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SOME DATA ON GAMETES PRESERVATION AND ARTIFICIAL INSEMINATION IN TELEOST FISH

by

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R E S U M E

— La durée de conservation des gamètes de poissons est généralement brève lorsqu'elle est pratiquée à des températures supérieures à 0°C (quelques heures à quelques jours). A des températures légèrement inférieures à 0°C (-2 à -4°C) la fécondance du sperme se maintient pendant plusieurs semaines, alors que l'aptitude à la fécondation des ovules ne se conserve pas plus de quelques jours. — Les techniques de congélation du sperme ont été pratiquées avec succès chez quelques espèces de poisson marins, mais restent à améliorer chez les salmonidés.

Des méthodes d'insémination artificielle ont récemment été établies pour plusieurs espèces de poissons de mer (Bar, Dorade) et d'eau douce (Truite, Brochet). Le milieu dans lequel se déroule normalement la fécondation (eau douce ou eau de mer) ne constitue pas l'environnement optimum pour la survie des gamètes. Une solution saline : pH 9,0, 250 mosmols constitue le dilueur le plus approprié pour les salmonidés, alors que l'eau de mer diluée de moitié convient mieux pour l'insémination chez le Bar et la Dorade.

A B S T R A C T

— Gametes preservation is usually short (several hours to several days) at temperature above 0°C. At subzero temperature sperm fertilizing ability may be kept for several weeks while egg fecondability does not exceed one week. Techniques of sperm cryopreservation in liquid nitrogen are now established for some marine fish species but have to be improved for salmonids. —

Methods of artificial insemination were lately set up for various species of freshwater fish (Trout, Pike), and marine fish (seabass, seabream) Medium in which egg fertilization normaly occurs (freshwater or sea water) is not the best environment for gametes survival. Saline solution : pH 9.0, 250 mosmols, is the most appropriate diluent for salmonids. Diluted sea water (around 20 ‰ salinity and pH 8 to 9) is successfully used for artificial insemination in seabass and seabream.

MOTS CLES : Conservation des gamètes - Insémination artificielle - Téléostéens.

KEY WORDS : Gametes preservation - Artificial insemination - Teleosts.

INTRODUCTION

Control of reproduction in fish includes the possibility of manipulation or preservation of gametes and their optimum utilisation by artificial insemination Few works were carried out so far in this field in Teleosts and this paper summarizes some data in vue of setting up a standard methodology for studying gametes preservation and artificial insemination in cultivated fish.

I. GAMETES PRESERVATION

1.1. Parameters used to estimate the viability of gametes

- Sperm

a) Fertilizing ability

A standard procedure was established to estimate the fertilizing ability of sperm in salmonids (BILLARD and GILLET, 1975) : briefly batches of about 200 fresh eggs taken from a pool of several females are mixed with 10 ml of diluent (BILLARD, 1977a) and inseminated with the sperm to be tested ; final dilution of sperm varies from 1/100 (10^{-2}) to 1/10,000 (10^{-4}). Percentage of fertilization are estimated by the percentage of embryonated eggs at 100 degrees-days.

b) Motility

Teleost sperm is usually non motile in the male genital tract. Motility is induced after dilution either in water or saline (6-10 ‰ salinity) for fresh-water fish (JASPERS, 1972 ; BILLARD, 1978) or in sea water, pure or diluted (salinity from 10 to 35 ‰, see Fig. 10) for marine fish. Spermatozoa motility can be assessed by motility score (HOYLE et al., 1968 ; SANCHEZ-RODRIGUEZ, 1975 ; GUEST et al., 1976) or by duration of the initial score observed immediatly after dilution (CARPENTIER and BILLARD, 1978). In the species studied so far intensity and duration of motility do not exhibit a strong correlation with fertilizing ability (CARPENTIER and BILLARD, 1978) except that non motile sperm never fertilize egg.

c) Others parameters

Some others parameters have been used to estimate the viability of sperm, such as respiration, mineral content of the seminal plasma, especially Na/K ratio (HWANG and IDLER, 1969 ; CARPENTIER and BILLARD, 1978) and enzymatic activities (BRETON et al., 1974). All the methods for the estimation of the sperm viability should be carefully standardized. Many factors may interfere such as nature of the extender, rate of dilution, temperature, oxygen availability (for instance in seabass sperm motility is inhibited under CO_2 athmosphere).

- Eggs

a) Fecundability

The above described procedure for testing fertilizing ability in sperm may also be used for estimating the fecondability of eggs. In that case pooled fresh sperm is used to inseminate experimental eggs.

b) Other parameters

Some indirect parameters may be used to estimate eggs viability ; for instance the composition of eggs and coelomic fluid (SATIA et al. 1974) or protein content (folin) in the coelomic fluid which varies from 1 to more than 20 mg/ml (BRETON and BILLARD, unpublished). In addition, in the coelomic fluid, YOSHIDA et NOMURA (1972) have identified a factor which enhance sperm motility.

1.2. Sperm preservation

- In vivo

In most of the species living in temperate zone spermatogenesis is seasonal and all spermatozoa are usually formed before the reproductive season. Sperm is stored either in the testis or in the vas deferent until spawning. Recent data showed that sperm motility in seabass (BILLARD et al., 1977) as well as fertilizing ability in rainbow trout (CHEMAYEL, 1975) decreased during the period of spermiation leading to the conclusion that some phenomenon of ageing occur. Therefore this decrease in sperm quality in the course of spermiation shows that in vivo preservation is somewhat deficient and do not keep the sperm at its initial state.

It is of interest to know how long sperm could survive in the testis of dead fish in case of milt availability rely on fish caught by gillnet. Survival depends on the temperature but does not exceed few hours (BLAXTER, 1955 ; BOONPRAKOB and DHEBTARANON, 1974)

- In vitro

a) At temperature above 0°C.

Many works were carried out studying the in vitro preservation of sperm, especially in salmonids. At room temperature sperm viability is usually short : few hours (Fig. 1) or few days (WITHLER and MORLEY, 1968). It is slightly prolonged when storage temperature is dropped in salmonids (CARPENTIER, 1977) and in *Fundulus* (KUCHNOW and FOSTER, 1976), but the main factor seems to be the oxygen availability as noticed by TRUSCOTT et al. (1968) and confirmed by HOLTZ et al. (1976) and STOSS et al. (1978). ZELL (1978) has indicated that fertilizing ability of two days old sperm rises from 7 and 13 % to 78 and 79 % after incubation in a Tris-citrate diluent (pH 8.9). A similar phenomenon was reported by GINSBURG (1963) who observed a recovery of fertilizing ability of diluted sperm in Ringer or coelomic fluid one hour after dilution. Therefore motility and fertilizing ability of stored or diluted sperm may be recovered provided adequate treatments are performed.

b) At subzero temperature

Salmonid sperm has been successfully stored for several weeks at subzero temperature with the addition of diluent and cryoprotector such as DMSO or Ethylene glycol (5 or 10 %) ; this was done at around -4°C (TRUSCOTT et al., 1968 ; SANCHEZ-RODRIGUEZ and BILLARD, 1977) or under O_2 athmosphere at -2°C (STOSS et al. 1978).

c) In liquid nitrogen or dry ice

Sperm cryopreservation in liqui dnitrogen was reported to be successful in some marine fish species : herring (BLAXTER, 1953, 1955), cod (MOUNIB et al., 1968), plaice (PULLIN, 1972). Some work was also carried out in our laboratory on cryogenic preservation of turbot, seabream and seabass sperm. A diluent modified after MOUNIB et al. (1968) was used : NaCl 19.5 g/l, glycine 6.25 g/l, $MgSO_4 7H2O$ 0. 25 g/l, $CaCl_2 HCO$ 0.25 g/l buffer Tris-HCl 0.02M, pH 8.5. The optimum rate of dilution is one volume of sperm + 1 volume of diluent including the cryoprotector (DMSO) whichshould be added at the concentration of 10 % either for seabream or seabass (Fig. 2, 3). Glycerol is also an effective cryoprotector at a concentration of 17 % v/v. Optimal results are obtained when the rate of temperature decrease is 10°C/mn (Fig. 2, 3). At the end of the spawning period when sperm quality is weak (BILLARD et al., 1977) an improved diluent should be used ; good results were obtained after the incorporation of protein (BSA, 10 mg/ml) to the diluent, or the use of a cell culture medium : Eagle or INRA-MENEZO B2 (MAUVIOT and BILLARD, unpublished). General procedure for deep freezing is summarized in Fig. 4. After sampling, sperm is stored in a refrigerator around 0°C during 15 mn ; diluent + cryoprotector kept also at 0°C are added to the sperm and the mixture is put in straws which are immediatly transfered into nitrogen vapor , above the liquid nitrogen level, at such a distance that the cooling rate of 10°C/mn is obtained. On the contrary no equilibration time is allowed as spermatozoa become motile after dilution. Therefore they should be frozen as quick as possible to avoid exhaution. When equilibration exceed 2 mn, fertilizing ability drops (Fig. 5). The freezing rate of 10°C/mn should be kept until temperature of the straws reaches -80°C ; at this temperature straws can be dropped directly into liquid nitrogen (Fig. 6). In conclusion, sperm cryopreservation has been acheived in most of the marine fish studied although limited success were reported in grey mullet by CHAO et al. (1975).

In salmonids controversial results have been reported. OTT and HORTON (1971a, b) claimed they succeeded in salmonid sperm cryopreservation although they had a limited success in Steelhead trout (GRAYBILL and HORTON, 1969). TRUSCOTT and IDLER (1969) in the atlantic salmon also had a limited success. STEIN (1978) obtained good results in rainbow and brown trout and Hucho. STOSS et al. (1978) reported variable results (from 2 to 80 % hatching). Similar results were observed in our laboratory (table 1) ; good fertilization ability is observed in some males and none in others. It seems that individual variations in sperm qua l ity may explain the limited success reported in the literature.

In other freshwater fish successful sperm cryopreservation was reported in pike (de MONTALEMBERT et al., 1978 ; STEIN, 1978) channel catfish (GUEST et al. 1976). Cyprinid sperm has been frozen successfully (MOCZARSKI, 1976,1977) ; however in carp STEIN (1978) reported recovery of motility but not of fertilizing ability and SIN (1974) had very limited success in silver carp and bighead. In conclusion, attempts of sperm cryopreservation have been more successfull in marine fish than in freshwater fish.

1.3. Egg preservation

- In vivo

After ovulation egg survival in the ovaries or in the body cavity varies according to the species. In rainbow trout eggs left in the body cavity after ovulation may keep their fecondability for 5 to 7 days (SAKAI et al., 1975) and 30 days in some cases (ESCAFFRE et al., 1977). In pike eggs fecondability in the ovarian cavity remains at its initial level for 2 days (de MONTALEMBERT et al. 1978).

- <u>In vitro</u>

a) At temperature above 0°C. In salmonids egg fecondability may be kept in the refrigerator for several days after stripping (BARRETT, 1951 ; POON and JOHSON, 1970 ; TAKANO et al., 1973). Similarly herring eggs have been stored at 4°C for a period up to eight days (BLAXTER, 1955).

b) At 0°C eggs fertility was maintained during at least 7 days for brown trout and 3 days for rainbow trout (CARPENTIER, 1977).

c) At temperature below 0°C research is in a preliminary stage. WHITTINGHAM and ROSENTHAL (1978) showed that herring embryos can withstand short exposure to -10°C. ZELL (1978) has reported successfull short term storage of trout egg at-20°C and eggs and embryos at -55°C.

2. ARTIFICIAL INSEMINATION

2.1. Salmonids and freshwater fish

Artificial insemination is commonly used in salmonid culture but the technique is not very efficient since sperm of one male is used to fertilize eggs from only 3 to 4 females. NURSALL and HASLER (1952) tryed to improve the "dry method" and showed that one male can fertilize no more than 20 females. This results from the use of water as an extender for fertilization. In water survival is very short either for spermatozoa or eggs (SMIRNOV, 1963 ; BUSS and CORL, 1966 ; BILLARD and JALABERT, 1974) which present profound ultrastructural modifications (BILLARD and BRETON, 1970 ; SZÖLLÖSI and BILLARD, 1975). ELLIS and JONES (1939) demonstrated the superiority of saline solution and coelomic fluid keeping sperm motility. NOMURA (1964) and BILLARD et al. (1974) used an isotonic salt solution as an extender for artificial insemination. The percentage of fertilized eggs was increased and it was shown that osmotic pressure of 250 mosmols and pH 9.0 were the most important factors (PETIT et al., 1973) (Fig. 7, 8). Deleterious effects of low pH has been shown by INABA et al. (1958) who also indicated that broken eggs decreased pH. Use of a diluent prevents the harmful effects of pollution by vitellus (Fig. 9), and rises the percentage of fertilization (Fig. 10). To set up a reliable technique of artificial insemination several other parameters had to be fixed ; buffer (BILLARD et al. 1974b) optimum gametes/ diluent ratio (BILLARD, 1975). It has also been shown that mixing eggs from several females or sperm from several males does not affect the percentage of fertilization (BILLARD, 1977b). The procedure for artificial insemination in salmonids is summarized in fig 11. In conclusion, a satisfactory technique of artificial insemination using a diluent is now available for salmonids ; in comparison with the draditional technique, dry or ultradry, it gives a higher percentage of fertilization and require only minute quantities of sperm allowing a reduction of male population in hatchery. However, from a genetic point of view, this population should be kept above a minimum level that is : $\frac{1}{N} = \frac{1}{N♂} \cdot \frac{1}{N♀}$ (N = total number of effective males and females). A similar technique is used for pike (BILLARD et al., 1976). For the other cultivated freshwater spercies, the way by which eggs fertilization is carried out remains primitive ; it consist simply of mixing eggs and sperm and adding water. Some improvement are due to WOYNAROVICH (1962) who used a mixture of 4 ‰ Nacl + 3 ‰ urea for removing the sticky layer of eggs in cyprinids. This solution also prolongs the fertilizing ability of sperm.

2.2. Marine fish

The so called artificial insemination in marine fish is practised in the same way as for freshwater fish. Stripped eggs are mixed with sperm and various amounts of sea water are added afterward. BOONPRAKOB and DHEBTARANON (1974) employed wet and dry method with equal success in Indo-Pacific mackerel. Some work carried out in the laboratory on seabass showed that optimum salinity for the diluent (based on motility of spermatozoa) is around 20 ‰. Optimum pH is 8 to 9 (fig. 12). The diluent used for cryogenic preservation (see § 1.2 c) was found to be satisfactory for artificial insemination in the following conditions : 10 ml diluent + 2000 eggs + 10 µl sperm. In conclusion, controlled reproduction of cultivated fish includes artificial insemination but this part has been somewhat neglected so far and need to be improved in most of the species candidate for aquaculture.

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BIBLIOGRAPHY

BARRETT . -1951- Fertility of salmonid eggs and sperm after storage. J.Fish.Res.Board.Can., 8, p. 125-133.

BILLARD R. -1975- L'insémination artificielle de la truite Salmo gairdneri Richardson. V - Effets de la dilution et définition du rapport optimum gamètes/dilueur. Bull.Fr.Piscic., 257, p. 122-135.

BILLARD R. -1977a- Utilisation d'un système Tris-glycocolle pour tamponner le dilueur d'insémination pour truite. Bull.Fr.Piscic., 264, p. 103-112.

BILLARD R. -1977b- A new technique of artificial insemination for salmonids using a sperm diluent. Fisheries, 2, p. 24-25.

BILLARD R. -1978- Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in medium of various salinities. Aquaculture, in press

BILLARD R., BRETON B. -1970- Modifications ultrastructurales et cytochimiques des spermatozoïdes après dilution chez les poissons d'eau douce. VIIè Congr.Int.Microsc.Electr., Grenoble, 637-638.

BILLARD R., JALABERT B. -1974- L'insémination artificielle de la truite Salmo gairdneri Richardson. II - Comparaison des effets de différents dilueurs sur la conservation de la fertilité des gamètes avant et après insémination. Ann.Biol.anim.Bioch.Biophys., 14, p. 6O1-6IO.

BILLARD R., PETIT J., JALABERT B., SZÖLLÖSI D. -1974a- Artificial insemination in trout using a sperm diluent. In J.H.S. BLAXTER, The early life history of fish, Springer Verlag, Berlin Heidelberg New York, p. 715-723.

BILLARD R., JALABERT B., BRETON B. -1974b- L'insémination artificielle de la Truite Salmo gairdneri Richardson. III - Définition de la nature et de la molarité du tampon à employer avec les dilueurs d'insémination et de conservation. Ann.Biol.anim.Bioch.Biophys., 14, p. 611-621.

BILLARD R., GILLET C. -1975- Effets de la température sur la fécondation et la survie des gamètes chez la truite ARc-en-ciel. Bull.Fr.Piscic., 259, P. 53-65.

BILLARD R., DEBRUILLE M., GERARD J.P., de MONTALEMBERT G. -1976- L'insémination artificielle du brochet. Bull.Fr.Piscic., 262, p. 30-34.

BILLARD R., DUPONT J., BARNABE 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, p. 363-367.

BLAXTER J.H.S. -1953- Sperm storage and cross-fertilization of spring and autumn spawning herring. Nature, 172, p. II89-II90.

BLAXTER J.H.S. -1955- Herring rearing. I - The storage of herring gametes. Marine Res.Scot., Home Dept. n° 3, 12 pp.

BOONPRAKOB U., DHEBTARANON Y. -1974- Experiments on artificial fertilisation and descriptive studies of eggs and larvae of the indo-pacific mackerel, Rastrelliger neglectus (Van Kampen). IPFS Procs, 15 (111), p. 144-158.

BRETON B., MENEZO Y., BILLARD R. -1974- Mise en évidence de quelques enzymes dans le sperme de la carpe (Cyprinus carpio L.) et de la truite (Salmo gairdneri R.) et dans le liquide coelomique de la truite. C.R.Acad.Sci. série D, Paris, 278, p. 1285-1288.

BUSS K., CORL K.G. -1966- The viability of trout germ cells immersed in water. Progr.Fish.Cult., 28, p. 142-145.

CARPENTIER P. -1977- Essais de conservation, de transport des gamètes et des oeufs fécondés et étude des conditions optimales de résorption vésiculaire chez les salmonidés.DEA, Univ. Paris VI, 55 p.

CARPENTIER P., BILLARD R. -1978- Conservation à court terme des gamètes de salmonidés à des températures voisines de 0°C. Ann.Biol.anim.Bioch.Biophys. 18, in press.

CHAO N.H., CHEN H.P., LIAO I.C. -1975- Study on cryogenic preservation ofgrey mullet sperm. Aquaculture, 5, p. 389-406.

CHEMAYEL M.-1975- Etude de la variabilité du pouvoir fécondant du sperme en relation avec ses caractéristiques chez la Truite Arc-en-ciel (Salmo gairdneri Richardson). Thèse 3è Cycle, Univ. Paris VI, 56 p., 52 fig.

ELLIS W.G., JONES J.W. -1939- The activity of the spermatozoa of Salmo salar in relation to osmotic pressure. J.Exp.Biol., 16, p. 530-534.

ESCAFFRE A.M., PETIT J., BILLARD R. -1977- Evolution de la quantité d'ovules récoltés et conservation de leur aptitude à être fécondés au cours de la période post-ovulatoire chez la Truite Arc-en-ciel. Bull.Fr.Piscic., 265,p.135-142

GINSBURG A.S. -1963- Sperm egg association and its relationship to the activation of the egg in salmonid fishes. J.Embryol.exp.Morph., 11, p. 13-33

GRAYBILL J.R., HORTON H.F. -1969- Limited fertilization of steelhead trout eggs with cryo-preserved sperm. J.Fish.Res.Board.Can., 26, p. 1400-1404.

GUEST W.C., AVAULT J.W., ROUSSEL J.D. -1976- Preservation of channel catfish sperm. Trans.Amer.Fish.Soc., 105, p. 469-474.

HOLTZ W., BÜYÜKHATIPOGLY S., STOSS J., OLDIGS B., LANGHOLZ H.J. -1976- Preservation of trout spermatozoa for varying periods. FOA Tech.Conf. Aquacult., Kyoto, 3 p.

HOYLE R.J., TRUSCOTT B., IDLER D.R. -1968- Studies on freezing sperm of atlantic salmon (Salmo salar). Fish.Res.Board.Can., Tech.Rep. n° 93, 42 p.

HWANG P.C., IDLER D.R. -1969-. A study of major cations, osmotic pressure and pH in seminal components of atlantic salmon. J.Fish.Res.Board.Can., 26, p. 413-419.

INABA D., NOMURA M., SUYAMA M. -1958- Studies on the improvement of artificial propagation in trout culture. II - On the pH values of eggs, milt, coelomic fluid and others. Bull.Jap.Soc.Sci.Fish., 23, p. 762-765.

JASPERS E. -1972- Some spermatological aspects of channel catfish, Ictalurus punctatus (Rafinesque).

KUCHNOW K.P., FOSTER R.S. -1976- Thermal tolerance of stored Fundulus heteroclitus gametes ; fertilizability and survival of embryos. J.Fish.Res.Board.Can. 33,p. 676-680. Ph.D. Thesis, Louisiana State University, 99 p.

MOCZARSKI M. -1976- Cryobiological factors in grass carp sperm preservation. VIIIème Congr. of An.Reprod.artif. Ins. Krakov.

MOCZARSKI M. -1977- Deep freezing of carp Cyprinus carpio L. sperm. Bull.Acad.Pol.Sci., Ser.Sci.Biol., 25, p. 187-189.

MOUNIB M.S., HWANG P.C., IDLER D.R. -1968- Cryogenic preservation of Atlantic cod (Gadus morrhua) sperm. J.Fish.Res.Board.Can., 25, p. 2623-2632.

NURSALL J.R., HASLER A.D., -1952- The viability of gametes and the fertilization of eggs by minutes quantities of sperm. Progr.Fish.Cult., 14, p. 165-168.

NOMURA M. -1964- Studies on reproduction of rainbow trout, Salmo gairdneri with special reference to egg takens. VI - The activities of spermatozoa in different diluents, and preservation of semen. Bull.Jap.Soc.Sci.Fish, 30, p. 723-733.

OTT A.G., HORTON H.F. -1971a-. Fertilization of chinook and coho salmon eggs with cryopreserved sperm. J.Fish.REs.Board.Can., 28, p. 745-748.

OTT A.G., HORTON H.F. -1971b- Fertilization of steelhead trout (Salmo gairdneri) eggs with cryo-preserved sperm. J.Fish.Res.Board.Can., 28, p. 1915-1918.

PETIT J., JALABERT B., CHEVASSUS B., BILLARD R. -1973- L'insémination artificielle de la truite (Salmo gairdneri Richardson). I - Effets du taux de dilution, du pH et de la pression osmotique du dilueur sur la fécondation. Ann.Hydrobiol., 4, p. 201-210.

POON D.C., JOHNSON A.K. -1970- The effect of delayed fertilization on transported salmon eggs. Prog.Fish Cult., 32, p. 81-84.

PULLIN R.S.V. -1972- The storage of plaice (Pleuronectes platessa) sperm at low temperatures. Aquaculture, 1, p. 279-283.

SAKAI K., NOMURA M., TAKASHIMA F., OTO H. -1975- The over-ripening phenomenon of rainbow trout. II - Changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. Bull.Jap.Soc.Sci.Fish., 41, p. 855-860.

SANCHEZ-RODRIGUEZ M. -1975- Contribution à l'étude de l'insémination artificielle de la truite (Salmo gairdneri) : les possibilités de manipulation des gamètes et de conservation du sperme.DEA, Univ. Paris VI, 21 p.

SANCHEZ-RODRIGUEZ M., BILLARD R. -1977- Conservation de la motilité et du pouvoir fécondant du sperme de truite Arc-en-ciel maintenu à des températures voisines de 0°C. Bull.Fr.Piscic., 265, p. 144-152.

SATIA B.P., DONALDSON L.R., SMITH L.S., NIGHTINGALE J.N. -1974- Composition of ovarian fluid and eggs of the University of Washington strain of rainbow trout (Salmo gairdneri). J.Fish.Res.Board.Can., 31, p. 1796-1799.

SIN A.W. -1974- Preliminary results on cryogenic preservation of sperm of silver carp (Hypophthalmichthys molitrix) and bighead (Aristichthys nobilis). Hong Kong Fish. Bull., 4, P. 33-36.

SMIRNOV A.I. -1963- Conservation dans l'eau de l'aptitude des oeufs et des spermatozoïdes du saumon Oncorhynchus gorbuscha (W.) à la fécondation (En Russe). Nauchn.Dokl.vyssh.Shk.Biol.Nauki, USSR, 3, P. 37-41.

STEIN H. -1978- Cryopreservation of the sperm of some freshwater teleosts. Ann.Biol.anim.Bioch.Biophys., 18, in press

STOSS J., BÜYÜKHATIPOGLU S., HOLTZ W. -1978- Short term and cryopreservation of rainbow trout (Salmo gairdneri Richardson) sperm. Ann.Biol.anim. Bioch.Biophys., 18, in press.

SZÖLLÖSI D., BILLARD R. -1975- The micropyle of trout eggs and its reactions to different incubation media. J.Microscopie, 21, p. 55-62.

TAKANO K., HIROI O., YASUKAWA M., SHETAKE T. -1973- STudies on the retention of gametes of salmonids fishes. I - On the fertility of chum salmon eggs after storage. Sci.Rep.Hokkaido Salmon Hatchery, 27, p. 31-37.

TRUSCOTT B., IDLER D.R., HOYLE R.J., FREEMAN H.C. -1968- Sub-zero preservation of atlantic salmon sperm. J.Fish.Res.Board.Can., 25, p. 363-372.

TRUSCOTT B., IDLER D.R. -1969- An improved exender for freezing atlantic salmon spermatozoa. J.Fish.Res.Board.Can., 26, p. 3254-3258.

WHITTINGHAM D.G., ROSENTHAL H. -1978- Attemps to preserve herring embryos at subzero temperatures. Helgoländer wiss Meeresuntersuchungen, 1, in press.

WITHLER F.C., MORLEY R.B. -1968- Effects of chilled storage on viability of stored ova and sperm of sokeye and pink salmon. J.Fish.Res.Board.Can., 25, p. 2695-2699.

WOYNAROVISH E. -1962- Hatching of carp eggs in "Zuger" glasses and breeding of carp larvae until an age of 10 days. Bamidgeh, 14, p. 38-42.

YOSHIDA T., NOMURA M. -1972- A substance enhancing sperm motility in the ovarian fluid of rainbow trout. Bull.Jap.Soc.Sci.Fish., 38, p. 1073

ZELL S.R. -1978- Cryopreservation of gametes and embryos of salmonid fishes. Ann.Biol.anim.Bioch.Biophys., 18, in press

Table I : Fertilizing ability of rainbow trout sperm stored during 26 days in liquid nitrogen. Extender MENEZO-INRA B_2 1 vol.-sperm 1 vol. Cryoprotector : DMSO 10 %. Rate of temperature decrease 5°C/mn. Control refers to egg fertility (insemination with fresh sperm). Frozen sperm were thawed in various diluents (1) buffered in Carbonate-bicarbonate, (2) buffered with Tris- Glycine (BILLARD, 1977).

Male n°	Treatment	Diluent for thawing	Percentage of fertilization
I	Cryopreserved	coelomic fluid	77.6
		DIA Carb-bic (1)	73.1
		DIA T_G (2)	78.8
2	Cryopreserved	DIA T_G	0
3	Control	DIA T_G	84.5

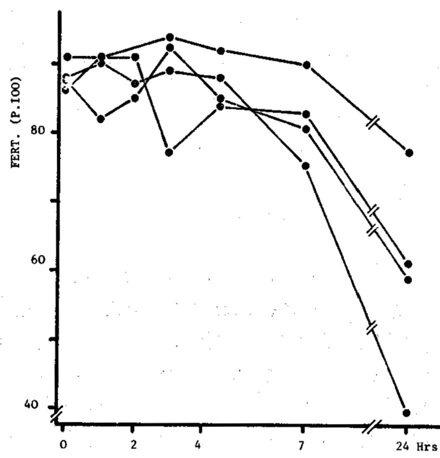

Figure 1 : Change in fertilizing ability of trout sperm stored at 10°C. Aliquots were taken at various intervals to inseminate batches of freshly collected eggs from 4 females over a period of 24 h (BALIK and BILLARD, unpublished).

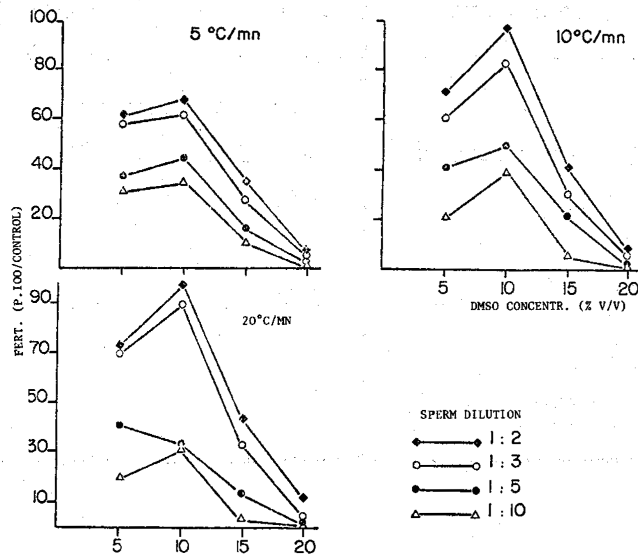

Figure 2 : Seabass sperm cryopreservation in liquid nitrogen : definition of DMSO concentration, sperm dilution and rate of temperature decrease.

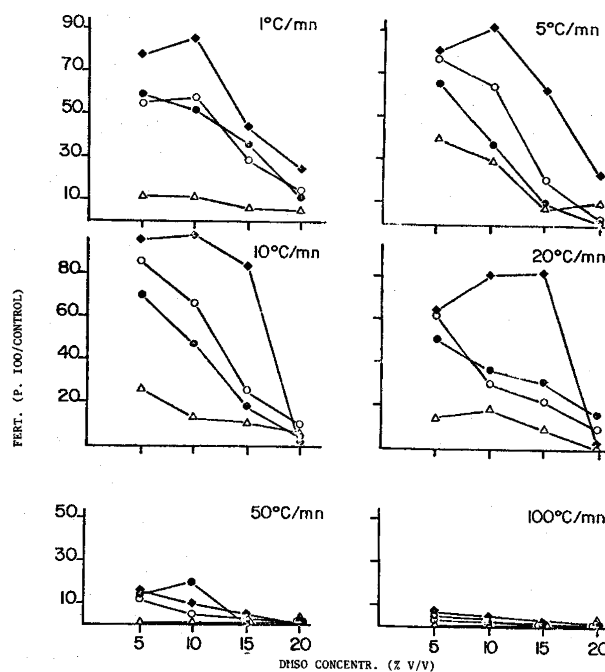

Figure 3 : Seabream sperm cryopreservation in liquid nitrogen : definition of DMSO concentration sperm dilution and rate of temperature decrease.

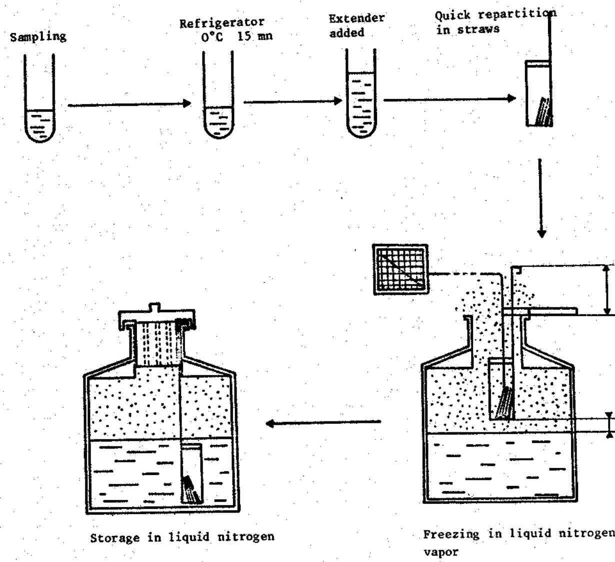

Figure 4 : General procedure for sperm cryopreservation in liquid nitrogen. If possible it is better to put the straws in a horizontal position during freezing.

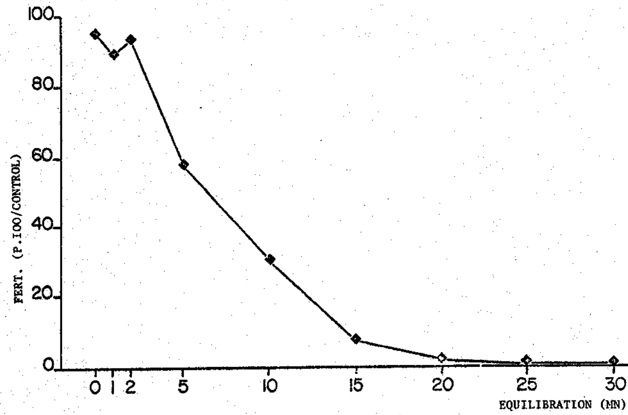

Figure 5 : Effects of the duration of equilibration (time elapsed between sperm dilution and freezing seabream).

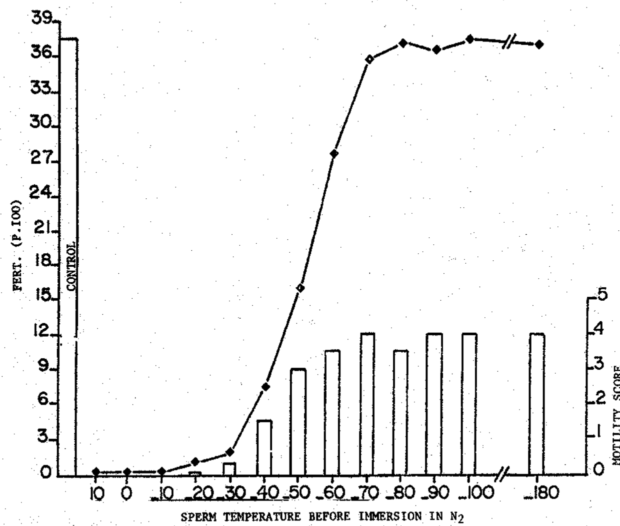

Figure 6 : Definition of the temperature at which straws can be dropped into liquid nitrogen.

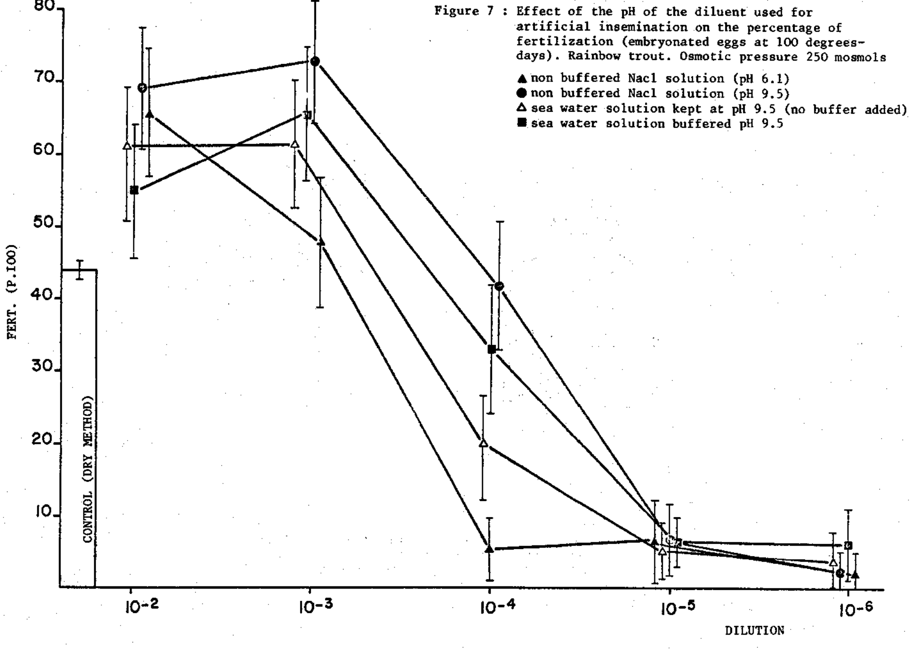

Figure 7 : Effect of the pH of the diluent used for artificial insemination on the percentage of fertilization (embryonated eggs at 100 degrees-days). Rainbow trout. Osmotic pressure 250 mosmols

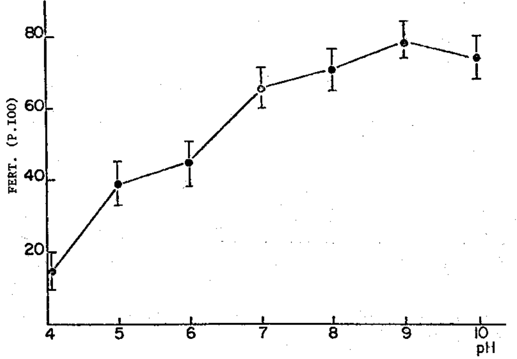

Figure 8 : Effect of the pH of the diluent on the percentage of fertilization on rainbow trout (osmotic pressure 250 mosmols).

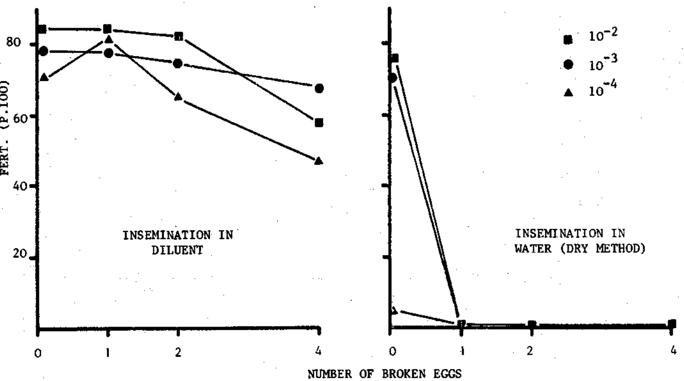

Figure 9 : Favourable effect of diluent on the percentage of fertilization as compared with water for insemination of brown trout eggs polluted by vitellus from broken eggs (number of broken eggs by batches of about 200 eggs)

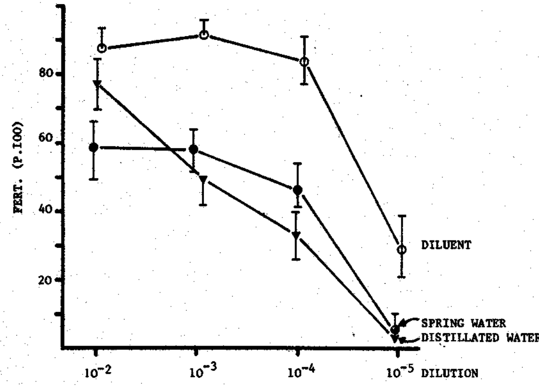

Figure 10 : Effects of various medium used for artificial insemination of rainbow trout eggs

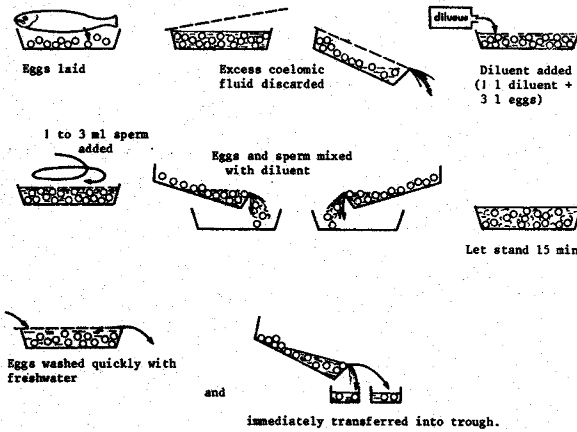

Figure 11 : Method of artificial insemination for salmonid

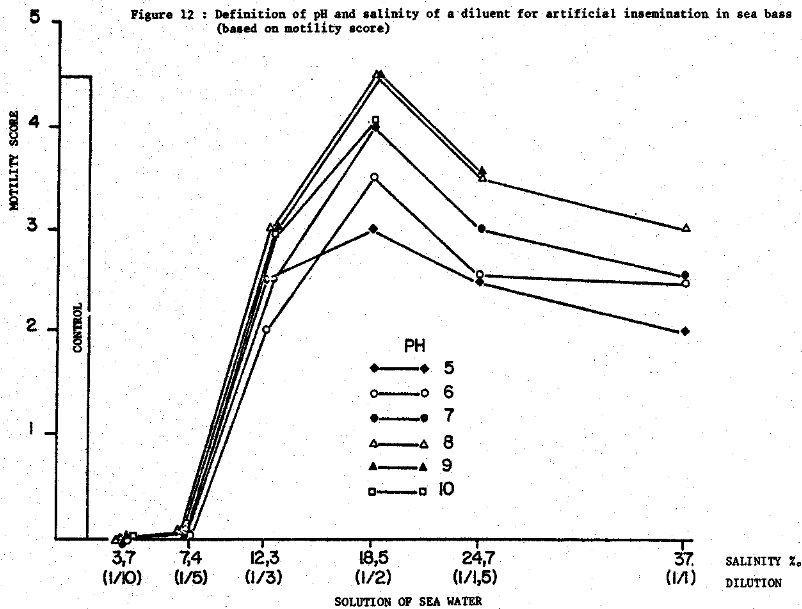

Figure 12 : Definition of pH and salinity of a diluent for artificial insemination in sea bass (based on motility score)