Spawning and management of gametes, fertilized eggs and embryos in Siluroidei

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Abstract

The structure of gonads and gametes is highly diversified in siluroid fishes; in some groups the testes are composite with an anterior part formed of spermatogenetic tissues and the posterior part of seminal vesicles which may or may not store spermatozoa. For catfish species of aquacultural interest, ovaries present the same general morphology as in other teleosts with oviducts. Spermatozoa are biflagellated in the Channel catfish (*Ictalurus punctatus*) and monoflagellated in the European catfish (*Silurus glanis*) and African catfishes (*Clarias gariepinus, Heterobranchus longifilis*). The egg size shows considerable intergroup differences, from 0.8-1.2 mm in some Pimelodidae and Clariidae up to 15-20 mm in Ariidae. In numerous cases, the eggs are adhesive and develop either a surrounding sticky layer (*Ictalurus, Chrysichthys, Silurus*) or an adhesive attachment disc (*Clarias, Heterobranchus*). Fertilization is in general external but internal fertilization is reported in some species. The annual sperm production was measured in European catfish and Channel catfish as 1.7×10^{11} and 1.8×10^{10} spermatozoa.kg^{-1} body weight, respectively. In females, the fecundity ranges from about 50 eggs per spawn in ariids up to more than 100000 eggs.kg^{-1} body weight, *e.g.* in pimelodids and clariids. A large variety of controlled reproduction systems are found in the siluroids. In some cases (*Ictalurus, Chrysichthys, Hoplosternum*), spawning occurs naturally in ponds or tanks provided that adequate spawning substrates are available; fertilized eggs are collected immediately after spawning and placed into incubators until hatching. But for most species, although natural or semi-natural spawning could be achieved in captivity, the tendency at the present time is to develop techniques using hormonal-induced ovulation and artificial insemination in order to control the various steps of reproduction and to allow gamete preservation and manipulation. Oocyte maturation and ovulation or spermiation can be induced in many species by a large variety of hormones (GnRHs, fish gonadotropins, hCG, various steroids etc). Some information is available on gamete biology and preservation. Sperm motility is short-lived, not exceeding 70-80 s of forward movement as in most other freshwater teleosts. A peculiarity of the European catfish sperm is the activation by urine during sampling which could be prevented by direct collection in an immobilizing solution (NaCl 200 mM, Tris HCl 30 mM, pH 7.0). Spermatozoa cryopreservation was successfully attempted in several species. Ova generally survive only a few hours after ovulation and fertilization must be carried out soon after. Methods for gamete collection, insemination and incubation of eggs are described for the most widely cultured siluroid species.

Keywords: Siluroidei, reproduction, gonads, gametes, natural spawning, induced spawning, fertilization, egg incubation.

Reproduction contrôlée et gestion des gamètes et des œufs chez les Siluroidei

Résumé

La structure des gonades et des gamètes est fortement diversifiée chez les Siluroidei ; dans quelques groupes les testicules sont composés d'une partie antérieure incluant les tissus spermatogénétiques et d'une partie postérieure avec des vésicules séminales pouvant ou non stocker les spermatozoïdes. Chez les espèces d'intérêt aquacole, les ovaires présentent une morphologie générale voisine de celle des autres poissons téléostéens possédant un oviducte. Les spermatozoïdes sont biflagellés chez le poisson-chat américain (*Ictalurus punctatus*) et monoflagellés chez le silure européen (*Silurus glanis*) et les poissons-chats africains (*Clarias gariepinus, Heterobranchus longifilis*). Les ovules montrent des différences de tailles considérables selon les groupes : de 0,8-1,2 mm chez certains Pimelodidae et Clariidae jusqu'à 15-20 mm chez les Ariidae. Dans de nombreux cas, les œufs sont adhésifs ; ils peuvent alors être entièrement enrobés d'une couche collante (*Ictalurus, Chrysichthys, Silurus*) ou posséder un simple anneau adhésif (*Clarias, Heterobranchus*). En général, la fécondation est externe mais l'existence d'une fécondation interne est signalée chez quelques espèces. La production annuelle de sperme a été mesurée chez le silure européen et le poisson-chat américain pour lesquels elle atteint $1{,}7 \times 10^{11}$ et $1{,}8 \times 10^{10}$ spermatozoïdes.kg^{-1} de poids corporel, respectivement. Chez les femelles, la fécondité varie entre environ 50 ovules par ponte chez les Ariidés et plus de 100 000 ovules.kg^{-1}, chez les Pimelodidés et Clariidés par exemple. Une grande diversité de méthodes de reproduction contrôlée ont été développées chez les Siluroidei. Dans quelques cas (*Ictalurus, Chrysichthys, Hoplosternum*), la ponte se produit naturellement en étang ou en bac à condition que des substrats de pontes appropriés soient disponibles, les œufs sont collectés immédiatement après la ponte et placés en incubateurs jusqu'à l'éclosion. Pour la plupart des espèces, bien que des pontes naturelles ou semi-naturelles puissent être obtenues, la tendance actuelle est au développement de techniques d'induction hormonale de l'ovulation et de fécondation *in vitro* qui permettent un meilleur contrôle des différentes étapes de la reproduction et autorisent la conservation et la manipulation des gamètes. La maturation ovocytaire et l'ovulation ou la spermiation peuvent être induites chez de nombreuses espèces par une grande variété d'hormones (GnRHs, gonadotropines de poissons, hCG, différents stéroïdes...). Quelques informations sont disponibles sur la biologie des gamètes et leur conservation. La motilité des spermatozoïdes est de courte durée, n'excédant pas 70-80 s de progression comme chez la plupart des autres téléostéens d'eau douce. Une particularité des spermatozoïdes de silure européen est leur activation par de l'urine au moment du prélèvement, cette activation peut toutefois être empêchée par une récolte directe dans une solution d'immobilisation (NaCl 200 mM, Tris HCl 30 mM, pH 7.0). La cryoconservation du sperme a été tentée avec succès chez plusieurs espèces. Les ovules survivent quelques heures après l'ovulation et leur fécondation doit être réalisée rapidement. Les méthodes utilisées pour la récolte des gamètes, l'insémination et l'incubation des œufs sont décrites pour les espèces de Siluroidei dont l'élevage est le plus répandu.

Mots-clés : Siluroidei, reproduction, gonades, gamètes, ponte, fécondation, incubation des œufs.

INTRODUCTION

Although some exceptions are reported, as is the case of *Hoplosternum littorale* who build a nest provided that vegetable material is available (Pascal *et al.*, 1994), catfishes or Siluroidei generally do not reproduce spontaneously under culture conditions. In most cases gametogenesis is completed but final oocyte maturation, ovulation and spawning do not occur. This situation, common in teleosts, indicates that the ovulatory mechanisms and spawning behaviour necessitate exogenous stimuli which are both distinct from those involved in the process of vitellogenesis and absent from the usual farming environment (Harvey and Hoar, 1980; Stacey, 1984; Legendre and Jalabert, 1988). When spawning is not possible, a massive atresia of all the oocytes may occur as reported for *Chrysichthys nigrodigitatus* (Nunez Rodriguez *et al.*, 1995). In clariid catfishes, the final stimulus to spawn appears to be associated with a rise in water level and flooding of marginal areas (Bruton, 1979). The artificial flooding of previously dry or shallow habitats has often been used for inducing *Clarias* species to spawn (*see* below). In the absence of such stimuli, the gonadotropin (GtH) surge, the event behind the processes that lead to oocyte maturation and ovulation in fishes (Jalabert, 1976; Goetz, 1983), does not occur. Most of the hormonal treatments aiming at inducing ovulation or stimulating spermiation in captive fishes either simulate (hypophysation, gonadropic hormones) or artificially induce (hypothalamic hormones) this endogenous discharge.

Unlike many orders of fishes, Siluroidei have representatives in all major breeding guild categories

(non-guarders, guarders and bearers) and exhibit a wide range of reproductive characteristics and spawning behaviour (Bruton, 1996). Depending on the reproductive biology and environmental spawning requirements of the species, techniques of controlled reproduction in catfish culture may involve various procedures of natural spawning (provided final spawning stimulus through adequate environmental cues including or in the absence of spawning substrate), hormonal-induced spawning, or hormonal-induced ovulation and artificial insemination. Nowadays, although natural spawning in captivity is still used on commercial fish farms (particularly for the Channel catfish, *Ictalurus punctatus*), hormonal induced-ovulation and artificial fertilization are attracting much attention in terms of research and applications as they allow the greatest control over the various steps of reproduction and allow gamete preservation and manipulation.

This review summarizes the available data on morphology of gonads, morphology and production of gametes, requirements for spawning and their implications for controlled reproduction of catfishes. Methods and constraints for gamete collection, insemination and incubation of eggs are presented for some of the most widely cultured Siluroid species. Present knowledge on the biology and preservation of gametes in this fish group is also reviewed.

GAMETE RELEASE AND COLLECTION

Gonad morphology

Ovaries

The ovaries of Siluroidei are generally paired elongated organs suspended in the dorsal body cavity by mesovaria and connected to the genital pore by short oviducts. In pre-spawning females of African Clariids (*Clarias gariepinus, Heterobranchus longifilis*), Richter and Van Den Hurk (1982) and Legendre *et al.* (1992) presented the ovary as a hollow, sac-like structure consisting of an outer wall with lamellae containing oogonia and oocytes in various stages of development (*i.e.* previtellogenic, vitellogenic, postvitellogenic and atretic follicles). A similar pattern was reported for *Clarias batrachus* (Lehri, 1968), *Silurus glanis* (Konstantinov, 1941; Hochman, 1967), *Heteropneutes fossilis* (Sundararaj and Sehgal, 1970) and *Ictalurus punctatus* (Grizzle, 1985). In mature pre-spawning siluroid females (0.1-25 kg body weight), the gonadosomatic index (GSI) ranges between 10 and 20% (*table* 1).

Testis

The morphology of the male testis is highly variable in the various groups of siluroids. An example of this variety was given by Loir *et al.* (1989) for seven South American catfish families (*Fig.* 1). The testis is composite: some families show testis entirely formed by spermatogenetic tissue; in some others the posterior part becomes sterile and organized in seminal vesicles which do not have germinal cells but may store spermatozoa at least in some cases. The sperm duct is also highly variable in length and structure and some species exhibiting internal fertilization possess a gonopodium. Several works have been devoted to the testis structure. A short description of the structure of *Clarias gariepinus* testis was given by Van Oordt *et al.* (1987) showing twisted seminiferous tubules running in an anterio-posterior direction in the gonad. In Ictaluridae *Ictalurus punctatus* Sneed and Clemens (1963) and Grizzle (1985) have described testis made up of lobes. They include spermatogenetic tissue in the anterior and middle part (3/4) and show annual changes in size and colour and secretory lobes in the posterior part (1/4) which are smaller and remain pink throughout the year and produce secretory material; spermatozoa are not stored in these posterior lobes

Table 1. – Usual body weight (BW), age and pre-spawning gonadosomatic index (GSI) of female broodfish, and relative fecundity and diameter of ova in some siluroid species.

Species	Female BW (kg)	Age (years)	GSI (%)	Fecundity (per kg BW × 10^3)	Oocyte/ova diameter (mm)	References
Galeichthys feliceps	–	not cultured	–	50 (*)	16	Tilney and Hecht (1993)
Ictalurus punctatus	1.5-4	3-6	15	8	3	Grizzle (1985); Tucker and Robinson (1990)
Chrysichthys nigrodigitatus	1.5-3	3-4	8-10	15-18	2.5-3	Hem (1986), Otémé (1993)
Silurus glanis	4-25	>3	3-15	10-25	2.5-2.9	Fijan (1975); Horvath and Tamas (1976); Wisniewolski (1988); Kouril *et al.* (1993)
Hoplosternum littorale	0.07-0.3	1	6-12	45-75	1.4	Hostache *et al.* (1993); Pascal *et al.* (1994)
Clarias gariepinus	00-3	1-3	15-20	60-150	1.2-1.5	Hogendoorn (1979); Richter *et al.* (1987) Legendre *et al.* (1992)
Clarias macrocephalus	0.1-0.2	–	10-12	20-50	1.3-1.6	Ali (1993); Tan-Fermin and Emata (1993)
Heterobranchus longifilis	2-6	2-4	15	30-120	1.2-1.6	Legendre (1986); Legendre and Otémé (1995)
Pseudoplatystoma coruscans	5-13	–	–	120-130	0.8	Cardoso (pers. comm.)

(*): absolute fecundity.

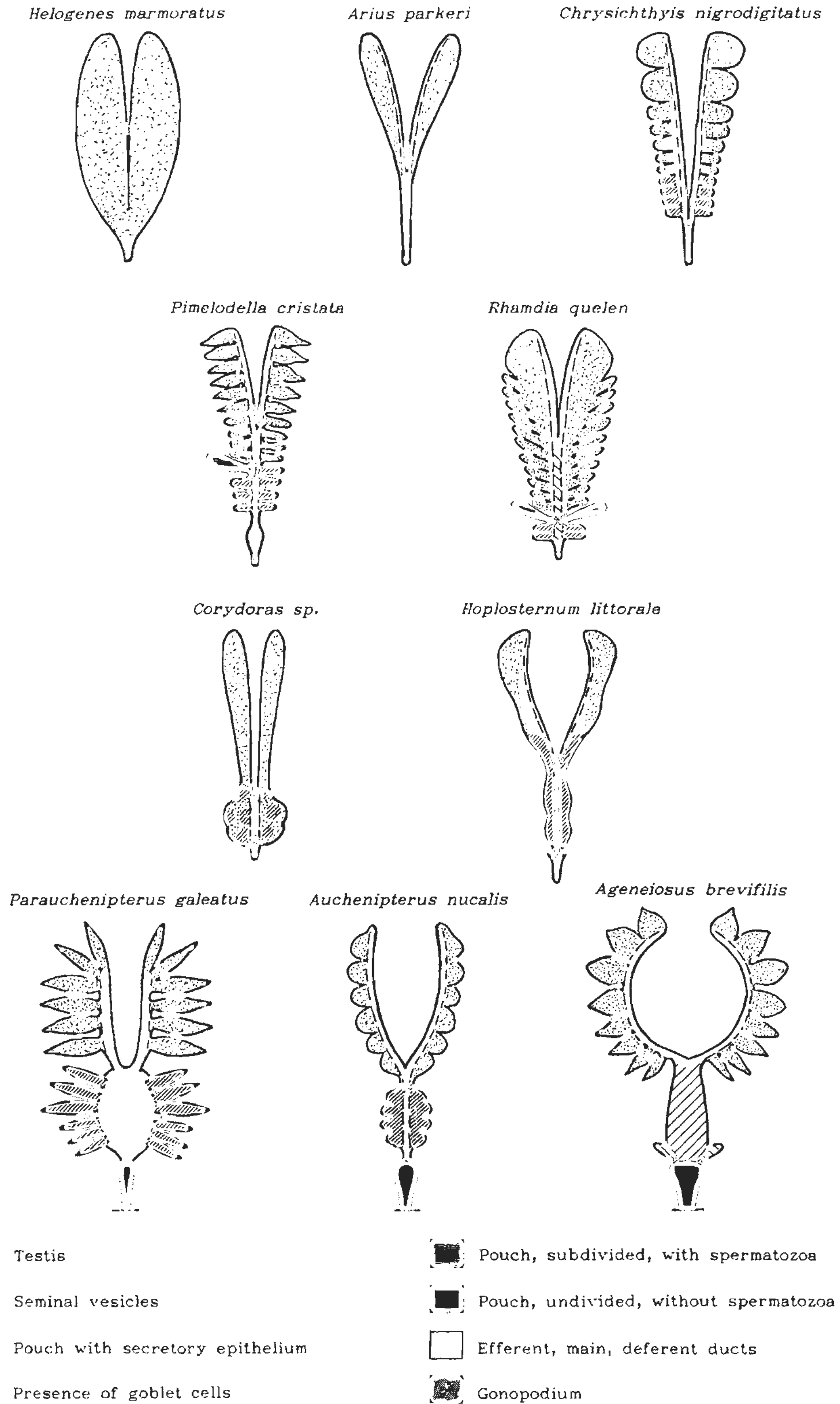

Figure 1. – Schematic representation of the testis of some siluroid species. After Loir *et al.* (1989).

and secretions are not essential to their viability. On the contrary in *Heteropneustes fossilis*, the seminal vesicle ensure a spermatozoa storage and secrete various compounds including sialic acid according to Senthilkumaran and Joy (1993) who suggested it may have a role in sperm survival. A detailed study by Fishelson *et al.* (1994) show that the seminal vesicles of *Clarias gariepinus* are formed after spermatogenesis has started; they are first simple protrusions from the sperm duct and become more complex with age. Spermatozoa are not usually found in the distal part of pouches and accumulate in the proximal part during spawning.

In *C. gariepinus* it was shown that the seminal vesicles have the capacity to produce glucuronidate steroid (Van den Hurk *et al.*, 1987*a, b*, Resink *et al.*, 1987*a*) and are the source of sex attractant for the females (Resink *et al.*, 1987*b*).

Gamete morphology and gamete production

Ova

Size and number of ova produced in an individual spawn show a very high interspecific variability in the siluroids. Diameter of ova ranges between 0.8 and 15-20 mm while relative fecundity varies from a few dozen up to 150 000 ova per kg female body weight (*table* 1).

The biggest eggs and the lowest fecundity are found in the ariids which display paternal buccal incubation (Rimmer and Merrick, 1983). Such extremely low fecundity constitutes a major constraint for their culture as a very large number of breeders would have to be maintained on a fish farm for a massive production of eggs and fry. This might explain why ariid catfishes have not been considered as candidate for aquaculture up to now, despite their high appreciation by consumers and high commercial value in many countries.

Although ova and eggs of some species are non adhesive, *e.g.* in *Pseudoplatystoma coruscans* (Cardoso *et al.*, 1995) or in *Silurus lithophilus* (Kobayakawa, 1985), most catfishes possess demersal eggs, spherical or slightly oval in shape, which become sticky after contact with water. Catfish eggs adhere together (forming an egg mass as in *Ictalurus punctatus* or *Chrysichthys nigrodigitatus*) or to substrates using different modes of attachment. In *S. glanis* and two Japanese *Silurus* species (*S. asotus, S. biwaensis*), *Rhamdia sapo* or *I. punctatus*, the eggs adhere via a thick mucous coating (Kobayakawa, 1985; Cussac and Maggese, 1986; Abraham *et al.*, 1993; Yemelyanova, 1993). In *Clarias gariepinus*, Richter and Van den Hurk (1982) and Riehl and Appelbaum (1991) reported the presence, around the micropyle and the future animal pole of ova, of an annular bulge ("attachment disc") consisting of numerous tiny attaching-filaments embedded in a cementing substance containing acidic mucopolysaccharides. A similar attachment system was reported in *Heterobranchus longifilis* (Legendre and Teugels, 1991). In *Sturisoma aureum*, the eggs are fastened by numerous, 25 μm long attaching-filaments, which arise at the animal pole (Riehl and Patzner, 1993).

Yemelyanova, (1993) discussed the formation of the oocytes envelopes in *I. punctatus*: the follicular cells, 15 μm high, are elongated in previtellogenic oocytes up to 350 μm and reach 20 μm in 1 500 μm oocytes. The vitelline membrane consists of two layers and the chorion progressively differentiates during the process of oocyte growth over the vitelline membrane reaching 60 μm in maturing oocytes. This author observed that the chorion results mostly from secretion of follicular cells, but some contribution of the oocytes cannot be excluded. In *C. gariepinus*, Richter and Van den Hurk (1982) reported that the " attachment disc" is secreted by the thickened ring of cylindrical follicular cells (60 μm in height) located around the micropyle. In *S. glanis*, the follicular cells proliferate during early vitellogenesis with an epithelial organization which becomes disrupted, and transformed into a large irregularly structured mass of muco-follicle cells forming a thick belt (up to 80-100 μm) and engaged in an intensive secretory activity (Abraham *et al.*, 1993). The mucus coat resulting from this process is made of acidic mucopolysaccharides and mucoproteins, which in contact with water swell and become bouncy and adhesive.

The eggs of *Sturisoma aureum* (2 mm in diameter) show a special surface pattern with 22 deep furrows, which run from the vegetatal pole to the animal pole and may function as a sperm guidance system (Riehl and Patzner, 1991).

Sperm

The spermatozoa morphology of Siluroidei was reviewed by Jamieson (1991) and detailed electron microscopic studies were published by Poirier and Nicholson (1982) and Emelyanova and Makeyeva (1991, 1992). The available information on siluroid spermatozoa indicates a large variability of structure especially in the shape of the head and Emelyanova and Makeyeva (1992) have studied the spermatozoa morphology of several species of catfishes. The nucleus is elongated in several families: Auchenipteridae and Ageneiosidae (Loir *et al.*, 1989). In *Tatia brunea* the head in slightly helical. The elongated shape of the nucleus goes with the presence of a gonopodium in males and an internal fertilization in the female genital tract. In most of the other known families the nucleus is spherical but there are reports of a slightly elongated head in *Clarias senegalensis* (Mattei, 1970). The head of spermatozoa of *Silurus glanis, Pseudobagrus fulvidraco, Liocassus ussuriensis* and *Ameiurus nebulosus* is spherical (Emelyanova and Makeyeva, 1992). A detailed study of the spermatozoa morphology of *Ictalurus punctatus* was given by Jaspers *et al.* (1976), Poirier and Nicholson (1982) and Emelyanova and Makeyeva (1991); the head

Table 2. - Measurements on spermatozoa of some siluroid species.

Species	Head (μm)	Mid- piece (μm)	Mitochondria	Flagella		References
				number	μm	
Silurus glanis	1.8	1.6 × 0.8	2-3	1	50	Emelyanova and Makeyeva (1992)
Pseudobagrus fulvidraco	1.8 × 1.7	1.3 × 0.4	4-5	1	60	Emelyanova and Makeyeva (1992)
Ameiurus nebulosus	1.7 × 1.8 1.1 (*)	2 × 1.8	up to 20	2		Emelyanova and Makeyeva (1992)
Ictalurus punctatus	1.8 × 2	1.5 × 3	up to 35	2	80-100 × 0.2	Emelyanova and Makeyeva (1991)
Ictalurus punctatus	2.3 ± 0.01 **2.4 ± 0.01	1.6 ± 0.01 **3.1 ± 0.01		1-2	94.9 ± 0.26	Jaspers *et al.* (1976)

(*) apical part, (**) mean ± SD.

diameter was 1.8-2.3 μm, the length and width of mid piece, 1.5-1.6 and 3.0-3.1 μm respectively and the flagellum length 80-100 μm (*table* 2). The flagellum had a classical 9+2 axoneme; the posterior part of the flagellum was twisted (Emelyanova and Makeyeva, 1992). Works by Emelyanova and Makeyeva (1991) and Poirier and Nicholson (1982) indicated that all spermatozoa have two flagella while Jaspers *et al.* (1976) reported that only 4.7% of the spermatozoa were biflagellar but comments by Emelyanova and Makeyeva (1992) suggest that the two flagella are in close contact with each other and twisted; this gives the impression of a single flagellum. Jamieson (1991) identified two flagella in the picture of *Rhamdia sapo* spermatozoa shown by Maggese *et al.*, 1984. In *Rhamdia sapo* the head is spherical and the mid piece is limited in size (Maggese *et al.*, 1984; Emelyanova and Makeyeva, 1991). Spermatozoa are usually free and even in the case of internal fertilization they are not organized into spermatophores, probably due to the presence of a gonopodium (Loir *et al.*, 1989).

In culture conditions, males of *S. glanis* have a low GSI (1-1.5%) and a low spermatogenetic production (1.7 10^{11} spz.kg^{-1} body weight) (Saad and Billard, 1995); GSI is also low (≈ 1%) in *S. glanis* found in the wild (Zholdasova and Guseva, 1987). In the channel catfish the GSI (0.25%) and the spermatogenetic production (1.8 10^{10} spz.kg^{-1} bw) are even lower (Guest *et al.*, 1976*a*). The GSI is also low, not exceeding 1%, in *Clarias gariepinus* (Van Oordt *et al.*, 1987) and *Heterobranchus longifilis* (Legendre, 1992), and 0.2-0.6% in *Hoplosternum littorale* (Hostache *et al.* 1993).

Intratesticular spermatozoa concentration in 10^9 spz.g^{-1} testis was measured by Guest *et al.* (1976*a*) in channel catfish (7.1±0.8) and by Saad and Billard (1995) (7.2±1.3) in *S. glanis* and, in 10^9 spz.ml^{-1}, by Legendre *et al.* (1992) in *H. longifilis* (3.0) and by Steyn and Van Vuren (1987*a*) in *C. gariepinus* (6.2). The concentration of spermatozoa in the milt of *S. glanis* is difficult to assess due to contamination by urine (Linhart *et al.*, 1986) explaining the wide differences found in the literature; in 10^9 spz.ml^{-1}: 0.6-1.6 (Linhart *et al.*, 1986), 7.2 (Saad and Billard, 1995), 0.2-3.5 (Redondo-Müller, 1990).

Pre-spawning conditions and choice of broodfish

After reaching sexual maturity in nature, *Ictalurus punctatus* (Busch, 1985), *Chrysichthys nigrodigitatus* (Hem, 1986) or *Silurus glanis* (Horvath, 1984) reproduce only once a year while *Clarias gariepinus* (Clay, 1979), *Rhamdia sapo* (Espinach Ros *et al.*, 1984) or *Hoplosternum littorale* (Pascal *et al.*, 1994) seem capable of multiple spawning.

In cultivated African Clariid species, the rainy season is the primary reproductive period. Nevertheless, sexually mature individuals can be found all year round in ponds or lagoon enclosures (Micha, 1976; Legendre 1986; Nwadukwe *et al.*, 1993), provided with adequate and unrestricted feeding (Pham and Raugel, 1977; Richter *et al.*, 1987). Under hatchery conditions, good quality eggs and sperm of *C. gariepinus* can be obtained continuously when water temperature is constantly maintained at 25°C; at 30°C the proportion of atretic oocytes increases and the testis regresses (Richter *et al.*, 1982). At 25°C, a same female can be stripped every 6-8 weeks (Hogeendoorn and Vismans, 1980).

Ictalurus punctatus begin to spawn when the minimum water temperature exceeds 21°C for several days; normal spawning temperature range from 21 to 29°C with 26°C considered as optimal, water temperatures of 30°C or higher can adversely affect egg development and fry survival (Busch, 1985; Zlatev, 1987). Channel catfish can mature as early as 2 years of age and as small as 340 g. However, broodfish of at least 3 years old and about 1.5 kg body weight are preferred for reliable spawning (Busch, 1985). Good broodstock care entails the maintenance of appropriate standing crop weights (density not exceeding 1 300 kg.ha^{-1}) and the provision of an adequate food supply (generally pelleted feed supplemented or not with beef liver).

In *S. glanis*, the sexes are separated when the water temperature reaches 12-15°C (April) in order

to prevent the males to injure females by biting. About 1-1.5 month before spawning, broodfish are held in special holding ponds and fed on forage fish (carp, tench, goldfish, bream etc.; Kouril *et al.*, 1981) stocked with 2 to 3 times the total weight of the fish (Kouril *et al.*, 1993). They readily accept pelleted feeds as well. The breeders can consume 30 % of their annual ration during that time (Horvath, 1984). Spawning can be started as soon as the pond water temperature reaches 20-22°C (Horvath, 1984; Kouril *et al.*, 1993). In the case of hormonal induced reproduction, broodfish are kept separate in tanks divided into individual compartments. If fish are stored in groups, the mouth can be closed with a piece of string passed through holes drilled in the nasal and chin bones after anesthesia. This procedure does not disturb the opercular movements and the processes of maturation (Horvath, 1984). Ribes and Ribes (1991) and Kohde and Jahnichen (pers. comm.) stated that it is possible to reproduce *S. glanis* in heated water (20-26°C) at any time of the year.

In *Chrysichthys nigrodigitatus*, which is a common fish in West African lagoons, the breeders are generally kept in lagoon enclosures at a low stocking density of 0.1 fish.m^{-2} and fed with a 35% crude proteins pelleted feed supplemented once a week with beef liver (Hem, 1986). Spawning begins at the end of the major rainy season when the salinity is lower than 5 g.l^{-1} and temperature around 27-29°C. In this catfish, a rise in water salinity up to 7 g.l^{-1} or in water temperature above 32°C induce oocyte atresia and regression of the ovaries in initially fully mature females (Hem *et al.*, 1994).

While preparing the fish for reproduction, mature males and females of *I. punctatus* (Tucker and Robinson, 1990) and *C. nigrodigitatus* (Hem, 1986) are easy to distinguish as secondary sexual characteristics are strongly pronounced. In both species, mature males display a broad, muscular head wider than the body and thickened lips, while head of females remains slender. These characteristics are commonly used as indicators of the ripeness of males. In Clariid catfishes, sex is easy to identify based on the shape of the genital papilla which is elongated and pointed in male, while in the female the vent is more rounded with a longitudinal cleft (De Kimpe and Micha, 1974; Mollah and Tan, 1983*a*; Legendre, 1986). However, in the absence of other evident secondary sexual characteristics and of semen emission during stripping, it is not possible to judge externally whether or not a male has developed mature testis. In *S. glanis*, sexual dimorphism is not very pronounced. Females have a comparatively more oval, convex and blunt genital papilla, while male present a more conical papilla and a brush part on the first spin ray of the pectoral fin (Kouril *et al.*, 1993), but difficulty in distinguishing differences may lead to errors in sexing. As an alternative, Fijan (1975) proposed the use of a slightly modified medical otoscope, the extremity of which is inserted through the genital papilla into the short duct for direct observation of the gonads. In some catfishes, such as *Pangasius hypophthalmus* (senior synonym of *P. sutchi*) (Thalathiah *et al.*, 1988) or *Pangasius bocourti* (Cacot, pers. comm.), no external characteristics allow for distinction of sexes. Males are identified only when sexually mature by emission of sperm upon hand-pressure onto the abdomen. Although in most species, macroscopic criteria (thickness and softness of the belly region, swollen and reddish genital papilla) have been used for determination of the sexual maturity stage of the females, the direct observation of oocytes (diameter, position of the germinal vesicle) sampled by canulation of the ovaries is now widely recognized as the most accurate and reliable method.

Usual body weight and age of broodfish are given in *table* 1 for some siluroid fishes commonly used in aquaculture.

Natural spawning

Natural spawning – *i.e.* reproduction in captivity without any use of hormonal treatment – was developed in *Ictalurus punctatus*, *Chrysichthys nigrodigitatus*, *Silurus glanis*, *Clarias batrachus* and *Hoplosternum littorale*.

In *Ictalurus punctatus*, reproductive traits and practices have been reviewed recently by Busch (1985) and Tucker and Robinson (1990). In nature, channel catfish spawn under edges, around or in submerged logs, stumps or roots and in cavities in the bank. The male typically prepares a nest by clearing soft mud and debris from an easily protected area. Channel catfish are sequential spawners. Periodically, the female deposits a layer of eggs and the male fertilizes them. Clemens and Sneed (1957) reported that it takes 4 to 12 hours for a brood fish pair to complete an egg mass with eggs being released five to ten times per hour. The male assumes care of the egg mass after oviposition.

The method of propagation most commonly practised is the "*pond method*" in which seasonally ripe brood fish held in ponds are provided with spawning containers and allowed to spawn at will. This method, requires minimal facilities and the less amount of time, labour and skill of the culturist. Spawning success (percentage of female spawning) ranges from less than 40 to over 80%, depending on water temperatures during the spawning season and condition of broodstock (Tucker and Robinson, 1990). As one male can spawn several times in one season, a sex ratio in favour of females was often used. However, Bondari (1983) obtained equal spawning success (in terms of spawns produced, egg mass weight or hatchery traits) in ponds stocked with male to female ratio of 1:1, 1:2, 1:3 and 1:4. However, the average number of days required for a female to produce an egg mass was longer for fish stocked at the 1:4 ratio versus those stocked at the 1:1 ratio. A 1:1 sex ratio was recommended by Smitherman *et*

al. (1983) in order to minimize inbreeding. A great variety of containers have been successfully used as nesting site (*e.g.* 38 l milk can and metal or wooden boxes). They are placed horizontally anywhere in the pond at 0.30 to 0.75 m water depth and are inspected each third to fourth day to determine whether eggs are present. Egg masses may be left in the pond or transferred to a hatchery. In this case temperature and diseases can be more readily controlled, predation on the eggs and fry can be eliminated and swim-up fry can be strayed on artificial rations.

The so-called "*pen method*" consisting of placing one pair of mature breeder in a pen (commonly 1.5 x 3 m) implemented in ponds is also used to some extent. It facilitates the use of selected fish for mating, protection against intruders and also allows the use of hormones for better control over the spawning act. The fish farmer must be able to accurately determine the sex and relative condition of his brood fish to successfully spawn channel catfish in pens (Busch, 1985).

The African claroteids, *Chrysichthys nigrodigitatus* and *C. maurus*, display a reproductive behaviour similar to that of *Ictalurus punctatus*. In nature, during the reproductive season, the eggs are released in cavities under rocks or pieces of wood where they are generally guarded by the parents until hatching. The technique of controlled reproduction which is now in use for *C. nigrodigitatus* on commercial fish farms in Ivory Coast was based on the observation of this breeding behaviour (Hem *et al.*, 1994). Pairs of mature breeder are chosen on the basis of morphological criteria and the size of oocytes determined after intraovarian biopsy. Each pair, constituted by one male and one female of similar size is then confined in a spawning container made of a piece of PVC tube (30 cm in diameter and 80 cm in length). During their stay in the container, the brooders are not fed and let in darkness. Spawning occurs generally after 7 to 30 days depending on the initial oocyte diameter. Hem (1986) reported successful oviposition in more than 80% of the females using this technique. A spawning procedure similar to the pen method described for *Ictalurus punctatus* (pair of brooders placed in small pen or tank with a PVC spawning container, but not confined in it) was also tested in *C. nigrodigitatus* (Otémé, 1993), but in that case the spawning success was lower and did not exceed 40%.

In central Europe a modification of the carp Dubravius methods (Dubravius, 1547) was used for natural spawning of *S. glanis* (Jasfalousi, 1954; Mihalik, 1956; Mares *et al.*, 1970). When the water temperature reaches 20 °C, groups of mature females and males are introduced into small ponds of 0.1-0.3 ha equipped with nests made of willow roots, pyramidal in shape and fixed strongly to the bottom of the pond. One nest is provided for each pair of broodfish (Wisniewolski, 1989; Woynarovich and Horvath, 1980). The male cleans the nest and after a period of courtship spawning takes place, generally when the temperature goes up to 22-24°C (Steffens pers. comm.). The fertilized eggs which adhere to the nest are guarded by the male. Half day before hatching the nests are transferred to another pond, or are taken apart, the egg-covered part being placed into net cages or boxes with a water renewal of 7-10 $l.min^{-1}$ at 20-22°C (Woynarovich and Horvath, 1980).

C. batrachus and *C. macrocephalus* have similar reproductive behaviour and spawn during the rainy season along the margin of water body or in rice fields. The females make small, round hollow nests, about 30 cm in diameter and 5-8 cm deep, in the grassy bottom in shallow waters. The eggs are deposited in the nest, and being adhesive, stick on the soil and surrounding grass and are guarded by the male (Pillay, 1993). However, contrary to *C. batrachus*, *C. macrocephalus* do not seem to spawn in confined waters of fish ponds and thus hormonal treatments are necessary (Carreon *et al.*, 1973). The technique for natural spawning of *C. batrachus* in Thailand uses spawning ponds from 4 000 to 32 000 m^2 in area and 50-70 cm in depth (Areerat, 1987). At the bottom of the ponds, holes (15-20 cm in diameter and separated by a 1 m distance) used as the nest and ditches used for holding the broodfish during the operation are dug, and rice or grass is grown. Before reproduction, broodfish are abundantly fed with a mixture of 90% ground trash fish mixed with 10% rice bran. Stocking rate is about 1 pair/6 m^2. When fish are ready to spawn, feeding is stopped and the water is pumped out of the ditch before new and fresh water is introduced to stimulate spawning. After refilling the pond, spawning occurs in the nesting holes within 1-2 days. After hatching, the fry remain in a school in the hole and are collected with small scoop nets 8-9 days after spawning. Areerat (1987) reported that using this method, the breeders can spawn 12 times a year and the fry production in each crop ranges from 1 250 000 to 2 500 000 $fry.ha^{-1}$. A similar method of environmentally induced spawning was developed in Indonesia using specialized breeding ponds and concrete boxes filled with fibrous matting obtained from palm trees as nesting sites; soon after spawning the eggs were removed from the pond and incubated in the hatchery (Knud-Hansen *et al.*, 1990).

In *Clarias gariepinus*, successful spawning can be achieved when ripe fish are placed in freshly filled ponds that had been dry for a time (De Kimpe and Micha, 1974; Van Der Waal, 1974). However, the number of fingerlings obtained is generally very poor (Richter, 1976). As *C. gariepinus* does not show any parental care (Bruton, 1979), the fry are not concentrated and cannot be collected as in *C. batrachus*. Cannibalism between the young fish and heavy predation by frogs, aquatic insects and other aquatic animals occur and can be considered as responsible for the low fry survival. Because of these limitations, such a breeding method has not much practical importance.

In *Hoplosternum littorale* spawning occurs in floating nests built by the male on the water surface with froth and vegetable fragments. The most particular steps of the reproductive behaviour are the oral milt collection by the female and its transfer to the foam bed before laying (Gauthier *et al.*, 1988). The male takes care of the nest until hatching, adding bubbles, and protects the eggs by vigorously attacking any intruder. A nest may contain egg spawned by several females (up to five) which are fertilized by the same male (Pascal *et al.*, 1994). On the other hand, these authors reported that a same female may spawn up to 14 times in one reproductive season and that a 100 g-female could lay 48 600 eggs representing about 180% of its body weight. In captivity, reproduction generally occurs in ponds, but is also possible in 1 m^3 pen or cage or even in 200 l aquaria if appropriate vegetable materials are provided for nest building (Luquet *et al.*, 1989; Pascal *et al.*, 1994). As parental care ends after hatching, the newly hatched larvae are susceptible to heavy predation in ponds. For this reason and for easier management of larvae, it is desirable to conduct the end of incubation out of the nest (Hostache *et al.*, 1992).

Hormonal-induced ovulation, spermiation and spawning

Female

Semi-natural spawning *–i.e.* the combination of hormonal-induced ovulation and spontaneous oviposition and fertilization of the eggs by a male in pond, tank or aquarium– have been practised to some extent in several catfishes, *e.g.*: *I. punctatus* (Busch, 1985), *S. glanis* (Grozev, 1968), *Heteropneustes fossilis* (Khan, 1972) or *C. batrachus* (Devaraj *et al.*, 1972). However, it proved unreliable for fingerling production in *C. gariepinus* due to heavy losses of egg and fry (Hogendoorn, 1979). Nowadays, hormonal-induced oocyte maturation and ovulation followed by the stripping of ova and artificial fertilization has been carried out for experimental purposes and/or commercial production in most of the Siluroid species considered for aquaculture. Hormonal treatments, latency time between injection and ovulation, and ovulation success in major cultivated catfishes are given in *table* 3.

In *I. punctatus*, hormone spawning remains seldom used in the commercial catfish industry as fry are usually obtained by natural spawning. Nevertheless, it may have important practical advantages as it allows prediction of spawning time and facilitates synchronous spawning of females (Dunham, 1993). After injection with carp pituitary, hCG (human chorionic gonadotropin) or LHRH analogue, the female is usually placed together with a male into an aquarium or tank were spawning and fertilization take place. Hand-stripping of eggs and artificial fertilization are mostly used when hybrids or triploids are to be produced. Stripping is done when the females begin depositing eggs. Dunham (1993) reported that, starting from this moment, the collection of all the eggs requires 3-5 stripping at 1-2-h intervals. To date, consistent use of hormones to spawn Channel catfish by control of oocyte maturation and ovulation is not considered as fully accomplished (Tucker and Robinson, 1990).

Induced-spawning through hormonal therapies is practised in *S. glanis* to a greater extent (Proteau and Linhart, 1993). The methodology of hypophysation varies mainly in relation to the preparation protocol of the pituitary extract, the injected dose and the number of injections. The females ovulated after one or two carp pituitary injections of 4-5 $mg.kg^{-1}$ body weight (Fijan, 1975; Horvarth and Tamas, 1976; Kouril and Hamackova, 1982). Ovulation was reported after injection of LHRHa alone (Kouril *et al.*, 1987) or combined with dopamine antagonists such as pimozide or domperidone (Epler and Bienarz, 1989). In the case of off-season reproduction, Proteau (pers. comm.) reported that after treatment with LHRHa ($100\pm\mu g.\ kg^{-1}$) associated with pimozide (5 $mg.kg^{-1}$), a supplementary injection of carp pituitary (5 $mg.kg^{-1}$) could be applied successfully when ovulation was not already obtained 40 h after the first injection.

Among siluroids, it is in the African catfish, *C. gariepinus*, that the greatest variety of hormonal treatments have been tested for artificially-induced breeding of female broodfish (*Table* 3). Based on previous studies carried out in the Indian catfish *Heteropneustes fossilis* (*see* Sundararaj and Goswami, 1977), 11-deoxycorticosterone-acetate (DOCA) was first used for artificial reproduction in *C. gariepinus* (De Kimpe and Micha, 1974; Pham and Raugel, 1977; Hogendoorn, 1979), but Richter and Van den Hurk (1982) demonstrated that this steroid could induce oocyte maturation only and not ovulation. As stripping of eggs could be possible after treatment with DOCA, these authors came to the conclusion that ovulation was evoked mechanically. More recently, 17α-hydroxyprogesterone was used successfully for inducing both oocyte maturation and ovulation, but two successive injections were required (Richter *et al.*, 1985). Haider and Rao (1994) have induced ovulation of *C. batractus* females after injection of 17α 20 β Pg plus salmon gonadotropin (SG-G100). The nature and role of steroid hormones implicated in the process of oocyte maturation and ovulation in the Siluroidei were discussed by Richter *et al.* (1987).

Oocyte maturation and ovulation of *C. gariepinus* were also induced after injection of pituitary extract from *Clarias* (Van der Waal, 1974; Micha, 1976; Schoonbe *et al.*, 1980; Hecht *et al.*, 1982), common carp (Hogendoorn, 1979; Hogendoorn and Vismans, 1980; Richter and Van Den Hurk, 1982), tilapia or *Heterotis* (Micha, 1976), or after injection of hCG alone (Eding *et al.*, 1982) or combined with carp pituitary extract (Shoonbee *et al.*, 1980). Hypothalamic

Table 3. – Hormonal treatment, water temperature, latency time (between last injection and ovulation) and percentage of ovulated females in cultivated catfishes.

Species	Hormonal treatment (doses per kg female body weight)	Temperature (°C)	Latency time (h)	Ovulation (%)	References
Ictalurus punctatus	CP, 2 mg (1st inj.) + 9 mg (after 12 h)	26-27	28-48	70-100	Bidwell *et al.* (1985); Dunham (1993)
	hCG, 1000-1760 I.U.		24-72	50-100	Sneed and Clemens (1959); Dupree *et al.* (1966)
	LHRHa, 0.01 mg (1st inj.) + 0.09 mg (after 12 h)			88	Bush (1985); Bush and Steeby (1990)
Silurus glanis	CP, 4-5 mg (in 1 or 2 inj.)	20-24	16-33	87-100	Fijan (1975); Horvath and Tamas (1976); Kouril and Hamackova (1977)
	LHRHa, 0.01-0.05 mg	22-23	30-33	100	Kouril *et al.* (1987)
	LHRHa, 0.05 mg + PIM, 10 mg	20	24-36	100	Epler and Bienarz (1989)
Clarias gariepinus	CP, 4 mg	25	11	100	Hogendoorn and Vismans (1980); Richter and Van den Hurk (1982)
	hCG, 4000 U.I.	25	14-16	70-80	Eding *et al.* (1982)
	D-Ala^6-LHRH, 0.05 mg + PIM, 5 mg	25	12.5	100	De Leeuw *et al.* (1985)
	17α-HP, 3 mg (1st inj.) + 5 mg (after 4 h)	25	12.5	100	Richter *et al.* (1985)
	DOCA, 50 mg	25	14	(1)	Richter and Van den Hurk (1982)
Clarias macrocephalus	hCG, 2000 I.U.	26-31	13-18	95	Mollah and Tan (1983*a*)
	D-Ala^6-LHRHa, 0.05 mg + PIM, 1 mg	30	16-20	100	Tan Fermin and Emata (1993)
Clarias batrachus	CP, 6 mg	25	17	100	Zonneveld *et al.* (1988)
	D-Ala^6-LHRH, 0.05 mg + PIM, 5 mg	25	18-21	86	Manickam and Joy (1989)
Clarias fuscus	hCG, 200 I.U. + 1800 I.U. (after 6 h)	25	16-19	100	Young *et al.* (1989)
Heterobranchus longifilis	hCG, 1500 I.U.	29	12	100	Legendre (1986); Legendre and Otémé (1995)
	CP, 6 mg	26-29	7-10	–	Nwadukwe *et al.* (1993)
	Frog pituitary, 7 mg	29	7	100	Nwadukwe (1993)
Pangasius hypophthalmus (2)	Clarias pituitary + hCG, 500-1000 U.I.	28-32	8-12	–	Potaros and Sitasit (1976)
	LHRHa, 0.02 mg + 0.03-0.05 mg (after 8 h)	–	–	70	Thalathiah *et al.* (1988)
Heteropneustes fossilis	D-Lys6-sGnRHa, 0.025 mg	25	14-18	100	Alok *et al.* (1993)
Pseudoplatystoma coruscans	CP, 0.8 (1st inj.) + 6 mg (after 13 h)	23-25	9	60	Cardoso *et al.* (1995)
Rhamdia sapo	*Prochilodus* pituitary, 0.75 - 6 mg	24	17	75	Espinach Ros *et al.* (1984)

(1): oocyte maturation but no ovulation (*see* text). (2): *ex P. sutchi.*
Inj.: injection. CP: carp pituitary. PIM: pimozide. 17αHP: 17α hydroxyprogesterone.

hormone analogues (LHRHa) proved efficient in inducing oocyte maturation and ovulation in this species but their potency was greatly increased when administered in combination with pimozide (De Leeuw *et al.*, 1985) or with other anti-dopamine antagonists (Goos *et al.*, 1987).

Resink *et al.* (1989) reported that ovulation could be successfully induced in 67% of *C. gariepinus* females when they were held in the presence of a male and another ovulated female in the same water but away from any visual or tactile contact. Successful ovulation (55%) was also observed when ovarian fluid of an ovulated female was administered as replacement of the ovulated female; a combination of female and male pheromones was considered responsible for the observed ovulation responses. Pheromonal induction of ovulation may therefore constitute an interesting approach for the control of reproduction in catfish culture in the future.

After treatment with carp pituitary suspension, Hogendoorn and Vismans (1980) found in *C. gariepinus* that stripping females as soon as free-running ova (called " unripe ova " by Richter and Van den Hurk, 1982) could be obtained was too early, leading to both low hatching percentages and high proportion of deformed larvae after fertilization. The best results were obtained about 10, 3 and 1 h later at 20, 25 and 30°C , respectively; time at which the eggs could be stripped more easily than before. These findings may suggest the existence of a short post-ovulatory maturation period of ova. By contrast, in both *H. longifilis*, (Legendre and Otémé, 1995)

and *Rhamdia sapo* (Espinach Ros *et al.*, 1984), the first free-running eggs were of good quality and led directly to high hatching rates with low proportion of deformed larvae. In *H. longifilis*, Legendre and Otémé (1995) found that, in an individual female, ovulation 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.

Male

The spontaneous sperm release during the breeding season is very low in the channel catfish (Guest *et al.*, 1976*a*) and in *S. glanis* (Hilge, 1983) and males have to be injected with hormones to stimulate spermiation. This is partly due to their low GSI and low spermatogenetic production. Various hormones have been used successfully to stimulate spermiation especially hCG in *S. glanis* (Redondo-Müller *et al.*, 1990), *Pangasius sutchi* and *C. macrocephalus* (Na-Nakorn *et al.*, 1993), *Eurotropius depressirostris* (Kruger and Polling 1984), *Ictalurus furcatus* (Dunham, 1993). Carp pituitary homogenates were used in *S. glanis* (Redondo-Müller *et al.*, 1990; Linhart and Billard, 1994). In *C. gariepinus*, Van der Waal (1985) stated that contrarily to untreated fish, it was possible to collect semen by stripping from males injected with *Clarias* pituitary homogenates. GnRH was injected alone or in combination with Domperidone (Ovaprim) in *S. glanis* with limited success (Redondo *et al.*, 1990). GnRH implants had also a limited effect (Linhart and Billard, 1994). Some performances of these various treatments are reported in *table* 4 for *S. glanis*. Tambasen-Cheong *et al.* (1995) found significant increase in semen volume and decrease in sperm density 24 h after treatment with Ovaprim in *C. macrocephalus*.

The steroids directly involved in spermiation are not precisely known. Hormonal injection usually results in a sequence of steroid secretion; in *C. batrachus* hCG induces first a peak of secretion in the blood of testosterone (and 11 KT) followed by progestins and again 11 KT (Zairin *et al.*, 1993). In *C. gariepinus* exhibiting spontaneous spermiation the production of testosterone glucuronide was considerably enhanced (Resink *et al.*, 1987*a*) and 5 β reduced steroids were found in spawning fish but not in non-spawning individuals (Schoonen *et al.*, 1987). After *in vitro* incubation, *testis* of *S. glanis* stimulated by carp pituitary homogenates produced 20 α Progesterone, $17\alpha 20\beta$ Progesterone and 11β hydroxyprogesterone (Kime *et al.*, 1993).

The use of intratesticular sperm

Many siluroid species are oligospermic (GSI ≤ 1%) and the volume of sperm collected even after hormonal stimulation is generally low and in case of insufficient sperm availability males are killed and the "milt" is extracted from testis cut in small pieces and either extended in a saline solution or pressed through a

Table 4. – Yield of induced spermiation and spermatozoa motility in *Silurus glanis* (body weight 2.5-7.3 kg) injected with various hormones: hCG (Chlorulon 1500 Ferring), CPE (carp pituitary powder, Argent), LHRHa (D-Ala6- LHRHa, Sigma), Ovaprim (Syndel Canada, 1 ml contains 20 μg GnRH (D-Arg6, Trp7, Leu8, Pro9, NET) and 10 mg Domperidone. After Redondo-Müller (1990 and unpublished).

Hormone	Dose per kg body weight	Number of males	Total number of spermatozoa $\times 10^9$/kg	Motility	
				%	Time (s).
hCG	0	4	0.4	50-80	58-65
	2 000 I.U.	4	1.22 ± 0.32	80-100	75-87
	4 000 I.U.	4	1.26 ± 0.16	80-100	88-95
	10 000 I.U.	4	1.25 ± 0.30	70-90	74-84
CPE	0	7	0.2	10-100	38-94
	4 mg	7	1.6 ± 0.51	90-100	75-95
	8 mg	7	1.87 ± 0.33	90-100	62-110
	12 mg	7	2.20 ± 0.39	60-100	62-105
LHRHa (*)	0	5	0.04	10-100	38-94
	50 μg	5	0.96 ± 0.66	90-100	75-95
	100 μg	5	0.33 ± 0.18	90-100	62-100
Ovaprim	0	5	0.4		
	0.5 ml	5	0.34 ± 0.18		
	1.0 ml	5	1.80 ± 2.26		
	1.5 ml	5	2.76 ± 1.64		

(*): +Domperidone 5 mg.kg^{-1}

net fabric into the eggs. This method is commonly reported in clariid species (Hogendoorn and Vismans, 1980; Mollah and Tan, 1983*b*; Legendre, 1986) as well as in *Silurus glanis* culture (Horvath, 1984; Hollebecq *et al.*, 1987). The physiology of the intratesticular sperm of *S. glanis* is not known; it is not clear if spermatozoa "maturation", *i.e.* the acquisition of the capacity of movement, occurs in the testis between the end of spermatogenesis and the beginning of spermiation as reported in salmonids by Miura *et al.* (1992). This point must be explored especially if males are taken in pre-spermiation stage. This technique is not always applicable for instance in the case of valuable selected males. An alternative way is to practise partial castration via surgical operation (Siwicki and Jeney, 1986).

GAMETE BIOLOGY AND ARTIFICIAL FERTILIZATION

Ova viability

Ova viability refers here to the time lapse during which fertilization of ovulated oocytes remains possible once they have been emitted by the female or exposed soon after stripping to water or various solutions. This aspect has received little attention in siluroids. Linhart and Billard (1995) shown that in *S. glanis*, the fertilizability of ova was entirely lost in less than 10 min after immersion in buffered fresh water or NaCl solutions (17 or 41 mM NaCl,

5 mM Tris-HCL, pH 7.0). However, after 2 and 4 min exposure, the hatching percentage was found significantly higher in NaCl solution compared to buffered fresh water. In *H. longifilis*, egg viability appeared even shorter: after immersion in buffered water or in NaCl solution (38 mM NaCl, 5 mM Tris-HCL, pH 8.0), fertility of ova was totally lost after 90 to 120 seconds only (unpublished data). In both species, the loss of fertility was probably due to the closing of the micropyle resulting from the expansion of ova envelopes.

Sperm motility

Information on sperm motility is available only in a few species: this is probably due to the oligospermic characteristic of most of the catfishes, the difficulty of sperm collection in significant amounts and because artificial insemination is not such a common practice in the cultivated siluroid species. Most of the information available show that the spermatozoa are immotile in the genital tract and are activated by a decrease of osmotic pressure when released in freshwater. The immotility in the semen results from the osmotic pressure of the seminal fluid as in cyprinid fish and does not seem due to K^+ (like in salmonids) as shown in *Rhamdia sapo* (Cussac and Maggese, 1988). The motility intensity of *I. punctatus* sperm measured on a 0-5 standard scale of arbitrary units was on average 3 (Guest *et al.*, 1976*a*) and duration of motility was about 60 s in pond water (Guest *et al.*, 1976*b*). In *S. glanis*, studies on motility were first made by Jasfalousi (1954), Linhart (1984) and Linhart *et al.* (1986, 1987). They observed that motility was triggered by urine collected at the same time as milt by the stripping procedure leading to a significant drop of fertilizing ability. To prevent this phenomenon the sperm was collected in a syringe partly filled with an "immobilizing solution" of the same osmotic pressure as the seminal fluid: NaCl (or KCl) 200 mM, Tris HCl 30 mM, pH 7.0. *Silurus glanis* spermatozoa showed the classical motility pattern of those of other freshwater teleost: immediately after dilution in freshwater or in an activating solution (NaCl 17 mM, Tris 5 mM, pH 8.0) 90-100 % of spermatozoa were motile with forward movement and declined progressively to nearly zero within an average 80 s (*fig.* 2). After they stop moving spermatozoa still exhibit some flagellum agitation for 100-180 s. Redondo-Müller (1990) reported duration of motility of 50 s in sperm of non-stimulated males and 75 s on sperm of males injected with LHRHa; the percentage of motile spermatozoa also increased after stimulation. A similar picture was given by Guest *et al.* (1976*b*) in *I. punctatus*; spermatozoa diluted in fresh water or distilled water stopped their progressive movement after 60-80 s. In a 0.65% NaCl solution these authors reported a motility of 7 min but this was probably due to a low dilution rate and asynchronous activation of motility occurring even after a long period of time. *Clarias gariepinus* spermatozoa diluted in distilled water showed total motility time of 118 s but moderate motility lasted only 33 s (Steyn and Van Vuren, 1987).

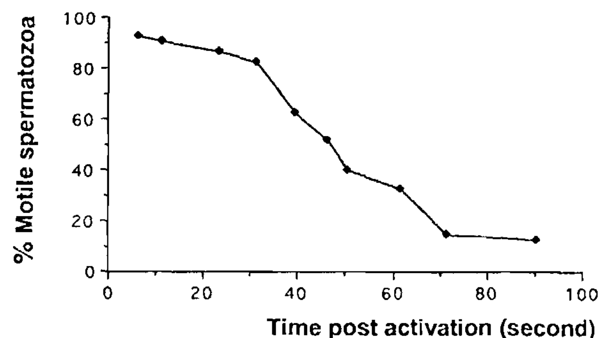

Figure 2. – Changes with time of the percentage of motile *Silurus glanis* spermatozoa sampled in the immobilizing solution (KCl 200 mM, Tris HCl 30 mM, pH 7.0) and activated in the activating solution (Nacl 17 mM, Tris Hcl 5 mM, pH 8.0).

Duration of mobility of 45 s (forward motion) or 75 s (vibratory movement) with a concomitant loss of fertilizing capacity was reported in *Rhamdia sapo* (Maggese *et al.*, 1984). As in other freshwater teleost species a disorganization of the sperm cells rapidly occurs after dilution in hypoosmotic media (*e.g.* in *Rhamdia sapo*; Maggese *et al.*, 1984). Disorganization was evident after 20 s in freshwater in the channel catfish (Guest *et al.*, 1976*b*).

The fertilizing capacity of spermatozoa sampled with urine (52.3±16%) was much less than for those sampled in the immobilization solution (85.1±9%); by comparison intratesticular spermatozoa (65.6±6.2%) were less fertile (Linhart *et al.*, 1987). This may indicate that a kind of maturation of spermatozoa occurs at the time of spermiation as observed in salmonids (Muira *et al.*, 1992).

Only limited information is available on the composition of the seminal fluid of siluroid fish. In *S. glanis*, the osmotic pressure in which the spermatozoa remain immotile is about 300 $mOsm.kg^{-1}$ but due to contamination with urine a large variability was reported 105-244 $mOsm.kg^{-1}$ (Saad and Billard, 1995). The pH of the seminal fluid of *S. glanis* was also found highly variable 7.8-8.8 (Saad and Billard 1995) but again a contamination was possible by urine which has a pH of 5.8-6. In *C. gariepinus* seminal plasma the pH was 7.73, the osmotic pressure 222 $mOsm.kg^{-1}$ and the ionic composition, in $mg.ml^{-1}$: 2.8 Na^+, 0.48 K^+, 0.05 Ca^{++}, 0.015 Mg^{++} (Steyn and Van Vuren, 1986).

Artificial insemination and fertilization

Artificial fertilization has been practised mostly in silurids and clariids. An improved procedure of gamete collection and fertilization was established in *S. glanis*

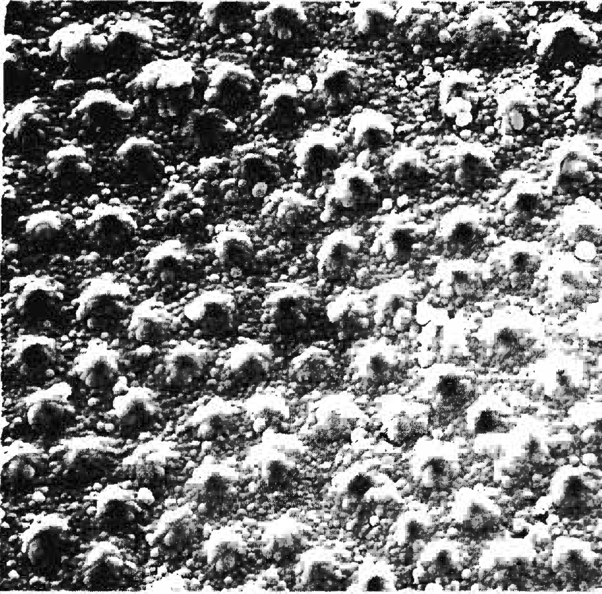

Figure 3. – Vitelline envelope of unfertilized ova of *Silurus glanis*; × 14 000 (1 cm = 0.71 μm). Kudo *et al.* (unpublished)

(Linhart *et al.*, 1987). The sperm is first collected either by stripping into an immobilizing solution with a dilution ratio superior to 0.9:1, or directly from testis of sacrificed or operated males. After stripping female, eggs (200 ml) and sperm (3 ml) are mixed together in a bowl and 100 ml of activating solution (17 mM NaCl, 5 mM Tris, pH 8) are added. After 2-5 min of gentle stirring, the eggs are transferred to incubators. Horvath and Tamas (1976) formerly used a 3 $g.l^{-1}$ NaCl solution for fertilization process.

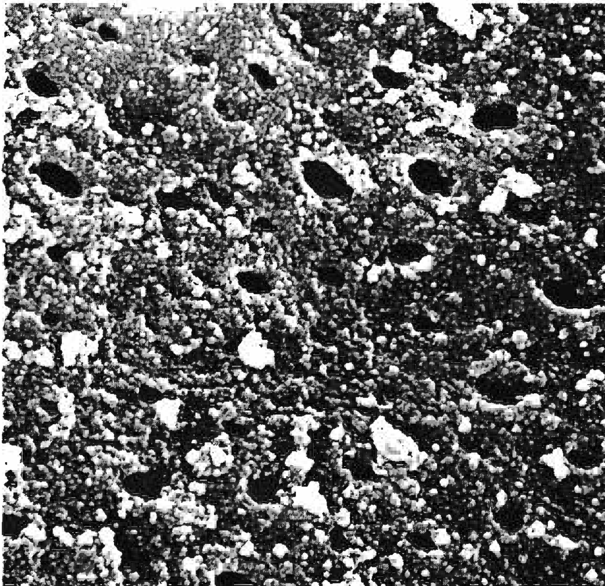

Figure 4. – Fertilizing envelope of *Silurus glanis* egg four-minute after insemination; × 14 000 (1 cm = 0.71 μm). Kudo *et al.* (unpublished).

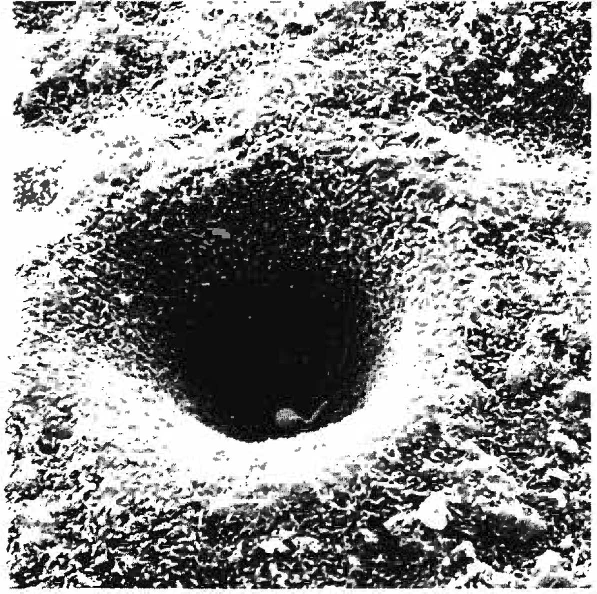

Figure 5. – A sperm-egg contact at the sperm entry site of *Silurus glanis*, immediatly under the internal aperture of the micropilar canal. The sperm is located in the central portion of a conical depression of the fertilizing enveloppe; 20 second after insemination; × 1 680 (1 cm = 5.95 μm). Kudo *et al.* (unpublished).

In the clariids, *C. gariepinus* and *H. longifilis*, intratesticular sperm is maintained inactivated in 155 mM NaCl solution (dilution 1:10); the time of contact between sperm and ova practised for fertilization is generally 1 min (Hogendoorn and Vismans, 1980; Legendre, 1986). With the view of maximizing the use of available sperm in *C. macrocephalus*, Tambasen-Cheong *et al.* (1995) found an optimum ratio of 25-50 μl of diluted sperm (1:3.5 in NaCl 155 mM) for 10 g of ova and 5 ml of water, corresponding to 4 000-8 000 spermatozoa per ovum. In *H. longifilis*, Legendre (1992) show that the fertilization success (assessed by hatching %) dropped significantly when the number of spermatozoa used during fertilization was decreased from 50 000 to 5 000 spz per ovum.

The process of sperm penetration into the egg of *S. glanis* has been investigated by scanning and transmission electron microscopy (Kudo *et al.*, 1994). After activation of the egg during fertilization, the vitelline envelope (*fig.* 3) undergoes considerable morphological changes resulting in the so-called fertilization envelope (*fig.* 4). The micropyle consists of a vestibule and a canal which opens in a conical depression of the egg envelope. Twenty seconds after insemination, one spermatozoon has reached the sperm entry site located at the bottom of the conical depression (*fig.* 5) and the sperm head membrane fused with the egg plasma membrane. At this stage, a cytoplasmic eminence is present under the fused sperm head. After 4 min, there is a complete penetration of the sperm head and the sperm flagellum has penetrated into the egg except for a short length

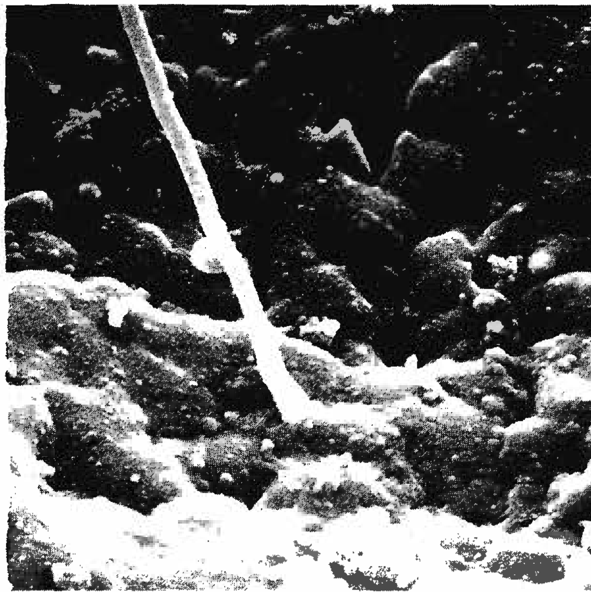

Figure 6. – Complete penetration of the sperm head and partial penetration of the sperm flagellum in *Silurus glanis*; four-minute after insemination; × 14 000 (1 cm = 0.71 μm). Kudo *et al.* (unpublished).

(*fig.* 6). During this process, sperm aggregation has been frequently observed in the upper part of the micropylar vestibule. In *S. glanis*, the internal aperture of the micropyle is wide enough to permit the entry of only one spermatozoon; this is possibly a way to prevent polyspermy (Ginsburg, 1987; Kudo, 1991).

Gamete preservation

Ova

In all siluroids studied so far, the phenomenon of ageing that leads to overripening of ova maintained within the ovaries occurs rapidly, within a few hours. Some data on the *in vivo* survival of ova are shown in *Table* 5. Survival time is estimated by the time lapse between ovulation and the moment at which the initial egg fertilizability (measured by hatching percentages) begin to drop. However, it should be underlined that in *Rhamdia sapo* and *H. longifilis*, noticeable increases in the proportion of deformed larvae were observed before such a drop in hatching percentages occurred (Espinach Ros *et al.*, 1984; Legendre and Otémé, 1995). Therefore, survival times during which ova are able to develop normally are even shorter than those given in *Table* 3. In *H. longifilis*, Legendre and Otémé (1995) showed that the proportion of deformed larvae increased significantly from 4 to 20% two hours after completion of ovulation and 2 h later the hatching percentages dropped from 92 to 36%. In *S. glanis*, Linhart and Billard (1995) found that the hatching rate of ovulated oocytes left within the ovaries remained unchanged for 2 h following ovulation and decreased after 4 and 6 h with a significant increase in the percentage of abnormal hatched larvae.

In *C. gariepinus*, histological observations made on the ovaries of females, injected with carp pituitary suspension and autopsied 22 h after ovulation (at 25°C), showed postovulatory follicles secreting huge amounts of eosinophilic material that not only filled up their own cavity, but also enveloped the ovulated eggs stored in the ovarian cavity. These eggs were in an advanced stage of atresia (Richter and Van den Hurk, 1982). To date, the factors and mechanisms governing overripening of ova maintained *in vivo* remain poorly understood in catfishes, as in other teleosts, and need to be further investigated. In practice, as the ova survival time decreases with increasing temperature (Hogendoorn and Vismans, 1980; Espinach Ros *et al.*, 1984), the correct timing of stripping becomes increasingly critical at higher temperatures.

In *S. glanis*, the survival of ova collected by stripping and stored *in vitro* under aerobic conditions at different temperatures was investigated by Linhart *et al.* (1991) and Linhart and Billard (1995). In comparison to control (eggs fertilized immediately after stripping), hatching was not significantly changed after 8.5 h storage at 19°C and 3.5 h at 25°C. However, at this time observed proportions of deformed larvae were already high (37 to 74% of hatched larvae). There was no ova survival at all after 3.5 h storage at 8°C, 12 h at 19°C and 8.5 h at 25°C .

Spermatozoa

Spermatozoa survival in the testis has not been studied in detail. In some species they are present all year round, *e.g.* in *H. longifilis*, (Otémé *et al.*, 1996), but their survival has not been assessed. The relationship that spermatozoa establish with the male and female genital tract has been discussed by Loir *et al.* (1989). In several species the spermatozoa are stored in the seminal vesicle for some time but this is not general; even if seminal vesicle are present sperm storage occurred in other structures: pouches in some Pimelodidae, large and highly secretory subdivided deferent duct in *Pseudopimelodus rassinus* and *Ageneiosus brevifilis*, secretions from the sperm duct epithelium or isolated cells with large nuclei found in the main sperm duct as in *Hoplosternum thoracatum* (Loir *et al.*, 1989). Spermatozoa storage is reported in several species exhibiting internal fertilization (*Pimelotus ornanus, P. galateus, A. brevifilis*), embedded in secretion but we do not know how long they survive.

Several works have reported *in vitro* sperm storage after sampling usually at 4-5°C in refrigerator, undiluted or diluted in an immobilizing solution. The fertilizing capacity of *C. gariepinus* spermatozoa stored during 24 h was slightly reduced (4%) in a non-diluted state but was not changed if diluted 1/100 or 1/1 000 in an immobilizing-solution (Hogendoorn and Vismans, 1980); the decrease was probably due to anoxic condition as it was shown by Redondo-Müller *et al.* (1989) and Redondo-Müller (1990) that the addition of antibiotics considerably improved the

Table 5. – *In vivo* survival of ova after hormonal induced ovulation in some siluroid species.

Species	Hormonal treatment (doses per kg female body weight)	Temperature (°C)	Survival time [1] (h)	References
Silurus glanis	Carp pituitary suspension, 5 mg.kg^{-1}	24	<4	Linhart and Billard (1995)
Clarias gariepinus	Carp pituitary suspension, 4 mg.kg^{-1}	20	<9 [2]	Hogendoorn and Vismans (1980)
		30	<2 [2]	"
Clarias macrocephalus	hCG, 2000 IU.kg^{-1}	26-31	<12	Mollah and Tan (1983*b*)
Heterobranchus longifilis	hCG, 1500 IU.kg^{-1}	29	<4	Legendre and Otémé (1995)
Rhamdia sapo	*Prochilodus* pituitary suspension, 3 mg.kg^{-1}	20	<9	Espinach Ros *et al.* (1984)
		24	<5	"

(1) the data indicate storage time at which the first signicant drop in hatching percentage is detected.
(2) estimations from graphs given by the authors.

spermatozoa survival in *S. glanis* (*fig.* 7). *Ictalurus punctatus* spermatozoa extended in saline solutions were still motile after 9 weeks of storage (Guest *et al.*, 1976*b*). Attempt at cold storage of *C. gariepinus* semen by Hecht *et al.* (1982) was not successful. *Pangasius* milt stored in syringes kept in a refrigerator showed a motility of 100% after 18 h storage and 10% after 18 h but motility was not given for semen left at ambient temperature (Withler, 1980).

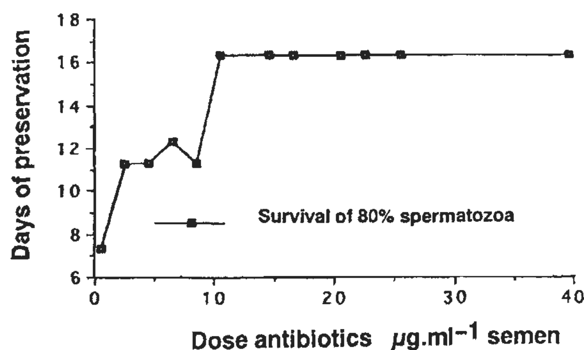

Figure 7. – Survival of *Silurus glanis* spermatozoa stored in the immobilizing solution (IM) (200 mM NaCl, 30 mM Tris-HCl, pH 7.0: 470 mosm.kg^{-1}) with increasing amount of antibiotics (Eurofloxacina II) 0 to 40 µg/ml of diluted semen (1 volume IM: 1 volume sperm). After Redondo-Müller (1990).

Cryopreservation of intratesticular sperm of *S. glanis* was successfully attempted by Marian and Kraznai (1987). Later Linhart *et al.* (1993) have set up a field technique with sperm sampled in the immobilizing solution using large pellets (5 ml) placed above liquid nitrogen vapour and achieved a fertilization rate of 45% (range 4-48%) compared to 51% (range 7-51%) in the control with about 260 000 spermatozoa/egg. Sperm of *Clarias gariepinus* was also successfully cryopreserved in liquid nitrogen by Steyn and Van Vuren (1987*b*) with high motility and satisfactory survival rate (after 14 days 51.2% fertilization vs 51% in controls with 49 000 spermatozoa/egg): this technique was proposed for the conservation of genetic diversity (Van der Walt *et al.*, 1993). Otémé *et al.* (1996) reported equivalent hatching rates (about 80%) in *H. longifilis* after fertilization with fresh sperm and sperm cryopreserved in liquid nitrogen for 1 h or 8 months after dilution in the Mounib medium supplemented with 10% egg yolk, 5% DMSO and 5% glycerol as cryprotectants. However, sperm was probably used in excess in their fertilization trials as a noticeably reduced motility was observed with the preserved sperm (20-30%) compared to control (70-80%). Sperms of *Heteropneustes fossilis* and *Clarias batrachus* have been preserved partially for several days up to one year at −70°C using glycerol as the cryoprotectant (Padhi and Mandal, 1995). Successful spermatozoa cryopreservation referring only to motility was reported by Guest *et al.* (1976*b*) in *I. punctatus*. The extenders was a saline solution plus fructose buffered at pH 7.3 and including 10% DMSO; motility of thawed spermatozoa was 2.47 vs 4 in control in a 0-5 motility scale. Cryopreservation of *Pangasius sutchi* spermatozoa by Withler (1982) was not successful, ending with 1% fertilization.

EGG INCUBATION

Depending on the characteristics of spawns and eggs, four main types of situation leading to different incubation practices can be distinguished in catfishes: egg stuck together forming an egg mass, egg stuck on spawning nest vegetable material (case of some natural spawning), independent sticky eggs spread in a single layer, or non-adhesive eggs incubated in funnels or jars. In this case, non-stickiness of eggs may be either a species characteristic or adhesive eggs whose sticky substances have been removed using chemical treatment to avoid their clumping together. Optimal range of incubation temperature, egg size and corresponding duration of embryonic development are given for some siluroids in *table* 6. The longest egg incubation period ever found in siluroids (75-80 days at 20-26°C) was reported in the ariid catfish, *Galeichthys feliceps* (Rimmer and Merrick, 1993).

Table 6. – Range of water temperature, egg size and duration of incubation of eggs of various siluroid species, with some indication of treatments to prevent fungi development.

Species	Temperature (°C)	Egg size [1] (mm)	Duration of incubation	Treatment during incubation	References
Galeichthys feliceps [2]	20-26	>16×13	75-80 days	–	Tilney and Hecht (1993)
Ictalurus punctatus	25-28	3.5	5-8 days	100 ppm of formalin (15 min) 25-50 ppm of Diquat or Betadine (15 min)	Tucker and Robinson (1990)
Chrysichthys nigrodigitatus	27-29	3.2	5 days	–	Hem *et al.* (1994)
Silurus glanis	22-25	3.5	2-3 days	1-2 times/day: 2 ppm of MG (5 min) 2-5 ppm of Wescodyne (2 min)	Kouril and Hamackova (1977); Kouril *et al.* (1993) Kouril (pers.comm).
Hoplosternum littorale	26-31	1.4	2-3 days	–	Luquet *et al.* (1989); Hostache *et al.* (1992)
Clarias gariepinus	20	1.6-1.9	48 hours	1 000 ppm formalin (15 min)	Hogendoorn (1979); Bruton, 1979
	30		18-20 hours	–	Haylor and Mollah (1995)
Clarias macrocephalus	26-30	1.7-1.9	22-34 hours	–	Mollah and Tan (1982)
Heterobranchus longifilis	25-29	1.7-2.1	20-29 hours	–	Legendre and Teugels (1991)
Pseudoplatystoma coruscans	23-25	1.3	19 hours	–	Cardoso *et al.* (1995)

(**1**): diameter of fertilized, hydrated eggs. (**2**): not cultured ariid catfish. MG: malachite green.

In *I. punctatus*, the whole egg masses are collected from the spawning containers immediately or a few days after spawning. The most commonly used hatchery equipment is 400 l hatching trough containing several shallow egg baskets and a paddle wheel, slowly rotated in order to maintain a water current that gently rocks each egg mass and circulate fresh water around and through. However, Ringle *et al.* (1992) reported that egg incubation carried out in jars, after chemical separation of egg masses using different solutions of Na_2SO_3, L-cysteine-HCL and papain alone or in combination, resulted in 20% higher hatching success than with the traditional method in troughs; chemical separation of eggs reduced fungal diseases problems and labour associated with egg incubation. Phelps and Walser (1993) found a positive effect of sea salt on hatching rate in *Ictalurus punctatus*: hatching percentage was of 58% for untreated eggs (fresh water) and 79% for eggs treated at salinity of 0.5-2.5 $g.l^{-1}$, higher salinity led to lower results. Environmental requirements and incubation facilities for Channel catfish eggs have been reviewed by bush (1985) and Tucker and Robinson (1990).

In *C. gariepinus*, Hecht *et al.* (1980) found that eggs can get rid of their stickiness by any one of the following treatments applied after fertilization: washing for 45 min with a urea solution (3 $g.l^{-1}$ urea+4 $g.l^{-1}$ NaCl); washing for 30 min in urea solution followed by 5-10 s rinsing in a full cream milk powder mixture (15-25 g per l of water); and continuous stirring for 35 to 40 min in a full cream milk powder mixture (15-25 $g.l^{-1}$) at a volume ratio of 1:20, eggs to milk. Following those treatments, eggs can be subsequently incubated in funnels (Zuger jar). However, Haylor (1993) stated that this method requires additional expense in time, labour and chemicals and is not without risk of mechanical or chemical damage to the eggs. He proposed to spread the fertilized eggs in a single layer on a horizontal 1-mm material mesh on which they adhere rapidly. After hatching, the larvae can pass through the net and it is then easy to separate them from the unfertilized eggs and egg shells. A similar incubation procedure, used since 1986 in the Ivory Coast, was found to be highly efficient for eggs of *H. longifilis* (Slembrouck and Legendre, 1988). In this species, incubation is carried out in darkness in well oxygenated stagnant water. The thermal tolerance limits of the eggs is situated between 22 and 35°C, no hatching being observed at either 21 or 36°C (Legendre and Teugels, 1991). Haylor and Mollah (1995) reported a wider zone of thermal tolerance for eggs of *C. gariepinus*. They observed no hatching at 15°C, while at 20 and 35°C high hatching percentages (around 70%) were still obtained, but the proportion of deformed larvae was not given. A temperature of 30°C was considered optimal for egg incubation in both *C. macrocephalus* and *C. gariepinus* (Mollah and Tan, 1982; Haylor and Mollah, 1995).

Eggs of *S. glanis* can be incubated in boxes or in a net in tanks (spawn collected from natural spawning) or in Weiss' or Zuger jars (after induced spawning). Treatment to eliminate stickiness are carried out either after 10-12 h incubation in jars with alcalase enzyme solution (Horvath, 1980) or immediately

after fertilization with a clay suspension (Kouril *et al.*, 1993), a tannic acid solution (Geldhauser, pers. comm.) or using proteolitic enzymes such as alcalase or pancreatic trypsin (Proteau pers. comm.). The inferior lethal temperature during incubation is 14°C. Oxygen consumption of eggs at temperatures of 19.7 and 22.5°C were found to be 3.3 and 6.5 $\mu g.egg^{-1}.h^{-1}$ after elimination of stickiness, and 32.4, 37.3 $\mu g.egg^{-1}.h^{-1}$ before hatching, respectively (Kouril *et al.*, 1993). The non-sticky pelagic eggs of pimelodids, *e.g. Pseudoplatystoma coruscans* (Cardoso *et al.*, 1995) and *P. fasciatum* (Kossowski, 1996), can be placed directly in funnel-type incubators after fertilization without any particular treatment.

The incubation technique used in *Hoplosternum littorale* is original and consists of drawing the aggregated egg cake from the nest and incubating the whole mass standing on nylon wires at a few centimetres above the surface of the water, just to maintain the eggs in humid and well oxygenated atmosphere. This incubation technique, which mimics the situation normally found in the floating nests, proved efficient and led to more than 85% hatching rate (Luquet *et al.*, 1989). The temperature at which incubation is carried out was found to influence significantly the sex ratio of the progeny (Hostache *et al.*, 1992).

CONCLUSION

Depending on the morphology of gonads and gametes, gamete production and spawning behaviour, a wide range of controlled reproduction methods was developed in the siluroids. They may involve either natural spawning in captivity or hormonal induced ovulation and stimulation of spermiation followed or not by artificial fertilization of the eggs. Numerous studies have sought to optimize conditions for the collection of viable gametes or eggs, necessary for subsequent massive production of fry. Optimal hormones and hormone doses to be used for inducing oocyte maturation and ovulation have received most attention. By contrast, studies on the biology of gametes and their requirement for improved viability, optimal management during artificial insemination and short and long-term preservation remains scarce. This is due, at least partly, to the relatively short history of siluroids in aquaculture, the actual potential of several of them being still under evaluation. With the foreseeable development of their culture, optimized management of gametes for successful artificial reproduction and need to manipulate them in the frame of genetic programs will most probably gain in importance in the future. Knowledge on gamete biology of catfishes needs to be improved in this perspective.

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