

**ANALYSIS OF PRECOCIOUS MORTALITY OF *PANGASIVS HYPOPHthalmus* LARVAE  
(SILURIFORMES, PANGASIIDAE) DURING THE LARVAL REARING  
AND PROPOSITION OF APPROPRIATE TREATMENTS<sup>1</sup>**

Jojo Subagja <sup>(1)</sup>, Jacques Slembrouck <sup>(2)</sup>, Le Thanh Hung <sup>(3,4)</sup> and Marc Legendre <sup>(2)</sup>

(1) RIFF, Jalan Raya 2, Sukamandi Subang 41256, Java Barat, Indonesia

(2) IRD (ex ORSTOM), Catfish Asia Project, Instalasi Penelitian Perikanan Air Tawar, Jalan. Ragunan-  
Pasar Minggu, P.O. Box 7220/jkspm, Jakarta 12540, Indonesia  
and GAMET, B.P. 5095, 34033 Montpellier Cedex 1, France

(3) Aquaculture Research Unit, CIRAD-EMVT, GAMET, BP 5095, 34033 Montpellier Cedex 1, France

(4) National University of Ho Chi Minh City, Faculty of Fisheries, Thu Duc, Ho Chi Minh City, Vietnam

### Abstract

Both in Indonesia and Vietnam, larval rearing of *Pangasius hypophthalmus* remains problematic due to variable and generally low survival rates obtained. The first week represents the most critical period for these larvae and up to now the cannibalistic behaviour was considered as the main cause of mortality.

In the present study, two experiments were carried out in order to better understand the evolution and causes of mortality of *P. hypophthalmus* larvae from hatching up to 8 days of age and find out measures to improve survival rates.

The first experiment was designed to evaluate the importance of cannibalism and differences in mortality when larvae from two different females were reared either in groups of 30 individuals or in isolated condition (30 larvae reared separately). In both cases, the culture was carried out either with or without antibiotic. The aim of the second experiment was to test Oxytetracycline and different disinfectants (Chloramine-T, formalin and "formalin + Malachite Green Oxalate") at different dosages to prevent bacterial outbreaks in the culture.

The results indicated that the survival rates of *P. hypophthalmus* larvae was dependant on the initial quality of larvae or eggs and that larval mortality was more a consequence of pathogenic infection than a direct effect of cannibalism. The present study demonstrated that the survival rates of larvae were systematically improved when rearing was carried out in water containing antibiotic (Oxytetracycline at a dose of 5 to 20 mg.L<sup>-1</sup>). Survival rates and final mean body weights of larvae as high as those obtained using antibiotic were also reached with applications of disinfectants such as Chloramine-T and formalin. The use of these disinfectants is recommended for an application in commercial *P. hypophthalmus* hatcheries.

### INTRODUCTION

Fish breeding in Indonesia has rapidly developed and is characterised by the emergence of numerous small-scaled hatcheries. Among cultured fish species, *Pangasius hypophthalmus* (Sauvage, 1878), synonymised with *Pangasius sutchi* since a recent systematic revision of Pangasiidae (Roberts & Widthayanon, 1991) and

originating from the Mekong River, was introduced from Thailand to Indonesia in 1972 (Hardjamulia *et al.*, 1981). The species 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) and in Indonesia in 1981 (Hardjamulia *et al.*, 1981). However, larval rearing

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of this species remains problematic because variable and low survival rates of larvae are generally experienced. In West Java, the survival rate of larvae after 2 weeks from hatching generally falls in the range of 10-15%. During the first week of larval rearing, Slembrouck (1996) observed high variations of survival rates (0-79%) at the Chau Doc hatchery, Vietnam. Similar variations of survival rate were also observed at the Can Tho University, Vietnam (unpublished data) and at the RIFF Sukamandi station in West Java, Indonesia (0-83%). A marked cannibalistic behaviour, sometimes leading to the disappearance of more than 90% of the population (Campet, 1997), was considered as the main cause of mortality of *P. hypophthalmus* larvae. Observations carried out during the larval rearing showed that cannibalism started as early as 40 hours post hatching, before complete absorption of yolk sack, and stopped 3 to 4 days after hatching (Slembrouck, 1996).

Although *P. hypophthalmus* has been cultivated for about 30 years, published information on the larval rearing of this species remains very scarce. It is known however that the first 8 days of life represent the most critical period, afterwards the mortality rate decreases (Yuniardi, 1987). Observations carried out at the Sukamandi station indicated that two peaks of mortality generally occur during this period. The first mortality peak was observed at 2-3 days of age during the period of cannibalism and represented about 30-50% of initial fish number, while the second peak, occurring at 5-7 days of age and representing 50-60% mortality, seemed to be due to other causes than cannibalistic behaviour. Previous works indicated that the cannibalistic behaviour and feed intake during the first 3-4 days of age were passive phenomena (Hardjamulia *et al.*, 1981) and that the survival rate could be increased when the fry were fed *Artemia* nauplii at a daily rate of 250% of fish biomass, with a minimum of 5 distributions per day during the first 5 days of rearing (Prihastowo, 1987; Yuniardi, 1987). However, after the first week of larval rearing, the survival rate still showed very high variability in both private and research stations, even when fry were abundantly and frequently fed with *Artemia* nauplii.

Therefore, the objectives of the present study were to better understand the evolution and causes of precocious mortality of *P. hypophthalmus*

larvae from hatching up to 8 days of age and find out feasible measures to improve survival rates.

## MATERIAL AND METHODS

The larvae were obtained from 3-5-years old *P. hypophthalmus* brooders held in ponds at the RIFF Sukamandi station (West Java, Indonesia). Ovulation was induced using two injections of salmon gonadotropin-releasing hormone analogue and domperidone (Ovaprim<sup>®</sup>, Syndel Laboratories, Canada) with a total dose of 0.9 ml.kg<sup>-1</sup> body weight. Males received a single Ovaprim injection (0.4 ml.kg<sup>-1</sup>) in order to increase the volume of milt collected. After stripping of gametes, artificial fertilisation was performed and eggs were incubated in happas placed in 5 m<sup>3</sup> concrete tanks. Twelve hours after hatching, the larvae were individually counted and transferred to their respective rearing containers. Two larval rearing experiments were carried out.

### Experiment 1

The first experiment was designed to evaluate the importance of cannibalism and differences in mortality when larvae were reared either in groups of 30 individuals or in isolated condition (30 larvae reared separately). In both cases, the culture was carried out either without antibiotic or in water containing permanently Oxytetracycline at a dose of 5mg.L<sup>-1</sup>. Therefore, the fish were placed in the following situations :

- isolated larvae with antibiotic,
- isolated larvae without antibiotic,
- group of larvae with antibiotic,
- group of larvae without antibiotic.

A supplementary treatment consisting in groups of larvae without feeding and without antibiotic was done for comparison of the evolution of daily mortality with other treatments tested.

Each treatment was tested on larvae obtained from the eggs of two different females fertilised with sperm pooled from two males. The groups of 30 larvae were reared in 300 ml plastic containers with 3 replications for each treatment x female combination. Each isolated larvae was placed in a 150 ml plastic container, 30 larvae being individually followed for each treatment x female combination.

### Experiment 2

The second experiment was designed to test the efficiency of Oxytetracycline and different disinfectants at different dosages to prevent bacterial outbreaks in the culture. The following treatments and doses were compared:

- Oxytetracycline at doses of 5, 10, 15 and 20 mg.L<sup>-1</sup>,
- Chloramine-T at doses of 1.5, 2.0 and 2.5 mg.L<sup>-1</sup>,
- formalin at doses of 1.5, 2.0 and 2.5 mg.L<sup>-1</sup>,
- solution of 4 g of Malachite Green Oxalate (MGO) in 1 litre of formalin at final doses of 1.5, 2.0 and 2.5 mg.L<sup>-1</sup>,
- untreated water (control).

All treatments were tested with 3 replications on groups of 30 larvae reared in 300 ml plastic containers and obtained from one female and one male. Oxytetracycline was applied as a permanent bath to the larvae from the first day up to 8-days of age, while all treatments using disinfectants were applied every two days during a period of 24 h.

In both experiments, the larvae were reared in stagnant spring water, and fed in excess with *Artemia* nauplii starting from 36 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.

Water of each plastic container was changed twice a day at 10:00 and 22:00. During the experiments, water temperature was measured continuously (Optic StowAway<sup>®</sup>) and fluctuated between 27.5 and 29.8°C. Dissolved oxygen and pH were measured at day 3, 5 and 7 before water changing and varied in the range of 4.6-6.3 mg.L<sup>-1</sup> and 7.0-7.4, respectively. Ammonia and nitrite concentrations were determined at the same time using Aquaquant<sup>®</sup> kits (Merck 14423, 14424) and ranged between 0.002 and 0.027 mg.L<sup>-1</sup>, and between 0.011 and 0.016 mg.L<sup>-1</sup>, respectively.

Died larvae were removed and counted twice a day, simultaneously to water changes, to estimate the percentage of observed mortality per period of 24 h. On the last day of experiment (day 8), all the remaining larvae were individually counted for calculation of actual survival rate. The percentage of missing larvae was calculated as: 100-survival (%) - total observed mortality (%).

At the end of the experiment, the larvae were weighed at an accuracy of 0.1mg, the fishes being

previously placed on paper towels in order to absorb adhering water.

Final mean body weights and survival rates of larvae were subjected to three way ANOVA (female x type of fish stocking x antibiotic) in the first experiment and to one way ANOVA followed by Duncan's multiple range test to determine significant differences among treatments in the second experiment. When necessary, angular transformation of data expressed as percentage was carried out in order to stabilise the residual variance.

## RESULTS

### Experiment 1

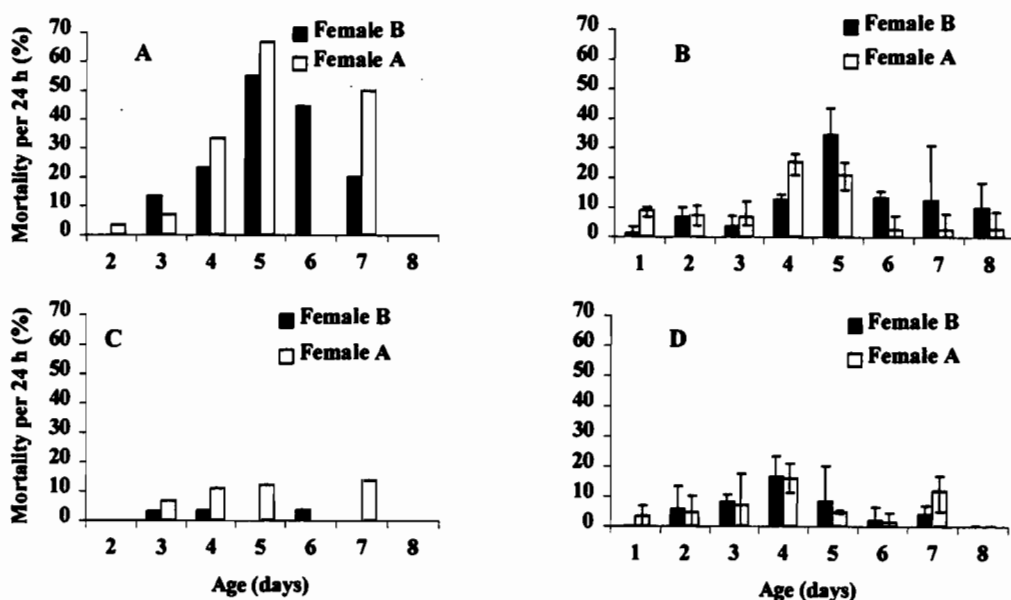
#### *Evolution of daily mortality.*

The evolution of mortality per period of 24 h for larvae obtained from the two different females, reared in group or isolated, with antibiotic or not, is given in Figure 1. The mortality of the isolated larvae was recorded from the second day only (Fig. 1A, 1C), while some dead larvae were already counted in the groups of fish (Fig. 1B, 1D). This is because the quasi totality of isolated larvae surprisingly died during the first night after stocking in their containers; these larvae were immediately replaced for a new starting at day 1.

From Figure 1, the mortality of isolated larvae from both females seemed to be higher than that observed in the group of larvae during the experimental period. However, this was not confirmed when the percentages of missing larvae were taken into account (see *infra* and Table I).

In the treatments without antibiotic, the percentage of dead fish per 24 h increased progressively from day 1 to day 5 and slightly decreased afterwards. The highest daily mortality was observed at 5 days of age for both isolated larvae (60-70%) and groups (20-35%). It should be noted that in this experiment, the first peak of mortality, which is generally observed at day 2 during larval rearing of *P. hypophthalmus*, did not occur (Fig. 1B).

The treatment with Oxytetracycline allowed a clear lowering of larval mortality (Fig. 1A, 1C). For the isolated larvae, the percentage of dead fish per period of 24 h remained very low during the whole experimental period, not exceeding 5% and 15% for larvae from female B and A, respectively.



Vertical bars indicate range between replicates.

**Figure 1:** Evolution of mortality per period of 24 h up to the age of 8 days in *P. hypophthalmus* larvae obtained from two different females and reared isolated (A) or in group (B) without antibiotic, and isolated (C) or in group (D) with antibiotic.

| Broodfish | Fish stocking | Feeding        | Antibiotic OTC (5 mg.L <sup>-1</sup> ) | Final body weight (mg) | Survival rate (%) | Missing larvae (%) |
|-----------|---------------|----------------|--|------------------------|-------------------|--------------------|
| Female A  | Isolated      | <i>Artemia</i> | No                                     | 18.0                   | 6.7               | 0.0                |
| Female B  | Isolated      | <i>Artemia</i> | No                                     | 15.3                   | 13.3              | 0.0                |
| Female A  | Isolated      | <i>Artemia</i> | Yes                                    | 21.9                   | 51.9              | 0.0                |
| Female B  | Isolated      | <i>Artemia</i> | Yes                                    | 21.6                   | 83.3              | 0.0                |
| Female A  | Group         | <i>Artemia</i> | No                                     | -                      | 0.0               | 43.3               |
| Female B  | Group         | <i>Artemia</i> | No                                     | 17.0                   | 10.0              | 25.6               |
| Female A  | Group         | <i>Artemia</i> | Yes                                    | 18.4                   | 30.0              | 30.0               |
| Female B  | Group         | <i>Artemia</i> | Yes                                    | 18.9                   | 53.3              | 10.0               |
| Female A  | Group         | No feeding     | No                                     | -                      | 0.0               | 51.0               |
| Female B  | Group         | No feeding     | No                                     | -                      | 0.0               | 31.0               |

**Table I :** Mean body weight, survival rate and percentage of missing fish after 8 days of rearing for *P. hypophthalmus* larvae obtained from two different females, reared in group or isolated with antibiotic or not in the first experiment.

Similarly, for larval groups, the daily mortality was generally lower than 10%, except on day 4 at which values of 16% were observed for both females.

#### Survival rate

The survival rates obtained at the end of the experiment ranged between 0% and 83% depending on rearing conditions and female parent (Table I). The results of analysis of variance indicated significant differences in larval survival

depending on the female parent ( $p < 0.01$ ), stocking conditions (isolated or grouped larvae;  $p < 0.05$ ) and antibiotic treatment ( $p < 0.001$ ). No interaction was found between these three factors.

The highest survival rate (83%) corresponded to the isolated larvae from female B reared using antibiotic. In all rearing situations, except in absence of feeding, the survival of larvae from female B was systematically higher (about 10 to 30%) than that of larvae from female A. Group rearing condition resulted in lower survival than

isolated rearing in water containing antibiotic or not. The use of Oxytetracycline led to a clear improvement of the survival of *P. hypophthalmus* larvae after 8 days of culture. The increases in survival rates when using antibiotic were of 45% and 30% for larvae from female A and of 70% and 43% for larvae from female B, in isolated conditions and in groups respectively.

No survival was observed for larvae from female A fed with *Artemia* and reared in groups without antibiotic, as well as for groups of larvae from both female A and B without feeding.

#### *Missing larvae*

When larvae were reared in isolated conditions, no missing fish were registered. In all group-rearing treatments, the percentage of missing fish was always about 20% higher for larvae from female A compared to larvae from female B (Table I). The percentage of fish that disappeared during the experiment were similar for both females in the groups without antibiotic fed with *Artemia* nauplii and in groups without feeding. Missing fish tended to be less numerous in the groups reared with antibiotic in comparison to groups without antibiotic (Table I).

#### *Growth*

At the end of the 8-days rearing period, the mean body weight of larvae was comprised between 15.3 and 21.6 mg and did not differ significantly between treatments (Table I).

### **Experiment 2**

#### *Evolution of daily mortality*

The evolution of daily mortality for all treatments during the experiment is given in Figure 2.

The control showed 2 peaks of mortality at day 2 (up to 41%) and at day 7 (up to 31%). These peaks were not observed when Oxytetracycline (maximum 14,2%) or Chloramine-T (maximum 13,2%) were used, whatever the dose applied.

At day 2, the highest mortality (up to 83%) was observed with the treatment "formalin + MGO" at 2 mg.L<sup>-1</sup> (Fig. 2). The lowest mortality obtained during the first 3 days using this medicine corresponded to the lowest dosage (1,5 mg.L<sup>-1</sup>).

The treatment with formalin at doses of 1.5 and 2 mg.L<sup>-1</sup> resulted in two peaks of mortality at day

2 and day 6 respectively. With a higher dose of formalin (2.5 mg.L<sup>-1</sup>), the daily mortality remained low (less than 10%) during the whole experiment (Fig. 2).

The mortality stopped completely at day 3 with Oxytetracycline at a dose of 20 mg.L<sup>-1</sup>, day 4 with Chloramine-T at 2.5 mg.L<sup>-1</sup>, day 7 with formalin at 2.5 mg.L<sup>-1</sup> and day 5 with "formalin + MGO" at a dose of 2,5 mg.L<sup>-1</sup>.

#### *Survival rate*

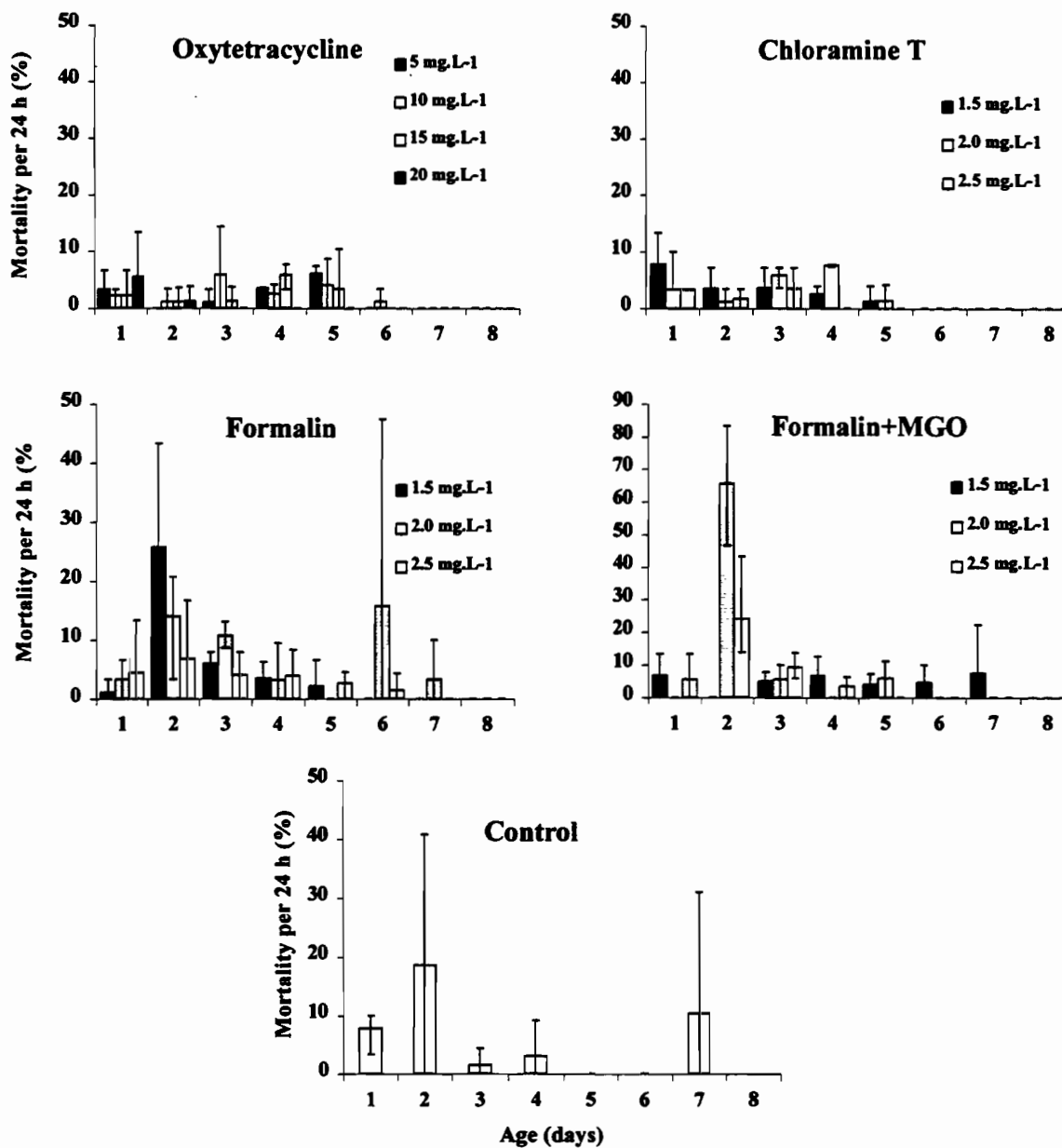
The survival rate of *P. hypophthalmus* larvae was not significantly different when using Oxytetracycline and Chloramine-T at any of the doses tested, and formalin at 1.5 or 2.5 mg.L<sup>-1</sup> (Table 2). The lower mean survival observed using formalin at 2.0 mg.L<sup>-1</sup> was due to a low value in one of the replicates. The best results were obtained with Oxytetracycline at all doses (81-87%), Chloramine-T at doses of 1.5 and 2.5 mg.L<sup>-1</sup> (81 and 87%) and formalin at 2,5 mg.L<sup>-1</sup> (76%). Survival rates obtained using Oxytetracycline at 5, 15 or 20 mg.L<sup>-1</sup> and Chloramine-T at 2.5 mg.L<sup>-1</sup> were significantly higher than that of the control groups (52%). Formalin at doses of 1.5, 2.0 and 2.5 mg.L<sup>-1</sup> did not led to significant difference from the control, even though higher dose gave a downward trend of mortality. Application of "formalin + MGO" at a dose of 2 mg.L<sup>-1</sup> led to the lowest survival rate (29%).

#### *Missing larvae*

The percentages of missing larvae were low for all treatments, ranging from 1 to 10% (Table 2). Although missing larvae tended to be more numerous in the control (10%), no significant difference was found between all treatments.

#### *Growth*

At the end of the 8-day experiment, the mean body weight of larvae reared with any doses of Oxytetracycline (21.5-22.2 mg), Chloramine-T (21.7-23.7 mg) and formalin (21.8-22.3 mg) were not significantly different from each other and from the control (21.3 mg). However, the mean larval body weight obtained with all doses of "formalin + MGO" (12.5-18.4 mg) were significantly lower than that of larvae reared with all other medicines tested (Table 2).



Vertical bars indicate range between replicates.

**Figure 2:** Evolution of mortality per period of 24 h up to the age of 8 days in *P. hypophthalmus* larvae reared with Oxytetracycline, Chloramine-T, formalin, formalin + MGO and without medicine.

## DISCUSSION

In this study, the results obtained in the control groups of the two experiments carried out (no prophylactic treatment, feeding with *Artemia nauplii*) illustrated once again the problems generally encountered during the larval rearing of *P. hypophthalmus*: low survival rates of larvae after one week with two peaks of mortality occurring generally at 2 and then 5-7 days of age. The present study demonstrated, however, that the survival rate of *P. hypophthalmus* larvae were systematically improved when rearing was carried

out in water containing antibiotic. The use of Oxytetracycline proved to be highly efficient in preventing the two peaks of mortality observed at 2 and 5-7 days of age in the control situation without prophylactic treatment. This result suggests that mortality of larvae at these stages was mostly a consequence of pathogenic infection and not of cannibalistic behaviour.

Several supplementary arguments indicated that the influence of cannibalistic behaviour was limited in the two experiments: 1) direct observations of cannibalism were only occasional, 2) the evolution of daily mortality in isolated and

| Treatment          | Dose (mg.L <sup>-1</sup> ) | Final body weight (mg) | Survival rate (%)   | Missing larvae (%) |
|--------------------|----------------------------|------------------------|---------------------|--------------------|
| Oxytetracycline    | 5                          | 22.0 <sup>d</sup>      | 83.5 <sup>cd</sup>  | 3.3 <sup>a</sup>   |
| Oxytetracycline    | 10                         | 22.2 <sup>d</sup>      | 81.1 <sup>bcd</sup> | 3.3 <sup>a</sup>   |
| Oxytetracycline    | 15                         | 21.5 <sup>d</sup>      | 83.5 <sup>cd</sup>  | 2.2 <sup>a</sup>   |
| Oxytetracycline    | 20                         | 21.6 <sup>d</sup>      | 86.7 <sup>d</sup>   | 6.7 <sup>a</sup>   |
| Formalin           | 1,5                        | 22.1 <sup>d</sup>      | 60.0 <sup>bcd</sup> | 5.6 <sup>a</sup>   |
| Formalin           | 2,0                        | 21.8 <sup>d</sup>      | 55.6 <sup>abc</sup> | 5.6 <sup>a</sup>   |
| Formalin           | 2,5                        | 22.3 <sup>d</sup>      | 75.5 <sup>bcd</sup> | 3.3 <sup>a</sup>   |
| Chloramine-T       | 1,5                        | 23.7 <sup>d</sup>      | 81.1 <sup>bcd</sup> | 1.1 <sup>a</sup>   |
| Chloramine-T       | 2,0                        | 23.2 <sup>d</sup>      | 71.1 <sup>bcd</sup> | 8.9 <sup>a</sup>   |
| Chloramine-T       | 2,5                        | 21.7 <sup>d</sup>      | 86.7 <sup>d</sup>   | 5.0 <sup>a</sup>   |
| Formaldehyde + MGO | 1,5                        | 18.4 <sup>bc</sup>     | 71.5 <sup>bcd</sup> | 1.1 <sup>a</sup>   |
| Formaldehyde + MGO | 2,0                        | 12.5 <sup>a</sup>      | 28.9 <sup>a</sup>   | 1.1 <sup>a</sup>   |
| Formaldehyde + MGO | 2,5                        | 17.0 <sup>b</sup>      | 58.1 <sup>abc</sup> | 4.4 <sup>a</sup>   |
| Control            | -                          | 21.3 <sup>cd</sup>     | 52.2 <sup>ab</sup>  | 10.0 <sup>a</sup>  |

Figures with the same superscripts in the same column are not significantly different ( $p < 0.05$ ).

**Table 2:** Survival rate, mean body weight and percentage of missing larvae as a function of prophylactic treatment for *P. hypophthalmus* after 8 days of larval rearing in the second experiment.

grouped larvae followed similar kinetics and 3) the fact that percentages of missing larvae were equivalent in fish fed *Artemia* or unfed (first experiment) tended to confirm that the missing larvae were not ingested by congeners. A reduction of cannibalism was reported in both *P. hypophthalmus* (Hardjamulia *et al.*, 1981) and *Clarias gariepinus* (Hecht & Appelbaum, 1988) when the larvae received adequate and abundant feeding.

In this investigation, Oxytetracycline was used at doses ranging between 5 mg.L<sup>-1</sup> and 20 mg.L<sup>-1</sup> during 8 days. No significant difference in survival rates and mean body weight of the larvae was found as a function of the dose used. However, the follow up of daily mortality (Figure 2) showed that the mortality stopped sooner (day 3) with a dose of 20 mg.L<sup>-1</sup> than with the other doses. The dosage recommended by De Kinkelin *et al.* (1985) for treatment of fish was also 20 mg.L<sup>-1</sup> during 6-8 days.

The pathogenic agent responsible of the infection of *P. hypophthalmus* larvae was identified as *Aeromonas hydrophila*, which could be isolated from the larvae as early as 2 days of age (Hambali *et al.*, 1999). *Aeromonas hydrophila* is widespread in the environment and can even be found in the intestinal flora of fish without pathogenic consequences. Generally, pathogenic effects become manifest when the fish are adversely effected by some other factor. The

primary prophylactic measure against *A. hydrophila* is stress avoidance. Stress can result from protozoan infection, inadequate hygiene, abundance of particulate matter in the water, handling and crowding, low oxygen content and chronic exposure to various pollutants (Kabata, 1985).

In the present experiments, the larvae were reared in limited living space in regularly changed stagnant water. However, it is assumed that these rearing conditions were not deleterious to the larvae because similar survival rates were observed in many occasions when larvae from the same spawn were reared in 30 L tanks supplied with water of high quality in a re-circulating system (unpubl. data).

It has been observed that at the age of 2-4 days the larvae search for food in a passive manner; they swim actively with the mouth open and close their jaws when meeting a prey or a congener (Hardjamulia *et al.*, 1981; unpublished observations). This behaviour may result in cannibalism, but very often the bitten congener escapes and continues to swim. It was hypothesised that wounded body of larvae resulting from this behaviour may facilitate the entrance of pathogens and subsequent mortality of larvae. In that case bacterial infection could be, at least for a part, a secondary consequence of fish behaviour. The fact that survival of larvae was significantly higher for isolated larvae than for

larvae reared in groups (exp. 1) tended to support this hypothesis. Nevertheless, further investigations on interaction between behaviour and survival of larvae remain necessary to fully clarify this question.

In any cases, treatments using antibiotic have to be administrated at the effective dosage and during enough time to ensure elimination of bacteria (De Kinkelin *et al.*, 1985). As a consequence of inappropriate use of an antibiotic, bacteria, such as *Aeromonas hydrophila* (Aoki *et al.*, 1971) and *Aeromonas salmonicida* (Popoff & Davaine, 1971), can develop resistance to this antibiotic which is transmitted to the next generations. Therefore the systematic use of antibiotics did not appear as a sustainable way for larval rearing of *P. hypophthalmus* at the production scale and alternative solutions had to be found.

The present study demonstrated that survival rates and final mean body weight as high as those obtained using antibiotics could be reached with applications of disinfectants such as Chloramine-T and formalin.

With Chloramine-T results obtained at doses of 1.5 and 2.0 mg.L<sup>-1</sup> did not significantly differed from the control. Therefore, the higher dose of 2.5 mg.L<sup>-1</sup> is recommended for a routine application of this disinfectant.

Although no significant differences in survival and body weight of larvae were found when using formalin at doses of 1.5 and 2.5 mg.L<sup>-1</sup>, the results tended to be improved at the highest dose. It should be noted that the peak of mortality observed at day 2 was clearly reduced with 2.5 mg.L<sup>-1</sup> formalin, while it remained quite high with the lower doses (Fig. 2). De Kinkelin *et al.* (1985) recommended treatments at a dose of 25-40 mg.L<sup>-1</sup> of formalin during 24-48 hours in closed water, although the corresponding species and age of fish were not given. It is thus possible that higher dose of formalin than those tested in the present study could lead to further improvement of the results. Complementary investigations remain to be performed in order to identify the optimal dose of formalin for larval rearing of *P. hypophthalmus*. This latter disinfectant presents a particular interest because it is inexpensive and very easy to obtain.

By contrast, the larvae of *P. hypophthalmus* appeared sensitive to "formalin + MGO", this disinfectant leading to similar or even lower survival than in the control and to the lowest final

body weights. In *Silurus glanis*, it was assumed that treatments containing malachite green were toxic for the larvae and could be administered at dosage not exceeding 0.1 mg.L<sup>-1</sup> for a maximum period of 1 hour (Schlumberger, 1993). De Kinkelin *et al.* (1985) recommended a dosage of 0.1 mg.L<sup>-1</sup> of MGO for a continuous treatment in closed water. Bastiawan, (1988) showed that *Saprolegnia* sp. were sensitive to MGO at doses of 1-5 mg.L<sup>-1</sup>. However, dose of 1 mg.L<sup>-1</sup> represented a threat for newly hatched carp larvae. In the present investigation, MGO was used in association with formalin at doses of 0.006, 0.008 and 0.01 mg.L<sup>-1</sup>, respectively. Therefore the dosages used were not high but the duration of treatment (24 h) appeared to be too long for the *P. hypophthalmus* larvae. For these reasons, "formalin + MGO" can hardly be recommended for an application during larval rearing of *P. hypophthalmus*, particularly when compared to other disinfectants such as Chloramine-T and formalin.

In this study, the survival rates at day 8 were respectively 52, 10 and 0% for larvae reared without antibiotic, and then 84, 53 and 30% when the same larvae were reared in water containing permanently 5 mg.L<sup>-1</sup> of Oxytetracycline. As the rearing conditions were strictly equivalent in the different experiments performed, these results indicate that the survival rates of *P. hypophthalmus* larvae was strongly dependent on the initial quality of the larvae or eggs. This may explain, at least for a part, the survival variability observed in the preliminary investigations and in productions on farms. The causes of these discrepancies are unknown and further investigations would be necessary to identify criteria that may allow assessment of the initial quality of the larvae.

## CONCLUSIONS

The present study indicated that bacterial disease and female parents had more influence on survival rates of *P. hypophthalmus* larvae than a direct effect of cannibalistic behaviour.

Survival rates and mean body weights of the larvae were considerably improved in water treated with either Oxytetracycline (5-20 mg.L<sup>-1</sup>) and Chloramine-T (2,5 mg.L<sup>-1</sup>) in comparison to



control situation without medicine. A clear tendency of improved survival rates was also observed with the use of formalin (2.5 mg.L<sup>-1</sup>). As it is known that the use of antibiotics may induce bacteria resistance when applied in an inappropriate manner, the use of these disinfectants is recommended for safer treatment or prevention against bacterial diseases in commercial *P. hypophthalmus* hatcheries.

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# THE BIOLOGICAL DIVERSITY AND AQUACULTURE OF CLARIID AND PANGASIID CATFISHES IN SOUTH-EAST ASIA



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Edited by :  
Marc LEGENDRE  
Antoine PARISELLE



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