A new technique of artificial insemination for salmonids using a sperm diluent

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A NEW TECHNIQUE OF ARTIFICIAL INSEMINATION FOR SALMONIDS USING A SPERM DILUENT

R. Billard

Artificial insemination has been practised for a long time in salmonids. Some studies to improve the technique were carried out by Nomura (1964) in Japan and Ginsburg (1968) in Russia. While the Russian workers suggested using a dry method, the Japanese employed an extender.

A French diluent has been recently produced for use on rainbow trout in fish farms. It was first shown that in the diluent made with NaCl, osmotic pressure and pH (Fig. 1) were the most important parameters, especially when the dilution rate was high (Petit et al. 1973). Other parameters were also studied, such

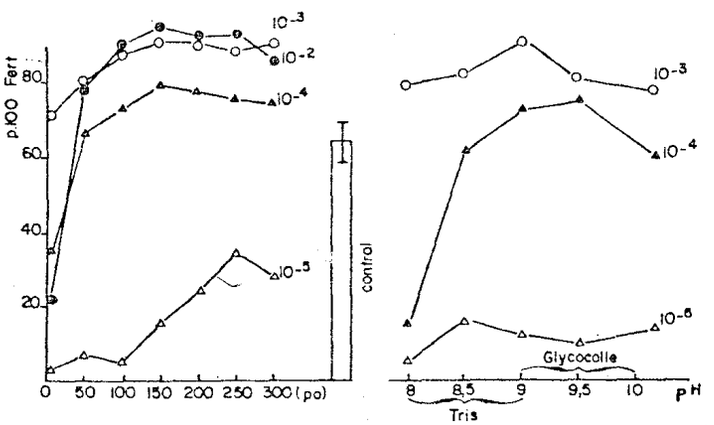

Figure 1. Effect of diluent osmotic pressure (left) and pH (right) on fertilization rate measured after 10-day incubation at 10 C. Buffers used were Tris-HCl and glycine. Batches of 200 eggs were inseminated in 10 ml diluent with varying amounts of sperm: 10 μl (10^{-3}), 1 μl (10^{-4}), 0.1 μl (10^{-5}).

as the type of buffer to be used and the optimum ratio of spermatozoa to eggs (Billard et al. 1975). Fertilization rate was generally higher when this diluent was used than with the dry method, probably because sperm survival was slightly prolonged after dilution (Fig. 2). This permits the eggs to be fertilized over a longer period, considering that ova may be fertilized 30 sec after mixing with the sperm (Fig. 3). This is much longer than the duration of sperm survival in water. The diluent also prevents precipitation of vitellus and inhibition of sperm motility occurring when water is mixed with ova polluted by vitellus from broken eggs, a phenomenon mentioned by Leitritz (1962). This makes a large difference in fertilization rate (Fig. 4).

Some other parameters, such as temperature, must also be considered during insemination (Fig. 5). In this experiment carried out in January, the end of the reproductive season, 4 to 10 C is the optimum temperature, but in other experiments at the beginning of the reproductive season high fertilization rates

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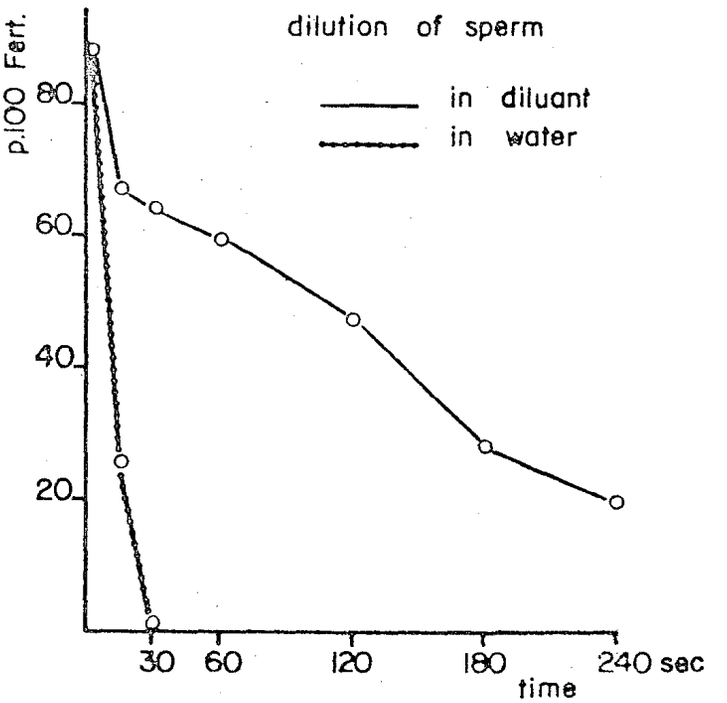

Figure 2. Sperm survival after dilution in extender DIA-; (Billard 1976) and water. Sperm is diluted (10^{-3}) and eggs are then inseminated at varying times after dilution.

are recorded also at 15 C. This is probably due to sperm aging, which seems to occur in fish (Billard and Breton 1976). The optimum ova/diluent ratio has been determined (Billard 1975); it varies from 10 to 20 ova/ml extender. Mixing eggs from

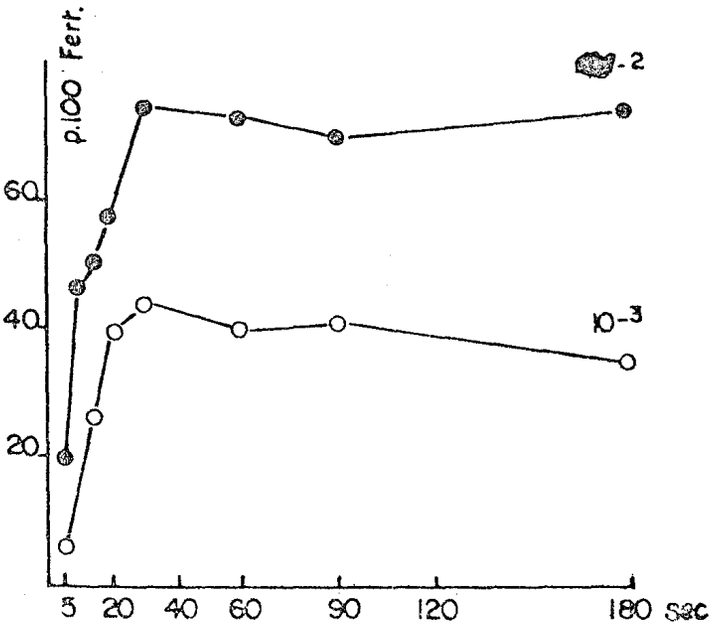

Figure 3. Determination of fertilization time after insemination. At varying times after insemination the medium containing eggs and sperm is poured into a 200-liter aquarium of fresh water; high dilution immediately inhibits sperm motility.

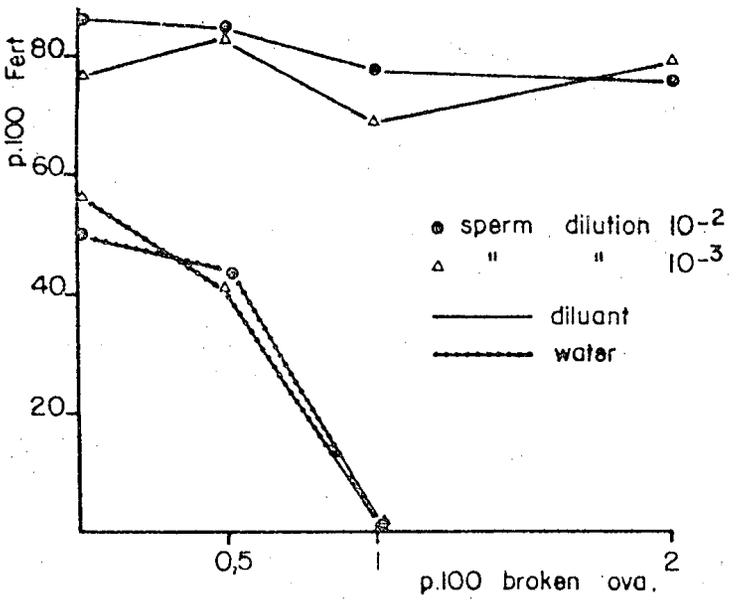

Figure 4. Favorable effect of diluent on fertilization rate as compared with water for insemination of batches of ova polluted by vitellus (broker ova).

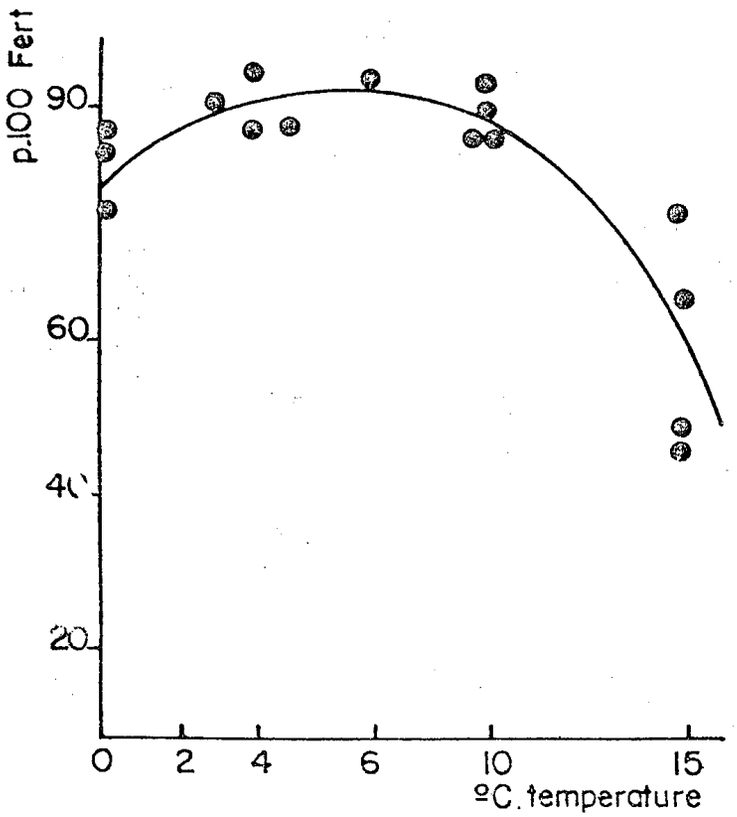

Figure 5. Effect of gamete temperature at insemination time on fertilization rate (incubation at 10 C for 10 days as in Figure 1).

several females does not change their fertilizing ability; the same is true for males (Fig. 6). The procedure for artificial insemination is summarized in Figure 7.

Eggs should be carefully collected, avoiding shock. Coelomic fluid varying in quality from one female to another (Dorier 1969) and not having the right pH (pH 8.0, Inaba et al. 1958) should be discarded and replaced by diluent (1 liter of diluent for 2 liters of ova). Sperm is then added: all ova are usually fertilized with a ratio of 300,000 spermatozoa·egg: i.e., 1 ml of sperm (10^{-3}) to 1 liter diluent + 2 liters of eggs. Spermatozoa sperm concentration varies from 6 to 25 · 10^9 spermatozoa/ml (Billard et al. 1971). Sperm and ova should be mixed immediately, simply by

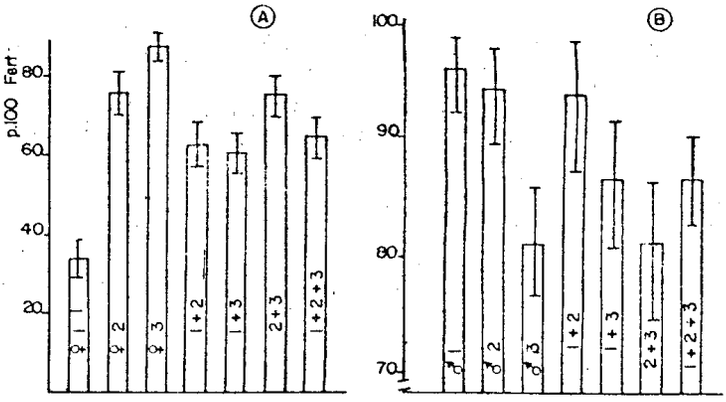

Figure 6. Effects of mixing eggs from several females (A) and sperm from several males (B) on the percentage of fertilization.

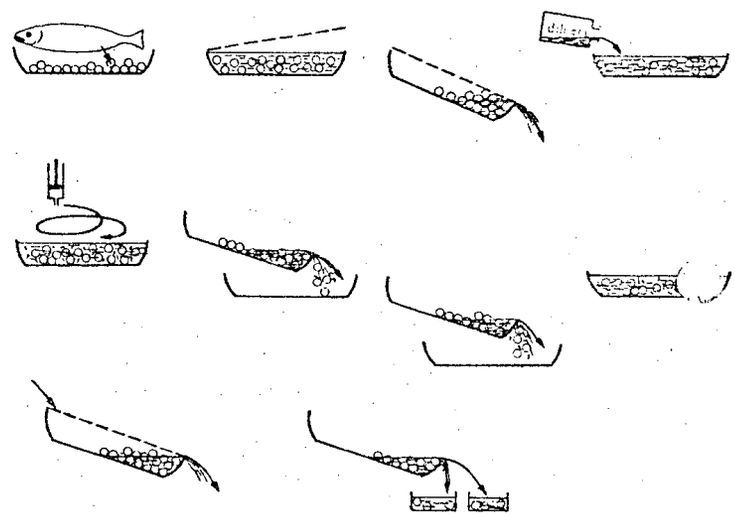

Figure 7. Summary of process of artificial insemination.

pouring sperm, ova, and diluent 2 or 3 times into another container. The mixture should sit for 15 minutes; after quick washing with freshwater, eggs are then poured into a trough.

This technique gives a higher fertilization rate than the usual dry method and requires only a minute quantity of sperm. Sire populations in hatcheries can be reduced because a few males can fertilize one hundred females. The diluent is now patented and produced by Lathevet, 23 rue des Fossés St. Jacques, Paris 75005.

BIBLIOGRAPHY

Billard, R., J. Petit, B. Jalabert, D. Szöllösi. 1974. OBAN. Pages 715–723 *in* Symposium on the early life history of fish, Blaxter, ed.

Billard, R. 1975. Bull. Franç. Pisc. 257:121–135.

Billard, R., B. Breton. 1976. 2e Congrès des Ichthyol. Europ., Paris, sous presse.

Billard, R. 1976. Ann. Hydrobiol., sous presse.

Dorier, A. 1949. Trav. Lab. Hydrobiol. Pisc. Univ. Grenoble 41:69–73.

Ginsburg, A. S. 1968. VIe Congr. Int. Reprod. anim. Insem. artif., Paris, II:1037–1040.

Inaba, D., M. Nomura, M. Suyuma. 1958. Bull. Jap. Soc. Sci. Fish. 23:762–765.

Leitritz, E. 1962. Fish. Bull. 107. 169 pp.

Nomura, M. 1964. Bull. Jap. Soc. Sci. Fish. 30:723–733.

Petit, J., B. Jalabert, B. Chevassus, R. Billard. 1973. Ann. Hydrobiol. 4:201–210.