

Effect of varying latency period on the quantity and quality of ova after hCG-induced ovulation in the African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae)

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Accepted June 8, 1995.

Legendre M., Z. Otémé. *Aquat. Living Resour.*, 1995, 8, 309-316.

Abstract

In captive *Heterobranchus longifilis* females, oocytes maturation and ovulation are induced by a single injection of human chorionic gonadotropin (hCG) at a dose of 1.5 I.U./g body weight. In the present study, three experiments were performed in order to specify the timing of ovulation and the effects of varying latency period on the quantity and quality of the collected ova. The ova quality was estimated by hatching percentage and proportion of deformed larvae obtained after artificial fertilization. The results showed that, in an individual female, ovulation of postvitellogenic oocytes is not synchronous and takes 3 to 4 hours to be completed, between 7-8 h and 11 h after hCG injection at 30°C. The quantity of ova that could be collected by handstripping increased with the latency period, from 18 000 ± 12 000 to 72 000 ± 29 000 ova per female at 8 h and 11 h after injection, respectively. However, the first ova obtained (8 h after injection) were of good quality (94% hatching) and showed no signs of incomplete final maturation. Eleven hours after injection, the percentages of ovulated oocytes within the whole ovarian population of postvitellogenic oocytes were high in all the females, being generally around 90%. After ovulation, aging of ova occurred rapidly: the proportion of deformed larvae increased significantly (from 4 to 20%) 2 h after completion of ovulation and, 4 h post-ovulation, hatching percentage dropped from 92 to 36%. In *H. longifilis*, the optimal latency period can be defined as the time, from injection to stripping, allowing the best compromise between quantity and quality of ova released. This latency period was found to be 11 h following hCG injection at 30°C, and corresponds approximately to the completion of ovulation.

Keywords: *Heterobranchus longifilis*, Siluriformes, aquaculture, ovulation, fecundity, overripening.

Influence du temps de latence après induction hormonale de l'ovulation sur la quantité et la qualité des ovules d'un silure africain, Heterobranchus longifilis (Teleostei, Clariidae).

Résumé

Chez les femelles de *Heterobranchus longifilis* maintenues en captivité, la maturation ovocytaire et l'ovulation sont provoquées par injection de gonadotropine chorionique humaine (hCG) à la dose de 1,5 U.I./g de poids vif de femelle. Trois expériences complémentaires ont été réalisées dans le but de préciser la chronologie de l'ovulation et d'évaluer l'incidence d'une variation du temps de latence sur la quantité et la qualité des ovules effectivement récoltés. La qualité des ovules a été évaluée par le pourcentage d'éclosion et par la proportion de larves déformées obtenues après fécondation artificielle.

Chez une même femelle, l'ovulation n'est pas synchrone mais s'étale sur une période de 3 à 4 h, entre 7-8 h et 11 h après injection de hCG à 30°C. La quantité d'ovules pouvant être récoltés par massage abdominal des femelles augmente avec le temps de latence, de 18 000 ± 12 000 à 72 000 ± 29 000 ovules par femelles à 8 h et 11 h après injection, respectivement. Cependant, les premiers ovules obtenus, dès 8 h après l'injection, sont de bonne qualité (94 % d'éclosion) et aucun signe de maturation incomplète

n'a été détecté. Onze heures après injection, la proportion des ovocytes ovulés par rapport au total des ovocytes post-vitellogéniques est élevée chez toutes les femelles (environ 90%). Après l'ovulation, les ovules s'engagent rapidement dans un processus de vieillissement: 2 h après l'achèvement de l'ovulation, la proportion de larves déformées augmente significativement (de 4 à 20%) et, après 4 h, les pourcentages d'éclosion chutent fortement (de 92 à 36%).

Chez *H. longifilis*, le temps de latence optimal peut être défini comme le délai entre l'injection et la collecte des ovules, permettant le meilleur compromis entre la quantité et la qualité des ovules récoltés. Après injection de hCG, ce délai est de 11 h à 30°C et correspond approximativement à l'achèvement de l'ovulation.

Mots-clés : *Heterobranchus longifilis*, Siluriformes, aquaculture, ovulation, fécondité, qualité des ovules.

INTRODUCTION

Hormonal induction of oocyte maturation and ovulation with *in vitro* fertilization of eggs are current practices in the controlled reproduction of numerous species used in aquaculture. The latency period, defined as the delay between hormonal injection and ova collection, is a key factor in the success of this type of operation (Woynarovitch and Horvath, 1980; Harvey and Carolsfeld, 1993).

Early collection of gametes can lead to low hatching rates and a large proportion of deformed larvae as is the case of *Clarias gariepinus* (Richter and Van den Hurk, 1982). Springate *et al.* (1984) also reported that in rainbow trout maximum fertilization rates are obtained four days after ovulation.

Inversely, delayed collection leads to overripening of ova which can result in low fertilization rates, increase in the number of deformed embryos, or increased mortality rates for embryos and larvae (Sakai *et al.*, 1975; Springate *et al.*, 1984). After ovulation, the maximum lapse of time assuring high fertilization rates and normal egg development varies according to species. This time lapse range from 6-30 days in the rainbow trout (Sakai *et al.*, 1975; Bry, 1981) to a few hours in the majority of teleosts studied, including several species of Siluriformes (Woynarovitch and Horvath, 1980; Hogendoorn and Vismans, 1980; Mollah and Tan, 1983; Espinach-Ros *et al.*, 1984; Fortuny *et al.*, 1988).

Besides their quality, the quantity of ova collected is also likely to vary according to the latency period (Escaffre *et al.*, 1977). This quantitative aspect, little discussed in the literature, raises the problem of possible asynchronous ovulation (McEvoy, 1984).

Heterobranchus longifilis Valenciennes, 1840, is a catfish of high potential interest for African aquaculture (Legendre 1983, 1992). In captive females of this species, oocytes maturation and ovulation do not occur spontaneously and are induced by injection of human chorionic gonadotropin (hCG). This ovulating agent was chosen because its activity is well standardized; it is also easy to preserve in tropical climates and has the advantage of being readily available in most developing countries. It proved to be highly efficient in *H. longifilis*, a single hCG injection induced ovulation in 100% of the females ($N > 200$)

when applied at between 1.0 and 2.5 I.U./g body wt. (Legendre 1986, 1992 and unpubl. data). The latency period between injection and ova collection vary little as a function of the hormone doses given but is dependent upon water temperature. For collection of good quality ova, Legendre (1992) proposed latency periods of 11 h at a water temperature of 30°C, 12 h at 29°C, 13.5 h at 28°C, 15 h at 27°C and 17 h at 26°C.

However, in practice, different constraints can lead the fish culturist to advance or delay ova collection. Therefore, the aim of this investigation was to evaluate the effect of varying latency periods on the yield of ova and to define the time interval during which high hatching rates with high percentages of normal larvae can be expected in *H. longifilis*. The results obtained are discussed in relation to the chronology of oocyte maturation and ovulation observed after hCG injection.

MATERIALS AND METHODS

Fish origin and maintenance

The *H. longifilis* broodstock used, ranging from 3 to 4 years old and sexually mature at one year of age, came from the Layo aquaculture research station, located 40 km west of Abidjan (Côte d'Ivoire). These fish were descended from wild stocks that spontaneously colonized the fish ponds of the station in 1982 (Legendre, 1983). The broodstock was raised in lagoon enclosures and fed 6 days a week with a 35% protein pelleted feed, distributed at a daily rate of 1-2% of fish biomass.

General experimental conditions

Oocyte maturation and ovulation were induced with a single intramuscular hCG injection of 1.5 I.U/g body weight (Legendre, 1986). After ovulation, with one exception (experiment 2), the ova were collected by stripping the females. Approximately 400 ova were weighed ($P \pm 0.1$ mg) to determine the mean weight of one ovum and the females' fecundity (number of ova harvested). Males received no hormonal treatment, the sperm was obtained by dissection of the testis and thereafter kept on ice after dilution in a 9 g.l⁻¹ NaCl solution (dilution rate 10⁻¹).

The quality of ova was evaluated from hatching rates obtained with batches of 200-300 eggs fertilized with 0.2 ml of diluted sperm; this corresponded approximately to a number of 10^5 spermatozoa for one ovum. Spermatozoa activation was obtained by addition of 6 ml freshwater. After 1 min. of gentle stirring, eggs were rinsed to remove excess milt and transferred for incubation in a plastic box containing 300 ml of standing water at ambient temperature (28-30°C). After hatching, the proportions of normal and deformed larvae were determined for each batch of eggs by observations and counting over an illuminated table.

Three complementary experiments were conducted to evaluate the influence of a variation in the latency period on the quantity and quality of ova obtained. In each experiment, the latency period between injection and ova collection determined as a function of water temperature by Legendre (1992) was used as a reference. During the latency period, the maintenance temperature of brooders was recorded every 2-3 h. The amplitude of thermal variations was generally less than 2°C. The first and third experiment were conducted during the major dry season (December-January) and the second was conducted at the beginning of the major rainy season (June).

Experiment 1: Effects of advanced or delayed ova collection

A group of 6 females weighing from 2.7 to 4.7 kg was used. Samples of approximately 30 follicles were taken from each female by intraovarian canulation before hCG injection, and then every 2 h up to 8 h following injection. After being fixed in Serra's solution (60% ethanol, 30% formalin, 10% acetic acid, by volume), these follicles were observed using a binocular microscope to determine the position of the germinal vesicle and the advancement of oocyte maturation.

In order to test the influence of advanced or delayed collection on the quantity and quality of ova, each female was stripped off the maximum of its ova successively at 8, 11, and 15 h after injection. As the average maintenance temperature of brooders was 30°C, the 11 h delay corresponded to the reference latency period. At each stripping, the number of ova collected was estimated by weighing (± 0.1 g), and their quality was evaluated by hatching rates obtained after fertilization of two subsamples of eggs from each female. The same sperm, pooled from two males killed one hour before starting the experiment, was used to fertilize all the ova collected.

Experiment 2: Ovulation rate

The aim of this experiment was to evaluate the ovulation rate after the reference latency period for females treated with hCG.

A new group of 6 females weighing between 3.4 and 5.6 kg was used. After a latency time of 11 h (reference latency period at 30°C), all the females were killed, their gonads removed, and the ova collected by direct stripping of the ovaries. Ova and remnant ovaries were weighed (nearest 0.1 g). The number of ova collected and their quality were evaluated as in experiment 1. For each female, the number of unovulated postvitellogenic oocytes (green eggs of diameter greater than 1 mm; Legendre, 1986) still present in the ovarian tissues after stripping was determined from three samples taken from the anterior, median and posterior portions of the gonad. These samples were individually weighed and preserved in 4% formalin.

The ovulation rate was evaluated by determining the proportion of ovulated oocytes within the total population of postvitellogenic oocytes present in the ovaries, and by the ovulatory response (R) calculated as follows: $R = 100 \text{ (weight of ova) / (weight of ova + weight of remnant ovaries)}$.

Experiment 3: Survival of ova maintained in ovario after ovulation

The duration for ova to remain viable in the ovarian cavity after ovulation was studied for a group of 6 females ranging 3.1 to 4.0 kg body weight. Starting from the reference latency time (12 h at 29°C), and then every two hours until 20 h post-injection, a partial collection of ova (approximately 10 g per stripping) was carried out for each female. For each individual stripping, ova quality was evaluated by hatching rates obtained after fertilization of two subsamples of eggs. To avoid possible lessening of spermatozoa fertilizing ability, sperm from two groups of two males were used for the fertilization trials. The first group of males, killed two hours before first stripping of females, was used to fertilize the ova collected 12 and 14 h after injection. The sperm of the second group was used immediately and served to fertilize ova stripped from 16 to 20 h after injection. Nevertheless, *H. longifilis* sperm remains generally viable for more than 10 h when preserved and used in the conditions of the current study.

Statistical analysis

Hatching percentages and percentages of deformed larvae were compared using Student T test after angular (arc-sine) transformation of data. In experiment 2, the number of unovulated postvitellogenic oocytes per g of gonad in samples taken from the anterior, median and posterior portions of the ovaries were compared using a mixed two-way ANOVA considering position in gonad as fixed effect and female as random effect.

RESULTS

At the time of hormonal treatment, the oocyte's germinal vesicle was in a central position for all the females. After hCG injection, oocyte maturation begun rapidly. The germinal vesicle migrated to the periphery of the oocyte in 2-4 h, and after 6 h vesicle breakdown occurred in 90% of the observed oocytes (fig. 1). The first ova were observed 7-8 h after injection.

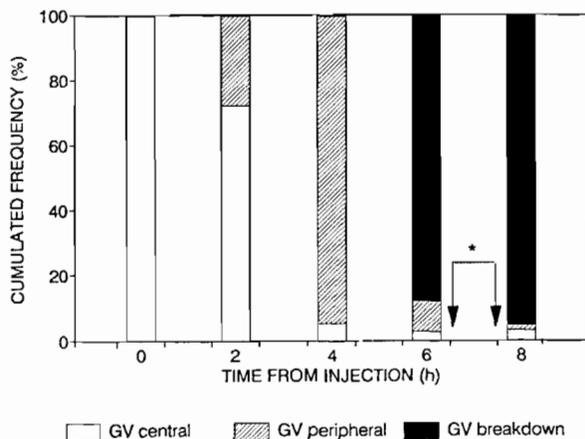


Figure 1. – Evolution of oocyte maturation after hCG injection in *H. longifilis*. Mean for six females in experiment 1 (average temperature: 30°C).

(*): observation of first ova.

GV: germinal vesicle.

Influence of advanced or delayed ova collection (table 1; fig. 2)

The total quantity of ova collected after complete stripping carried out successively at 8, 11, and 15 hour latency period in experiment 1, ranged between 11,000 and 43,000 per female kg.

The proportion of ova obtained at first stripping (8 h after injection) in the total of ova individually collected was generally low, indicating that there was partial ovulation at this time. A high individual variability

Table 1. – Total number of ova collected from six *H. longifilis* females successively stripped off the maximum of their ova after 8 h, 11 h and 15 h latency period, and percentages of ova obtained after the first (8 h) and the second stripping (8 h+11 h; cumulated data) in experiment 1 (average temperature: 30°C).

Female (No.)	Body weight (kg)	Weight of one ovum (mg)	Total No. of ova collected	Ova collected after the first stripping		Ova collected after the second stripping	
				No.	%	No.	%
1	2.7	1.3	116,000	23,000	19.8	104,000	89.6
2	4.7	1.3	50,000	26,000	52.0	31,000	62.0
3	3.1	1.3	90,000	22,000	24.4	76,000	84.4
4	4.2	1.6	86,000	29,000	33.7	65,000	75.6
5	4.4	1.3	139,000	2,000	1.4	99,000	71.2
6	3.7	1.3	71,000	3,000	4.2	55,000	77.5

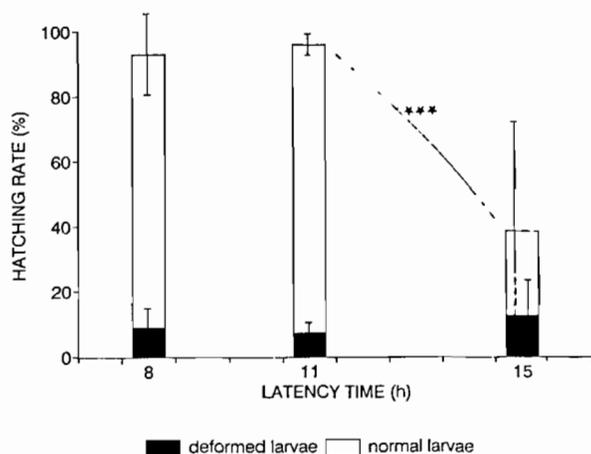


Figure 2. – Hatching rates of *H. longifilis* eggs as a function of latency period from hCG injection in experiment 1 (average temperature: 30°C).

Means for six females emptied from the maximum of their ova at each stripping.

Vertical bars refer to the confidence interval ($p=0.05$).

(1.4 to 52.0%) was observed (table 1). However, the quantity of ova collected 11 h after injection compensated for the individual variations observed at 8 h in such a way that the cumulated proportions of ova collected after the two strippings (8 h+11 h) was high for all the females ($77 \pm 10\%$, table 1). Stripping 15 h after injection yielded approximately 23% more ova on average.

The quality of ova obtained after 8 or 11 h latency period was excellent for all the females. Mean hatching rates were greater than 90% with a proportion of deformed larvae not exceeding 10% (fig. 2). By contrast, hatching rates of eggs collected 15 h after injection were greatly reduced and were, on average, about 2.5 times lower ($p < 0.001$) than hatching rates recorded from eggs collected 8 or 11 h after injection. Nevertheless, there was some variation between individuals and a hatching rate greater than 85% was recorded for one female. This indicates that the drop in mean hatching rates observed at 15 h latency time corresponded to aging of ova, and not to

Table 2. – Proportion of ovulated oocytes, ovulatory response (R), gonadosomatic index (GSI), hatching rate and proportion of deformed larvae obtained at the reference latency period (11 h at 30°C) for the six *H. longifilis* females in experiment 2. GSI=(weight of the ovary after ovulation/fish body weight) × 100.

Female (No.)	Body weight (kg)	Weight of one ovum (mg)	No. of ova collected	No. of unovulated oocytes	% of ovulated oocytes	R (%)	GSI (%)	Hatching rate (%)	Deformed larvae (%)
1	4.5	1.8	416,000	43,000	90.6	77.1	21.1	91.1	5.1
2	5.0	1.6	464,000	159,000	74.5	61.6	23.4	95.6	6.9
3	3.4	1.5	349,000	16,000	95.6	80.6	19.2	96.1	2.4
4	3.6	1.7	257,000	13,000	95.2	79.6	15.0	92.0	5.2
5	5.3	1.8	244,000	32,000	88.4	71.8	11.6	81.0	7.2
6	5.6	1.7	449,000	87,000	83.7	71.7	18.9	92.7	10.5

an alteration in fertilizing ability of spermatozoa. No relationship was found between the quantity and the quality of ova obtained within each of these different strippings.

Ovulation rate at the reference latency period (table 2)

In experiment 2, gonado-somatic index (GSI) and fecundity were high for all females. The number of ova collected after the reference latency period (11 h at 30°C) by direct stripping of the ovaries varied between 46,000 and 103,000 per female kg. The quality of ova was as good as in experiment 1, despite the absence of intermediate stripping. The mean hatching rate was high (91 ± 6%) and the percentage of deformed larvae remained low (6 ± 3%).

The numbers of unovulated postvitellogenic oocytes remaining in the ovarian tissues after ova collection were not significantly different as a function of the anterior, median, or posterior origin of the samples. Thus, the data obtained from the three samples taken from each female were pooled.

The ovulation rate was high in most females (table 2). The proportion of ovulated oocytes within the whole ovarian population of postvitellogenic oocytes was approximately 90% in all cases except for one female the percentage of which was lower (75%). The ovulatory response (R) ranged between 62 and 81% (mean = 74 ± 7%).

Survival of ova maintained in ovario after ovulation (fig. 3)

Mean hatching rates obtained after partial stripping in experiment 3 were high (90 to 92%) until 14 h after injection, and then decreased abruptly ($p < 0.001$) after 16 h (36%). No hatching occurred after a 20-h latency period. The mean proportion of deformed larvae, low at 12 h (4%), greatly increased ($p < 0.01$) after 14 h of latency (20%). A highly significant negative correlation ($r = 0.91$) was found between proportions of deformed larvae (deformed larvae/hatched eggs) and hatching rates.

It was noticed that ovarian fluid was almost nonexistent in ova collections carried out at the

reference latency period. However, the amount of fluid increased progressively as the latency time was extended. This tended to facilitate ova emission and, 20 h after injection, the ova run out spontaneously from the female. After 16 h of latency, the advanced overripening state of ova became directly visible by a large increase in the number of degenerating gametes (white ova) in the samples.

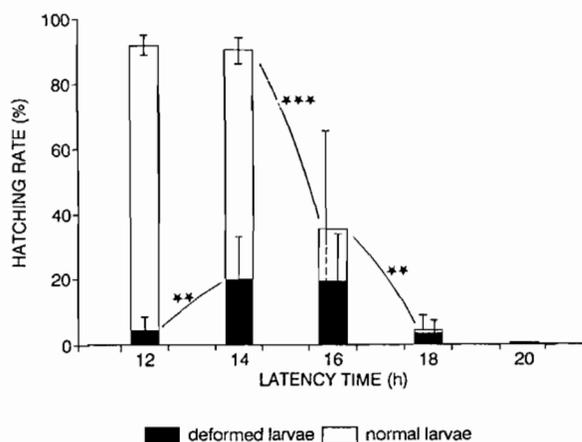


Figure 3. – Hatching rates of *H. longifilis* eggs as a function of latency time from hCG injection in experiment 3 (average temperature: 29°C).

Means for six females partially emptied from their ova at each stripping. Vertical bars refer to the confidence interval ($p = 0.05$).

DISCUSSION

In brooders of *H. longifilis* administration of hCG is highly efficient in inducing oocyte maturation and ovulation; 100% response was still obtained in the current study. After hormonal treatment, ovulation is asynchronous for the follicles of individual females and occurs over a period of 3-4 h (7-8 to 11 h after injection at 30°C). As a matter of fact limited proportions of ova were collected 8 h after injection, compared to the total quantity of ova that could be collected after supplementary stripping of the females at 11 and 15 h latency time. This suggests

differences in the sensitivity of follicles to the hormonal stimulation or possible differences in time for the hormone to reach the different follicles within the gonad. In turbot (*Scophthalmus maximus*), McEvoy (1984) reported that it takes 4-6 h for a complete batch of eggs to be ovulated.

The asynchrony of ovulation reported here for *H. longifilis* may indicate that, under conditions of natural spawning, all the ova would not be laid in a single batch. From observations of the breeding habits carried out in aquaria after hormonal treatment, Van Der Waal (1974) has shown that, in *Clarias gariepinus*, the eggs are laid in 15 to 50 successive batches of a few hundred, with a time interval between egg-laying ranging from 2 to 20 min.

A high ovulatory response and a proportion of ovulated oocytes of around 90% were observed at the reference latency period (11 h at 30°C). At this time, no difference was found in the numbers of unovulated oocytes for the samples taken from the different portions of the ovary, indicating that ovulation was homogeneous throughout the entire gonad. Complementary observations (unpublished), made under similar conditions with a group of 10 females, showed that when the females were killed 3-4 h later than the reference latency time (14-15 h after injection at 30°C), the ovulatory response averaged $77 \pm 6\%$ (ranging from 63 to 84%) and did not noticeably differ from the ovulatory response obtained in this study ($74 \pm 7\%$). These observations indicate that the majority of postvitellogenic oocytes that were not ovulated after the reference latency time, would not ovulate even if the latency time was extended. Therefore, it can be assumed that the reference latency periods correspond approximately to the completion of ovulation of females treated with hCG.

In experiment 1, the observation that 20% of the ova could be still collected 15 h after injection, 4 hours after completion of ovulation, has most probably a mechanical origin associated with the collection method. The force applied onto the abdomen of the female during stripping can only be moderate and, because of this, the ova collection made at the reference latency time (11 h at 30°C) is incomplete. The increase in the amount of ovarian fluid observed when the latency time was extended may explain the ease of extraction of the residual ova after 15 h.

The variation in the total quantity of ova collected from *H. longifilis* females in experiment 1 carried out in December (11,000 to 43,000 per female kg) and in experiment 2 carried out in June (46,000 to 103,000 per female kg) corresponds to seasonal changes in fecundity. These results are highly compatible with the range of relative fecundity observed so far during the dry season (2,000-78,000), when egg production is clearly lower than during the rainy season (31,000-97,000) (Legendre 1986, 1992).

In their study on follicle maturation in *C. gariepinus*, Richter and Van Den Hurk (1982) defined the optimum latency time as the delay allowing the greatest hatching percentages with the lowest proportion of deformed larvae. For this species, the optimal latency time after injection with carp pituitary extract is 11-14 h at 25°C. When ova are collected before or after the optimum period, hatching rates are low with a high proportion of deformed larvae (Hogendoorn and Vismans, 1980; Richter and Van Den Hurk, 1982). This evolution in egg viability was not observed in *H. longifilis*. The first ova that could be obtained by handstripping were of good quality (8 h after hCG injection) and led directly to high hatching rates with small proportions of deformed larvae. However, at this stage ovulation was still at its beginning and the number of ova collected was low. On the other hand, aging of ova began rapidly after ovulation. A delay of 2 h in ova collection already led to a noticeable increase in the number of abnormal embryonic development, and after two more hours hatching rates were greatly reduced. Therefore, in *H. longifilis*, the optimal latency time can be defined as the delay allowing the best compromise between the quantity and the quality of ova collected.

Quite high variations between individual females were observed for both the quantity of ova collected at the beginning of ovulation (8 h after injection) and the quality of ova collected 4 h after ovulation ends. However, it should be stressed that at the reference latency time the results were constantly good with low individual variation, either in terms of ovulation or hatching rates. This confirms the validity of the latency periods proposed previously for this species (Legendre, 1992).

Escaffre *et al.* (1977) reported that in rainbow trout the survival period of ova may depend on the quantity of ova left in the general cavity of the female. However, in *H. longifilis*, the drop observed in hatching rates 4 h after the completion of ovulation was of the same order of magnitude in experiments 1 and 3 in which the methods of ova collection were different (partial or total collection; *fig. 2 and 3*). This suggests that in this catfish the aging process is independent from the quantity of ova remaining within the ovarian cavity.

Although several hypothesis were advanced, the factors and mechanisms responsible for the decrease in viability of ova preserved *in vivo* after ovulation remain poorly understood in fish. From a study on *Roccus saxatilis*, Stevens (1966) suggested that the loss of egg viability may be the result of hypoxia within the ovary after ovulation, caused by a change in the ovarian fluid. In rainbow trout, ova retention leads to major morphological changes (Nomura *et al.*, 1974), which according to Sakai *et al.* (1975) could be the cause of decreased egg viability. Hirose *et al.* (1977) showed that the decrease in hatching rates for eggs of *Plecoglossus altivelis* fertilized one day after ovulation was associated with decreased sodium

and potassium concentrations in the ova, suggesting a change in membrane permeability. Renard *et al.* (1987) hypothesized that the loss of viability of over-matured ova could be due primarily to the closing of the micropyle after an elevation of the chorion. Craik and Harvey (1984) reported that in rainbow trout a severe decrease in hatching rates can occur without significant modification in the biochemical composition of the ova and suggested that egg viability after ovulation may be hormonally controlled. Finally, Boulekbache *et al.* (1989) indicated that the unfavorable effects of aging may be associated with a decrease in ATP reserves which may compromise embryonic development through a disorganization in the cellular structure.

The causes of the loss in viability of ova aged *in vivo* were not investigated directly in the present study. However, a point of relevance is that the first oocytes to ovulate (7-8 h after injection) do not appear to engage in the process of overripening unless ovulation has been completed. In experiment 2 and 3, hatching rates higher than 90% and low proportions of deformed larvae (around 6%) were indeed obtained for egg collected at the reference latency period, despite ova were not stripped off the gonads 8 h after injection

as in experiment 1. Similarly, the lowering in egg quality observed 4 h after the reference latency period remained roughly the same in experiment 1 and 3, although in the latter there was no early collection of the ova. This may indicate that an environment favorable to ova survival is maintained within the gonad during the process of ovulation and that this environment changes rapidly when ovulation has been completed.

In summary, the results of this investigation show that in *H. longifilis* ovulation is asynchronous after hormonal treatment with hCG, and that the total quantity of ova able to be collected by stripping increases with an increased latency period. However, after ovulation ends, aging of ova occurs rapidly. As a consequence, the optimal period for collection of ova, *i.e.* the most likely to lead to the best compromise between egg quantity and quality, is narrow. It has been found to correspond to the completion of ovulation and fits with the latency periods recommended previously for this species. In practice, because a delay of two hours in ova collection is already leading to a sharp increase in embryonic developmental anomalies, these latency periods should be respected to within one hour.

Acknowledgments

The authors are grateful to Prof. Roland Billard (MNHN, Paris) and Dr. Bernard Jalabert (INRA, Rennes) for critical comments on the manuscript, and to Mr. Jacques Slembrouck (ORSTOM) for his technical assistance.

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