys ingested by *P. hypophthalmus* larvae between day 6 and day 8 (Fig. 4). It was noticed however that at 8 days of age, the larvae regurgitated a part of the ingested *Artemia* nauplii when they were fixed in 5% formalin. This reaction, which biased the counting of ingested preys at this moment, was not observed during the samplings of the 2nd, 4th and 6th days. This phenomenon was concomitant to morphological changes in the digestive tract, corresponding to the development of an individualised stomach. However, a detailed investigation remains necessary to precise the ontogeny of the digestive tract in *P. hypophthalmus*. In an other pangasiid species, *Pangasius bocourti*, the stomach attains its functional and physiological achievement 3 days after the first feeding (Hung *et al.*, 1999).

The specific growth rate of larvae calculated over the whole experimental period was increased at higher feeding rate. However, examining the evolution of SGR by period of two days showed that the growth differential observed between larvae fed with ration R1, R2 and R3 concerned mostly the first four days of larval rearing (see Fig. 3). The same trend was also observed when considering the number of *Artemia* ingested: the differential in number of prey ingested between larvae fed the different rations was reduced in older fish in comparison to younger ones. These results suggest that feed rationing would be more efficient (i.e. leading to high growth rates for a lower total quantity of *Artemia* distributed) if an initial high ration (R3) is reduced to R2 between 4 and 6 days of age, than reduced to R1 between 6 and 8 days of age.

CONCLUSION

The present study showed that both *Artemia* nauplii and *Moina* sp., which led to similar survival rates, could be used as a first feed for larval rearing of *P. hypophthalmus*. However, larvae fed *Artemia* nauplii systematically displayed a faster growth rate than those fed *Moina*. *Pangasius hypophthalmus* larvae were rather fragile and sensitive to strong water aeration used as a mean to homogenise the distribution of preys in the water column.

In the range tested, the feeding level using *Artemia* had a predominant effect on larval growth and survival in comparison to fish stocking density or prey accessibility. This positive relationship between feeding level and larval growth and survival was associated to an increase in the mean number of *Artemia* nauplii ingested by the larvae. In the most favourable conditions, the larvae showed a very high growth rate, reaching up to 50 mg mean body weight at 8-days of age.

Although, growth performance and survival rates increased according to the feeding level, the ration R3 (corresponding 27 *Artemia* per larvae per feeding, or 360% of fish biomass, on the first day of exogenous feeding) appeared as the maximal efficient ration for *P. hypophthalmus* larvae. Further investigations are presently carried out to precise the optimal feeding level according to the age and body development of the larvae.

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