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

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Marc LEGENDRE
Antoine PARISELLE

EGG QUALITY OF AN ASIAN CATFISH OF THE MEKONG RIVER (*PANGASIUS HYPOPHTHALMUS*) DURING THE PROCESS OF MATURATION INDUCED BY HCG INJECTIONS

Marc Campet [(1)], Philippe Cacot [(1)], Jérôme Lazard [(1)],Tran Quoc Dan [(2)], Do Thanh Muon [(2)] and Pham Thanh Liem [(3)]

(1) Aquaculture Research Unit, CIRAD-EMVT, GAMET, BP 5095, 34033 Montpellier Cedex 1, France
(2) AGIFISH Co, Xi Nhiep Chanh Huong, Chau Doc, An Giang Province (Vietnam).
(3) UCT, Faculty of Fisheries, Can Tho (Vietnam).

Abstract

In captive *Pangasius hypophthalmus*, oocyte maturation and ovulation are induced by hormonal injection following a given protocol: preparatory injections (500 UI.kg^{-1}) and decisive injection (2000 UI.kg^{-1}).

The aim of this work is to specify the timing of ovulation and the effects of varying latency period on the quality of ova. Oocytes and ova were collected by intra-ovarian biopsy and hand stripping. Ova quality was estimated by fertilisation rate, hatching rate and proportion of deformed larvae. Ovulation occurred as a synchronous process 8h30 to 9h30 after the last hormonal injection. The first ova obtained (8h30 after injection) were of good quality (85% hatching). At this time, the ovulation rate was 100%.

Three hours after ovulation, ageing of ova started to occur: the proportion of deformed larvae increased (24%) and hatching rate collapsed (35%).

In *P. hypophthalmus*, the optimised latency period was found to be 8h30 and corresponded approximately to the completion of ovulation.

INTRODUCTION

Pangasius hypophthalmus, an Asian catfish from *Pangasiidae* family, is widely cultivated in South Vietnam (Peignen, 1993). A method for artificial propagation of this specie has been recently set up.

The latency period defined as the delay between the last hormonal injection and ova collection is an essential matter in reference to ova quality (Bromage, 1995). Ovulated eggs of oviparous Teleost become overripe if retained in the body cavity and these eggs show a progressive reduction in viability for many species. Early or late collection of gametes can lead to low hatching rate and large proportion of deformed larvae caused by low rate of ovulated oocytes or overripe ova (Legendre & Otémé, 1995). After ovulation the latency period leading to eggs of optimal quality is specific to each specie. This period vary from one hour to 4-6 days (Table 1).

In captive females of *Pangasius hypophthalmus*, final oocyte maturation and ovulation do not occur spontaneously and are hormonally induced with human chorionic gonadotropin (hCG).

The aim of this investigation was to assess the latency period to obtain the best egg quality in terms of hatching rate and proportion of normal larvae.

MATERIALS AND METHODS

P. hypophthalmus were fished in the wild (0.5-200 g) and reared in floating cages during 7 years in order to provide brood fishes for artificial propagation. Brood stock, described in Table 2, consisting of 78 females and 22 males was held in floating cages (50 m^3) on the Mekong River at density of one fish per m^3 (t°= 24-34°C).

The brood-stock was fed once a day with a 40-45% protein (dry matter) dry pelleted feed distributed at a rate of 1.5% of fish biomass (Table 3). Intra-ovarian canulation and binocular lens measure of the oocytes diameter was used to assess the maturity stage of females. Fishes showing more than 60% of oocytes with a diameter of at least 0.9 mm were selected and individually transfer to 1.5 m^3 tank with 10.4 l.mn^{-1} water exchange.

Oocyte maturation and ovulation were induced with intra-muscular hCG injections as it is described in Figure 1.

Males received a single intra-muscular injection of 2000 UI.kg^{-1} of body weight (Eeckhoutte, 1996). The sperm was obtained by stripping and kept in a refrigerator at 2–5 °C after dilution (dilution rate 1:2) in 9 g.l^{-1} NaCl solution adjusted at pH 7 with basic TRIS buffer. The sperm was pooled from three males for fertilising ova. Sperm motility was assessed every hour (Sanchez-Rodriguez & Billard, 1977) to insure optimal conditions.

Oocytes maturation and quality was assessed during a period of 9 hours which starts five hours after the last hormonal injection. Two sampling method were used on this purpose.

Species	Time (h.)	References
Oreochromis niloticus	1h	Rana (Unpublished data)
Prochilodus platensis	1h	Fortuny *et al.* (1988)
Roccus saxatalis	1h	Stevens (1966)
Carassius auratus	2-3h	Formacion & Lam in Formacion (1991)
Macculochella peeli	2-3h	Rowland (1988)
Misgurnus anguilicaudatus	3-8h	Suzuki (1975)
Hippoglossus hippoglossus	4-6h	Kjorsvik *et al.* (1990), Bromage *et al.* (1994), Holmefjord (1991), Norberg *et al.* (1991)
Rhamdia sapo	5-9h	Espinach Ros *et al.* (1984)
Gadus morhua	9-12h	Kjorsvik & Lonning (1983)
Clarias macrocephalus	10h	Mollah & Tan (1983)
Scophthalmus maximus	10-20h	McEvoy (1984), Howell & Scott (1989)
Plecoglossus altivelis	1-2 days	Hirose *et al.* (1979)
Limanda yokahamae	2-3 days	Hirose *et al.* (1979)
Salvelinus alpinus	5 days	Gillet (1991)
Oncorhynchus mykiss	4-6 days	Springate *et al.* (1984)
Clupea harengus	14 days	Hay (1986)
Oncorhynchus kisutch	20 days	Fitpatrick *et al.* (1987)

Table 1: Latency period for different species (from Bromage, 1995).

	Stocking density in cage (kg.m^{-3})	Feeding rate (%)	Weight (kg)	Length (cm)	Body condition index
Male	7.2	1.5	5.8 ± 1.2 [4.0-7.1]	70.9 ± 4.1 [66.0-77.0]	1.3 ± 0.1 [1.2-1.5]
Female			5.5 ± 0.9 [4.5-6.9]	71.4 ± 3.4 [68.5-76.5]	1.5 ± 0.1 [1.4-1.8]

Table 2: Brood fishes characteristics.

Raw material		Nutritional value [a]	
Blood meal	17.6%	Moisture	10.9%
Soya oil	3.0%	Protein	50.7%
Vitamin	0.8%	Lipid	7.2%
Broken rice	9.8%	Carbohydrates	22.6%
Fish meal	35.4%	Fibre	2.7%
Soya meal	33.4%	Hash	16.8%

[a] percent are expressed from dry material except for moisture which is expresses from total material.

Table 3: Composition of the diet and nutritional value.

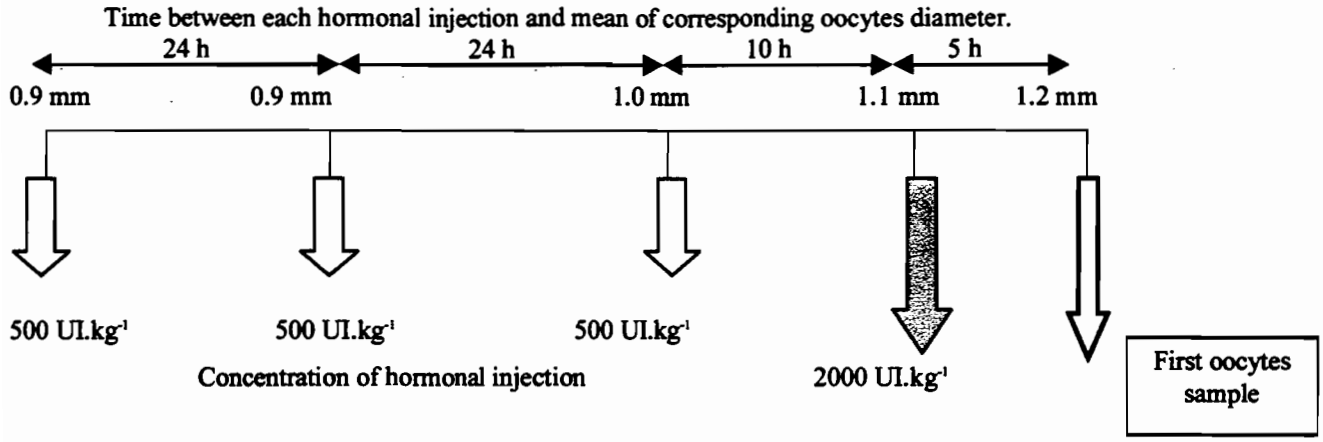


Figure 1: Schematic representation of the hormonal treatment for *P. hypophthalmus* females.

Manual removing (stripping) of eggs which is only efficient once ovulation has occurred and gonadal biopsie which can be used at any time.

Samples of 40 gametes were fixed in Serra's solution (60% ethanol, 30% formalin, and 10% acetic acid, in volume) and were observed using a binocular lens to determine the position of the germinal vesicle (GV). The diameter of 30-50 gametes was also measured under binocular lens. Ovulation rate was evaluated by determining the proportion of ova sticking to their support after been wet with mineral water.

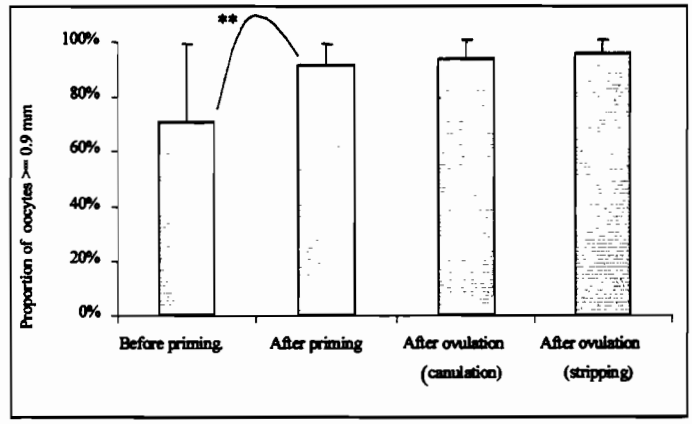
Egg quality assessment was based on fertilisation rate, hatching rate and proportion of deformed larvae obtained from batches of 200-300 eggs. Eggs were fertilised with diluted sperm (1%) in plastic box leading to a ratio of 2 10^6 spermatozoa for one ova. Activation was obtained by addition of 6 ml of fresh water. After 1 mn of gentle stirring, eggs were washed with clean water and placed in glass tanks (t° = 29-30°C) with closed water system for incubation (19–22h).

Fertilisation and hatching rate were respectively assessed 8h and 24h after fertilisation. Proportion of deformed larvae was evaluated when 100% hatching occurred.

RESULTS

The hormonal treatment is composed of two mains steps. First the preliminary treatment composed of two injections of 500 UI.kg^{-1}at 24h interval. Those injections induce the growth of oocyte diameter (Fig. 2). Thus the average oocyte diameter increase from 0.9 mm to 1 mm. This increase concerns oocytes from 0.5 mm and allowed them to be receptive to the ovulation treatment. The second step of the treatment (500 and 2000 UI.kg^{-1} with an interval of 10h) induces the last phenomena of maturation and oocytes diameter reach the size of mature ova (1.2 mm). The germinal vesicle is in central position for 100% of oocytes observed at the time of the last hormonal injection and migrate towards the periphery before breaking down (GVBD). Five hours after the last hormonal injection the GVBD has occurred for 90% of the case.

The ovulation process is achieved 11h after the last hormonal injection.

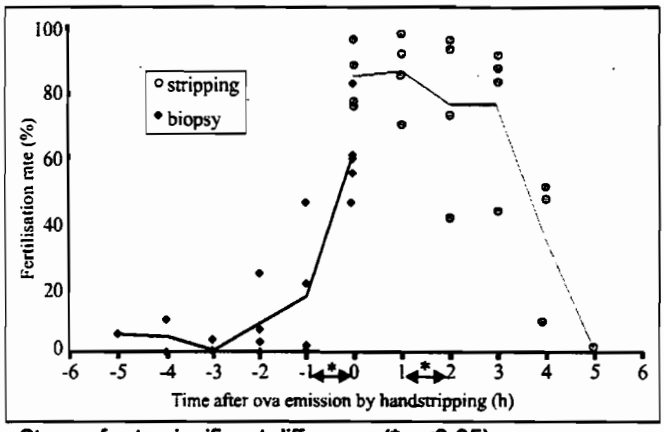


Stars refer to significant difference (** p<0,01).

Figure 2: Influence of hormonal treatment on oocyte diameter.

All the females hormonally induced had a positive response and spawn under artificial conditions.

The optimal quality of ova is obtained as soon as ovulation has occurred, fertilisation rate remains greater than 90% and proportion of deformed larvae lower than 10% for two hours (Fig. 3). The absolute hatching rate (hatching eggs/total eggs) exceeds 80% from 8h30 to 9h30 after the last hormonal injection which underlines the good embryonic development.

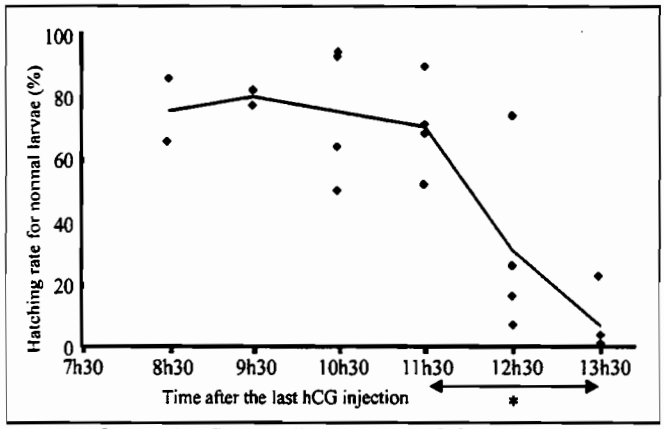


Stars refer to significant difference (* p<0,05).

Figure 3: Influence of the latency period on the fertilisation rate of *Pangasius hypophthalmus*.

After a latency period of 11h30 the viability of eggs start to be affected. Hatching rate and proportion of normal larvae are the main indicator of this decline. Thus the hatching rate of normal larvae dropped down to 30% 12h30 after the last hormonal injection (Fig. 4). One hour later 50% of the larvae hatched are deformed (Fig. 5).

Quality of ova obtained from stripping was always higher than for ova collected by gonadal biopsy (Fig. 6) however the evolution followed the same kinetic for the two sampling methods.

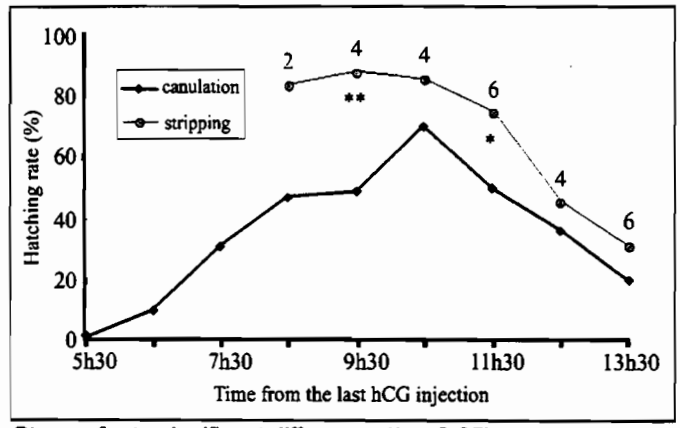


Stars refer to significant difference (* p<0,05).

Figure 4: Influence of the latency period on the production of normal larvae.

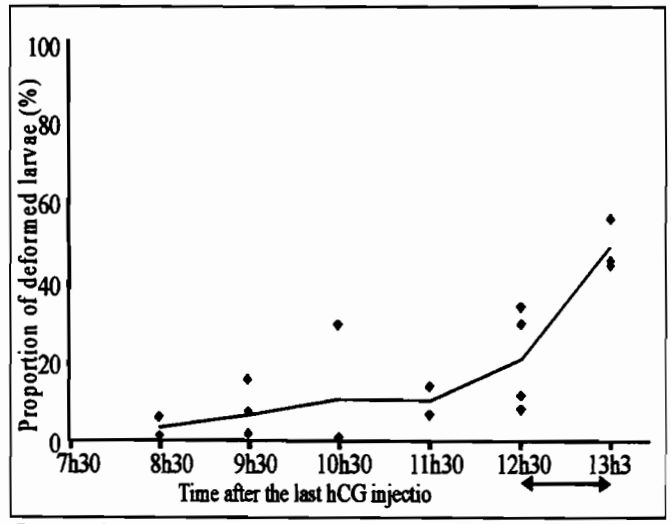
CONCLUSION

In summary the results of this investigation show that ovulation takes place from 8h30 to 12h30 hours after the last hormonal injection. This process is rapidly followed by emission of ova characterised by high hatching rate (hatching rate of 82% between 8h30 to 12h30 of latency) and low proportion of deformed larvae. Then the quality of ova decrease with an increased of latency period



Stars refer to significant difference (* p<0,05)..

Figure 5: Influence of the latency period on the proportion of deformed larvae.



Stars refer to significant difference (* p<0,05, ** P<0,01). Canulation was used on a group of 12 females each hours. The number of females sampled by stripping is mentioned above each point.

Figure 6: Influence of the sampling method on the absolute hatching rate. Ova are collected by intraovarian canulation or stripping.

and fertility declines drastically 12h30 after the last hormonal injection. Therefore eggs should be stripped as soon as ovulation has occurred which in this study correspond to a latency period ranging from 8h30 to 12h30. Further study should be conducted to determine origin of this variation as ovulation appears as a major factor to produce eggs of good quality.

Gonadal biopsy has always been providing lower quality of eggs than stripping. However this sampling method remains the only way to assess the sexual maturity level of fish as long as ovulation has not occurred. Thus results from this method should be considered with special attention.

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