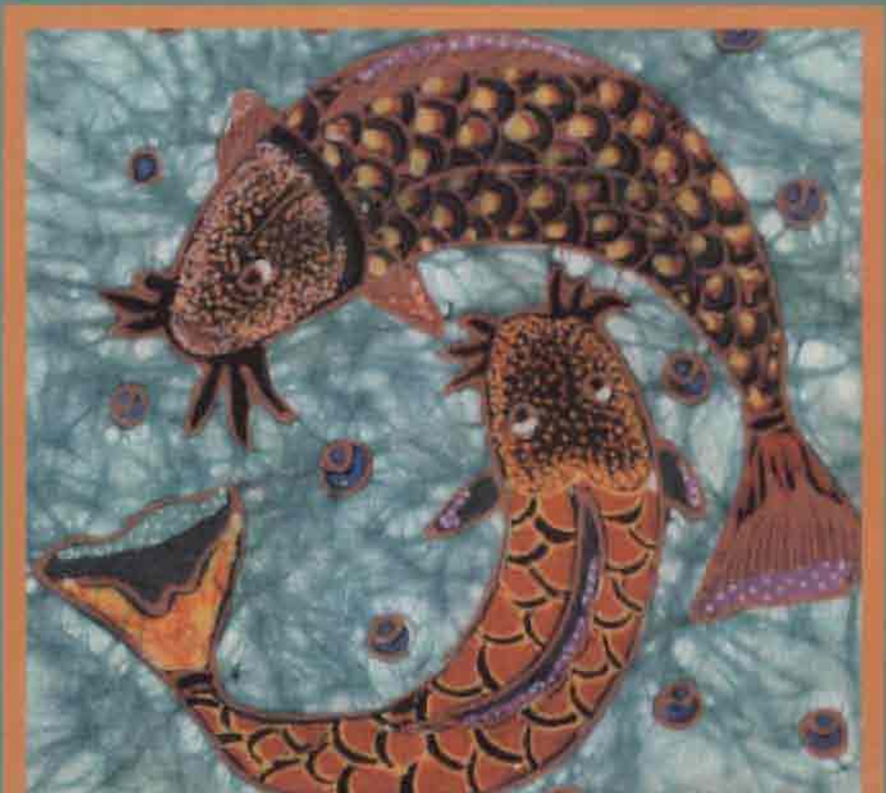
THE BIOLOGICAL DIVERSITY AND AQUACULTURE OF CLARIID AND PANGASIID CATFISHES IN SOUTH-EAST ASIA



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DESCRIPTION OF THE SEXUAL CYCLE RELATED TO THE ENVIRONMENT AND SET UP OF THE ARTIFICIAL PROPAGATION IN *PANGASIUS BOCOURTI* (SAUVAGE, 1880) AND *PANGASIUS HYPOPHTHALMUS* (SAUVAGE, 1878), REARED IN FLOATING CAGES AND IN PONDS IN THE MEKONG DELTA

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Abstract

First artificial propagation of *Pangasius hypophthalmus* was reported in Vietnam in 1980, but results did not allow extension for large scale production of fry. Otherwise, for *P. bocourti*, no reproduction was yet reported in 1994. Then, investigations were carried out on the reproduction of the two species in two rearing conditions, floating cages and ponds.

Climate in South Vietnam is characterised by two alternate seasons, dry (December-April) and rainy (May-November). Development of oocyte shows an annual cycle in the two species. In *P. bocourti*, maturity of females peaks before the rainy season, while in *P. hypophthalmus* maturity period is a bit delayed, as it occurs at the end of dry season and the beginning of the rainy season. For the two species, development of oocyte do not differ between the two environments, leading to conclusion that rise of water temperature and/or photoperiod probably induce the oocyte development. In males, mature fish produce milt when stripped. In the two species, mature male are observed at the same time as the females. However, in *P. bocourti*, some males can produce milt all year round. In the two species, sexual maturity peaks before, or at the early beginning, of the water flow in the Mekong River.

As no female was observed with ovulated oocyte in captivity, oocyte maturation was induced with hCG treatment. Females of *P. bocourti* require a long preliminary treatment with 3 to 10 injections at a low dose (530 $UI.kg^{-1}$). Afterwards oocyte are larger and more sensitive to the resolving treatment, including one or two injections (2020 or 1460–2070 $UI.kg^{-1}$). By contrast, females of *P. hypophthalmus* do not require a preliminary treatment, as they have naturally large oocyte sensitive to the resolving treatment, including one (2530 $UI.kg^{-1}$), two (2530–2520 $UI.kg^{-1}$) or three injections (490–1000–1500 $UI.kg^{-1}$). Application of such treatments led to ovulation and ova collection by stripping in 59% and 88% of the treated females, in *P. bocourti* and *P. hypophthalmus* respectively. Ova collected are smaller in *P. hypophthalmus* than in *P. bocourti*, 1.0 and 1.9 mm diameter respectively. One gram of ova in *P. bocourti* and *P. hypophthalmus* contains respectively 251 and 1437 $ova.g^{-1}$. The relative fecundity is lower in *P. bocourti* than in *P. hypophthalmus*, respectively 4.7 10^3 and 48.8 10^3 ova per kg of body weight. Influence of the rearing conditions and the fish morphology and fattening are discussed. Ovulation was usually induced once a year but preliminary data showed that 30% of females of the two species can be induced twice per year.

In males, compared to the natural spermiation per kilogram of body weight, a single injection of hCG (2000 $UI.kg^{-1}$) allows to collect 10 and 5 times more milt respectively in *P. bocourti* and *P. hypophthalmus*. In *P. bocourti*, a single injection of LHRHa (30 $\mu g.kg^{-1}$) associated with domperidone (3 $mg.kg^{-1}$) induces also a rise of milt collected, but lower than hCG. Generally, volumes of milt collected from *P. bocourti* (5–495 $\mu l.kg^{-1}$) are lower than in *P. hypophthalmus* (11–2092 $\mu l.kg^{-1}$). The same figure is observed about the spermatozoa concentration, lower in *P. bocourti* (2.4 10^9–36.5 10^9 $spz.ml^{-1}$) than in *P. hypophthalmus* (25.7 10^9–63.4 10^9 $spz.ml^{-1}$).

Milt of both species, diluted twice in 155 mM NaCl solution (9 $g.l^{-1}$) buffered at pH 7, can be stored during 24 h without change of its fertility. Motility of spermatozoa is brief and stops before one minute in tap water. So better fertilisation is obtained for *P. hypophthalmus* with a 34 mM NaCl activation solution (2 $g.l^{-1}$). The optimal dilution of milt in the activation solution is 10^2-10^4 and 10^2 times for *P. bocourti* and *P. hypophthalmus* respectively.

INTRODUCTION

Pangasius bocourti and *P. hypophthalmus* are catfishes of the Pangasiidae family belonging to the *Pangasius* genus. Both originated from the Mekong River and the Chao Phraya River in Thailand (Roberts & Vidthayanon, 1991). *Pangasius hypophthalmus* was sprayed to other area such as Malaysia, Indonesia and China (Pan & Zheng, 1983). *Pangasius hypophthalmus* is able to breath air through the swim bladder which allows the fish to stand in water with low level of dissolved oxygen (Browman & Kramer, 1985). *Pangasius bocourti* shows also the same characteristic. Moreover, fast growth and omnivorous regime make these species interesting for aquaculture (Lenormand, 1996). *Pangasius bocourti* has been reared in floating cages on the Mekong River since 1960, whereas this fish is never found in pond (Cacot, 1993). Now its amount of production in Vietnam reaches 15000 tons per year, which represents 75% of the production in floating cages. Most of this production is destined to the frozen filet processing and exportation, for an annual income of 22 billions USD. On the opposite side, *P. hypophthalmus* has been reared in the Mekong delta in ponds probably for centuries (Peignen, 1993). Now its amount of production is estimated to 20000 tons per year which are locally consumed, fresh or dried.

In 1994, the rearing of the two species was based on the catch of juveniles from the Mekong River, mostly in the Cambodian area. At least 20 billions of fingerlings of each species were needed per year in Vietnam. Drop of the catch and increasing of the demand since 1990 led to an important increase of the juvenile price for the Vietnamese fish aquaculturist (0.6 USD per 100 g juvenile). Faced to this bottle neck, production of fry by artificial propagation was necessary. The first reproduction of *P. hypophthalmus* was reported in Thailand in 1959 by Boonbrahm and later in Indonesia (Hardjamulia *et al.*, 1981) and in Malaysia (Thalathiah *et al.*, 1983). Now in these countries, the supply of fry for production is provided only by the artificial propagation and nursing. In Vietnam, the first artificial propagation was obtained in 1981 (My Anh *et al.*, 1981), but results were not sufficient to allow a large scale production of fry. For *P. bocourti*, no artificial propagation was reported in the world in 1994.

The presented research started in early 1994 in the frame of a PhD course of the author. Investigations have covered several fields of experiments that will be reported in a complete thesis report. Based on this work, the present paper aims to summarise the main results obtained on the reproductive cycle, induced breeding, gamete production and management for both *P. bocourti* and *P hypophthalmus* reared in floating cages or ponds in the Mekong delta.

MATERIAL AND METHODS (Figure 1)

Broodstocks

Since the systematic revision of Pangasiidae by Roberts and Vidthayanon (1991), the *Pangasius* species previously called *Pangasius pangasius* in Vietnam, should now be called *Pangasius bocourti* (Sauvage, 1880). The *Pangasius* species previously called *Pangasius micronema* in Vietnam and *Pangasius sutchi* (Fowler, 1937) in Thailand, Indonesia and Malaysia, should now be called *Pangasius hypophthalmus* (Sauvage, 1878).

In Vietnam, *P. bocourti* and *P. hypophthalmus* are locally called respectively "Ca ba sa" and "Ca tra". "Ca ba sa" means fish with three layers of fat, and experimental results on fish growth tend to confirm such appellation (see Le Thanh Hung *et al.*, 1999). "Ca tra" is in fact the genus name given locally to the pangasiid species in the Mekong delta, among which *P. hypophthalmus* is the most abundant species.

In 1994, the AGIFISH company was rearing large fishes in floating cages on the Mekong River in order to perform artificial propagation. These fishes had an estimated age of 3 and 6 years respectively in *P. bocourti* and *P. hypophthalmus*. Some large *P. hypophthalmus* were also available in a pond at the Can-Tho University. All these fishes are wild fish as they were caught at the juvenile stage in the Mekong River. The two species do not show any sexual dimorphism, as already mentioned by Roberts (1991).

However, in the broodfish used, body weight and fork length are respectively 30 and 8% bigger in females than in males.

Rearing conditions

Part of broodstocks available in 1994 were transferred into the experimental floating cages and ponds. The use of two rearing conditions allowed to assess the effect of the environment on the sexual cycle in the two species. In the two

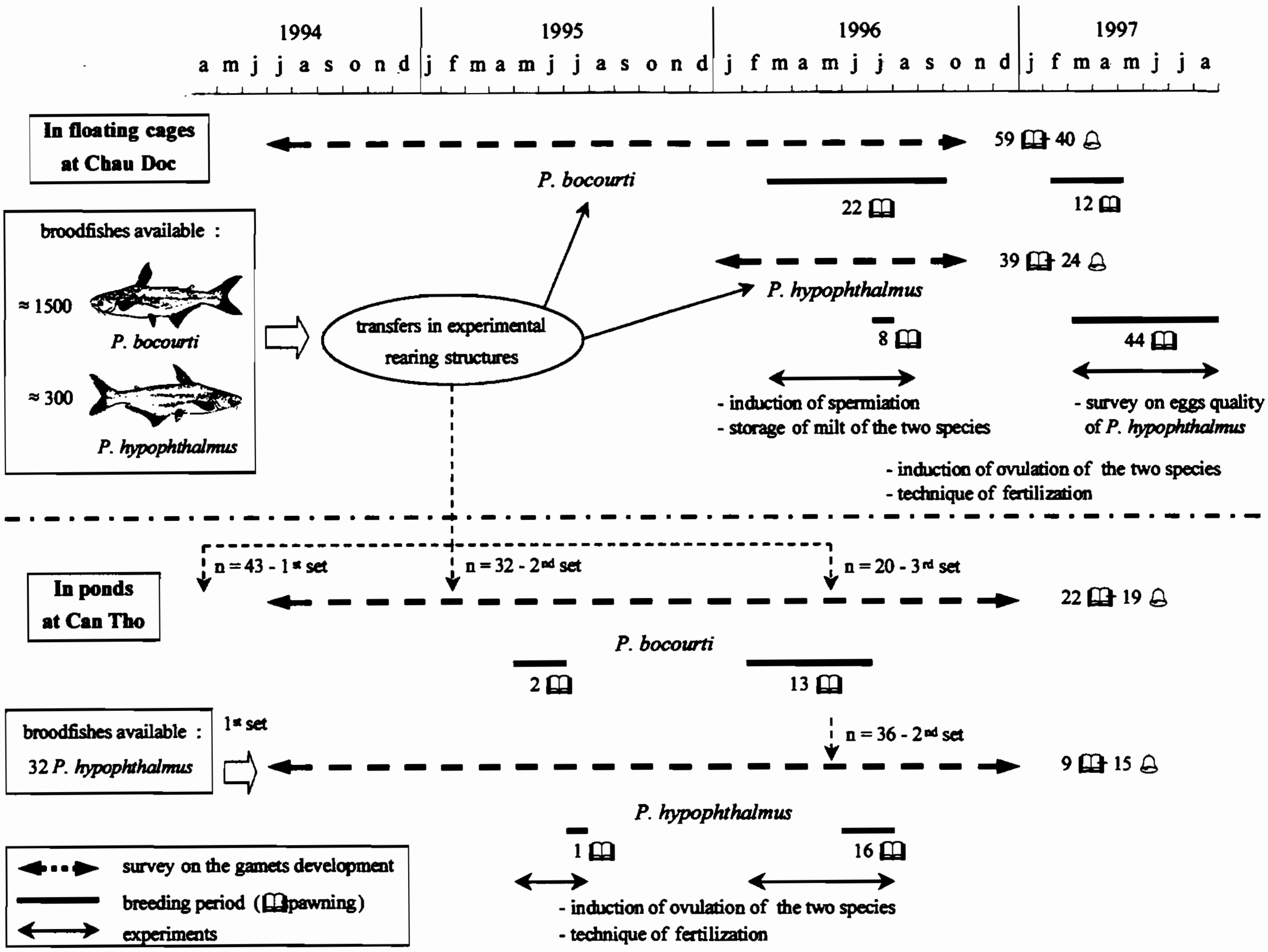


Figure 1: Distribution of broodstocks and experiments.

rearing structures, fish density is low in regard to those in growing conditions (Table 1). So broodfish in ponds could feed the natural food such as snail. Broodfish were also fed artificial feed given as moist or dry pellet. Each food was made with 2 to 6 raw materials. Twelve raw materials were used to prepare the pellet, considered as protein source (fish meal, cow liver, blood four), carbohydrate source (broken rice, corn), together protein and carbohydrate sources (soybean cake, rice bran, industrial food named PROCONCO C50) , and vitamin and mineral source (soybean and rice germs, industrial premix). Raw materials with high carbohydrate content were considered as source of energy as well as pellet binder. In moist pellet, leaves of the kapok tree *Ceiba pantandra* was also used specifically as pellet binder. The main ingredient was the local fish meal, despite its poor quality (44.6 and 37.4% of protein and ash on the dry matter). The average protein content of the food was 38.3 and 47.0% for the fish reared in cages and in ponds (Table 2). The fish were fed twice a day, morning and afternoon, at a feeding rate higher for *P. bocourti* than for *P. hypophthalmus* (Tableau 3). This difference was due first to the higher feed intake in *P. hypophthalmus* and to the higher fat content in *P. bocourti.*

Consequently, the quantity of protein provided was higher for *P. hypophthalmus* than for *P. bocourti*, respectively of 3.3–3.4 and 5.5–4.7 grams per kilo and per day (in floating cages–in ponds). In both species, carbohydrates were considered as energy source, as the study of fish in nature showed that fish have an omnivorous regime (Lenormand, 1996).

Survey on the development of gametes

In order to get information about the development of gonads, broodfishes of *P. bocourti* and *P. hypophthalmus* were caught regularly, respectively every 28–66 and 22–69 days (in ponds

	P. bocourti	P. hypophthalmus
In floating cages :		
-broodfishes per cage	50	50
-volume in m^3	45 and 54	27 and 54
-fish per m^3	1.1	1.6
-fish biomass $kg.m^3$	6.4	8.1
In ponds :		
-broodfishes per pond	36	31
-surface in m2	658 and 725	416 and 600
-surface (m2) per fish	19.5	15.2
-fish biomass in $g.m^2$	304.7	455.1

Tableau 1: Rearing structures (average values).

		In floating cages	In ponds
Moister (% TM) :			
- in moist pellet		34.5	43.7
- in dry pellet		12.9	10.2
Proteins	%DM	47.0	38.3
Lipids	%DM	6.9	7.5
Carbohydrates	%DM	18.5	16.0
Cellulose	%DM	4.4	3.4
Other (*)	%DM	-	10.7
Ash	%DM	23.2	24.0

* : carbohydrate and cellulose when the two components are not analysed.

Tableau 2: Composition of feed (average values).

		In floating cages		In ponds	
		P. bocourti	P. hypophthalmus	P. bocourti	P. hypophthalmus
Feeding rate (% biomass per day)		1.0	1.5	1.2	1.5
Proteins	$g.kg^{-1}.day^{-1}$	3.4	5.6	3.5	4.7
Lipids	$g.kg^{-1}.day^{-1}$	0.5	0.9	0.8	1.0
Carbohydrates	$g.kg^{-1}.day^{-1}$	1.2	2.2	1.2	1.6

Tableau 3: Nutrients provided by the feed (average values).

–in floating cages). In females, oocytes were sampled by ovarian biopsy with a flexible pipe ("Pipelle de Cormier"). Then oocyte diameter was measured under binocular lens, the modal diameter being used as the main criterion of oocyte development. Such method was applied to study the seasonal development of oocyte in *Heterobranchus longifilis* in Ivory Coast (Legendre, 1986). In males, presence of milt was checked by stripping of the fish. For both males and females, these observations were made on anaesthetised fish, by standing in solution of 0,25 $ml.l^{-1}$ phenoxy-2-ethanol. Fishes were tagged with blue spots on the skin of the belly, with saturated alcian solution injected using a "dermojet". Fishes were also tagged using intra-muscular electronic pit tag. So the gamete development was individually assessed.

Artificial propagation

Artificial propagation was conducted on selected mature fish. Mature females can respond to hormonal induction of ovulation. This stage is reached when mode of the oocyte diameter is 1.3 and 1.0 mm respectively in *P. bocourti* and *P. hypophthalmus*. Mature males produce milt when stripped. Mature broodfish were stood in tanks (3.5 m^3) with water exchange and water aeration during the hormonal treatment.

Three kinds of hormones were used :

- pituitary extracts of local common carp and Clariidae,
- human Chorionic Gonadotropin (hCG) produced by ORGANON,
- analogue of LHRH produced by BACHEM: ($Des\text{-}Gly^{10}$, $D\text{-}Arg^6$, Trp^7, Leu^8, $Pro\text{-}NHRt^9$) – LHRH, associated with two kinds of dopamine antagonists: pimozide or domperidone.

In females, first ovulation was obtained with hCG and then the use of this hormone was assessed during the following experiments. Use of hCG to induce oocyte maturation is common in other species of tropical catfishes such as *Heterobranchus longifilis* (Legendre, 1986), and *Clarias macrocephalus* (Carreon *et al.*, 1976, in Donaldson & Hunter, 1983). In *P. hypophthalmus*, use of hCG was reported in Vietnam (Huy *et al.*, 1990) and in Malaysia (Thalathiah *et al.*, 1988). In both cases, this hormone was associated with pituitary extracts of common carp, and, only in Indonesia, with homoplastic pituitary extract. In

Indonesia, use of hCG associated with pituitary extracts of common carp was also reported to induce oocyte maturation in the locally named "Ikan Patin", *Pangasius pangasius* (Meenakarn, 1986).

Hormonal treatments were also performed in males in order to enhance the milt production, with hCG in *P. bocourti* and *P. hypophthalmus*, and with LHRHa associated with domperidone in *P. bocourti* only.

Assessment of the quality of gametes

Quality of milt and ova collected was assessed by fertilisation trials made with about 300 ova set in half litre box. Incubation took place in these boxes in standing or current water. Motility of spermatozoa was checked before use of sperm, using criterion established by Sanchez–Rodriguez (Sanchez-Rodriguez & Billard, 1977). Fertilisation trials were made in standard conditions, with hundred times diluted milt in the activation solution (6 or 10 ml). Fertilisation and hatching rates were measured during incubation and percentages of abnormal larvae after hatching.

RESULTS

Reproductive cycle related to the environment (Figure 2)

The Mekong delta is a tropical area located at 10° North latitude. This area has a monsoon climate, with well marked dry and rainy seasons. Photoperiod has a range of 1 h 15. Air temperature ranges from 21.0 to 35.5°C, with an average value of 26.6 ± 1.4°C. The Mekong River is a typical flood plain river (Welcomme, 1979) with a low and high water time during the year. During the low water time, the flow is affected by the tide in the South Chinese Sea – named "East Sea" in Vietnam – causing balance of the flow alternatively from upstream and downstream four times per day. During the high water time, the flow runs only from upstream. These conditions are observed in both Chau-Doc and Can-Tho, where the water has nil salinity throughout the year.

In the two different rearing environments, sexual maturation of the two species shows a yearly marked cycle.

In *P. bocourti*

Oocyte development occurs when temperature increases from 26 to 31°C, respectively in January and April. Afterwards, proportion of mature females decreases with the temperature. This evolution is similar in both ponds and floating cages, whereas water transparency shows different patterns in the two environments. Indeed, in ponds and in floating cages, sexual maturation reaches a pick when transparency is respectively low and high. Water flow is also different between the two environments, as it is nearly nil in ponds whereas it ranges from 0 to 37.5 $m.mn^{-1}$ in the river in April, at the sexual maturation pick. Whatever the environment, sexual maturation reaches a pick during the dry season, before the rainy season. So comparison between ponds and cages leads to conclusion that temperature is probably the main parameter inducing the gonad development. However, photoperiod can also be involved as the oocyte development occurs also when the daytime increases.

In *P. hypophthalmus*

Oocyte development is delayed in regard to *P. bocourti* as it starts since March or April, when the water temperature is already high (30–31 °C). Mature females were observed in floating cages in various environmental conditions: in the dry and rainy season, when water transparency was high or low, during the low or high water periods. Sexual cycle shows also a similar schedule in both ponds and floating cages. However, in this species, more accurate information is needed especially by checking broodfish within shorter intervals (monthly).

In both species, males show the same periods of sexual maturation as the females in the two rearing conditions. Nevertheless in *P. bocourti* some males can produce milt throughout the year.

***Maturation in female* P. bocourti** (Figure 3)

Oocyte development was studied individually in 11 females. Out of the reproduction season, only small oocytes are observed, with very few oocytes larger than 0.6 mm. About 2 months later, oocytes are larger and show a single and marked mode. However, several trials failed to induce ovulation at such maturity stage. In fact, only large oocytes – from 1.60 mm of diameter – are sensitive to an hCG treatment. These called "sub-mature" females required a preliminary treatment involving several injections of hCG at low a dose, once per day.

Number of injections ranges from 3 to 10 and depends on the initial oocyte size, as females with well developed oocytes need less injections than

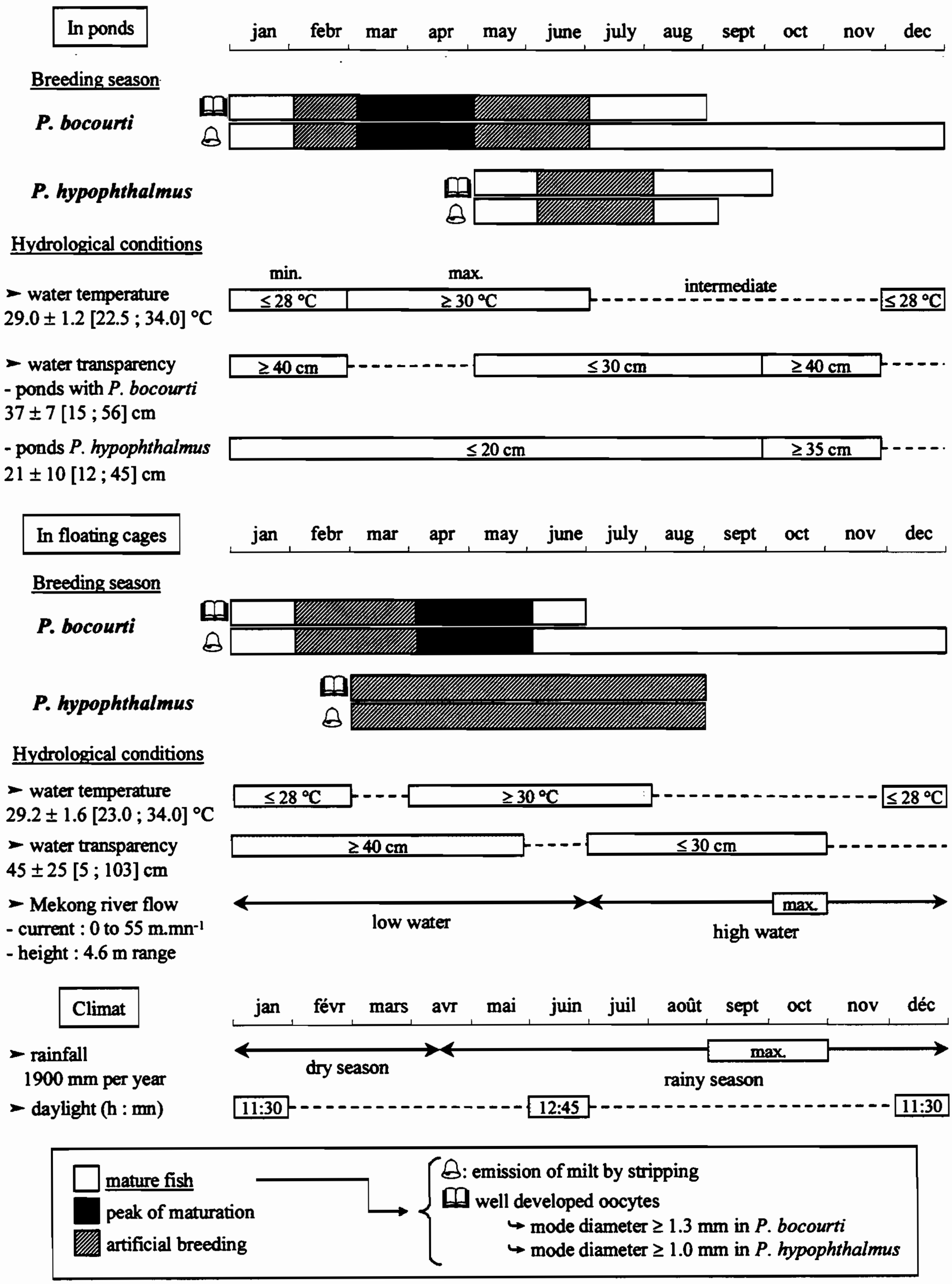


Figure 2: Reproductive cycle related to the environment.

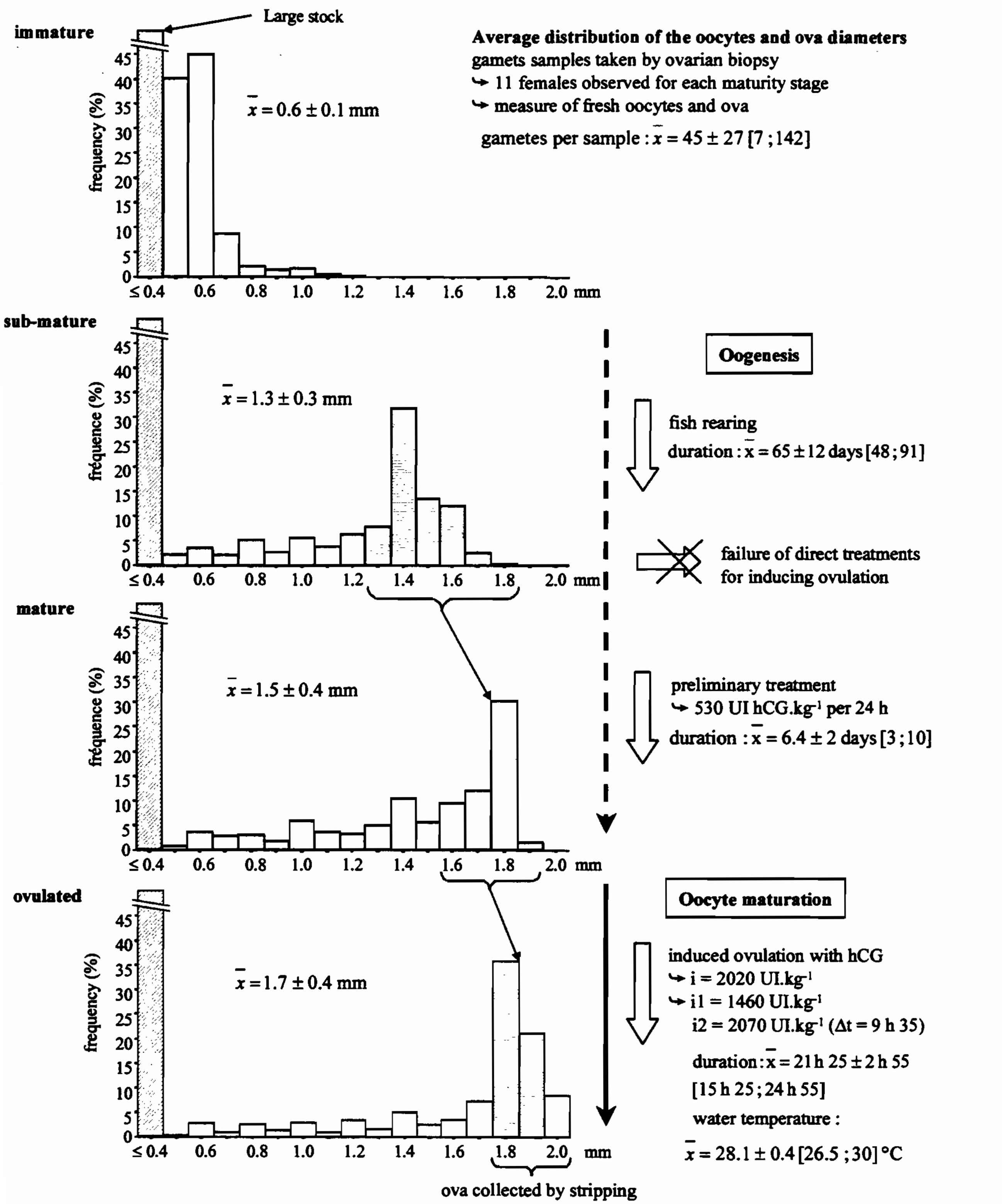


Figure 3: Oocyte development in females *P. bocourti* reared in ponds.

those having smaller oocytes. This treatment leads to an oocytes size increasing, and the proportion of oocytes from 1.60 mm to above increases from 14 to 53%. At this stage, females are considered as mature or sensitive to the second step of the treatment which will induce the oocyte maturation followed by ovulation. It involves one or two injections of hCG, at a higher doses and shorter interval between the two injections than for the preliminary treatment.

***Maturation in female* P. hypophthalmus** (Figure 4)

As in *P. bocourti*, oocyte development was studied individually in 17 females. Only 5 females were observed at the resting stage as the oocytes sampling is usually uneasy at this stage, due to the small size of the ovary. At this stage, out of the reproduction period, only small oocytes are observed, with very few oocytes larger than 0.5 mm. About 3 months later, oocytes are larger and show a single and marked mode. However, this duration has to be assessed with more precision, by checking fish within shorter interval (monthly). At this stage, females are sexually mature as they respond positively to an hormonal treatment inducing oocyte maturation followed by ovulation. In this species, oocytes are sensitive to the hormonal treatment from 1.0 mm diameter. At the mature stage such large oocytes represent 63% of the oocytes samples in average. The treatment applied involves one, two or three injections of hCG. An optional and short preliminary treatment can be applied before, with one or two injections of hCG at a lower dose. Even if it leads to an increasing of the oocyte size, this preliminary treatment does not seem to be necessary.

Process of oocyte maturation (Figure 5)

In both *P. bocourti* and *P. hypophthalmus* mature females, oocytes sampled by biopsy are surrounded by their follicular envelopes which can be seen by the presence of small capillary blood vessels (Figure 4). Also the germinal vesicle stands at the central position in all oocytes. The oocyte maturation induced by the hCG treatment is characterised by the migration of the germinal vesicle at the oocyte periphery, followed by the germinal vesicle breakdown. Considering all the females induced, reared in ponds and in floating cages, the success rate is 59% (n = 76) in *P. bocourti* and 88% (n = 67) in *P. hypophthalmus*.

Fecundity of females (Figure 6)

Cases of first spawning and single spawning

One gram of ova contains 5 times more ova in *P. hypophthalmus* than in *P. bocourti*. This difference is related to the smaller size of ova in *P. hypophthalmus*. After the first treatment of the year, the relative fecundity is twice higher in *P. hypophthalmus* than in *P. bocourti* when considering the weight of ova collected, and 10 times higher when considering the amount of ova. However in both species the relative fecundity is widely variable, as the coefficient of variation is 68% in both *P. bocourti* and *P. hypophthalmus*. In *P. bocourti*, the relative fecundity is two times higher in fishes reared in ponds than in floating cages. This lower fecundity in floating cages is associated with a preliminary treatment shorter and a higher stoutness (condition factor) than in broodfish reared in ponds. Otherwise, in broodfishes reared in floating cages, the relative fecundity is negatively related to the stoutness and the fattening (fat content in the belly).

On the opposite side, in *P. hypophthalmus* reared in floating cages, the relative fecundity is positively related to the stoutness. In this species, the fattening is much lower than in *P. bocourti*, however there is not enough data to assessed any relationship between fattening and the fish fecundity. In both species, into each rearing conditions, there is no relationship between the absolute or the relative fecundity and the fork length and the body weight.

Case of multiple spawning

Preliminary data show that some of the broodfish in both species are able to spawn twice a year. A second spawning occurred in the *P. bocourti* females getting high fecundity at the first spawning, compared to the single spawning females. In *P. hypophthalmus*, a second spawning occurred in half of the females in which the first spawning occurred during the first two months of the breeding season. In *P. bocourti*, fecundity at the second spawning is 6 times lower than at the first spawning. By contrast, in *P. hypophthalmus*, the second spawning fecundity is 75% higher than the first one. In both species, cumulated fecundity of the two successive spawns is higher than fecundity in the case of a single spawning, of 2.4 and 3.1 times in *P. hypophthalmus* and *P. bocourti* respectively. Finally, in one female *P. hypophthalmus* three spawning were obtained at 50 and 66 days of interval.

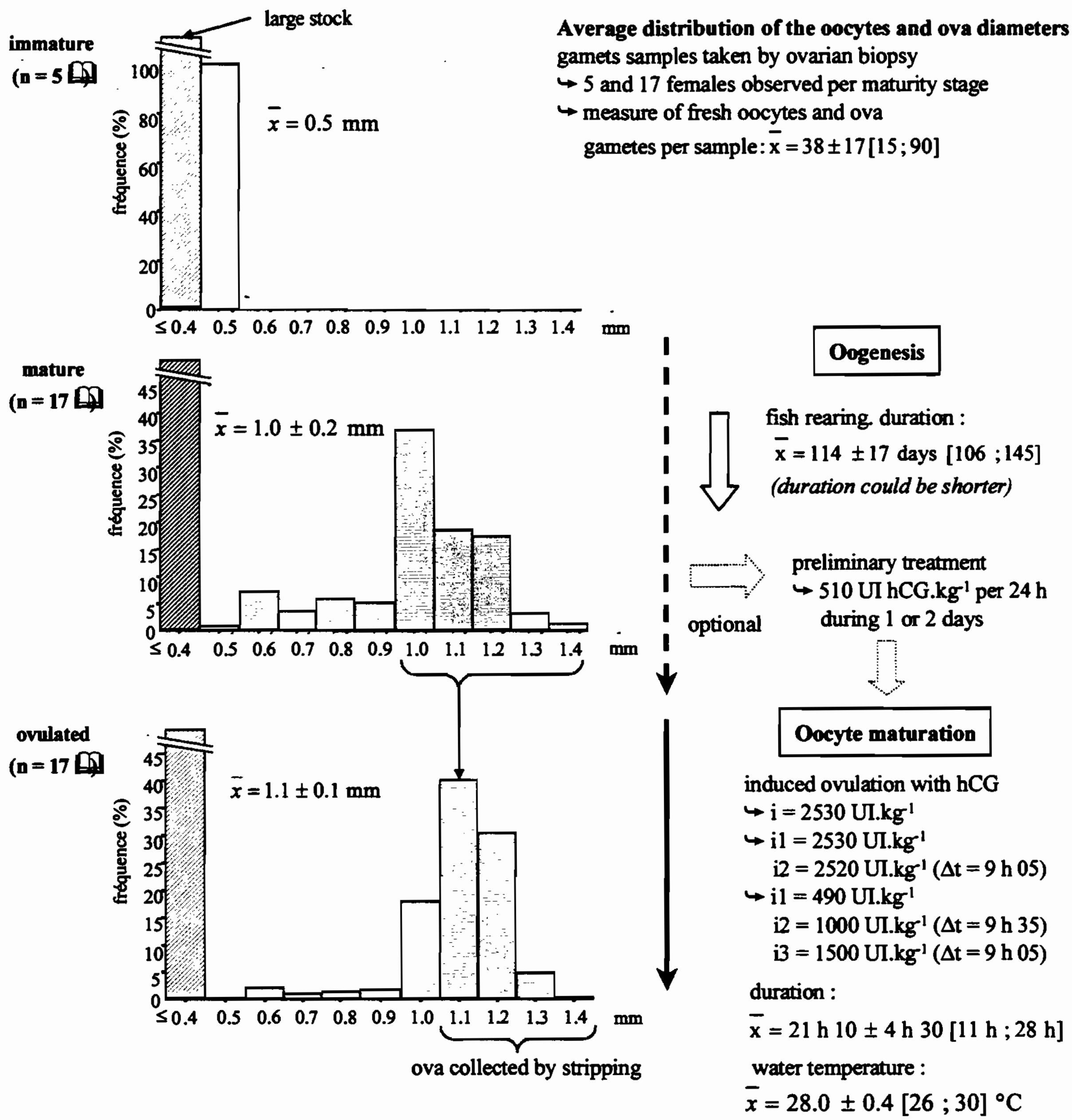


Figure 4: Oocyte development in females *P. hypophthalmus* reared in ponds.

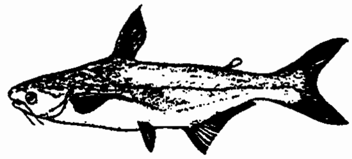
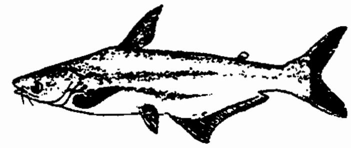
Characteristics of milt (Figure 7)

Mature males produce naturally milt when stripped. Volume of milt per kilo of body weight and spermatozoa concentration are respectively 4.5 and 3.6 higher in *P. hypophthalmus* than in *P. bocourti*. Milt production can be enhanced by hCG injection. Then the volume of milt collected is 10.4 and 5.4 higher than naturally, respectively in *P. bocourti* and *P. hypophthalmus*. In *P. bocourti*, injection of LHRHa associated with domperidone induces also, but at a lower level than hCG, a production of milt which is 5.6 times higher than naturally. In the case of induction with hCG, the volume of milt collected increase rapidly to a pick reached 12 h after injection, and then drops. On the opposite side, LHRHa plus domperidone induces a progressive increase of the volume until 48 h to maybe more.

In both species, the duration of spermatozoa motility is short in pure water. However, with sperm of *P. bocourti*, motility of spermatozoa is twice longer into 2 g.l^{-1} salt water than in pure water.

Management of gametes and fertilised eggs

All the procedures used for the collection of gametes, artificial fertilisation and egg incubation are presented in Figure 8.

		
	P. bocourti	*P. hypophthalmus*
Body weight : average value ± SD [min. ; max]	7,0 ± 1,1 kg [4,8 ; 9,7] per ♀	7,3 ± 1,6 kg [5,0 ; 13,3] per ♀
Ova per gram of ova :	251 ova [222 ; 280] .g^{-1}	1437 ± 90 ova [1316 ; 1524] .g^{-1}

from 1995 to 1997, with broodfishes reared in floating cages and in ponds

	P. bocourti		*P. hypophthalmus*
induced females (hCG)	76 ♀		67 ♀
First & single spawning	n = 45 ♀(59 %)	priming ⊕ ? ; stoutness ⊖ / ⊕ ; fattening ⊖	n = 59 ♀(88 %)
10³ ova.kg⁻¹	**4,7 ± 3,2 [0,4 ; 16,7]**		**48,8 ± 33,6 [5,5 ; 145,7]**
grams ova.kg⁻¹	*18,6 ± 13,3 [1,4 ; 66,4]*		*34,0 ± 23,4 [3,8 ; 101,4]*

	fishes in ponds in 1996	fishes in floating cages in 1997
induced females (with hCG)	10 ♀	33 ♀
Single spawning	n = 7 ♀(70 %)	n = 24 ♀(73 %)
10³ ova.kg⁻¹	**4,9 ± 1,7 [2,3 ; 6,6]**	**52,8 ± 32,4 [10,1 ; 134,9]**
grams ova.kg⁻¹	*19,5 ± 6,6 [9,0 ; 26,2]*	*36,7 ± 22,6 [7,0 ; 93,8]*
1ˢᵗ spawning	n = 3 ♀(30 %)	n = 9 ♀(27 %)
10³ ova.kg⁻¹	**13,3 ± 3,1 [10,4 ; 16,7]**	**47,3 ± 33,0 [24,0 ; 127,3]**
g ova.kg⁻¹	*52,9 ± 12,5 [41,6 ; 66,4]*	*32,9 ± 22,9 [16,7 ; 88,6]*
Characteristics	♀with the highest fecundity	♀with early spawning
2ⁿᵈ spawning	n = 3 ♀	n = 9 ♀
interval	53 ± 25 days [25 ; 75]	73 ± 26 days [40 ; 117]
10³ ova.kg⁻¹	**2,2 ± 1,2 [1,0 ; 3,4]**	**82,9 ± 40,7 [37,2 ; 170,2]**
grams ova.kg⁻¹	*8,7 ± 4,8 [3,9 ; 13,6]*	*57,7 ± 28,3 [25,9 ; 118,4]*
1ˢᵗ + 2ⁿᵈ spawnings	n = 3 ♀	n = 9 ♀
10³ ova.kg⁻¹	**15,5 ± 4,0 [12,6 ; 20,1]**	**130,2 ± 69,3 [73,6 ; 297,5]**
grams ova.kg⁻¹	*61,6 ± 16,0 [50,1 ; 79,9]*	*90,6 ± 48,2 [51,2 ; 207,0]*

Figure 5: Compared fecundity of females *P. bocourti* and *P. hypophthalmus* related to the rank of spawning.

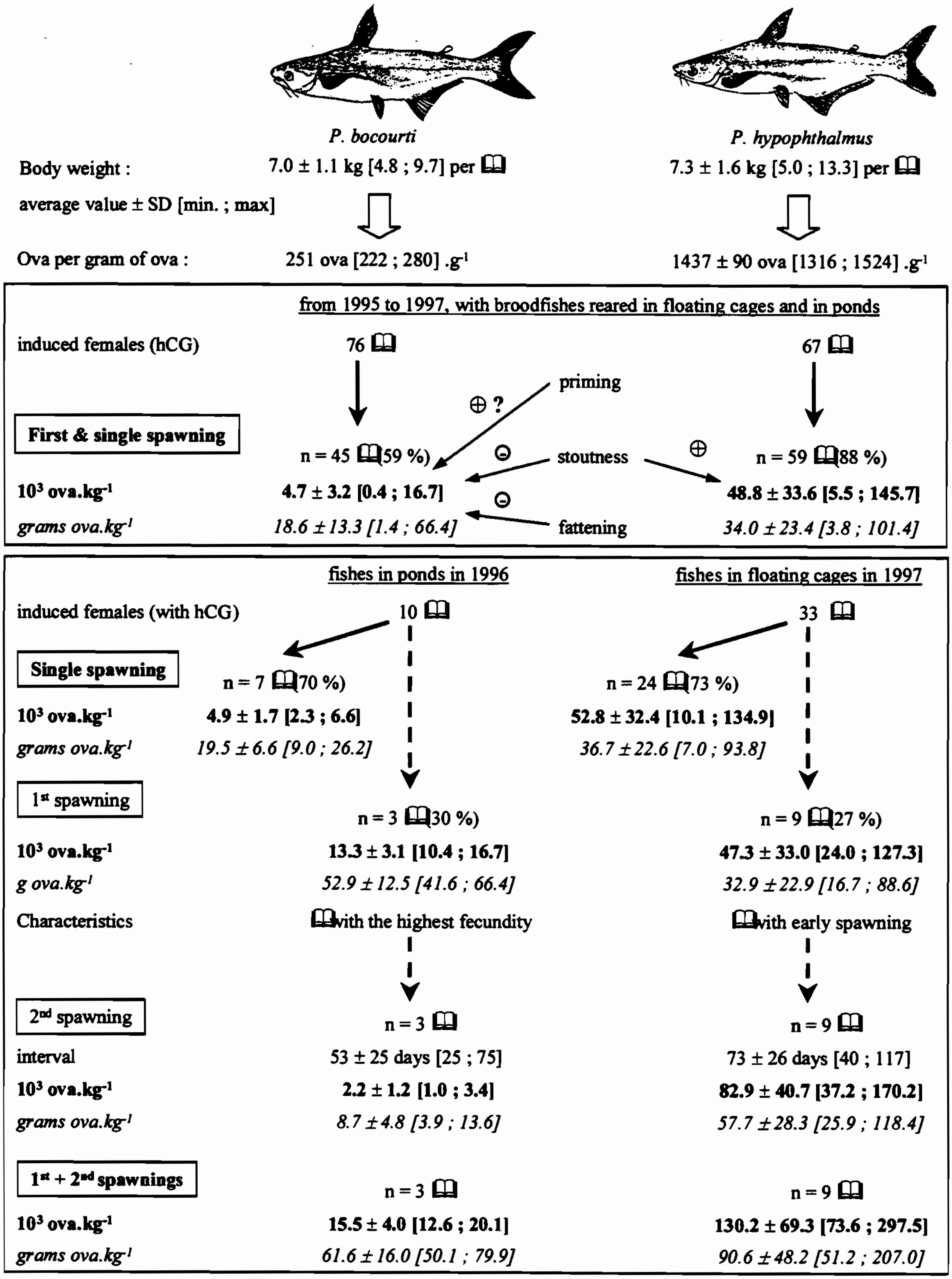
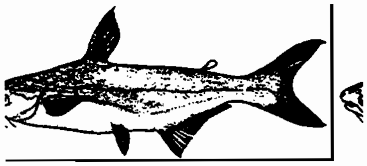
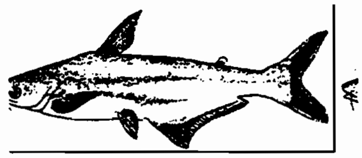


Figure 6: Relative fecundity in females *P. bocourti* and *P. hypophthalmus*.

		
	P. bocourti	*P. hypophthalmus*
Body weight:	5.2± 1.0 kg [3.3 ; 6.9] per♂	5.5± 1.1 kg [3.9 ; 6.7] per♂
average value± SD [min ; max]	⇩	⇩
	broofishes rearedn floatingcages	

Volume & concentration ofemen

Naturalspermiation :	n = 8♂	n = 4♂
volume (µl.kg^{1})	**33 ± 32 [5 ; 90]**	**151 ± 258 [11 ; 537]**
concentration (10 spz.ml^{1})	*13.3 ± 10 [2.4 ; 27.3]*	*48.0 ± 18.6 [25.7 ; 65.5]*
Inducedspermiation :		
➢ **2000 UIhCG.kg^{1}**	n = 4♂	n = 4♂
optimalperiod of latency	12 h	24 h
volume (µl.kg^{1})	**345 ± 125 [237 ; 495]**	**823 ± 850 [338 ; 2092]**
concentration (10 spz.ml^{1})	*26.9 ± 13.4 [7.0 ; 36.2]*	*57.2 ± 6.8 [47.5 ; 63.4]*
➢ **30 µg LHRHa**	n = 4♂	-
+ 3 mg domperidonekg^{1}		
optimalperiod of latency	(48 h)	-
volume (µl.kg^{1})	**187 ± 147 [75 ; 385]**	-
concentration (10 spz.ml^{1})	*15.8 ± 6.3 [9.3 ; 24.0]*	-
Overall volume & concentration of semen are	independent	positively related
Water temperature	[28.1 ; 30.4]°C	[27.8 ; 29.5]°C

Motilityof spermatozoa

In two activatingsolutions :		
➢ pure water	57 ± 12 seconds [40 ; 98]	45 ± 9 seconds [30 ; 69]
➢ 2 g.l^{1} NaCl	112 ± 41 seconds [39 ; 210]	-

( ) : datawhichhaveto be confirmed

- : lackingdata

Figure 7: Characteristics of the semen collected in males *P. bocourti* and *P. hypophthalmus*.

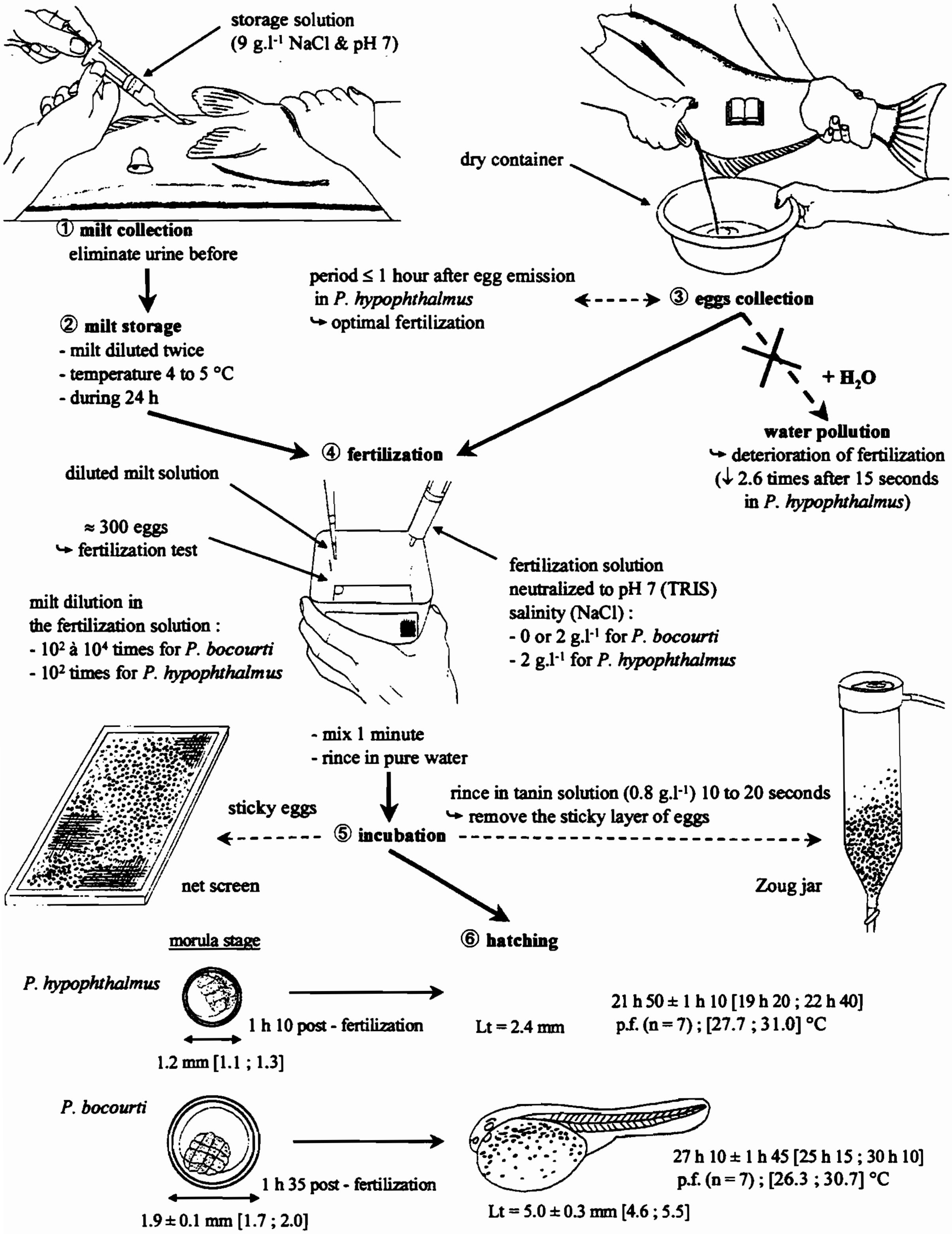


Figure 8: Management of gametes, from collection of semen and ova to the hatching of eggs.

DISCUSSION

Type of oocyte development and spawning frequency

Out of the breeding season, females of both species have only one group of small oocytes. Later, during the breeding season, oocytes distribution still shows an important group of small oocytes but also a new and single marked group of large oocytes. Large oocytes are spawned during one spawning season, whereas the stock of small oocytes will be available for the following breeding seasons. However, in some cases and especially in *P. hypophthalmus*, a new recruitment of the small oocytes can occur during the same breeding season, leading to a second spawning. Such pattern indicates that females of the two species are group–synchrone maturation fish. This pattern is intermediate to the synchronous and asynchronous way of oocyte development (Vlaming, 1983). In synchronous species, such as European Eel *Anguilla anguilla* and Sockeye Salmon *Onchorynchus nerka*, there is only a single group of oocytes in the ovary whatever the stage of oogenesis. In such species, spawning usually occurs only once a life. In asynchronous species, such as Tilapia and *Blennius pholis*, there is at the same time oocytes at different stages of oogenesis in ovary, without any marked groups.

In both *P. bocourti* and *P. hypophthalmus*, hCG treatment induces a single spawning of all the large oocytes group. In cases of partial ovulation of the large oocytes, ova produced cannot be collected by stripping. Otherwise, in *P. hypophthalmus*, the quality of ova drops quickly after ovulation, showing that ova has to be collected rapidly – 1 hour after emission of ova – to guaranty a good fertility (Campet, 1997). So, in nature and in the two species, oocyte maturation is probably a synchronous process concerning all the large oocytes in the ovary. Then, at least in *P. hypophthalmus*, it is rapidly followed by a single – or several near time – spawning.

Sexual cycle related to the environment

In both *P. bocourti* and *P. hypophthalmus*, gametes development show a yearly cycle which is related to the evolution of environment. Role played by the water temperature and/or the photoperiod fluctuations are probably the main parameter(s) involved in this process. On the opposite side, fluctuations of water flow and water transparency did not appear to be preponderant in regard to the development of gametes. Otherwise, gamete development in *P. hypophthalmus* occur two months after *P. bocourti*, suggesting that *P. hypophthalmus* requires higher temperature and/or longer daytime than *P. bocourti* to start sexual maturation.

Contrasting with the situation observed in Vietnam, in *P. hypophthalmus* reared in ponds in Indonesia, the oocyte development does not show such yearly evolution as the average oocyte diameter stands high throughout the year (see Legendre *et al.*, 1999). Moreover, in these conditions females are able to reproduce three to four times per year. The environment in this sub-equatorial area is relatively stable as the temperature stands high, ranging from 28 to 31°C, the photoperiod range is only half an hour and the rainy season is little marked. Such observations tend to confirm the role of the environmental fluctuation in the yearly reproduction cycle in *P. hypophthalmus* in the South Vietnam area.

In the presented study and in the two rearing conditions, spawning were obtained in *P. bocourti* mainly during the low water time in the Mekong River. In *P. hypophthalmus*, it occurred a bit later, mainly at the beginning of the water flow period. Roberts (1983) has reported migration for reproduction in several species of Pangasiidae in the Mekong River, among which *P. bocourti* and *P. hypophthalmus*. Migration occurs from May to July at the Khone waterfalls in Southern Laos, upstream the Mekong delta. This period corresponds to the beginning of the water flow in the river. So the spawning in both captivity and nature probably occur at the same time in *P. hypophthalmus*. However, in *P. bocourti*, the spawning in captivity is probably advanced in regard to the spawning in nature. This delay could be due to the preliminary treatment applied in this fish to achieved the oocyte development. According to this hypothesis, several days of treatments in captivity could replace several weeks in nature to reach the optimal stage of maturity.

Spawning before flow was reported by Welcomme (1979) to be a common behaviour in fish living in the flood plain river such as the Mekong. This strategy provides good conditions for the larvae as they develop in the floodplain, where food is abundant and predation risk is low. Indeed, fingerlings of both *P. bocourti* and *P. hypophthalmus* spray in the Mekong delta from June to September, when the water flow increases (Lenormand, 1996).

***Differences of reproduction performances between* P. bocourti *and* P. hypophthalmus**

In both ponds and floating cages, females of *P. bocourti* do not achieved the oogenesis, as they require a preliminary treatment before inducing ovulation. Such treatment was reported in female Orinnoco cachama (*Colossoma oculus*), in order to advance the gonadal development up to the pre-ovulation stage (Woynarovitch & Horvath, 1980). In this case, the preliminary treatment involved 5 injections of pituitary extracts, at 5–10% of the decisive dose, with 24 hours between two injections. Afterwards the decisive dose is given within two injections, at respectively 40 and 60% of the dose.

On the opposite side, females *P. hypophthalmus* held in culture conditions reach naturally an advanced stage of oogenesis which allows a simple treatment to induce the oocyte maturation. That is probably why artificial propagation provides better results in *P. hypophthalmus* than in *P. bocourti*, both in terms of success rate and relative fecundity. The two species differ also in term of lipid storage as the broodfish of *P. bocourti* can develop an important fat tissue in the abdominal cavity, whereas *P. hypophthalmus* seems to be a lean fish. Such phenomenon was also observed in 10 g fingerlings of *P. bocourti* by Le Thanh Hung (personal communication), in case of excess feeding with a 60% of dry matter protein feed. In female broodfish of *P. bocourti*, the fat tissue is probably used during oogenesis for the ovary growth, as there is a negative relationship between the development of the two tissues.

Two hypothesis can be advanced to explain this poor gonad development observed in *P. bocourti* compared to the one in *P. hypophthalmus*.

Firstly, the environment may be not fully suitable in the rearing conditions, as it could lack some stimuli involved in the gonad development, such as fluctuation of the water flow. According to Roberts (1993), spawning probably occurs in a waterfall area, with a strong water flow and then hydrological conditions differing a lot from those in ponds or in floating cages. In some other species living in floodplain river, role of the hydrological fluctuations in reproduction was demonstrated. In the catfish *Pimelodus maculatus*, an increasing of both water temperature and water flow are necessary to induce the gonad development in the Jaguari River (Basile Martins, 1975, in Welcomme, 1979). In the Parana River, *Prochilodus platensis* isolated fish in the flood plain cannot mature, whereas fish in the main channel of the river can achieved their sexual maturation (Bonetto, 1975, in Welcomme, 1979).

Secondly, for most of the species living in floodplain river, feeding mainly occur during the high water time, when food is abundant, whereas the low water time is a starvation period (Chevey & Le Poulain, 1940; Welcomme, 1979). In *Clarias gariepinus* living in the Shire river, the feeding intake is three times higher during the high water time than during the low water time (Willoughby & Walker, 1977, in Welcomme, 1979). So in such rivers, fish are able to constitute and to mobilise alternatively their body reserve, such as lipid, during the high and low water time. In *Alestes leuciscus* living in the delta of Niger, the growth in weight is reversible as the condition factor can decrease of 30% within one year (Daget, 1957, in Welcomme, 1979). In the Mekong River, Pangasiidae probably follow such ecological figure, which could be accentuated in broodfish, as they have to migrate and to constitute their gonads during the starvation period. However, in rearing conditions, broodfish were fed continuously throughout the year, without alternative periods of feeding and starvation. Consequently, fish probably store more reserve than they can use for growth and reproduction. Finally, the fat tissue probably interferes with the development of gonad in the abdominal cavity because of congestion. This hypothesis is reinforced by the fact that in floating cages from 1994 to 1996, the reducing of feeding rate led to a reducing of the fat content in the body cavity of broodfish, from 12.1 to 6.4% of body weight, whereas the gonado-somatic index increased from 0.5 to 3% in the same time. Finally, negative effects of both constraints, environments and feeding regime, can be cumulated in the rearing conditions.

Pangasius hypophthalmus appears as a more tolerant species concerning its environment in regard to the reproduction performances. Such hypothesis is reinforced by the presence of *P. hypophthalmus* in small canals in the Mekong Delta, where water quality is poor, whereas the presence of *P. bocourti* is restricted to the main river channel (Lenormand, 1996). It could be a reason why fecundity in *P. hypophthalmus* does not differ between the two rearing conditions, whereas fecundity in *P. bocourti* is twice higher in ponds than in cages. About the feeding regime related to reproduction, *P. hypophthalmus* could also show a different figure than *P. bocourti*. This species probably feed throughout the year, or with a shorter period of starvation than in *P. bocourti*. According to this hypothesis, fish do not need large reserve to go on the starvation time. Otherwise, broodfish of *P. hypophthalmus* probably spawn in the river not as far as *P. bocourti*, as for the former species very small fry are collected in the Mekong delta (Lenormand, 1996), then fewer energy has to be spent for migration.

Difference of fecundity between the two species could also depend on the genetic aspect. The relative fecundity is ten times higher in *P. hypophthalmus* than in *P. bocourti*, respectively 48800 and 4700 ova per kilo of body weight. However the survival rate of larvae in conditions of mass production at the AGIFISH station is about 1 and 75% respectively in *P. hypophthalmus* and *P. bocourti* one month after hatching (Vo Phuoc Hung, AGIFISH Co, pers. comm.). Thus, considering the fact that fertilisation rate is about the same in the two species, the number of one month old fingerlings per kilo of female broodfish is 7 times higher in *P. bocourti* than in *P. hypophthalmus*. Even if results are different in natural conditions, larvae of *P. hypophthalmus* seem to be weak in comparison to *P. bocourti*. Consequently, in *P. hypophthalmus*, emission of high number of small ova could compensate the low survival rate of larvae.

Two other aspects related to the fish physiology could also affect the fecundity in *P. bocourti*. First, broodfish of both species have been reared in floating cages from the fingerling stage (50 g and several months old). During the first two years, those fish were reared at a high density – up to 200 $kg.m^{-3}$ – and fed at satiety with moist pellet containing mostly carbohydrates from rice bran. Such conditions led to very fat fish in *P. bocourti* (Cacot, 1993). It can also affect the formation of gonad and then the future fecundity of fish. It was shown in Plaice fish that biotic and abiotic factors experienced during the beginning of fish life determine later the fish fecundity (Simpson, 1951, in Kartas & Quignard, 1984). Secondly, fecundity could be affected by the age of fish if too old, as broodfish of *P. bocourti* were 6 to 9 years old when the present study was carried out. Such negative effect of age was reported in Sturgeon, common Carp and Pike (Nikolsky, 1969, in Kartas & Quignard, 1984). However the age at the first maturity remains unknown in *P. bocourti*.

High variability of the fecundity in the two species

In both species fecundity is highly variable. Such variability can have genetic cause as broodfish used are wild fish, initially caught in the Mekong River. In *P. hypophthalmus*, important migration for reproduction were reported, from the Great Lake "Ton-Le-Sap" to upstream, below or even above the Khone waterfall (Bardach, 1959) and (Sao Leang & Saveum, 1955, in Welcomme, 1979). On the opposite side, very small fry of *P. hypophthalmus* are caught on the bank of the Ton-Le-Sap (Bazir, SAMADHI, pers. comm.), suggesting that reproduction takes place on the flooded banks. Consequently, it could exist migratory and sedentary strains in *P. hypophthalmus*, characterised by two different levels of fecundity. Such figure was shown in the Malma trout living in Alaska (*Salvelinus malma*), where the migratory strain has higher fecundity than the sedentary strain (Blackett, 1973, in Kartas & Quignard, 1984). Otherwise, some strains may be more or less adapted to the rearing conditions in regard to their reproduction performances.

Concentration of milt

In both species, hormonal treatments induce an increasing of the volume of milt collected, whereas the spermatozoa concentration in milt does not decrease, or even tends to increase. Such response suggests that hormones stimulate the production of both seminal liquid and spermatozoa. Usually the hormonal treatment induces an increase of the volume collected, together with a reduced spermatozoa concentration resulting from dilution in the seminal liquid, as reported in common Carp (Saad & Billard, 1987).

Otherwise, concentration of spermatozoa in milt of *P. bocourti* and *P. hypophthalmus* are high compared to those in other species (Tableau 4).

CONCLUSION

Research carried out since 1994 on *P. bocourti* and *P. hypophthalmus* reproduction have led to the development of artificial propagation techniques in the two species. Extension of results has been carried out and allowed a mass production of fingerlings in Chau-Doc, where 30 000, 350 000 and 1 000 000 of fingerlings where produced respectively in 1995, 1996 and 1997. However,

	Concentration of milt (10^9 spermatozoa per ml)	References and authors
Ca ba sa: *P. bocourti*	13 – 26.9	(Eeckhoutte, 1996)
Ca tra: *P. hypophthalmus*	48 – 70.1	(Eeckhoutte, 1996)
Silure: *Silurus glanis*	7.18 ± 1.3	(Saad & Billard, 1995)
Tilapia: *Oreochromis niloticus, O. aureus, O. mossambicus*	2.8	(Rana & Mc Andrew, 1989)
Carpe: *Cyprinus carpio*	24.0 ± 3.5	(Saad & Billard, 1987)
African catfish: *Heterobranchus longifilis*	2.94 [1.26 – 4.26]	(Legendre *et al.*, 1992)
African catfish: *Clarias gariepinus*	4.00 [2.32 – 7.02]	(Legendre *et al.*, 1992)

Tableau 4: Concentration of spermatozoa in milt of some species.

more extension has still to be done to reach the 50 millions of fingerlings required in South Vietnam.

Production of juveniles faced now to two main bottle necks in each species. First, the availability of broodfish in the two species, restricted because fish probably reach sexual maturity after several years. So it involves to rear fish during a longer time than in other species artificially reproduced in the Mekong Delta, like Cyprinidae. But yet we do not know the age at the first maturity and if it is affected by the rearing conditions. In Indonesia, *P. hypophthalmus* reared in ponds can reach sexual maturity within the second year (Legendre, pers. comm.). However, in other places, broodfish of *P. hypophthalmus* were at least three years old. The second problem in *P. bocourti* is the low fecundity associated to the high fat content in broodfish. In *P. hypophthalmus*, the major constraint is the high mortality of larvae occurring from 3 to 6 days after hatching.

The two species studied can artificially crossbreed. Then hybrids are usually produced for rearing in floating cages since 1995, using ova of *P. hypophthalmus* fertilised with sperm of *P. bocourti*. Production of this hybrid allows the production of numerous offspring, due to both the high fecundity of *P. hypophthalmus* and the high survival rate of *P. bocourti*. Hybrid has also a white flesh inherited from *P. bocourti*, which is appreciated by the export market. However, clear data about the zootechnical performances and the fertility of the hybrid are still lacking.

PERSPECTIVES

In order to get more knowledge on the reproduction performances of both *P. bocourti* and *P. hypophthalmus*, further experiments will focus on the effects of broodfish feeding related to the fecundity and the quality of ova. Also a preliminary survey will start on the hybrid between the two species, in order to evaluate its growth potential and reproduction characteristics. These experiments will be carried out in floating cages on the Mekong River, from 1998 to 2000.

Beside the study in rearing conditions, survey aiming to precise the fish ecology in the Mekong River is also planned. This second approach will provide better understanding of the fish physiology and reproduction strategy in nature.

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