EFFECT OF EGGS INCUBATION TECHNIQUE ON HATCHING RATE, HATCHING KINETIC AND SURVIVAL OF LARVAE IN THE ASIAN CATFISH *PANGASIUS HYPOPHTHALMUS* (SILURIFORMES, PANGASIIDAE)

Anang Hari Kristanto [1], Jojo Subagja [1], Jacques Slembrouck [2] and Marc Legendre [2]

(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*

Abstract

The influence of incubation system on embryos development and subsequent survival of larvae was evaluated on eggs from two *P. hypophthalmus* females. All tests were performed at a same temperature of 28.5-29.5°C, given by the water of a re-circulating system to which all incubation devices were connected. The following situations were tested: 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type incubator (stickiness of eggs being suppressed with a clay suspension); 4- Plastic basket with water from the re-circulating system (stickiness of eggs being suppressed with a clay suspension); 5- Plastic basket with water from the re-circulating system (adhesive eggs); 6- Plastic basket with mineral water (adhesive eggs). No significant difference was found in hatching rates whatever the conditions of egg incubation. The development time and hatching kinetic were very similar in all systems; at 28.5-29.5°C, the first hatched larvae were observed between 19 and 21h post-fertilisation and the duration of the hatching period lasted 6 to 8 h. However, hatching tended to occur slightly earlier in the McDonald type incubator, probably as a consequence of mechanical agitation of eggs. Up to the age of 4 days, no significant difference was found in the survival of larvae as a function of the type of incubator from which they were issued. All together, the results indicate that, when properly managed, the incubation methods can hardly be responsible for the variability of hatching rates or survival of larvae often observed in different reproduction trials or from farm to farm.

INTRODUCTION

Pangasius hypophthalmus (Sauvage, 1878) (senior synonym of *P. sutchi*; Roberts & Vidthayanon, 1991) was introduced to Indonesia in 1972. Its hormonal induced-breeding was reported for the first time in this country by Hardjamulia *et al.* (1981). Since then the fry production has been developed by fish farmers.

In fish, the incubation techniques developed depend on the specific characteristics and requirement of eggs (adhesive or not, buoyant or not) (Woynarovitch & Horvath, 1980; Legendre *et al.*, 1996). The eggs of *P. hypophthalmus* are negatively buoyant, spherical or slightly oval in shape and become sticky after contact with water. They adhere to each other or to any substrate via a sticky mucous coating covering all their surface. Due to these characteristics, several incubation systems were used for this species by fish farmers in Indonesia: egg were incubated in monolayer in stagnant or running water, or in funnels (McDonald or Zuger jars) after suppression of stickiness by covering their surface with clay particles. McDonald type incubators are round-bottomed containers in which a downward water flow allows the eggs to rotate gently in the water column. The procedures of egg incubation vary largely from one farm to another. In some production systems, using large-sized McDonald or Zuger Jars, the duration of incubation tended apparently to be shorter than in stagnant water incubation systems. However no clear conclusions could be made from these observations because they concerned eggs from different individuals incubated in different conditions of water temperature. The question of the influence of an earlier hatching time on subsequent survival of

larvae was also raised.

The aim of this study was to compare the duration of the incubation period, hatching kinetic, hatching rate and early survival of larvae after incubation of the same *P. hypophthalmus* eggs in various conditions and systems.

MATERIAL AND METHODS

Experiments on incubation of *P. hypophthalmus* eggs were carried out at the Sukamandi Station of the Research Institute for Freshwater Fisheries (West Java, Indonesia). The *P. hypophthalmus* brooders used in these experiments were 4-years-old and 3.0 to 4.0 kg individual body weight. The procedures of induced breeding, gametes management and artificial fertilisation of eggs corresponded to those described by Legendre *et al.* (1999). Oocyte maturation and ovulation were induced with two successive Ovaprim® injections of 0.3 $ml.kg^{-1}$ female BW and 0.6 $ml.kg^{-1}$ given at 8 h interval. The males received a single Ovaprim® injection of 0.3-0.4 $ml.kg^{-1}$ applied at the moment of first injection of females.

In a **first experiment**, ova from two different females were stripped after induced ovulation and immediately fertilised with sperm pooled from 4 different males. The sperm was diluted directly in a 0.9% NaCl solution (dilution rate of 1/5) at stripping, then preserved at 5°C before use. For each female, batches of 200-300 eggs were fertilised with 0.2 ml of diluted sperm. Spermatozoa activation was obtained by addition of 10 ml freshwater. After 1 min of gentle stirring, eggs were rinsed to remove excess milt and transferred for incubation in the different situations tested. In some treatments, clay was used to suppress egg stickiness. In this case, after 1 min of gametes contact as indicated above, one spoon of clay suspension was added to the fertilisation medium for one supplementary minute of gentle stirring, before rinsing and transferring eggs to incubators.

Six incubation treatments, involving mini-incubators of 0.3-0.5 L each, were tested (3 replications per treatment) on eggs from the two different females. As all incubators were connected, immersed or placed to float in a same re-circulating water system, the temperature during incubation was strictly equivalent in all treatments (28.5-29.5°C). The incubation methods tested were the followings:

1. Happa made of fine mesh net placed in the tanks of the re-circulating system (adhesive eggs, no agitation of eggs, unrestricted water exchange).
2. Floating screen net placed in the tanks of the re-circulating system (adhesive eggs, no agitation of eggs, water exchange restricted to bottom).
3. McDonald type incubator connected to the re-circulating water system (stickiness of eggs suppressed with a clay suspension, agitation of eggs, unrestricted water exchange).
4. Plastic box filled with water from the re-circulating system and floating on it (stickiness of eggs suppressed with a clay suspension, no agitation of eggs, absence of water exchange).
5. Plastic box filled with water from the re-circulating system and floating on it (adhesive eggs, no agitation of eggs, absence of water exchange).
6. Plastic box filled with spring water and placed to float on water of the re-circulating system (adhesive eggs, no agitation of eggs, absence of water exchange).

All together, 36 groups of eggs were followed (6 treatments x 3 replicates x 2 females). The water quality in the different incubation systems was followed regularly during the experiment, pH varied between 7.1 to 8.5 and dissolved oxygen was in all cases higher than 5 $mg.L^{-1}$. Ammonia and nitrite concentrations were determined using Aquaquant® kits (Merck 14423, 14424) and ranged between 0,2 to 0,4 $mg.L^{-1}$, and between 0,012 to 0,05 $mg.L^{-1}$, respectively.

Hatching kinetics were followed up on two batches of eggs per female and per incubation treatment. In each situation, between the moment at which the first hatching was observed and the end of the hatching period, new hatched larvae were counted every hour, removed from the incubator and placed in a separate recipient.

After hatching has been completed in each group of eggs, all hatched larvae were counted and hatching percentages were determined from the initial number of ova used. For each female, the weight of one ova was determined by weighing about 0.5 g of ova collected after stripping and counting them after fixation in formalin 5% (two replications per female). Individual weight of ova was 0.64 mg in one female and 0.69 mg in the other. From these data, the number of ova used in each incubation trial was estimated by weighing them ($p \pm 0.1$ mg) before fertilisation.

In order to test for a possible effect of incubation method on subsequent survival of larvae, three replicated groups of 30 larvae issued from the incubation treatments n°1, 2, 3, and 5 were followed up to 4-days of age. Each group of 30 larvae was reared in spring water in a 300 ml plastic container and fed in excess with *Artemia* nauplii starting from 36 hours after hatching. 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 four times per day and dead larvae were removed and counted at the same time. On the last day of experiment (day 4), all the remaining larvae were individually counted for calculation of actual survival rate.

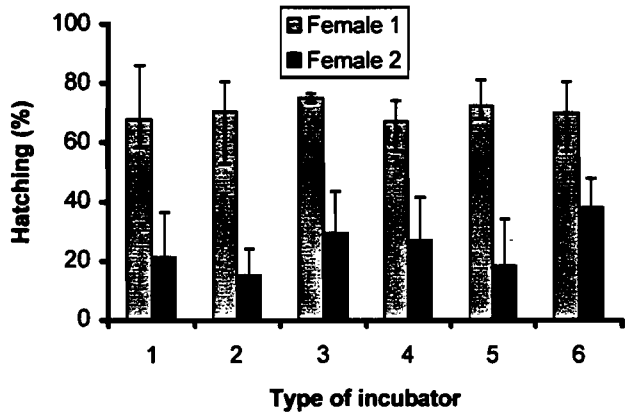
The hatching percentages and the survival rates of 4-days-old larvae obtained as a function of the method used for egg incubation were compared using two way ANOVA (incubation treatment x female). When necessary, angular transformation of data was carried out in order to stabilise the residual variance.

A **complementary experiment** was carried out to in conditions closer to those generally observed in *Pangasius* hatcheries. It aimed also to assess more accurately the possible effect of egg agitation on the duration of the incubation period. For this, using the eggs of two supplementary females, three different situations were compared: incubation in happas (60 x 60 x 80 cm, no egg agitation), incubation in mini-McDonald type incubators (0.5 L in volume, egg maintained in movement with a water flow of 15 $mL.s^{-1}$ as in first experiment) and incubation in bigger McDonald type incubators (10 L in volume, egg maintained in movement with a water flow of 45 $mL.s^{-1}$). About 200-300 eggs were placed in the mini-McDonald incubators while the number of eggs was about 50 times greater in the happas and big McDonald incubators. Each treatment was tested with 3 replications per female. The procedures of induced breeding and egg fertilisation were equivalent to those described for the first experiment. For incubation carried out in McDonald type incubators, clay was used to suppress egg stickiness as previously indicated. All the incubators were implemented or connected in a same re-circulating water system, allowing a same water temperature (28.5–29.5°C) in all situations.

RESULTS AND DISCUSSION

Hatching rates

The hatching rates obtained as a function of the different situations and systems of egg incubation tested are presented in Figure 1 for the two females used in the first experiment. A clear difference was found in the egg quality of the two females, mean hatching rates ranging from 67 to 75% and from 15 to 38% in female 1 and 2, respectively. By contrast, hatching rates did not significantly differ between treatments. Thus limitation of water exchange, treatment with clay to avoid stickiness or gentle agitation of eggs did not affect embryos survival in the conditions applied in this study.



Vertical bars indicate range between replicates.

Figure 1: Effects of the type of incubator used on the hatching rate of eggs from two *P. hypophthalmus* females. The conditions of egg incubation were the followings : 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type (stickiness of eggs being suppressed with a clay suspension); 4- Plastic box with water from the re-circulating system (stickiness of eggs being suppressed with a clay suspension); 5- Plastic box with water from the re-circulating system (adhesive eggs); 6- Plastic box with mineral water (adhesive eggs).

In the supplementary experiment, in which larger quantities of eggs were involved, fungus (*Saprolegnia*) started to develop on the eggs a few hours before hatching. However this fungal attack was much stronger on the eggs incubated in happas than on the ones covered with clay particles and slightly agitated in the McDonald type incubators. Fungus development remained very limited in all situations tested in the first experiment.

Development time and hatching kinetic

The development time of fish eggs is temperature dependent (Woynarovitch & Horvath, 1980; Mac Intosh & Little, 1995). It was therefore of prime importance to compare the development time of *P. hypophthalmus* eggs placed in the different incubation systems at a same temperature. This was done by implementing or connecting all the incubation systems tested in a same re-circulating water system.

The hatching kinetic was followed on two replicated groups of egg from two different females, in each of the various incubation situations tested (see Fig. 2 for examples). From these observations, the time lapse between fertilisation and first hatching or 50% hatching, and the total duration of hatching (between the first and last eggs to hatch) were determined. These data are presented in Table 1 for the eggs of the two females used in the first experiment. In all cases, the development time of *P. hypophthalmus* eggs was very similar, the curves of hatching kinetic being superposed. At 28.5-29.5°C, the first hatched larvae were observed between 19 and 21 h post-fertilisation and the duration of the hatching period lasted 6 to 8 h. The suppression of egg stickiness by covering them with clay particles did not affect embryos development nor development time. The only detected difference was for the eggs incubated in the McDonald type incubator which hatched 1 or 2 h earlier than in other incubation systems, in one female but not in the other (Fig. 2, Table 1).

In the complementary experiment carried with the eggs of two other females, the development time was also faster by about 2 h in the McDonald type incubators than in happas. This earlier hatching in

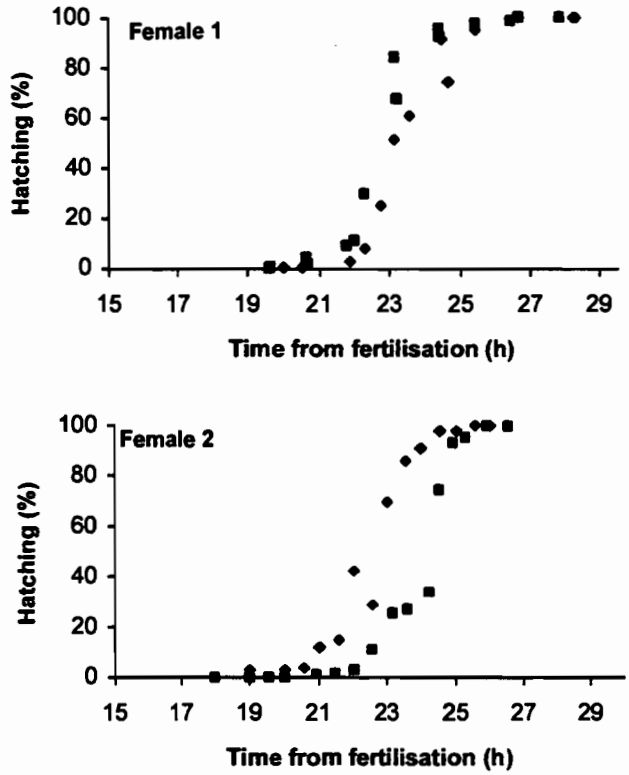


Figure 2: Hatching kinetic of eggs from two *P. hypophthalmus* females incubated in McDonald type incubator (♦) or in plastic box with stagnant water (■) (temperature: 28.5-29.5°C). Observations plotted from two replicates per treatment and female.

funnels was assumed to be a consequence of the slight mechanical agitation of eggs. By contrast, no difference was found between the "mini" and the bigger McDonald incubators. Therefore the mini system could be considered as representative of the bigger incubators generally used in hatcheries. Rana (1986) reported large variations in the embryos

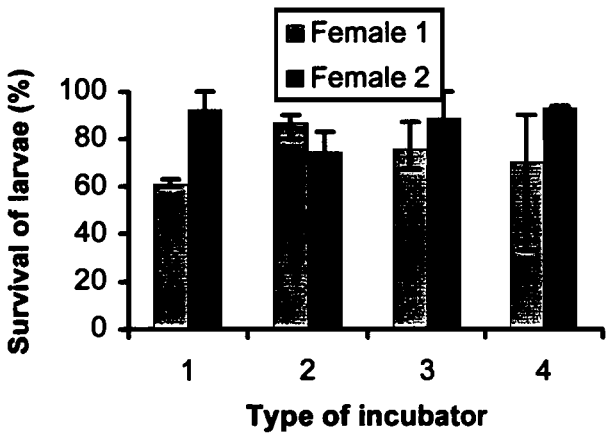
Type of incubator	Female 1			Female 2		
	First hatching (h post-fertilisation)	50% hatching (h post-fertilisation)	Total duration of hatching period (h)	First hatching (h post-fertilisation)	50% hatching (h post-fertilisation)	Total duration of hatching period (h)
1	20	24	7	21	24	6
2	20	24	8	20	24	8
3	20	23	8	19	23	7
4	20	24	8	21	25	8
5	20	23	6	21	24	6
6	20	23	8	21	24	6

Table 1: Development time and duration of hatching period in eggs of two *P. hypophthalmus* females incubated in the following conditions and systems: 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type (stickiness of eggs being suppressed with a clay suspension); 4- Plastic box with water from the re-circulating system (stickiness of eggs being suppressed with a clay suspension); 5- Plastic box with water from the re-circulating system (adhesive eggs); 6- Plastic box with mineral water (adhesive eggs). (Observations from two replicates per treatment and female).

survival and development time of *Oreochromis niloticus* and *O. mossambicus* eggs as a function of the shape of the incubator used. The development time of these species was slower in round-bottomed containers than in conical ones (90-102 h compared with 48-72 h).

Survival rate of larvae

After 4 days of rearing, the mean survival rate of *P. hypophthalmus* larvae issued from the different incubation systems tested in the first experiment varied between 61 and 87% in one female and between 74 and 93% in the other (Figure 3).



Vertical bars indicate range between replicates.

Figure 3: Survival rate of *P. hypophthalmus* larvae obtained from eggs of two different females incubated in the following conditions : 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type (stickiness of eggs being suppressed with a clay suspension); 4- Plastic box with water from the re-circulating system (adhesive eggs).

No significant effect of incubation techniques was found on the subsequent survival of larvae up to 4-days of age.

CONCLUSION

This study confirmed that, at a same water temperature, hatching of *P. hypophthalmus* eggs tented to occur slightly earlier (1 to 2 hours) in the McDonald type incubators than in all other incubation systems without mechanical agitation of the eggs. However, this slight shortening of the incubation period was not associated to a lowering in hatching rates or subsequent survival of larvae. All together, the results indicate that, when properly managed, the incubation methods can hardly be responsible for the variability of hatching rates or survival of larvae often observed in different reproduction trials or from farm to farm.

REFERENCES

Hardjamulia A., Djajadiredja R., Atmawinata S. & Idris D. (1981) Pembenihan jambal siam (*Pangasius sutchi*) dengan suntikan ekstraks kelenjar hipofise ikan mas (*cyprinus carpio*). *Bull. Pen. Perik Darat.*, **1**, 183-190.

Legendre M., Linhart O. & Billard R. (1996). Spawning and management of gametes, fertilized eggs and embryos in Siluroidei. *In* M. Legendre & J.P. Proteau (eds), The biology and culture of catfishes. *Aquat. Living Resour.*, **9**, Hors série, 59-80.

Legendre M., Slembrouck J., Subadja J. & Kristanto A.H. (1999) Effects of varying latency period on the *in vivo* survival of ova after Ovaprim- and hCG-induced ovulation in the Asian catfish *Pangasius hypophthalmus* (Siluriformes, Pangasiidae). *Proceedings of the mid-term workshop of the Catfish Asia project*, this volume.

Mac Intosh D.J. & Little D.C. (1995) Nile tilapia (*Oreochromis niloticus*). p. 277-320, *In* Bromage N.R., Roberts R.J., (eds), *Broodstock management and egg and larval quality.* Blackwell Science, 424 p.

Rana K.J. (1986) An evaluation of two types of containers for the artificial incubation of *Oreochromis* eggs. *Aquaculture and Fisheries Management*, **17**, 139-145.

Roberts T.R. & Vidthayanon C. (1991) Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **143**, 97-144.

Woynarovich E. & Horvath L. (1980) The artificial propagation of warm-water finfishes - A manuel for extension. *FAO Fish. Techni. Pap.*, **201**, 183 p.

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

Proceedings of the mid-term workshop of the
"Catfish Asia Project"
Cantho, Vietnam, 11-15 May 1998

Edited by :
Marc LEGENDRE
Antoine PARISELLE

CONTENTS

PATHOLOGY