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Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish *Heterobranchus longifilis* (Valenciennes, 1840)

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Abstract

The morphological and physiological characteristics of the testes and the sperm of the catfish *Heterobranchus longifilis* (Val.) are presented. The effect of cryopreservation on the fertilizing capacity of the sperm was also evaluated. Testicular structure and spermatogenesis are described using histological techniques. The coexistence in the lobules of spermatozoa and all the spermatogenic stages indicates that this species is able to perform continuous reproduction. No seasonal trend was noticed in the evolution of the gonadosomatic index and in the quantity of the sperm produced over a year's period. However, maximum sperm production was observed in April and September.

Different cryopreservation trials were conducted using a cryoprotective solution composed of 5% dimethyl sulphoxide (DMSO), 5% glycerol, 10% hen's eggyolk and 80% Mounib's solution. Fresh and cryopreserved semen gave equivalent hatching rates (81.1%, 83.4% and 78.9% respectively for the fresh, the 1-hour cryopreserved and the 8-month cryopreserved sperm). Percentages of normal and deformed larvae were not affected by sperm cryopreservation.

Introduction

The aquaculture of *Heterobranchus longifilis* has gained interest since the mid 1970s (Micha 1973; Bard, De Kimpe & Lessent (1976). Since then, significant contributions have been made towards the development of techniques for the reproduction and the culture of this catfish (Legendre 1983, 1986, 1991; Nunez, Otémé & Hem 1995). Extensive work has been reported on its nutrition and feeding (Kerdchuen & Legendre 1991; Kerdchuen 1992; Otémé & Gilles 1995). Other aspects of the biology and the culture of *H. longifilis*, including the effects of water chemistry on growth and survival, and the optimization of growth and feed conversion rates, have recently been investigated (Luquet, Otémé & Métongo 1993; Luquet, Otémé & Cissé 1995).

H. longifilis is a species that presents a high fecundity (up to 120 000 ovules per kg of body weight) and a very high growth rate (8–12 g day^{-1} for average body weights ranging from 200 to 900 g). A study carried out at Layo Aquaculture station (Legendre, Teugels, Cauty & Jalabert 1992) showed that *H. longifilis* displayed a much faster growth rate than *Clarias gariepinus* (Burchell), reaching about 700 g in 254 days as opposed to 370 g for *C. gariepinus*. Successful hybridization has been performed between *H. longifilis* and *C. gariepinus* (Hecht & Lublinkhof 1985; Hecht, Lublinkhof & Kenmuir 1991; Legendre *et al.* 1992), offering the possibility of improving growth, dress-out percentage, feed conversion efficiency and disease resistance of the species.

In aquaculture the availability of gametes throughout the year is important to ensure a constant supply of fish. From that point of view, *H. longifilis* presents a definite interest for aquaculture because its gametogenesis is continuous once sexual maturity is reached. However, the males have to be killed and the testes dissected out to collect the sperm as the semen cannot easily be obtained by stripping, unlike the ovules in females.

Several means, including long-term storage of gametes or embryos, may be used to improve fish farm management. Cryopreservation of sperm can facilitate artificial insemination and allow crossing or hybridization of geographically distant fish. The interest of sperm preservation is indisputable, especially when genetic studies are considered. In a recent study, Agnèse, Otémé & Gilles (1995) investigated the effects of domestication on genetic variability, fertility, survival and growth rate of *H. longifilis* and reported that this species was highly sensitive to inbreeding. The subsequent loss of polymorphism led to a significant decrease in fry survival rates and favoured a greater variability in growth rates. The cryopreservation of sperm could thus constitute a means of restoring genetic variability through a cryopreserved gene bank.

The first successful attempt to cryopreserve African catfish sperm was achieved by Steyn, Van Vuren, Schoonbee & Chao (1985) on *Clarias gariepinus*. The techniques used were improved in later experiments by Steyn & Van Vuren (1987), who studied the optimal cryodiluents and freezing rates to be used. Cryopreservation was then used as a support for genetic studies on *C. gariepinus* (Van der Bank & Steyn 1992; Van der Walt, Van der Bank & Steyn 1993).

To the best of our knowledge, no work has yet been reported on the spermatogenesis and the cryopreservation of *Heterobranchus longifilis* sperm. The aim of this work was therefore to present some general aspects of the spermatogenic development along with the annual sperm production in *H. longifilis* and to assess the fertilizing capacity of the sperm after cryopreservation.

Materials and methods

Experimental fish

The present study was conducted with gametes collected from sexually mature individuals. The *H. longifilis* brooders used in this work were 3–5 years old and came from an F_1 generation that descended from wild stock caught in the Ebrié Lagoon area around the Layo Station (Côte d'Ivoire). The fish were maintained in a brackish-water pond (salinity ranging from 1‰ to 3‰) at Layo Station, and were sexually mature at 11 and 15 months of age respectively for the males and the females.

Histology

A male was killed and the whole gonad, including the seminal vesicles, was removed for the study of the anatomy. The testes of two such males were dissected out every month, cut into several small pieces (1 mm thick) and allowed to fix in alcoholic Bouin's solution for six days. Paraffin embedding and sectioning of the pieces were performed using standard histological techniques (Martoja & Martoja 1967; Gabe 1968). Sections 7 μm thick were mounted on slides and coloured using hemalun–eosin. Observations of the slides were made using a light microscope after mounting with Canada balm.

Determination of gonadosomatic index and sperm production

Up to 11 males were dissected per month from January 1990 to March 1995. The testes were removed, weighed and the sperm collected in a graduated tube to determine the volume of sperm from each individual male.

All the data were pooled on a monthly basis to determine the overall yearly variation in the quantity of sperm produced and the gonadosomatic index (GSI = (gonad weight/body weight) × 100). The weight of the seminal vesicles was not taken into account in the gonad weight.

Gamete-collection techniques

Five males of *H. longifilis* were killed on 6 April 1994 and the testes were removed. The sperm was collected by dissecting and squeezing out the anterior lobes of each testis into a graduated glass tube. The sperm from all the males was pooled to minimize any 'intersperm' variation in sperm fitness for cryopreservation generally observed in other fish species. A sample of the pooled milt was deep-frozen in liquid nitrogen for 8 months prior to the beginning of the breeding experiment.

The milt from a second group of five males was collected on 3 January 1995 and pooled using the procedure described above. A sample of this milt was deep-frozen in liquid nitrogen for 1 h prior to insemination. Another sample of the same milt was stored in a 14-ml glass tube kept sealed on crushed ice (4°C) until use as fresh milt.

Ovulation was artificially induced with human chorionic gonadotrophins (hCG) in one female. This fish was selected on the basis of the swelling and the softness of the belly as well as the modal diameter (greater than 1 mm) of oocytes collected by intra-ovarian biopsy before inducing the ovulation. The

ovules were stripped out of the female after an 11-h latency period at 30°C.

Sperm cryopreservation techniques

The diluent tested in the present study was based on that of Mounib (1978): 125 mM sucrose, 100 mM potassium bicarbonate, 6.5 mM reduced glutathion. Different cryopreservation trials were conducted using a combination of extenders and cryoprotective agents in order to evaluate the diluent's suitability as cryodiluent for *H. longifilis* sperm. These trials were performed with the Mounib's medium to which were added 5% DMSO (dimethyl sulphoxide), 5% glycerol and 10% hen's eggyolk. The sperm was mixed with the diluent at a ratio of 1:3 as suggested by Legendre & Billard (1980), placed in 5-ml straws (IMV, ref. AA 303) and allowed to freeze 3 cm above the level of liquid nitrogen for 20 min as recommended by Maisse (1994). The straws were then stored in liquid nitrogen.

The motility was evaluated before and after freezing by estimating the proportion of motile spermatozoa and the duration of their movement, and considering the mass progressive motility while most of them were still swimming actively with progressive movement. The degree of motility was estimated as the percentage of motile spermatozoa. Aliquots of the semen samples were inspected for spermatozoa motility before and after dilution with fresh dechlorinated tap water. Motility was estimated on a glass slide under a light microscope (× 250 magnification) at room temperature (25°C). The estimation was started immediately, from approximately 10 s after dilution to the end of the active swimming of the spermatozoa.

Artificial insemination and hatching of ovules

The fertilizing ability of the sperm was evaluated using hatching percentages on batches of 200 to 300 ovules collected from one female *H. longifilis* and artificially inseminated respectively with fresh sperm, or with sperm (from the same pool) thawed after 1 h of cryopreservation, or with sperm cryopreserved in liquid nitrogen for 8 months. The cryopreserved sperm was thawed in a water bath (25°C) just before artificial insemination. An insemination ratio of 0.2 ml of milt (diluted 1:10 in 0.9% NaCl solution) for 0.4 g ovules was used for the different types of sperm. This corresponded to a minimum of 50 000 spermatozoa per ovule.

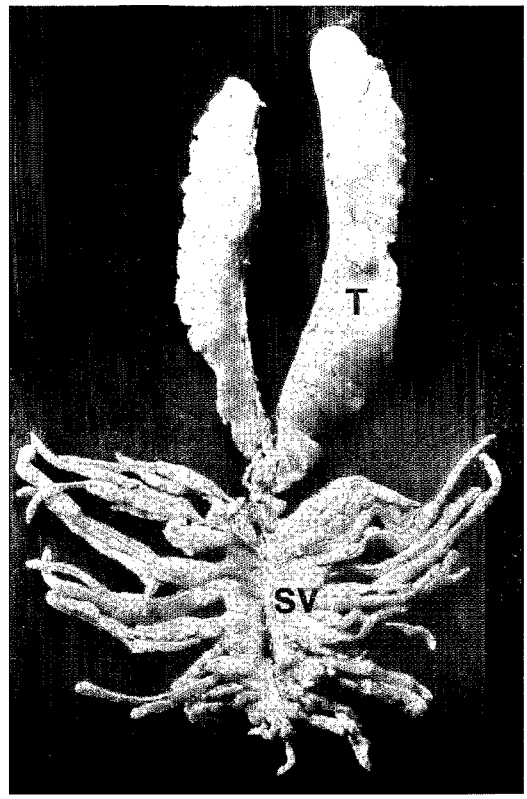


Figure 1 Testicular anatomy of *H. longifilis*. The testes (T) are well differentiated from the seminal vesicles (SV).

After 1 min of moderate agitation in 5 ml of dechlorinated tap water, the eggs were rinsed off and placed in plastic containers where incubation took place in darkness at 25°C in 300 ml of stagnant water. Twenty-four hours after fertilization, the total hatching and the proportions of normal and deformed larvae from each batch were determined by direct observation and counting over a light table. Hatching rates, and the percentages of normal and deformed larvae, from the fresh and cryopreserved milts were compared using a one-way analysis of variance at the 5% level of significance.

Results

Testicular structure

In adult males of *H. longifilis*, the testes appear as two elongated lobes (Fig. 1) the size and weight of which vary greatly from one fish to another independent

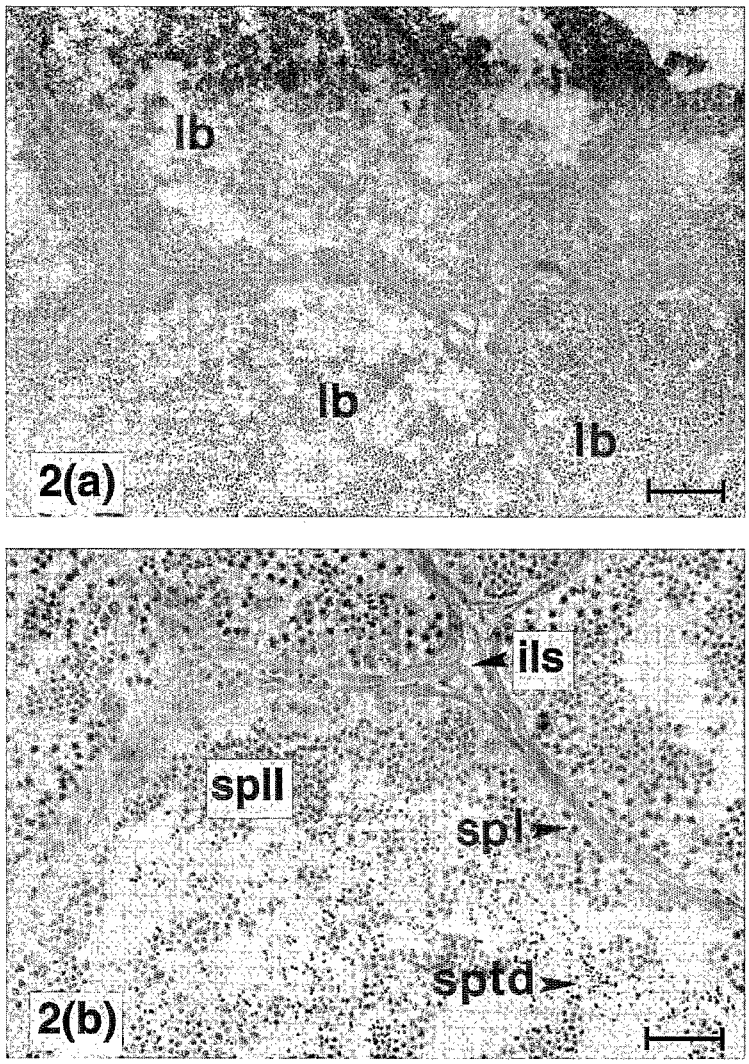


Figure 2 Transverse section through the testis of *H. longifilis*. (2a) General aspect of lobule organization (250 μm). (2b) Partial view of lobule section showing the distribution of the different spermatogenetic stages (100 μm).

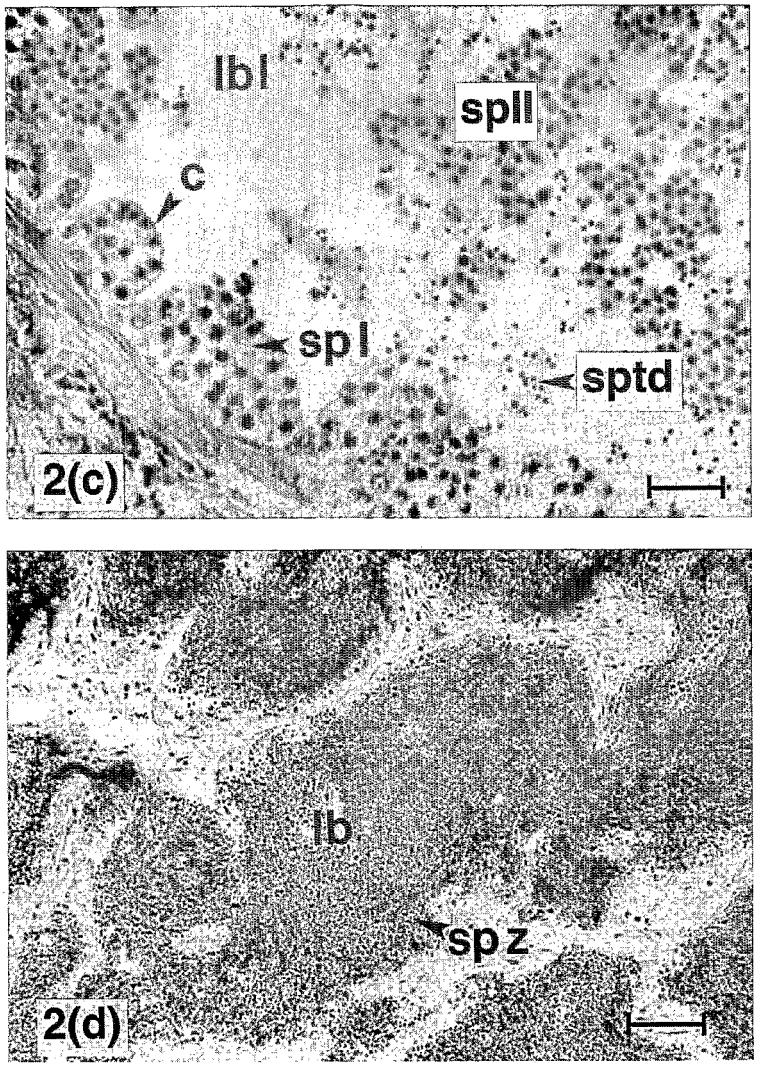
of the weight of the individual considered. They lie dorsally and to the rear of the abdominal cavity along with their associated seminal vesicles which are more or less developed and branched.

Spermatogenesis

The testes of *H. longifilis* are the lobular type, as defined by Billard, Fostier, Weil & Breton (1982). The observation of the histological sections showed a marked asynchronous development in the spermatogenesis within the same testis. The sections revealed irregularly shaped lobules of varying dimensions (Fig. 2a). All development stages could be observed inside these lobules (Fig. 2b). At the edge of the lobules and within individual cysts, the spermatocytes I are slightly bigger than the spermatocytes II and have dense and less-rounded nuclei. The spermatids, even smaller than the spermatocytes II, have very dense, rounded nuclei (Fig. 2c). In the inner part of the lobules, spermatozoa are packed and fill the lobular lumen (Fig. 2d).

Gonadosomatic index and annual sperm production

The monthly variations of the gonadosomatic index (GSI), along with the volume of sperm produced are



(2c) Higher magnification of the peripheral zone of a lobule showing the distribution of individual spermatocyte II cysts (63 µm). (2d) Section in the inner part of a lobule filled up with spermatozoa (100 µm). Labels: c, cyst; ils, interlobular space; lb, lobule; lbl, lobular lumen; sp I, spermatocyte I; sp II, spermatocyte II; sptd, spermatid; spz, spermatozoa.

shown in Fig. 3. No evidence of seasonal variation was found in the GSI, which fluctuated from 0.20% to 0.65%, with high individual variations (not shown). Likewise, no seasonal trend was observed in the mean monthly sperm production, although relatively large quantities of intratesticular semen were collected in April (19.6 ml) and September (21.2 ml). A considerable variability was also observed as attested by the standard deviation bars.

Sperm cryopreservation and motility

H. longifilis spermatozoa were immotile after collection. Motility was induced by dilution with fresh water. Motility levels decreased rapidly from vigorous swimming immediately after dilution with water to vibratory (and restricted forward) movement by 70 s. The sperm motility measured before and after cryopreservation showed that the motility was altered by the freezing–thawing process. Fresh sperm exhibited a percentage of motile spermatozoa ranging from 70% to 80% 10 s after dilution, while cryopreserved sperm only showed motility percentages between 20% and 30%.

Hatching rates using fresh and cryopreserved milt, and percentages of normal and deformed larvae, are presented in Fig. 4. It appeared that cryopreserved milt was as effective as fresh sperm in fertilization

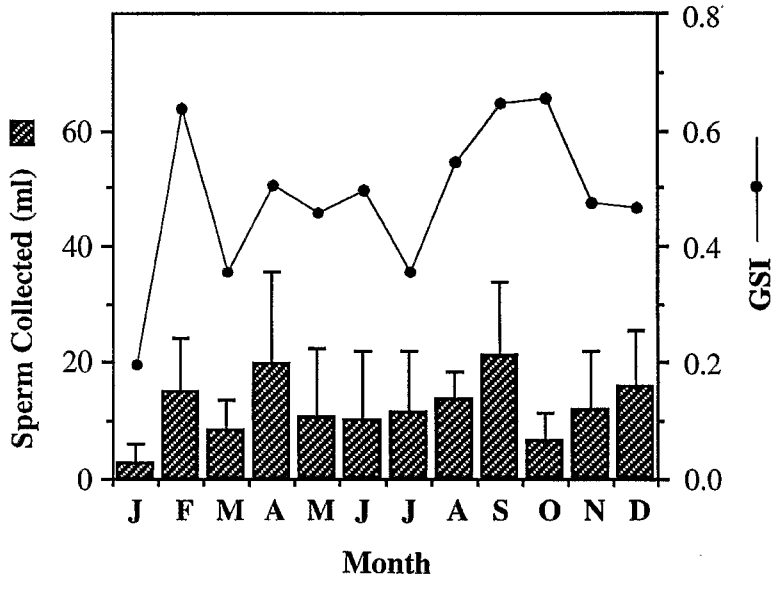


Figure 3 Monthly variation of the gonadosomatic index and the volume of sperm produced in *H. longifilis* (vertical bars indicate standard deviation of the mean).

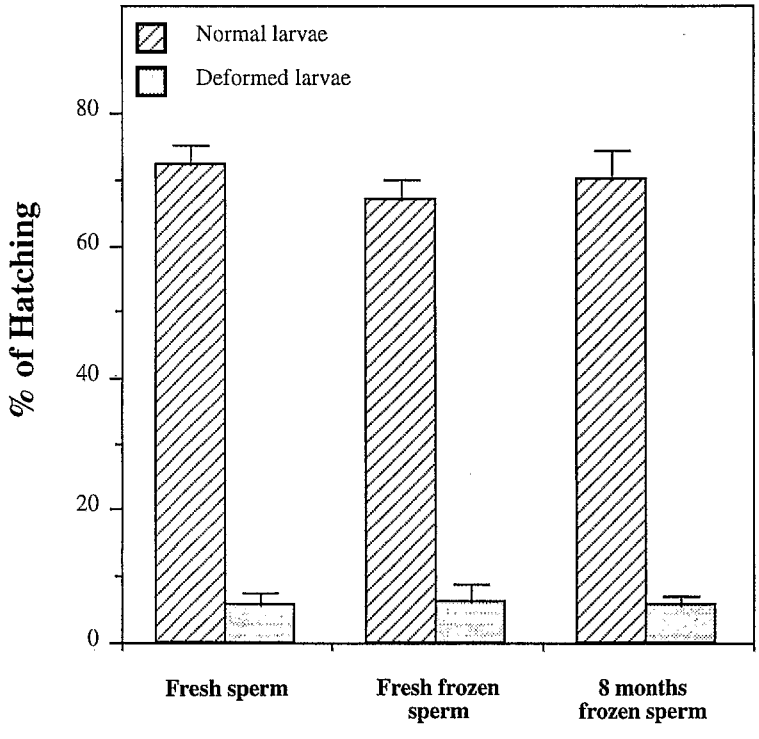


Figure 4 Hatching percentage of *H. longifilis* ovules fertilized with fresh and cryopreserved sperm (vertical bars indicate standard deviation of the mean).

and hatching trials at the dilution used, yielding 81.1% and 83.4% total hatched ovules for the sperm cryopreserved in liquid nitrogen for the 1-h and the 8-month periods respectively, as opposed to 78.9% for the fresh milt. The observed hatching rates of deformed larvae were 5.8%, 6.0% and 6.2%, respectively, for the sperm cryopreserved in liquid nitrogen for 1 h, 8 months and the fresh sperm. The differences between the total hatching values were not statistically significant ($P < 0.05$). No

significant difference ($P < 0.05$) was observed in the hatching rates of deformed larvae as well.

Discussion

The histology of the testes showed the coexistence of all the spermatogenic stages. The marked asynchronism of the development of spermatogenesis indicates that in captivity, this species is able to perform continuous spermiation all year round even though the preferred reproductive season is the rainy season (Legendre 1986). These observations confirm the permanent sexual activity of male *H. longifiis* reported by Legendre (1991). However, although spermatogenesis is continuous, there was a slight variation in sperm production showing high values in April (19.6 ml) and September (21.2 ml), corresponding to the dry seasons in Côte d'Ivoire. Similar seasonal variations in gamete production were reported in *H. longifilis* and in closely related species such as *C. gariepinus* (Legendre 1991). It is therefore possible to produce thousands of larvae year round with a limited number of brooders. However, several males have to be killed for semen at each reproduction as the sperm cannot easily be collected by abdominal massage, and because at present no objective criteria allow the identification of spermiating individuals.

It is difficult to assess the percentage of surviving spermatozoa after cryopreservation. Probably more than half of the spermatozoa survived, but the rate of motility was strongly reduced, going from 80% (fresh sperm) to 30% (cryopreserved sperm). After water is added, *H. longifilis* spermatozoa motility is brief, lasting 60–70 s. Varying motility times have been reported in other species. Trout *Salmo gairdneri* (Richardson) spermatozoa activated after dilution in fresh water or saline solution (300 mOsmol kg^{-1} exhibits a short period (20–25 s at 20°C) of progressive motility (Cosson, Billard, Gatti & Christen 1985). In Carp *Cyprinus carpio* (L.), motility initiated with fresh water or saline solution (<200 mOsmol kg^{-1}) lasts for 45 s (Redondo-Muller, Cosson, Cosson & Billard 1991). In marine fishes including European sea bass, *Dicentrarchus labrax* (L.), and turbot, *Scophthalmus maximus* (L.), total motility durations of 3–26 min have been reported (Billard, Dupont & Barnabé 1977; Suquet, Omnes & Fauvel 1982).

Decreased motility and freezing fitness have already been reported in trout *Salmo gairdneri* (Richardson) (Billard & Breton 1976) and European sea bass (Billard *et al.* 1977) and were related to sperm senescence. At the end of the spermiation period for these species, the ability of sperm to be deep-frozen decreases, which may be caused by sperm ageing phenomena. It has also been shown that sperm fitness for deep-freezing varied from male to male and that pooled sperm of several males gave better fertilization rates than the mean of individual values (Legendre & Billard 1980; Maisse, Pinson & Loir 1988; Maisse 1994). The results of the present study did not indicate the existence of a well-defined spermiation cycle in *H. longifilis*. Also, the semen used in the different trials came from pooled sperm from several males. Consequently, the observed decrease in motility of sperm after freezing cannot be attributed to sperm ageing during spermiation as seen in other species of fish; nor can it be attributed to the quality of individual sperms.

The cryopreservation trials were carried out with the Mounib's diluent used in previous studies (Legendre & Billard 1980; Maisse 1994). This diluent was improved by the addition of 10% eggyolk as suggested by Legendre & Billard (1980), who reported that the addition of eggyolk to the Mounib's diluent improved sperm motility after thawing. Our results showed that the milt of *H. longifilis* preserved for either short (1 h) or long (8 months) periods in liquid nitrogen (at −196°C) was just as effective as fresh milt at the sperm dilution (1:10) used in our experiments.

Legendre & Billard (1980) observed a lower fertilizing ability of cryopreserved rainbow trout sperm due to the freezing itself and not to an additional, deleterious effect of the diluent. The combination of Mounib medium + yolk + DMSO used in their study did not entirely preserve the performance of the fresh sperm, contrary to the one tested in the present work which did not seem to affect the performance of the sperm.

It appeared from the results of this study that the sperm of *H. longifilis* can be cryopreserved for at least several months with no deleterious effect on its fertilizing ability. Similar results were achieved by Steyn & Van Vuren (1987), who reported that sperm of *C. gariepinus* preserved for up to 28 months in liquid nitrogen had the same fertilizing capacity as fresh sperm, and showed the potential for the use of cryopreservation of African catfish sperm in selection and conservation programmes.

The fertilizing ability of *H. longifilis* sperm is not affected by the freezing and thawing process. This offers the possibility not only of limiting the quantity

of male individuals killed for reproduction, but also of constituting a gene bank in order to limit inbreeding, to maintain, and if necessary to improve, the quality of the fish population through selection programmes. Indeed, this species has been reported to be highly sensitive to inbreeding, exhibiting a significant variability in growth performances from the third generation (Agnèse *et al.* 1995).

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References

Agnèse J.F., Otémé Z.J. & Gilles S. (1995) Effects of domestication on genetic variability, fertility, survival and growth rate in a tropical siluriform: *Heterobranchus longifilis* Valenciennes, 1840. *Aquaculture* **131**, 197–204.

Bard J., De Kimpe, P. & Lessent P. (1976) Nouveaux poissons et nouvelles méthodes d'élevage en Afrique. *CIFA Technical Paper* **4** (Suppl. 1: Symposium on Aquaculture in Africa, Accra, Ghana, 1975) 365–372.

Billard R. & Breton B. (1976) Sur quelques problèmes de physiologie du sperme chez les poissons téléostéens. *Revue des Travaux de Institut des Pêches Maritimes* **40**, 501–503.

Billard R., Dupont J. & Barnabé G. (1977) Diminution de la motilité et de la durée de conservation du sperme de *Dicentrarchus labrax* L. (poisson, téléostéen) pendant la période de spermiation. *Aquaculture* **11**, 363–367.

Billard R., Fostier A., Weil C. & Breton B. (1982) The endocrine control of spermatogenesis in teleost fish. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 65–79.

Cosson M.P., Billard R., Gatti J.L. & Christen R. (1985) Rapid and quantitative assessment of trout spermatozoa motility using stroboscopy. *Aquaculture* **46**, 71–75.

Gabe M. (1968) *Techniques Histologiques*. Masson, Paris, 1113 pp.

Hecht T. & Lublinkhof W. (1985) *Clarias gariepinus* × *Heterobranchus longifilis* (Clariidae : Pisces): a new hybrid for aquaculture? *South African Journal of Science* **81**, 620–621.

Hecht T., Lublinkhof W. & Kenmuir D. (1991) Induced spawning of the vundu *Heterobranchus longifilis*, and embryo survival rates of pure and reciprocal clariid crosses. *South African Wildlife Research* **21** (4), 123–125.

Kerdchuen N. (1992) *L'alimentation artificielle d'un silure africain*, Heterobranchus longifilis *(Teleostei : Clariidae). Incidence du mode d'alimentation et première estimation des besoins nutritionnels.* Thèse de Doctorat, Paris 6; 182 pp.

Kerdchuen N. & Legendre M. (1991) Influence de la fréquence et de la période de nourrissage sur la croissance et l'efficacité alimentaire d'un silure africain, *Heterobranchus longifilis* (Teleostei, Clariiddae). *Aquatic Living Resources* **4**, 241–248.

Legendre M. (1983) Examen préliminaire des potentialités d'un silure africain *Heterobranchus longifilis* (Valenciennes, (1840) pour l'aquaculture en milieu lagunaire. *Documents Scientifiques du Centre de Recherches Océanographiques* Abidjan **14**, 97–107.

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. (1991) *Potentialités aquacoles de Cichlidae* (Sarotherodon melanotheron, Tilapia guineensis) *et Clariidae* (Heterobranchus longifilis) *autochtones des lagunes ivoiriennes.* Thèse de Doctorat, Université Montpellier II, 83 pp. + annexes.

Legendre M. & Billard R. (1980) Cryopreservation of rainbow trout sperm by deep-freezing. *Reproduction, Nutrition, Développement* **20**, 1859–1868.

Legendre M., Teugels G.G., Cauty C. & Jalabert B. (1992) A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus*, *Heterobranchus longifilis* and their reciprocal hybrids (Pisces, Clariidae). *Journal of Fish Biology* **40**, 59–79.

Luquet P., Otémé Z.J. & Métomgo S.B. (1993) Elevage du silure *Heterobranchus longifilis* Val. en bassins sans renouvellement d'eau: effets de l'évolution de quelques paramètres chimiques de l'eau sur la croissance et la survie. *Journal Ivoirien d'Océanologie et de Limnologie* **2** (2), 43–53.

Luquet P., Otémé Z.J. & Cissé A. (1995) Mise en évidence de la croissance compensatrice chez *Heterobranchus longifilis*. *Aquatic Living Resources* **8** (4), 389–394.

Maisse G. (1994) Comparaison de l'effet cryoprotecteur de différents glucides sur le sperme de truite arc-en-ciel (*Oncorhynchus mykiss*). *Aquatic Living Resources* **7**, 217–219.

Maisse G., Pinson A. & Loir M. (1988) Caractérisation de l'aptitude à la congélation du sperme de truite arc-en-ciel (*Salmo gairdneri*) par des critères physico-chimiques. *Aquatic Living Resources* **1**, 45–51.

Martoja R. & Martoja M. (1967) *Initiation aux Techniques de l'Histologie Animale.* Ed. Masson et Cie, Paris, 345 pp.

Micha J.C. (1973) Etudes de populations piscicoles de l'Ubangui et tentatives de sélection et d'adaptation de quelques espèces à l'étant de pisciculture. *Centre Technique Forestier Tropical*, Paris, 110 pp.

Mounib M.S. (1978) Cryogenic preservation of fish and mammalian spermatozoa. *Journal of Reproduction and Fertility* **53**, 13–18.

Nunez Rodrigez J., Otémé Z.J. & Hem S. (1995) Comparative study of vitellogenesis in two African catfish species *Chrysichthys nigrodigitatus* (Claroteidae) and *Heterobranchus longifilis* (Clariidae). *Aquatic Living Resources* **8** (4), 291–296.

Otémé Z.J. & Gilles S. (1995) Elevage larvaire du silure africain *Heterobranchus longifilis*: evaluation quantitataive des besoins en prioes vivantes des larves. *Aquatic Living Resources* **8** (4), 351–354.

Redondo-Muller C., Cosson M.P., Cosson J. & Billard R. (1991) *In vitro* maturation of the potential for movement of carp spermatozoa. *Molecular Reproduction and Development* **29**, 259–270.

Steyn G.J. & Van Vuren J.H.J. (1987) The fertilizing capacity of cryopreserved sharptooth catfish (*Clarias gariepinus*) sperm. *Aquaculture* **63**, 187–193.

Steyn G.J., Van Vuren J.H.J., Schoonbee H.J. & Chao N.-H. (1985) Preliminary investigations on the cryopreservation of *Clarias gariepinus* (Clariidae : pisces) sperm. *Water S.A.* **11** (1), 15–18.

Suqet M., Omnes M.H. & Fauvel C. (1982) Rapid assessment of sperm quality in turbot *Scophthalmus maximus*. *Aquaculture* **101**, 177–185.

Van der Bank F.J. & Steyn G.J. (1992) The effect of cryopreservation and various cryodiluents on alozyme variation of glucose phosphate isomerase in the F1 progeny of African catfish (*Clarias gariepinus*). *Comparative Biochemistry and Physiology* **103B**, 641–643.

Van der Walt L.D., Van der Bank F.H. & Steyn G.J. (1993) The suitability of using cryopreservation of spermatozoa for the conservation of genetic diversity in African catfish (*Clarias gariepinus*). *Comparative Biochemistry and Physiology* **106A**, 313–318.