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Larval rearing of an Asian catfish *Pangasius hypophthalmus* (Siluroidei, Pangasiidae): Analysis of precocious mortality and proposition of appropriate treatments

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Abstract – Both in Indonesia and Vietnam, larval rearing of *Pangasius hypophthalmus* remains problematic due to the variable and generally low survival rates obtained. The first week represents the most critical period for these larvae and up to now, 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 d of age and to find 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 in order to prevent bacterial outbreaks in the culture. The results indicated that the survival rates of *P. hypophthalmus* larvae was dependent 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 $mg \cdot L^{-1}$). Survival rate and final mean body weight 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 commercial *P. hypophthalmus* hatcheries. © Ifremer/Elsevier, Paris

Asia / catfish / *Pangasius* / larval rearing / mortality / cannibalism / prophylaxis

Résumé – Élevage larvaire d'un poisson-chat asiatique *Pangasius hypophthalmus* (Siluroidei, Pangasiidae) : analyses des mortalités précoces et propositions de traitements appropriés. En Indonésie et au Vietnam, l'élevage larvaire de *P. hypophthalmus* reste un problème majeur car les taux de survie obtenus sont variables et généralement faibles. La première semaine d'élevage est la période la plus critique. Jusqu'à présent, le comportement cannibale a été considéré comme la cause principale des mortalités. Dans cette étude, deux expériences ont été effectuées dans le but de mieux comprendre l'évolution et les causes de mortalité des larves de *P. hypophthalmus* depuis l'éclosion jusqu'à l'âge de 8 j, puis de trouver des solutions pour améliorer la survie. La première expérience a été réalisée en vue d'évaluer l'importance du cannibalisme et les différences de mortalité obtenues lorsque les larves proviennent de deux femelles différentes. Les larves ont été élevées en groupes de 30 individus ou isolées (30 larves élevées séparément), avec et sans antibiotique. L'objectif de la seconde expérience était de tester l'oxytétracycline et différents désinfectants (chloramine-T, formol et « formol + oxalate de vert malachite ») à différentes doses afin de prévenir le développement des bactéries dans l'élevage. Les résultats indiquent que le taux de survie des larves de *P. hypophthalmus* dépend de la qualité initiale des larves ou des œufs et que la mortalité larvaire est davantage la conséquence d'une infection pathogène que d'un effet direct du cannibalisme. Le taux de survie des larves est systématiquement amélioré lorsque l'élevage est réalisé dans un bain d'antibiotique (oxytétracycline à une dose de 5 à 20 $mg \cdot L^{-1}$). Des taux de survie et des poids moyens finals des larves aussi élevés que ceux obtenus en présence d'antibiotique peuvent également être atteints en utilisant des désinfectants tels que la chloramine-T et le formol. L'utilisation de ces désinfectants est recommandée dans les écloseries de *P. hypophthalmus* à but commercial. © Ifremer/Elsevier, Paris

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1. INTRODUCTION

Fish breeding in Indonesia has developed rapidly 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 [11] and originating from the Mekong River, was introduced from Thailand to Indonesia in 1972 [6]. The species has adapted well to local conditions and is appreciated by consumers.

Induced spawning of *P. hypophthalmus* was initially reported in Thailand in 1976 [4] and in Indonesia in 1981 [6]. However, larval rearing of this species remains problematic because of variable and low survival rates of larvae. In West Java, the survival rate of larvae 2 weeks after hatching generally falls in the range of 10–15 %. During the first week of larval rearing, Slembrouck [13] observed high variations in survival rates (0–79 %) at the Chaudoc hatchery, Vietnam. Similar variations in survival rate were also observed at Cantho University, Vietnam (unpubl. 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 [3], was considered as the main cause of mortality of *P. hypophthalmus* larvae. Observations carried out during larval rearing showed that cannibalism started as early as 40-h post-hatching, before complete absorption of yolk sack, and stopped 3 to 4 d after hatching [13].

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 d of life represent the most critical period, afterwards the mortality rate decreases [14]. 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 d of age during the period of cannibalism and represented about 30–50 % of initial fish number, while the second peak, occurring at 5–7 d of age and representing 50–60 % mortality, seemed to be due to causes other than cannibalistic behaviour. Previous studies indicated that the cannibalistic behaviour and feed intake during the first 3–4 d of age were passive phenomena [6] 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 d of rearing [10, 14]. 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 d of age and to find feasible measures to improve survival rates.

2. MATERIALS 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©, Syndel Laboratories, Canada) with a total dose of 0.9 $mL \cdot kg^{-1}$ body weight. Males received a single Ovaprim injection (0.4 $mL \cdot kg^{-1}$) 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^3 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.

2.1. 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 dosage of 5 $mg \cdot L^{-1}$. 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 three replications for each treatment × female combination. Each isolated larvae was placed in a 150-mL plastic container, 30 larvae being individually followed for each treatment × female combination.

2.2. 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 $mg \cdot L^{-1}$;
- chloramine-T at doses of 1.5, 2.0 and 2.5 $mg \cdot L^{-1}$;
- formalin at doses of 1.5, 2.0 and 2.5 $mg \cdot L^{-1}$;

– solution of 4 g of malachite green oxalate (MGO) in 1 L of formalin at final doses of 1.5, 2.0 and 2.5 mg·L^{-1};

– untreated water (control).

All treatments were tested with three 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 d 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 h after hatching up to 8 d of age. The feeding frequency was of eight meals per day at 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00 and 06:00 hours.

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

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

100 – survival (%) – total observed mortality (%).

At the end of the experiment, the larvae were weighed with an accuracy of 0.1 mg, 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 × type of fish stocking × 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.

3. RESULTS

3.1. Experiment 1

3.1.1. 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 were recorded from the second day only (*figure 1*A, C), while some dead larvae were already counted in the groups of fish (*figure 1*B, D). This is because the quasi-totality of isolated larvae surprisingly died during the first night after being stocked in their containers; therefore, these larvae were immediately replaced, restarting 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

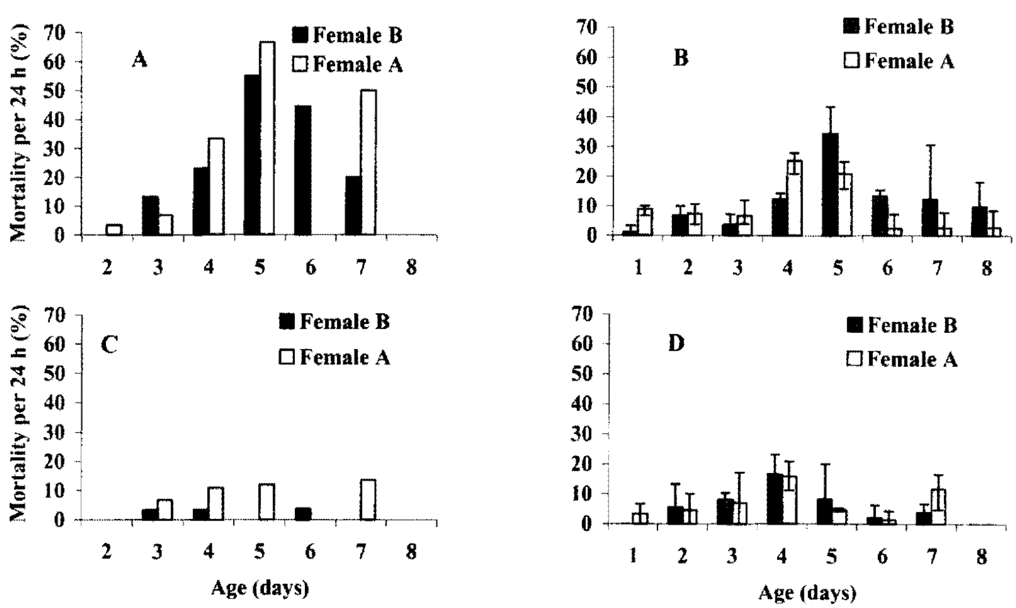

Figure 1. Evolution of mortality per period of 24 h up to the age of 8 d in *P. hypophthalmus* larvae obtained from two different females and reared isolated (A) or in group (B) without antibiotic, and isolated (C) or in group (D) with antibiotic. Vertical bars indicate range between replicates.

of missing larvae were taken into account (see following 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 d 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 (*figure 1*B).

Treatment with oxytetracycline clearly lowered larval mortality (*figure 1*A, C). 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. 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.

3.1.2. 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 the 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 the 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 conditions 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 d 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.

3.1.3. 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 was 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*).

3.1.4. Growth

At the end of the 8-d 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*).

3.2. Experiment 2

3.2.1. 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, no matter the dose applied.

At day 2, the highest mortality (up to 83 %) was observed with the treatment 'formalin + MGO' at 2 mg·L^{-1} (*figure 2*). The lowest mortality obtained during the first 3 d of using this treatment corresponded to the lowest dosage (1.5 mg·L^{-1}).

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

Broodfish	Fish stocking	Feeding	Antibiotic OTC (5 mg·L^{-1})	Final body weight (mg)	Survival rate (%)	Missing larvae (%)
Female A	Isolated	*Artemia*	No	18.0	6.7	0.0
Female B	Isolated	*Artemia*	No	15.3	13.3	0.0
Female A	Isolated	*Artemia*	Yes	21.9	51.9	0.0
Female B	Isolated	*Artemia*	Yes	21.6	83.3	0.0
Female A	Group	*Artemia*	No	–	0.0	43.3
Female B	Group	*Artemia*	No	17.0	10.0	25.6
Female A	Group	*Artemia*	Yes	18.4	30.0	30.0
Female B	Group	*Artemia*	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

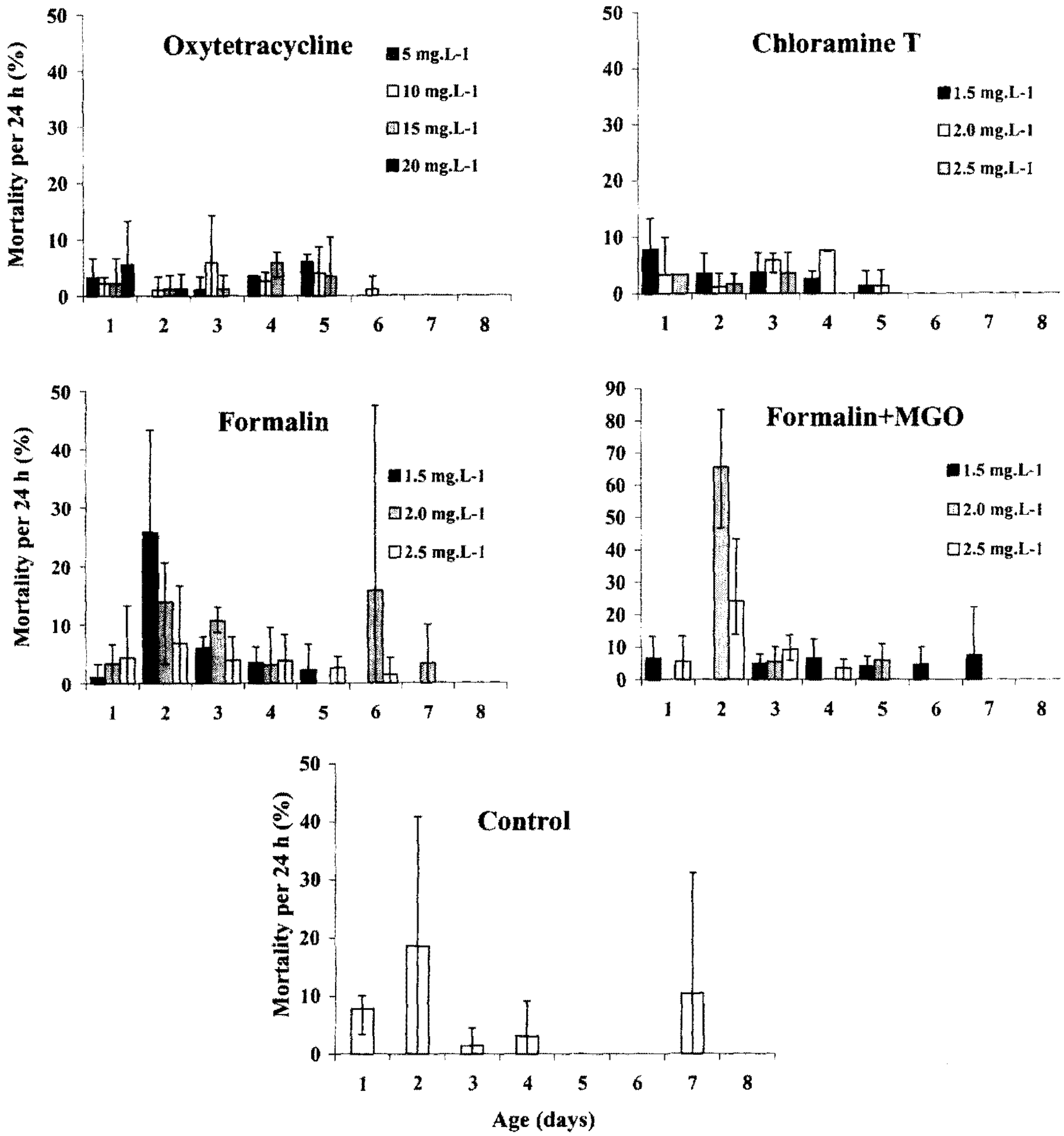

Figure 2. Evolution of mortality per period of 24 h up to the age of 8 d in *P. hypophthalmus* larvae reared with oxytetracycline, chloramine-T, formalin, formalin + MGO and without prophylactics. Vertical bars indicate range between replicates.

The treatment with formalin at doses of 1.5 and 2 mg·L^{-1} resulted in two peaks of mortality at day 2 and day 6, respectively. With a higher dose of formalin (2.5 mg·L^{-1}), the daily mortality remained low (less than 10 %) during the whole experiment (*figure 2*).

Mortality stopped completely at day 3 with oxytetracycline at a dose of 20 mg·L^{-1}, day 4 with chloramine-T at 2.5 mg·L^{-1}, day 7 with formalin at 2.5 mg·L^{-1} and day 5 with 'formalin + MGO' at a dose of 2.5 mg·L^{-1}.

3.2.2. *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^{-1} (*table II*). The lower mean survival observed using formalin at 2.0 mg·L^{-1} 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^{-1} (81 and 87 %) and formalin at 2.5 mg·L^{-1} (76 %). Survival rates obtained using oxytetracycline at 5, 15 or 20 mg·L^{-1} and chloramine-T at 2.5 mg·L^{-1} were significantly higher than that of control groups (52 %). Formalin at doses of 1.5, 2.0 and 2.5 mg·L^{-1} did not lead to significant difference from the control, even though a higher dose gave a downward trend of mortal-

Table II. Survival rate, mean body weight and percentage of missing larvae as a function of prophylactic treatment for *P. hypophthalmus* after 8 d of larval rearing in the second experiment. MGO: Malachite green oxalate.

Treatment	Dose ($mg \cdot L^{-1}$)	Final body weight (mg)	Survival rate (%)	Missing larvae (%)
Oxytetracycline	5	22.0^{d}	83.5^{cd}	3.3^{a}
Oxytetracycline	10	22.2^{d}	81.1^{bcd}	3.3^{a}
Oxytetracycline	15	21.5^{d}	83.5^{cd}	2.2^{a}
Oxytetracycline	20	21.6^{d}	86.7^{d}	6.7^{a}
Formalin	1.5	22.1^{d}	60.0^{bcd}	5.6^{a}
Formalin	2.0	21.8^{d}	55.6^{abc}	5.6^{a}
Formalin	2.5	22.3^{d}	75.5^{bcd}	3.3^{a}
Chloramine-T	1.5	23.7^{d}	81.1^{bcd}	1.1^{a}
Chloramine-T	2.0	23.2^{d}	71.1^{bcd}	8.9^{a}
Chloramine-T	2.5	21.7^{d}	86.7^{d}	5.0^{a}
Formaldehyde + MGO	1.5	18.4^{bc}	71.5^{bcd}	1.1^{a}
Formaldehyde + MGO	2.0	12.5^{a}	28.9^{a}	1.1^{a}
Formaldehyde + MGO	2.5	17.0^{b}	58.1^{abc}	4.4^{a}
Control	–	21.3^{cd}	52.2^{ab}	10.0^{a}

Values with the same superscript in the same column are not significantly different ($P < 0.05$).

ity. Application of 'formalin + MGO' at a dose of 2 $mg \cdot L^{-1}$ led to the lowest survival rate (29 %).

3.2.3. Missing larvae

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

3.2.4. Growth

At the end of the 8-d 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 prophylactics tested (*table II*).

4. 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 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 d 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 d 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 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 in cannibalism was reported in both *P. hypophthalmus* [6] and *Clarias gariepinus* [7] when the larvae received adequate and abundant feeding.

In this investigation, oxytetracycline was used at doses ranging between 5 and 20 $mg \cdot L^{-1}$ during 8 d. 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 $mg \cdot L^{-1}$ than with the other doses. The dosage recommended by De Kinkelin [5] for treatment of fish was also 20 $mg \cdot L^{-1}$ during 6–8 d.

The pathogenic agent responsible for the infection of *P. hypophthalmus* larvae was identified as *Aeromonas hydrophila*, which could be isolated from the larvae as early as 2 d of age (unpubl. data). *Aeromonas hydrophila* is widespread in the environment and can even been found in the intestinal flora of fish without pathogenic consequences. Generally, pathogenic effects become manifest when the fish are adversely affected 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 [8].

In the present experiments, the larvae were reared in a 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 on many occasions when larvae from the same spawn were reared in 30-L tanks supplied with water of high quality in a recirculating system (unpubl. data).

It has been observed that at the age of 2–4 d, the larvae search for food in a passive manner; they swim actively with their mouth open and close their jaws when meeting a prey or a congener ([6] and unpubl. obs.). This behaviour may result in cannibalism, but very often the bitten congener escapes and continues to swim. It was hypothesised that the 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 in 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 interactions between behaviour and survival of larvae remain necessary to fully clarify this question.

In any case, antibiotics have to be administered at the effective dosage and long enough to ensure elimination of bacteria [5]. As a consequence of inappropriate use of an antibiotic, bacteria, such as *Aeromonas hydrophila* [1] and *Aeromonas salmonica* [9], can develop resistance to this antibiotic which is transmitted to the next generations. Therefore, the systematic use of antibiotics did not appear sustainable 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^{-1} did not significantly differ from the control. Therefore, the higher dose of 2.5 mg·L^{-1} 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^{-1}, 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^{-1} formalin, while it remained quite high with the lower doses (*figure 2*). De Kinkelin [5] recommended treatments at a dose of 25–40 mg·L^{-1} of formalin during 24–48 h in closed water, although the corresponding species and age of fish were not given. It is thus possible that a 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', which gave similar or even lower survival rates than in the control and 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 a dosage not exceeding 0.1 mg·L^{-1} for a maximum period of 1 h [12]. De Kinkelin [5] recommended a dosage of 0.1 mg·L^{-1} of MGO for continuous treatment in closed water. Bastiawan [2] showed that *Saprolegnia* sp. were sensitive to MGO at doses of 1–5 mg·L^{-1}. However, a dose of 1 mg·L^{-1} 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^{-1}. 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 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 permanently containing 5 mg·L^{-1} 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 preliminary investigations and in farm productions. 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.

5. CONCLUSION

The present study indicated that bacterial disease and the female parent had more influence on the survival rate of *P. hypophthalmus* larvae than the 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^{-1}) and chloramine-T (2.5 mg·L^{-1}) in comparison to control situations without prophylactics. A clear tendency towards improved survival rates was also observed with the use of formalin (2.5 mg·L^{-1}). As it is known that the use of antibiotics may induce bacterial 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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REFERENCES

[1] Aoki T., Egusa S., Ogata Y., Watanabe T., Detection of resistance factors in fish pathogen *Aeromonas liquefaciens*, J. Gen. Microbiol. 65 (1971) 343–349.

[2] Bastiawan D., Effectiveness of malachite green oxalate to control infection of *Saprolegnia* on common carp eggs, Bull. Pen. Perik. Darat, Bogor (Indonesia) 5 (1988) 62–65.

[3] Campet M., Qualité des ovules d'un poisson chat élevé en cages flottantes dans le delta du Mekong (*Pangasius hypophthalmus*) durant le processus de maturation ovocytaire, Mémoire DAA, ENSA-Rennes, France (1997) 31 p. + annexes.

[4] Charoen Panil, Artificial breeding of *Pangasius sutchi* Fowler by hormone injection in Thailand, in: Annual report 1977 of the Tak Fisheries Station, Freshwater Fisheries Division, Bangkok, Thailand, 1977, pp. 28–30.

[5] De Kinkelin P., Michel C., Ghittino P., Précis de pathologie des poissons, Inra-OIE éd., 1985, 340 p.

[6] Hardjamulia A., Djajadiredja R., Atmawinata S., Idris D., Pembenihan jambal siam (*Pangasius sutchi*) dengan suntikan ekstraks kelenjar hipofise ikan mas (*Cyprinus carpio*), Bull. Pen. Perik Darat, Bogor (Indonesia) 1 (1981) 183–190.

[7] Hecht T., Appelbaum S., Observations on intraspecific aggression and coeval sibling cannibalism by larval and juvenile *Clarias gariepinus* (Clariidae: Pisces) under controlled conditions, J. Zool. Lond. 214 (1988) 21–44.

[8] Kabata Z., Parasites and disease of fish cultured in the tropics, Taylor & Francis Inc., London and Philadelphia, 1985, 318 p.

[9] Popoff M., Davaine Y., Facteurs de résistance transférable chez *Aeromonas salmonicida*, Ann. Inst. Pasteur 121 (1971) 337–342.

[10] Prihastowo H., Pengaruh frekuensi pemberian makanan terhadap kelangsungan hidup burayak Jambal siam (*Pangasius sutchi*, Fowler), Graduate student thesis, Institut pertanian Bogor falkultas perikanan (Indonesia), 1987, 39 p.

[11] Roberts T.R., Vidthayanon C., Systematic revision of the asian catfish family Pangasiidae with biological observations and descriptions of three new species, Proc. Acad. Natl. Sci. Phila. 143 (1991) 97–144.

[12] Schlumberger O., Mémento de pisciculture d'étang, 'Études' du Cemagref, Série ressources en eau, n°7, Cemagref-Dicova, Antony TEC et DOC, Cachan, 1993, 166 p.

[13] Slembrouck J., Élevage intensif de larves de *Pangasius bocourti* et de *Pangasius hypophthalmus* dans une écloserie du delta du Mékong (Vietnam), Mémoire Creufop-univ. Montpellier-II, Orstom-Gamet, 1996, 46 p.

[14] Yuniardi A., Pengaruh susunan makanan alami *Artemia salina* dalam kombinasi dengan *Daphnia carinata* terhadap kelangsungan hidup larva jambal siam (*Pangasius sutchi* Fowler), Graduate student thesis, Institut pertanian Bogor fakultas perikanan (Indonesia), 1987, 46 p.