

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

Marc Legendre <sup>(1)</sup>, Otomar Linhart <sup>(2)</sup> and Roland Billard <sup>(3)</sup>

<sup>(1)</sup> ORSTOM, GAMET, Groupe aquaculture continentale méditerranéenne et tropicale, BP 5095, 34033 Montpellier Cedex 1, France.

Address for correspondence: ORSTOM, Instalasi Penelitian Perikanan Air Tawar, Jalan Ragunan, Pasar Minggu, P.O. Box 7220/Jksp, Jakarta 12540, Indonesia.  
E-mail: legendre@idola.net.id

<sup>(2)</sup> Division de Biotechnologie, Reproduction et Génétique des Poissons, Université d'Angers, 49033 Angers Cedex, France.

<sup>(3)</sup> Laboratoire d'Ichtyologie générale et appliquée, Muséum National d'Histoire Naturelle, 43, rue Cuvier, 75231 Paris Cedex 5, France.

Accepted October 17, 1996.

---

Legendre M., O. Linhart, R. Billard. In: The biology and culture of catfishes. M. Legendre, J.-P. Proteau eds. *Aquat. Living Resour.*, 1996, Vol. 9, Hors série, 59-80.

### 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 spermatogenic 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  $1.7 \times 10^{11}$  and  $1.8 \times 10^{10}$  spermatozoa.kg<sup>-1</sup> body weight, respectively. In females, the fecundity ranges from about 50 eggs per spawn in ariids up to more than 100 000 eggs.kg<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup>, 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, guardians 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 spermatogenic 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 antero-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 spermatogenic 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 <sup>3</sup> )	Oocyte/ova diameter (mm)	References
<i>Galeichthys feliceps</i>	–	not cultured	–	50 (*)	16	Tilney and Hecht (1993)
<i>Ictalurus punctatus</i>	1.5-4	3-6	15	8	3	Grizzle (1985); Tucker and Robinson (1990)
<i>Chrysichthys nigrodigitatus</i>	1.5-3	3-4	8-10	15-18	2.5-3	Hem (1986), Otémé (1993)
<i>Silurus glanis</i>	4-25	>3	3-15	10-25	2.5-2.9	Fijan (1975); Horvath and Tamas (1976); Wisniewolski (1988); Kouril <i>et al.</i> (1993)
<i>Hoplosternum littorale</i>	0.07-0.3	1	6-12	45-75	1.4	Hostache <i>et al.</i> (1993); Pascal <i>et al.</i> (1994)
<i>Clarias gariepinus</i>	00-3	1-3	15-20	60-150	1.2-1.5	Hogendoorn (1979); Richter <i>et al.</i> (1987); Legendre <i>et al.</i> (1992)
<i>Clarias macrocephalus</i>	0.1-0.2	–	10-12	20-50	1.3-1.6	Ali (1993); Tan-Fermin and Emata (1993)
<i>Heterobranchus longifilis</i>	2-6	2-4	15	30-120	1.2-1.6	Legendre (1986); Legendre and Otémé (1995)
<i>Pseudoplatystoma coruscans</i>	5-13	–	–	120-130	0.8	Cardoso (pers. comm.)

(\*): absolute fecundity.

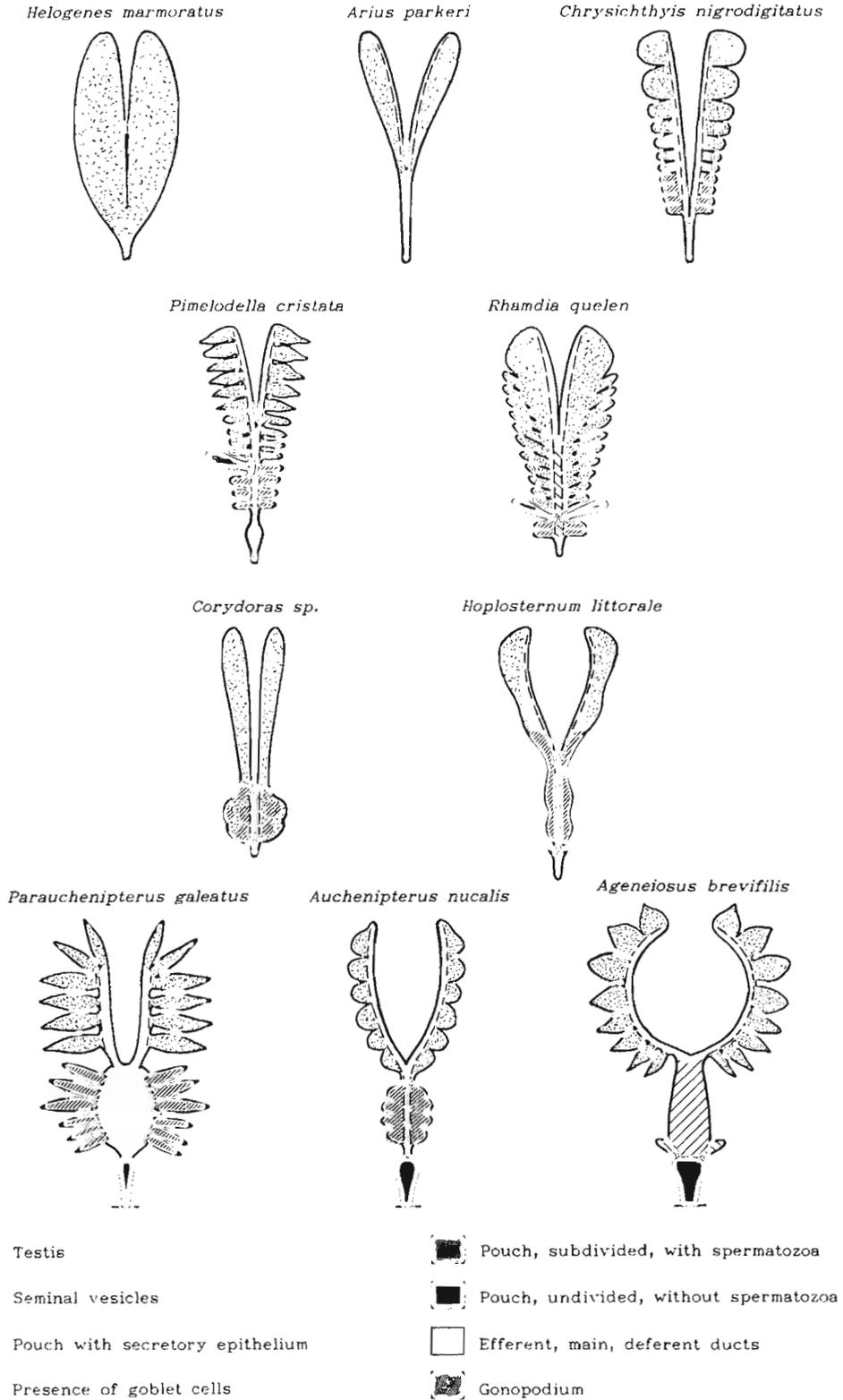


Figure 1. – Schematic representation of the testis of some siluroid species. After Loir *et al.* (1989).

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.*, 1987a, b, Resink *et al.*, 1987a) and are the source of sex attractant for the females (Resink *et al.*, 1987b).

### 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 150000 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  $\mu\text{m}$  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  $\mu\text{m}$  high, are elongated in previtellogenic oocytes up to 350  $\mu\text{m}$  and reach 20  $\mu\text{m}$  in 1500  $\mu\text{m}$  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  $\mu\text{m}$  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  $\mu\text{m}$  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  $\mu\text{m}$ ) 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 vegetal 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 is 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 ( $\mu\text{m}$ )	Mid- piece ( $\mu\text{m}$ )	Mitochondria	Flagella		References
				number	$\mu\text{m}$	
<i>Silurus glanis</i>	1.8	1.6 $\times$ 0.8	2-3	1	50	Emelyanova and Makeyeva (1992)
<i>Pseudobagrus fukiensis</i>	1.8 $\times$ 1.7	1.3 $\times$ 0.4	4-5	1	60	Emelyanova and Makeyeva (1992)
<i>Ameiurus nebulosus</i>	1.7 $\times$ 1.8	2 $\times$ 1.8	up to 20	2		Emelyanova and Makeyeva (1992)
	1.1 (*)					
<i>Ictalurus punctatus</i>	1.8 $\times$ 2	1.5 $\times$ 3	up to 35	2	80-100 $\times$ 0.2	Emelyanova and Makeyeva (1991)
<i>Ictalurus punctatus</i>	2.3 $\pm$ 0.01	1.6 $\pm$ 0.01		1-2	94.9 $\pm$ 0.26	Jaspers <i>et al.</i> (1976)
	**2.4 $\pm$ 0.01	**3.1 $\pm$ 0.01				

(\*) apical part, (\*\*) mean  $\pm$  SD.

diameter was 1.8-2.3  $\mu\text{m}$ , the length and width of mid piece, 1.5-1.6 and 3.0-3.1  $\mu\text{m}$  respectively and the flagellum length 80-100  $\mu\text{m}$  (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 spermatogenic production (1.7  $10^{11}$  spz.kg<sup>-1</sup> body weight) (Saad and Billard, 1995); GSI is also low ( $\approx$  1%) in *S. glanis* found in the wild (Zholdasova and Guseva, 1987). In the channel catfish the GSI (0.25%) and the spermatogenic production (1.8  $10^{10}$  spz.kg<sup>-1</sup> bw) are even lower (Guest *et al.*, 1976a). The GSI is also low, not exceeding 1%, in *Clarias gariepinus* (Van Oordt *et al.*, 1987) and *Heterobranchius longifilis* (Legendre, 1992), and 0.2-0.6% in *Hoplosternum littorale* (Hostache *et al.* 1993).

Intratesticular spermatozoa concentration in  $10^9$  spz.g<sup>-1</sup> testis was measured by Guest *et al.* (1976a) in channel catfish (7.1  $\pm$  0.8) and by Saad and Billard (1995) (7.2  $\pm$  1.3) in *S. glanis* and, in  $10^9$  spz.ml<sup>-1</sup>, by Legendre *et al.* (1992) in *H. longifilis* (3.0) and by Steyn and Van Vuren (1987a) 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<sup>-1</sup>: 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 1300 kg.ha<sup>-1</sup>) 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<sup>-2</sup> 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<sup>-1</sup> and temperature around 27-29°C. In this catfish, a rise in water salinity up to 7 g.l<sup>-1</sup> 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, 1983a; 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 × 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<sup>-1</sup> 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<sup>2</sup> 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<sup>2</sup>. 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<sup>-1</sup>. 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<sup>3</sup> 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 where 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<sup>-1</sup> 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±µg.kg<sup>-1</sup>) associated with pimozide (5 mg.kg<sup>-1</sup>), a supplementary injection of carp pituitary (5 mg.kg<sup>-1</sup>) 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. batrachus* 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
<i>Ictalurus punctatus</i>	CP, 2 mg (1st inj.) + 9 mg (after 12 h) hCG, 1000-1760 I.U.	26-27	28-48 24-72	70-100 50-100	Bidwell <i>et al.</i> (1985); Dunham (1993) Sneed and Clemens (1959); Dupree <i>et al.</i> (1966)
	LHRHa, 0.01 mg (1st inj.) + 0.09 mg (after 12 h)			88	Bush (1985); Bush and Steeby (1990)
<i>Silurus glanis</i>	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 LHRHa, 0.05 mg + PIM, 10 mg	22-23 20	30-33 24-36	100 100	Kouril <i>et al.</i> (1987) Epler and Bienarz (1989)
<i>Clarias gariepinus</i>	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 <i>et al.</i> (1982)
	D-Ala <sup>6</sup> -LHRH, 0.05 mg + PIM, 5 mg	25	12.5	100	De Leeuw <i>et al.</i> (1985)
	17 $\alpha$ -HP, 3 mg (1st inj.) + 5 mg (after 4 h) DOCA, 50 mg	25	12.5 14	100 (1)	Richter <i>et al.</i> (1985) Richter and Van den Hurk (1982)
<i>Clarias macrocephalus</i>	hCG, 2000 I.U.	26-31	13-18	95	Mollah and Tan (1983a)
	D-Ala <sup>6</sup> -LHRHa, 0.05 mg + PIM, 1 mg	30	16-20	100	Tan Fermin and Emata (1993)
<i>Clarias batrachus</i>	CP, 6 mg	25	17	100	Zonneveld <i>et al.</i> (1988)
	D-Ala <sup>6</sup> -LHRH, 0.05 mg + PIM, 5 mg	25	18-21	86	Manickam and Joy (1989)
<i>Clarias fuscus</i>	hCG, 200 I.U. + 1800 I.U. (after 6 h)	25	16-19	100	Young <i>et al.</i> (1989)
<i>Heterobranchus longifilis</i>	hCG, 1500 I.U.	29	12	100	Legendre (1986); Legendre and Otémé (1995)
	CP, 6 mg	26-29	7-10	–	Nwadukwe <i>et al.</i> (1993)
	Frog pituitary, 7 mg	29	7	100	Nwadukwe (1993)
<i>Pangasius hypophthalmus</i> (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 <i>et al.</i> (1988)
<i>Heteropneustes fossilis</i>	D-Lys6-sGnRHa, 0.025 mg	25	14-18	100	Alok <i>et al.</i> (1993)
<i>Pseudoplatystoma coruscans</i>	CP, 0.8 (1st inj.) + 6 mg (after 13 h)	23-25	9	60	Cardoso <i>et al.</i> (1995)
<i>Rhamdia sapo</i>	<i>Prochilodus</i> pituitary, 0.75 - 6 mg	24	17	75	Espinach Ros <i>et al.</i> (1984)

(1): oocyte maturation but no ovulation (*see text*). (2): *ex P. sutchi*.

Inj.: injection. CP: carp pituitary. PIM: pimozone. 17 $\alpha$ HP: 17 $\alpha$  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 pimozone (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.*, 1976a) 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 spermatogenic 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 *Clarius* 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.*, 1987a) and 5  $\beta$  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  $\alpha$  Progesterone, 17 $\alpha$ 20 $\beta$  Progesterone and 11 $\beta$  hydroxyprogesterone (Kime *et al.*, 1993).

### The use of intratesticular sperm

Many siluroid species are oligospermic (GSI  $\leq$  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  $\mu$ g GnRH (D-Arg6, Trp7, Leu8, Pro9, NfT) 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 $\pm$ 0.32	80-100	75-87
	4 000 I.U.	4	1.26 $\pm$ 0.16	80-100	88-95
	10 000 I.U.	4	1.25 $\pm$ 0.30	70-90	74-84
CPE	0	7	0.2	10-100	38-94
	4 mg	7	1.6 $\pm$ 0.51	90-100	75-95
	8 mg	7	1.87 $\pm$ 0.33	90-100	62-110
LHRHa (*)	12 mg	7	2.20 $\pm$ 0.39	60-100	62-105
	0	5	0.04	10-100	38-94
	50 $\mu$ g	5	0.96 $\pm$ 0.66	90-100	75-95
Ovaprim	100 $\mu$ g	5	0.33 $\pm$ 0.18	90-100	62-100
	0	5	0.4		
	0.5 ml	5	0.34 $\pm$ 0.18		
	1.0 ml	5	1.80 $\pm$ 2.26		
	1.5 ml	5	2.76 $\pm$ 1.64		

(\*): +Domperidone 5 mg.kg<sup>-1</sup>

net fabric into the eggs. This method is commonly reported in clariid species (Hogendoorn and Vismans, 1980; Mollah and Tan, 1983b; 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

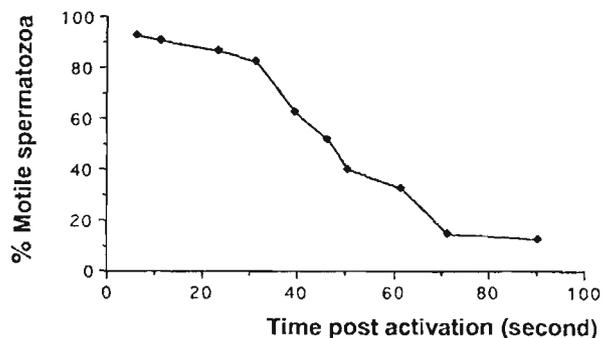
### Ova viability

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 silurid 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.*, 1976a) and duration of motility was about 60 s in pond water (Guest *et al.*, 1976b). In *S. glanis*, studies on motility were first made by Jafaloussi (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.* (1976b) 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



**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).

period of time. *Clarius 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).

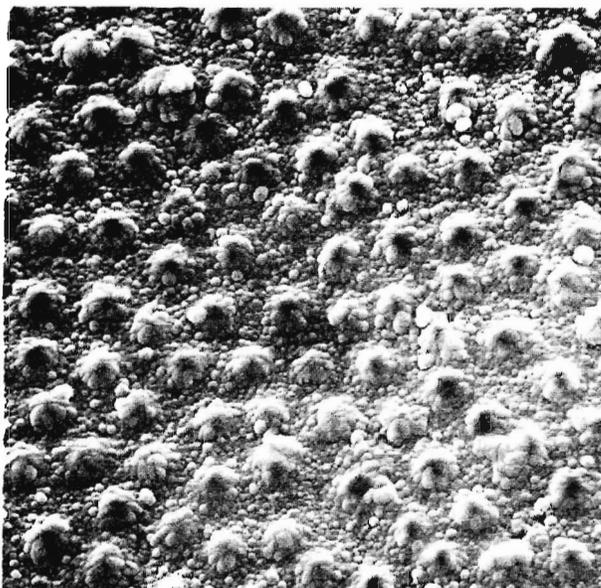
Duration of mobility of 45 s (forward motion) or 75 s (vibratory movement) with a concomitant loss of fertilizing capacity was reported in *Rhamdia sapo* (Maggese *et al.*, 1984). As in other freshwater teleost species a disorganization of the sperm cells rapidly occurs after dilution in hypoosmotic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

The fertilizing capacity of spermatozoa sampled with urine ( $52.3 \pm 16\%$ ) was much less than for those sampled in the immobilization solution ( $85.1 \pm 9\%$ ); by comparison intratesticular spermatozoa ( $65.6 \pm 6.2\%$ ) 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 (Muir *et al.*, 1992).

Only limited information is available on the composition of the seminal fluid of silurid fish. In *S. glanis*, the osmotic pressure in which the spermatozoa remain immotile is about  $300 \text{ mOsm.kg}^{-1}$  but due to contamination with urine a large variability was reported  $105\text{-}244 \text{ mOsm.kg}^{-1}$  (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  $222 \text{ mOsm.kg}^{-1}$  and the ionic composition, in  $\text{mg.ml}^{-1}$ :  $2.8 \text{ Na}^+$ ,  $0.48 \text{ K}^+$ ,  $0.05 \text{ Ca}^{++}$ ,  $0.015 \text{ Mg}^{++}$  (Steyn and Van Vuren, 1986).

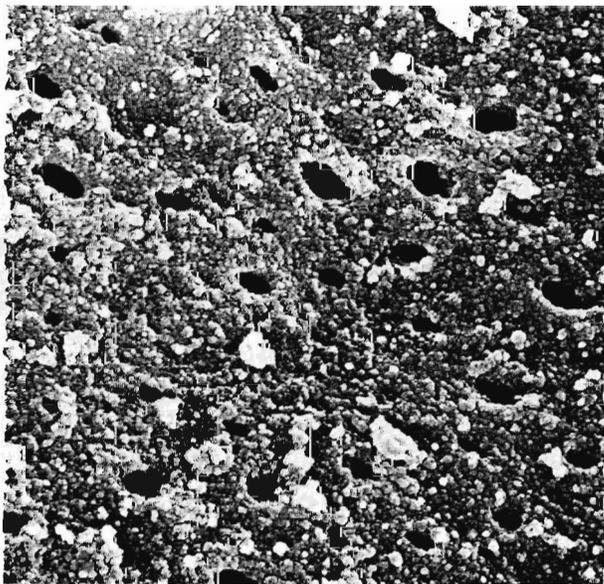
### Artificial insemination and fertilization

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*;  $\times 14\,000$  (1 cm = 0.71  $\mu\text{m}$ ). 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<sup>-1</sup> NaCl solution for fertilization process.



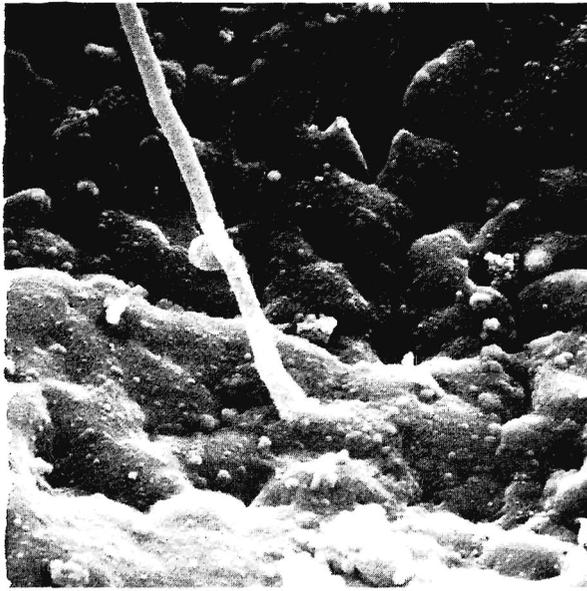
**Figure 4.** – Fertilizing envelope of *Silurus glanis* egg four-minute after insemination;  $\times 14\,000$  (1 cm = 0.71  $\mu\text{m}$ ). Kudo *et al.* (unpublished).



**Figure 5.** – A sperm-egg contact at the sperm entry site of *Silurus glanis*, immediately under the internal aperture of the micropilar canal. The sperm is located in the central portion of a conical depression of the fertilizing envelope; 20 second after insemination;  $\times 1\,680$  (1 cm = 5.95  $\mu\text{m}$ ). 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*, Tamhasen-Cheong *et al.* (1995) found an optimum ratio of 25-50  $\mu\text{l}$  of diluted sperm (1:3.5 in NaCl 155 mM) for 10 g of ova and 5 ml of water, corresponding to 4000-8000 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 50000 to 5000 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;  $\times 14\,000$  (1 cm = 0.71  $\mu\text{m}$ ). 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 <sup>(1)</sup> (h)	References
<i>Silurus glanis</i>	Carp pituitary suspension, 5 mg.kg <sup>-1</sup>	24	<4	Linhart and Billard (1995)
<i>Clarias gariepinus</i>	Carp pituitary suspension, 4 mg.kg <sup>-1</sup>	20	<9 <sup>(2)</sup>	Hogendoorn and Vismans (1980)
		30	<2 <sup>(2)</sup>	"
<i>Clarias macrocephalus</i>	hCG, 2000 IU.kg <sup>-1</sup>	26-31	<12	Mollah and Tan (1983b)
<i>Heterobranchus longifilis</i>	hCG, 1500 IU.kg <sup>-1</sup>	29	<4	Legendre and Otémé (1995)
<i>Rhamdia sapo</i>	<i>Prochilodus</i> pituitary suspension, 3 mg.kg <sup>-1</sup>	20	<9	Espinach Ros <i>et al.</i> (1984)
		24	<5	"

(1) the data indicate storage time at which the first significant 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.*, 1976b). 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).

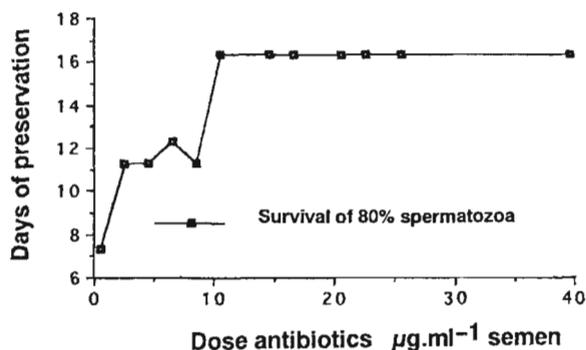


Figure 7. – Survival of *Silurus glanis* spermatozoa stored in the immobilizing solution (IM) (200 mM NaCl, 30 mM Tris-HCl, pH 7.0; 470 mosm.kg<sup>-1</sup>) with increasing amount of antibiotics (Eurofloxacin II) 0 to 40 µg/ml of diluted semen (1 volume IM: 1 volume sperm). After Redondo-Müller (1990).

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 260000 spermatozoa/egg. Sperm of *Clarias gariepinus* was also successfully cryopreserved in liquid nitrogen by Steyn and Van Vuren (1987b) with high motility and satisfactory survival rate (after 14 days 51.2% fertilization vs 51% in controls with 49000 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 cryoprotectants. 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.* (1976b) 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 silurid species, with some indication of treatments to prevent fungi development.

Species	Temperature (°C)	Egg size (1) (mm)	Duration of incubation	Treatment during incubation	References
<i>Galeichthys feliceps</i> (2)	20-26	>16×13	75-80 days	–	Tilney and Hecht (1993)
<i>Ictalurus punctatus</i>	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)
<i>Chrysichthys nigrodigitatus</i>	27-29	3.2	5 days	–	Hern <i>et al.</i> (1994)
<i>Silurus glanis</i>	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 <i>et al.</i> (1993) Kouril (pers.comm).
<i>Hoplosternum littorale</i>	26-31	1.4	2-3 days	–	Luquet <i>et al.</i> (1989); Hostache <i>et al.</i> (1992)
<i>Clarias gariepinus</i>	20 30	1.6-1.9	48 hours 18-20 hours	1 000 ppm formalin (15 min) –	Hogendoorn (1979); Bruton, 1979 Haylor and Mollah (1995)
<i>Clarias macrocephalus</i>	26-30	1.7-1.9	22-34 hours	–	Mollah and Tan (1982)
<i>Heterobranchus longifilis</i>	25-29	1.7-2.1	20-29 hours	–	Legendre and Teugels (1991)
<i>Pseudoplatystoma coruscans</i>	23-25	1.3	19 hours	–	Cardoso <i>et al.</i> (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<sub>2</sub>SO<sub>3</sub>, 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<sup>-1</sup>, 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<sup>-1</sup> urea+4 g.l<sup>-1</sup> 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<sup>-1</sup>) 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 proteolytic 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  $\mu\text{g.egg}^{-1}.\text{h}^{-1}$  after elimination of stickiness, and 32.4, 37.3  $\mu\text{g.egg}^{-1}.\text{h}^{-1}$  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.

## Acknowledgements

The authors acknowledge Dr Helena Linhart and T. Miltaud for technical assistance. This work was a part of a project on reproduction and genetics of Silurids of Europe supported by the European Commission (grant CIPA-CT93-0274).

## REFERENCES

- Abraham M., V. Hilge, R. Riehl, Y. Iger 1993. Mucollicle cells of the jelly coat in the oocyte envelope of the sheatfish (*Silurus glanis* L.). *J. Morphol.* **217**, 37-43.
- Ali A.B. 1993. Aspects of the fecundity of the feral catfish, *Clarias macrocephalus* (Gunther), population obtained from the rice fields used for rice-fish farming, in Malaysia. *Hydrobiologia* **254**, 81-89.
- Alok D., T. Kishnan, D. Salunke, H. Gupta, G.P. Talwar, L.C. Garg 1993. Precocious ovarian recrudescence and induced off-season spawning of *Heteropneustes fossilis* by D-Lys<sup>6</sup> salmon gonadotropin releasing hormone analog (sGnRH-A). *J. Fish Biol.* **45**, 909-915.
- Areerat S. 1987. *Clarias* culture in Thailand. *Aquaculture* **63**, 355-362.
- Bidwell C.A., C.L. Chrisman, G.S. Libey 1985. Polyploidy induced by heat-shock in channel catfish. *Aquaculture* **51**, 25-32.
- Bondari K. 1983. Efficiency of male reproduction in channel catfish. *Aquaculture* **35**, 79-82.
- Bruton M.N. 1979. The breeding biology and early development of *Clarias gariepinus* (Pisces; Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias*. *Trans. Zool. Soc. Lond.* **35**, 1-45.
- Bruton M.N. 1996. Alternative life-history of catfishes. In: the biology and culture of catfishes. M. Legendre, J.P. Proteau eds. *Aquat. Living Resour.* Hors Série, 35-41.
- Busch R.L. 1985. Channel catfish culture in ponds. In: Channel catfish culture C.S. Tucker ed. Amsterdam, Elsevier, 13-84.
- Busch R.L., J.A. Steeby 1990. An evaluation of a luteinizing hormone releasing hormone analog to induce spawning of channel catfish. *J. World Aquac. Soc.* **21**, 10-15.
- Cardoso E.L., M.S.D. Alves, R.M.A. Ferreira, H.P. Godinho 1995. Embryogenesis of the neotropical freshwater Siluriforme *Pseudoplatystoma coruscans*. *Aquat. Living Resour.* **8**, 343-346.
- Carreon J.A., R.F. Ventura, G.F. Almazan 1973. Notes on the induced breeding of *Clarias macrocephalus* Gunther. *Aquaculture* **2**, 5-16.

- Clay D. 1979. Sexual maturity and fecundity of the African catfish (*Clarias gariepinus*) with an observation on the spawning behavior of the Nile catfish (*Clarias lazera*). *Zool. J. Linn. Soc.* **65**, 351-365.
- Clemens H.P., K.E. Sneed 1957. The spawning behavior of the channel catfish *Ictalurus punctatus*. U.S. Fish and Wildlife Serv. Spec. Scient. Rep. Fish. 219.
- Cussac V.E., M.C. Maggese 1986. Oogenesis in *Rhamdia sapo*, (Pisces, Pimelodidae). Stages of the oocyte, egg's envelopes and effects of the human chorionic gonadotropin. *Rev. Brasil. Biol.* **46**, 139-147.
- Cussac V.E., M.C. Maggese 1988. Effects of salt solutions on the fertilizing ability and activation of gametes in the catfish *Rhamdia sapo*, (Pisces, Pimelodidae). *Rev. Brasil. Biol.* **48**, 203-211.
- De Kimpe P., J.C. Micha 1974. First guidelines for the culture of *Clarias lazera* in Central Africa. *Aquaculture* **4**, 227-248.
- De Leeuw R., H.J.Th. Goos, C.J.J. Richter, E.H. Eding 1985. Pimozide-LHRHa-induced breeding of the African catfish, *Clarias gariepinus* (Burchell). *Aquaculture* **44**, 295-302.
- Devaraj K.V., T.J. Varghese, G.P. Satyanarayana Rao 1972. Induced breeding of the fresh water catfish *Clarias batrachus* (L.) by using pituitary glands from marine catfish. *Curr. Sci.* **41**, 868-870.
- Dubravius J. 1547. De piscinis, (orig.publ.1547). Rev. ed. Sb.filosoficki, - SAV,1953,1:11-75 [in Czech and Latin].
- Dunham R. A. 1993. Observations on controlled artificial spawning of channel catfish. *Progress. Fish Cult.* **55**, 60-61.
- Dupree H.K., O.L. Green, K. E. Sneed 1966. Techniques for the hybridization of catfish. U.S. Fish and Wildlife Service, Southeastern Fish Cultural Lab., Marion, Alabama Rep. 221.
- Eding E.H., J.A.L. Janssen, G.G.J. Kleine Staarman, C.J.J. Richter 1982. Effects of human chorionic gonadotropin (HCG) on maturation and ovulation of oocytes in the ovary of the African catfish, *Clarias lazera* (C. & V.). In: Proc. Int. Symp. Reproductive Physiology of Fish. C.J.J. Richter, H.J.Th. Goos eds. Wageningen, The Netherlands, PUDOC, Wageningen, p. 195.
- Emelyanova N.G., A.P. Makeyeva 1991. Morphology of gametes in the channel catfish *Ictalurus punctatus*. *J. Ichthyol.* **31**, 10-16.
- Emelyanova N.G., A.P. Makeyeva 1992. Ultrastructure of the spermatozooids of some representative catfishes. *J. Ichthyol.* **32**, 127-134.
- Epler P., K. Bieniarz 1989. Gonad maturation and hormonal stimulation of spawning in the wels (*Silurus glanis* L.). *Pol. Arch. Hydrobiol.* **36**, 417-429.
- Espinach Ros A., V.G. Amutio, J P. Mestre Arceredillo, G. Orti, A. Nani 1984. Induced breeding of the south American catfish, *Rhamdia sapo* (C. & V.). *Aquaculture* **37**, 141-146.
- Fijan N. 1975. Induced spawning, larval rearing and nursery operations (*Silurus glanis*). EIFAC, FAO TP **25**, 130-138.
- Fishelson L., J.H.J. Van Vuren, A. Tyran 1994. Ontogenesis and ultrastructure of seminal vesicles of the catfish, *Clarias gariepinus*. *J. Morphol.* **219**, 59-71.
- Gauthier J. Y., P. Planquette, Y. Rouger 1988. Étude éthologique de la relation mâle-femelle au cours du cycle de reproduction chez *Hoplosternum littorale*. *Rev. Ecol. (Terre Vie)* **43**, 389-398.
- Ginsburg A.S. 1987. Egg cortical reaction during fertilization and its role in block to polyspermy. *Sov. Sci. Rev. Physiol. Gen. Biol.* **1**, 307-375.
- Goetz F.W. 1983. Hormonal control of oocyte final maturation and ovulation in fishes. In: Fish Physiology, W.S. Hoar, D.J. Randall, E.M. Donaldson eds. **9 B**, Reproduction. Academic Press. 117-170.
- Goos H.J. Th., K.P. Joy, R. De Leeuw, P.G.W.J. Van Oordt, A.M.L. Van Delft, J.Th. Gielen 1987. The effect of luteinizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with anti-dopamine and anti-serotonine properties on gonadotropin release and ovulation in the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 143-156.
- Grizzle J.M. 1985. Reproductive biology (*Ictalurus punctatus*). In: Channel catfish culture. C.S. Tucker ed. Elsevier, 229-282.
- Grozev G. 1968. The artificial spawning and rearing of sheat-fish. *Ribno Stopanstvo* **6-7**, 28-29.
- Guest W.C., J.W. Avault Jr., J.D. Roussel 1976a. A spermatology study of channel catfish *Ictalurus punctatus*. *Trans. Am. Fish. Soc.* **105**, 463-468.
- Guest W.C., J.W. Avault Jr., J.D. Roussel 1976b. Preservation of channel catfish sperm. *Trans. Am. Fish. Soc.* **105**, 469-474.
- Haider S., N.V. Rao 1994. Induced spawning of maturing Indian catfish (*Clarias batrachus* L.), using low doses of steroid hormones and salmon gonadotropin. *Aquac. Fish. Manag.* **25**, 401-408.
- Harvey B.J., W.S. Hoar 1980. The theory and practice of induced breeding in fish. Int. Dev. Res. Center, Ottawa, Ontario, 48 p.
- Haylor G.S. 1993. Controlled hatchery production of African catfish, *Clarias gariepinus* (Burchell): an overview. *Aquac. Fish. Manag.* **24**, 245-252.
- Haylor G.S., M.F.A. Mollah 1995. Controlled hatchery production of African catfish, *Clarias gariepinus*: the influence of temperature on early development. *Aquat. Living Resour.* **8**, 431-438.
- Hecht T., J.E. Saayman, L. Polling 1982. Further observations on the induced spawning of the sharp tooth catfish, *Clarias gariepinus* (Clariidae Pisces). *Water S.A.* **8**, 101-107.
- Hem S. 1986. Premiers résultats sur la reproduction contrôlée de *Chrysichthys nigrodigitatus* en milieu d'élevage. In: Aquaculture research in the African region. FIS Seminar, PUDOC, Wageningen, 189-205.
- Hem S., M. Legendre, L. Trebaol, A. Cissé, Z.J. Otémé, Y. Moreau 1994. L'aquaculture lagunaire. In: J.R. Durand, P. Dufour, D. Guiral and S.G.F. Zabi eds. Environnement et ressources aquatiques de Côte d'Ivoire. Tome II: Les milieux lagunaires. Editions de l'ORSTOM, Paris, **50**, 455-505.
- Hilge V. 1983. Investigation of induced breeding of the European catfish *Silurus glanis* L. and of the African catfish, *Clarias lazera* (C. & V.) (in German). *Inf. Fischwirtsch.* **30**, 149-151.
- Hochman L. 1967. Fertility in the sheatfish (*Silurus glanis*). *Acta Univ. Agric. Fac. Agron.* **15**, 333-335.
- Hogendoorn H. 1979. Controlled propagation of the African catfish *Clarias lazera*. Reproductive biology and field experiments. *Aquaculture* **17**, 323-333.

- Hogendoorn H., M.M. Vismans 1980. Controlled propagation of the African catfish, *Clarias lazera* (C & V). II. Artificial reproduction. *Aquaculture* **21**, 39-53.
- Hollebecq M.G., A. Saad, E. Pionnier, A. Heymann, R. Billard 1987. Études expérimentales sur la reproduction artificielle du silure glane, *Silurus glanis* L. *Aqua Revue Bordeaux* **17**, 22-24.
- Horvath L. 1980. Use of a protelytic enzyme to improve incubation of eggs of the European catfish. *Progress. Fish Cult.* **42**, 110-111.
- Horvath L. 1984. European catfish-sheafish (*Silurus glanis* L.) culture in carp farms. Chapt. 3. Akad. Kiado Budapest and Halver Corp. Seattle, 100-123.
- Horvath L., G. Tamas 1976. The reproduction of the sheaf fish (*Silurus glanis* L.) and raising of the sheaf fish forced fry. *Halasz. Tudom. Marlttek*, **2**, 11-13 [in Hungarian].
- Hostache G., M. Pascal, M. Kernén, C. Tessier 1992. Température et incubation chez l'atipa, *Hoplosternum littorale* (Teleostei, Siluriformes). *Aquat. Living Resour.* **5**, 31-39.
- Hostache G., M. Pascal., P. Planquette 1993. Saisonnalité de la reproduction chez l'atipa *Hoplosternum littorale*, (Callichthyidae, Siluriformes, Teleostei) par l'analyse de l'évolution du rapport gonado-somatique. *Aquat. Living Resour.* **6**, 155-162.
- Jalabert B. 1976. *In vitro* oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Carassius auratus*). *J. Fish. Res. Board Can.* **33**, 974-988.
- Jamieson B.G.M. 1991. Fish evolution and systematics: evidence from spermatozoa. Cambridge Univ. Press. 319 p.
- Jasfalousi L. 1954. Semi-artificial rearing of European catfish. *Ceskoslovenske rybarstvi* **2**, 26-28.
- Jaspers E.J., J.W. Avault Jr., J.D. Roussel 1976. Spermatozoal morphology and ultrastructure of channel catfish, *Ictalurus punctatus*. *Trans. Am. Fish. Soc.* **105**, 475-480.
- Khan H.A. 1972. Induced breeding of air-breathing fishes. *Indian Farming* **22**, 44-45.
- Kime D.E., S. Bhattacharya, M. Koldras, K. Bieniarz 1993. Steroidogenesis by ovaries and testes of the European catfish, the wels (*Silurus glanis*) *in vitro*. *Fish Physiol. Biochem.* **10**, 389-398.
- Knud-Hansen C.F., T.R. Batterson, C.D. McNabb, Y. Hadiroseyani, D. Dana, H.M. Eidman 1990. Hatchery techniques for egg and fry production of *Clarias batrachus* (Linnaeus). *Aquaculture* **89**, 9-19.
- Kobayakawa M. 1985. External characteristics of the eggs of Japanese catfishes (*Silurus*). *Jpn. J. Ichthyol.* **32**, 104-106.
- Konstantinov K.G. 1941. Nerest soma. *Zool. Zhurnal* **20**, 583-586 [in Russian].
- Kossowski C. 1996. Perspectives de l'élevage des poissons-chats (Siluroidei) en Amérique du Sud. In: The biology and culture of catfishes M. Legendre, J. P. Proteau eds. *Aquat. Living Resour.* Hors Série, 189-195.
- Kouril J., J. Hamackova 1977. The first artificial spawning of sheaf fish in CSSR. *Rybarstvi*, **12**, 268-269 [in Czech].
- Kouril J., J. Hamackova 1982. Artificial spawning, egg incubation and forced fry rearing of the sheaf-fish (*Silurus glanis*). *Prace VURH Vodnany* **2**, 119-126.
- Kouril J., J. Hamackova, T. Kepr 1981. Artificial reproduction of European catfish. In: *Reprod., Genet. Hybrid. fish. SZPIS, Vodnany*, 128-134 [in Czech].
- Kouril J., T. Barth, J. Hamackova 1987. Stripping the females of sheaf fish (*Silurus glanis* L.) with LHRH analog induction. *Prace VURH Vodnany*, **16**, 62-68.
- Kouril J., O. Linhart, J. Hamackova 1993. Artificial propagation of *Silurus glanis* L. *Metodika VURH Vodnany*, 14 p. [in Czech].
- Kruger E.J., L. Polling 1984. First attempts at artificial breeding and larval rearing of the butter catfish, *Eutropius depressirostris* (Schilbeidae: Pisces). *Water S. A.*, 97-104.
- Kudo S. 1991. Fertilization, cortical reaction, polyspermy-preventing and anti-microbial mechanisms in fish eggs. *Bull. Inst. Zool. Academia Sinica* **16**, 313-340.
- Kudo S., O. Linhart, R. Billard 1994. Ultrastructural studies of sperm penetration in the egg of the European catfish (*Silurus glanis*). *Aquat. Living Resour.* **7**, 93-98.
- Legendre M. 1986. Seasonal changes in sexual maturity and fecundity, and HCG-induced breeding of the catfish *Heterobranchus longifilis* Val. (Clariidae), reared in Ebrié lagoon (Ivory Coast). *Aquaculture* **55**, 201-213.
- Legendre M. 1992. Potentialités aquacoles des Cichlidae (*Sarotherodon melanotheron*, *Tilapia guineensis*) et Clariidae (*Heterobranchus longifilis*) autochtones des lagunes ivoiriennes. *ORSTOM, TDM* **89**, 83 p.
- Legendre M., B. Jalabert 1988. Physiologie de la reproduction. In: *Biologie et écologie des poissons africains d'eau douce*. Lévêque C., M.N. Bruton, G.W. Ssentengo eds. *ORSTOM, Trav. Doc.* **216**, 153-187.
- Legendre M., G.G. Teugels 1991. Développement et tolérance à la température des œufs de *Heterobranchus longifilis*, et comparaison des développements larvaires de *H. longifilis* et de *Clarias gariepinus*. *Aquat. Living Resour.* **4**, 227-240.
- Legendre M., Z.J. Otémé 1995. Effect of varying latency period on the quantity and quality of ova after hCG-induced ovulation in the African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae). *Aquat. Living Resour.* **8**, 309-316.
- Legendre M., G.G. Teugels, C. Cauty, B. Jalabert 1992. A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus*, *Heterobranchus longifilis* and their reciprocal hybrids (Pisces, Clariidae). *J. Fish Biol.* **40**, 59-79.
- Lehri G.K. 1968. Cyclical changes in the ovary of the catfish *Clarias batrachus* (Linn.). *Acta Anat.* **69**, 105-124.
- Linhart O. 1984. Evaluation of pike and wels sperm. *Bul. VURH Vodnany* **20**, 22-33 [in Czech with English summary].
- Linhart O., R. Billard 1994. Spermiation and sperm quality of European catfish (*Silurus glanis*) after implantation of GnRH analogues and injection of carp pituitary extract. *J. Appl. Ichthyol.* **10**, 182-188.
- Linhart O., R. Billard 1995. Survival of ovulated oocytes and ova in the European catfish (*Silurus glanis*) after *in vivo* and *in vitro* storage or exposure to various solutions. *Aquat. Living Resour.* **8**, 317-322.
- Linhart O., J. Kouril, J. Hamackova 1986. The motile spermatozoa of wels, *Silurus glanis* L. and tench, *Tinca tinca* L. after sperm collection without water activation. *Prace VURH Vodnany* **15**, 28-41.

- Linhart O., J. Kouril, J. Hamackova 1987. Increased rate of egg fertilization in artificial propagation of sheatfish (*Silurus glanis* L.) by means of suppressing the movements of spermatozoa with immobilization solution. *Aquaculture* **65**, 353-358.
- Linhart O., R. Billard, J.P. Proteau 1993. Cryopreservation of European catfish (*Silurus glanis*) spermatozoa. *Aquaculture* **115**, 347-359.
- Loir M., C. Cauty, P. Planquette, P.Y. Le Bail 1989. Comparative study of the male reproductive tract in seven families of South-American catfishes. *Aquat. Living Resour.* **2**, 45-56.
- Luquet P., T. Boujard, P. Planquette, Y. Moreau, G. Hostache 1989. The culture of *Hoplosternum littorale*: state of the art and perspectives. In: Advances in tropical aquaculture. AQUACOP-IFREMER, Paris, 511-516.
- Maggese M.C., M. Cukier, V.E. Cussac 1984. Morphological changes, fertilizing ability and motility of *Rhamdia sapo* (Pisces, Pimelodidae) sperm induced by media of different salinities. *Rev. Brasil. Biol.* **44**, 541-546.
- Manickam P., K.P. Joy 1989. Induction of maturation and ovulation by pimozone-LHRH analogue treatment and resulting high quality egg production in the Asian catfish, *Clarias batrachus* (L.) *Aquaculture*, **83**, 193-199.
- Mares J., J. Suchy, L. Hochman 1970. Rybnikarstvi, SZN, Praha, 300 p. [in Czech].
- Marian T., Z.L. Kraznai 1987. Cryopreservation of European catfish (*Silurus glanis* L.) sperm. In: Selection, hybridization and genetic engineering. Tiews K. ed. *Aquaculture*, **2**, 253-260. FAO/EIFAC Hamburg.
- Mattei X. 1970. Spermiogenèse comparée des poissons. In: Comparative spermatology Baccetti B. ed. Acad. Press. New York, 57-69.
- Micha J.C. 1976. Synthèse des essais de reproduction, d'alevinage et de production chez un silure africain: *Clarias lazera* Val. In: Symp. Aquac. Africa, CIFA Tech. Pap. **4**, Suppl. 1, 450-73.
- Mihalik J. 1956. Zkusenosti s poloumelym vyterem sumca. *Ceskoslovenske Ryb.* **4**, 50-52 [in Slovak].
- Miura T., K. Yamauchi, H. Takahashi, Y. Nagahama 1992. The role of hormones in the acquisition of sperm motility in salmonid fish. *J. Exp. Zool.* **261**, 359-363.
- Mollah M.F.A., E.S.P. Tan 1982. Effects of incubation temperature on the hatching of the catfish (*Clarias macrocephalus* Gunther) eggs with an illustration on the larval stages. *Malay. Nat. J.* **36**, 123-131.
- Mollah M.F.A., E.S.P. Tan 1983a. HCG induced spawning of the catfish, *Clarias macrocephalus* (Gunther). *Aquaculture* **35**, 239-247.
- Mollah M.F.A., E.S.P. Tan 1983b. Viability of catfish (*Clarias macrocephalus*, Gunther) eggs fertilized at varying post-ovulation times. *J. Fish Biol.* **22**, 563-566.
- Na-Nakorn U., W. Rangsin, S. Witchasunkul 1993. Suitable conditions for induction of gynogenesis in the catfish, *Clarias macrocephalus*, using sperm of *Pangasius sutchi*. *Aquaculture* **118**, 53-62.
- Nunez Rodriguez J., Z.J. Otémé, S. Hem 1995. Comparative study of vitellogenesis of two catfish species *Chrysichthys nigrodigitatus* (Claroteidae) and *Heterobranchus longifilis* (Clariidae). *Aquat. Living Resour.* **8**, 291-296.
- Nwaduwe F.O. 1993. Inducing oocyte maturation, ovulation and spawning in the African catfish, *Heterobranchus longifilis* Valenciennes (Pisces: Clariidae), using frog pituitary extract. *Aquac. Fish. Manag.* **24**, 625-630.
- Nwaduwe F.O., O.A. Ayinla, N.J. Abby-Kalio 1993. Effects of various doses of acetone-dried powdered carp pituitary extract and season on hatchery propagation of *Heterobranchus longifilis* (Val., 1840) (Pisces: Clariidae). *J. Aquac. Trop.* **8**, 33-40.
- Otémé Z.J. 1993. Reproduction et fécondité du machoiron *Chrysichthys nigrodigitatus* en élevage. *J. Ivoir. Océanol. Limnol. Abidjan* **2**, 53-59.
- Otémé Z.J., J. Nunez Rodriguez, C.K. Kouassi, S. Hem, J.F. Agnese 1996. Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish *Heterobranchus longifilis* (Valenciennes, 1840). *Aquac. Res.* in press.
- Padhi B.K., R.K. Mandal 1995. Cryopreservation of spermatozoa of two Asian freshwater catfishes, *Heteropneustes fossilis* and *Clarias batrachus*. *J. Aquac. Trop.* **10**, 23-28.
- Pascal M., G. Hostache, C. Tessier, P. Vallat 1994. Cycle de reproduction et fécondité de l'atipa, *Hoplosternum littorale* (Siluriforme) en Guyane Française. *Aquat. Living Resour.* **7**, 25-37.
- Pham A., B. Raugel 1977. Contribution à l'étude de la reproduction provoquée des femelles de *Clarias lazera* (Val.) (Pisces: Clariidae). CTFT, Nogent-sur-Marne, **15**, 27-33.
- Phelps R.P., C.A. Walser 1993. Effect of sea salt on the hatching of Channel catfish egg. *J. Aquat. Anim. Health* **5**, 205-207.
- Pillay T.V.R. 1993. Aquaculture, principles and practices. Fishing News Books, 575 p.
- Poirier G.R., N. Nicholson 1982. Fine structure of the testicular spermatozoa from the channel catfish, *Ictalurus punctatus*. *J. Ultrastr. Res.* **80**, 104-110.
- Potaros M., P. Sitatsit 1976. Induced breeding of *Pangasius sutchi* (Fowler) FAO, IPFC. Proc. **17**, 349-353.
- Proteau J.P., O. Linhart 1993. *Silurus glanis* L.: Actual state of the techniques of production. In: Aquaculture of Freshwater species (except salmonids). Eur. Aquac. Soc. P. Kestemont, R. Billard eds. Spec. Publ. **20**, 12-15.
- Redondo-Müller Ch. 1990. Biología del esperma de la carpa común, *Cyprinus carpio* L. y del siluro europeo *Silurus glanis* L. Thesis Dr. Univ. Zaragoza, Spain. 193 p.
- Redondo-Müller Ch., V. Hilge, R. Billard 1989. Artificial insemination of catfish (*Silurus glanis* L.). Bredene, Belgique. 1989. *Europ. Aquac. Soc. Spec. publ.* **10**, 211-212.
- Redondo-Müller Ch., R. Billard, E. Espinosa, A. Josa 1990. Inducción a la espermiación del siluro europeo, *Silurus glanis* L., mediante la aplicación de extractos hipofisarios de Carpa (EHC). Repr. Anim. Ins. Artif. Zaragoza, Spain. 211-214.
- Resink J.W., W.G.E.J. Schoonen, R.R. Van den Hurk, W.J.A.R. Viveen, J.G.D. Lambert 1987a. Seasonal changes in steroid metabolism in the male reproductive organ-system of the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 59-76.
- Resink J.W., R. Van den Hurk, R.F.O. Groeninx Van Zoelzn, E.A. Huisman 1987b. The seminal vesicle as source of sex attracting substances in the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 155-127.
- Resink J.W., T.W. M. Van den Berg, R. Van den Hurk, E.A. Huisman, P.G.W.J. Van Oordt 1989. Induction of

- gonadotropin release and ovulation by pheromones in the African catfish, *Clarias gariepinus*. *Aquaculture* **83**, 167-177.
- Ribes A., G. Ribes 1991. Reproduction artificielle du silure glane en toutes saisons sur le site géothermique de la Castillonne. *Aqua Revue* **37**, 27-29.
- Richter C.J.J. 1976. The african catfish, *Clarias lazera* (C. & V.), a new possibility for fish culture in tropical regions? *Misc. Pap. Landbouwhogeschool, Wageningen* **13**, 51-70.
- Richter C.J.J., R. Van den Hurk 1982. Effects of 11-desoxycorticosterone-acetate and carp pituitary suspension on follicle maturation in the ovaries of the African catfish, *Clarias lazera* (C. & V.). *Aquaculture* **29**, 53-66.
- Richter C.J.J., E.H. Eding, S.E.W. Leuven, J.G.M. Van Der Wijst 1982. Effects of feeding levels and temperature on the development of the gonad in the African catfish *Clarias lazera* (C. & V.). In: Proc. Symp. Reproductive Physiology of Fish. C.J.J. Richter, H.J.Th. Goos eds. PUDOC, Wageningen, 147-150.
- Richter C.J.J., E.H. Eding, A.J. Roem 1985. 17  $\alpha$ -hydroxyprogesterone-induced breeding of the African catfish, *Clarias gariepinus* (Burchell), without priming with gonadotropin. *Aquaculture* **44**, 285-293.
- Richter C.J.J., W.J.A.R. Viveen, E.H. Eding, M. Sukkel, A.J. Rothuis, M.F.P.M. Van Hoof Van, F.G.J. Der Berg, P.G.W.J. Van Oordt 1987. The significance of photoperiodicity, water temperature and an inherent endogenous rhythm for the production of viable eggs by the African catfish, *Clarias gariepinus*, kept in subtropical ponds in Israel and under Israeli and Dutch hatchery conditions. *Aquaculture* **63**, 169-185.
- Riehl R., S. Appelbaum 1991. A unique adhesion apparatus on the eggs of the catfish *Clarias gariepinus* (Teleostei, Clariidae). *Jpn. J. Ichthyol.* **38**, 191-197.
- Riehl R., R. Patzner 1993. Breeding, egg structure and larval morphology of the catfish *Sturisoma aureum* (Steindachner) (Teleostei, Loricariidae). *J. Aquaricult. Aquat. Sci.* **6**, 1-6.
- Rimmer M.A., J.R. Merrick 1983. A review of reproduction and development in the fork-tailed catfishes (Ariidae). *Proc. Linn. Soc. N. S. W.* **107**, 41-50.
- Ringle J.P., J.G. Nickum, A. Moore 1992. Chemical separation of catfish egg masses. *Progress. Fish-Cult.* **54**, 73-80.
- Saad A., R. Billard 1995. Production et gestion des spermatozoïdes chez le poisson-chat européen *Silurus glanis*. *Aquat. Living Resour.* **8**, 323-328.
- Shoonbee H.J., T. Hecht, L. Polling, J.E. Saayman 1980. Induced spawning of and hatchery procedures with the sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). *South Afr. J. Sci.* **76**, 364-367.
- Schoonen W.G.E.J., J.C.M. Granneman, J.G.D. Lambert, P.G.W.J. Van Oordt 1987. Steroidogenesis in the testes and seminal vesicles of spawning and non-spawning African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 77-88.
- Senthilkumaran B., K.P. Joy 1993. Annual cyclic, and castration and cyproterone acetate-induced, changes in sialic acid content of the seminal vesicle of the catfish, *Heteropneustes fossilis* (Bloch). *Fish Physiol. Biochem.* **10**, 425-430.
- Siwicki A., Z. Jeney 1986. Surgical intervention in wels (*Silurus glanis* L.) during artificial propagation. *Aquacultura Hungarica* (Szarvas) **5**, 55-58.
- Smitherman R.O., R.A. Dunham, D. Tave 1983. Review of catfish breeding research 1969-1981 at Auburn University. *Aquaculture* **33**, 197-205.
- Sneed K.E., H.P. Clemens 1959. The use of human chorionic gonadotropin to spawn warm-water fishes. *Progress. Fish-Cult.* **21**, 117-120.
- Sneed K.E., H.P. Clemens 1963. The morphology of the testes and accessory reproductive glands of the catfishes (Ictaluridae). *Copeia* **4**, 606-611.
- Stacey N.E. 1984. Control of the timing of ovulation by exogenous and endogenous factors. In: Fish Reproduction - Strategies and tactics. Potts G.W., R.J. Wootton eds. Academic Press, London, p. 208-222.
- Steyn G.J., J.H.J. Van Vuren 1986. The role of the blood-testis barrier in the chemical composition of the seminal plasma of the freshwater teleost *Clarias gariepinus*. *Comp. Biochem. Physiol.* **83A**, 421-425.
- Steyn G.J., J.H.J. Van Vuren 1987a. Some physical properties of the semen from artificially induced sharptooth catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* **86**, 315-317.
- Steyn G.J., J.H.J. Van Vuren 1987b. The fertilizing capacity of cryopreserved sharptooth catfish (*Clarias gariepinus*) sperm. *Aquaculture* **63**, 187-193.
- Slembrouck J., M. Legendre 1988. Aspects techniques de la reproduction contrôlée de *Heterobranchus longifilis* (Clariidae). *Cent. Rech. Océanogr. Abidjan, NDR 02/88*, 19 p.
- Suchomelova K. 1952. Mnozství jiker sumce obecneho. *Ceskoslov. Rybar.* 114 p. [in Czech].
- Sundararaj B.I., S.V. Goswami 1977. Hormonal regulation of *in vivo* and *in vitro* oocyte maturation in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.* **32**, 17-28.
- Sundararaj B.I., A. Seghal 1970. Effects of a long or an increasing photoperiod on the initiation of ovarian recrudescence during the preparatory period in the catfish, *Heteropneustes fossilis* (Bloch). *Biol. Reprod.* **2**, 413-424.
- Tambasen-Cheong Ma V.P., J.D. Tan-Fermin, L.M.B. Garcia, R.B. Baldevarona 1995. Milt-egg ratio in artificial fertilization of the Asian freshwater catfish, *Clarias macrocephalus*, injected salmon gonadotropin-releasing hormone analogue and domperidone. *Aquat. Living Resour.* **8**, 303-307.
- Tan-Fermin J.D., A.C. Emata 1993. Induced spawning by LHRHa and pimoziide in the Asian catfish *Clarias macrocephalus*. *J. Appl. Ichthyol.* **9**, 89-96.
- Thalathiah B.S., A.A.B. Othman, M.S. Zaini 1988. Induced spawning techniques practised at Batu Berendam, Melaka, Malaysia. *Aquaculture* **74**, 23-33.
- Tilney R.L., T. Hecht 1993. Early ontogeny of *Galeichthys feliceps* from the south east coast of South Africa. *J. Fish Biol.* **43**, 183-212.
- Tucker G.S., E.H. Robinson 1990. Channel catfish farming handbook. Avi Book, New York, 454 p.
- Van den Hurk R., J.W. Rcsink, J. Peute 1987a. The seminal vesicle of the African catfish, *Clarias gariepinus*. A histological, histochemical, enzyme-histochemical, ultrastructural and physiological study. *Cell. Tissue Res.* **247**, 573-582.

- Van den Hurk R., J.W. Resink, P.K. Woorthuis 1987b. An enzyme-histochemical study concerning the localization of steroid glucuronide production in the reproductive organs of African catfish *Clarias gariepinus*. *Aquaculture* **63**, 89-96.
- Van der Waal B.C.W. 1974. Observations on the breeding habits of *Clarias gariepinus* (Burchell). *J. Fish Biol.* **6**, 23-27.
- Van der Waal B.C.W. 1985. Stripping male *Clarias gariepinus* standard. *Aquaculture* **43**, 137-142.
- Van der Walt L.D., F.H. Van der Bank, G.J. Steyn 1993. The suitability of using cryopreservation of spermatozoa for the conservation of genetic diversity in African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol. A* **105**, 313-318.
- Van Oordt P.G.W.J., J. Peute, R. Van Den Hurk, W.J.A.R. Viveen 1987. Annual correlative changes in gonads and pituitary gonadotropes of feral African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 27-41.
- Wisniewolski W. 1988. Fecundity of catfish (*Silurus glanis* L.) from the rivers Vistula and Bug. *Acta Ichthyol. Piscat.* **13**, 25-34.
- Wisniewolski W. 1989. Zuchtmöglichkeiten des Welses in Teichen, in Polen. *Roczniki Nauk Rolniczych, Seria H.T.* **102**, 131-167.
- Withler F.C. 1980. Chilled and cryogenic storage of gametes of thai carps and catfishes. *Can. Tech. Rep. Fish. Aquat. Sci.* **943**: 15 p.
- Withler F.C. 1982. Cryopreservation of spermatozoa of some freshwater fishes cultured in South and Southeast Asia. *Aquaculture* **25**, 395-398.
- Woynarovich E., L. Horvath 1980. The artificial propagation of warm-water finfishes - a manual for extension. FAO Fish. Tech. Pap. 201.
- Yemlyanova N.G. 1993. Formation of egg membranes in Channel catfish, *Ictalurus punctatus*. *J. Ichthyol.* **33**, 85-92.
- Young M.J.A., A.W. Fast, P.G. Olin 1989. Induced maturation and spawning of the Chinese catfish *Clarias fuscus*. *J. World Aquac. Soc.* **20**, 7-11.
- Zairin M., K. Asahina, K. Furukawa, K. Aida 1993. Plasma steroid hormone profiles in HCG injected male walking catfish *Clarias batrachus*. *Zool. Sci.* **10**, 329-336.
- Zholdasova I.M., L.N. Guseva 1987. Oogenesis and cyclic changes in the gonads of male and female catfish, *Silurus glanis*, in the lower Amudar'ya basin. *J. Ichthyol.* **27**, 1-8.
- Zlatev S. 1987. A study on working fertility of channel catfish (*Ictalurus punctatus*). *Anim. Sci.* **24**, 84-86, in Bulgar with English summary.
- Zonneveld N., Rustida, W.J.A.R. Viveen, Wayan Mudana 1988. Induced spawning and egg incubation of the Asian catfish, *Clarias batrachus*. *Aquaculture* **74**, 41-47.