# STUDY OF MILT IN PUYE *GALAXIAS MACULATUS* (Jenyns, 1848)(SALMONIFORMES: GALAXIDAE)

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## ABSTRACT

The sperm density, motility and fertility of milt in "puye" or "angula" (*Galaxias maculatus*, landlocked form) were studied. For this, we used adult specimen taken from Southern Chile´s water and kept in captivity for aproximately two years. Once they were mature, twenty specimens (7.33 $\pm$ 0.61cm average total longitude and 1.37 $\pm$ 0.27g average total weight) were anaesthetized and their milt was extracted by stripping. A visual test, sperm recount, motility and fertility evaluation was done to it. The results showed that the puye´s milt is not abundant, it is whitish, and has a great viscocity. An average of 61.68 x $10^9$ sperm/ml and a motility graded 1, on a 0 to 5 scale (elaborated by Sanchez-Rodríguez & Billard, 1977) in a period of up to 24 hours after it got in contact with freshwater. The fertility average obtained was 92.56 $\pm$ 9.25%. The correlation between fertilization rate with sperm density was 0.177.

Key words: fish, reproduction, milt, sperm, motility, fertility.

## INTRODUCTION

The *Galaxias maculatus*, commonly known as "puye or angula", is a small native fish whose crystalline juvenile state (whitebait) is intensely exploited in order to satisfy the national and international market´s demand. This has put the species in danger of being extinct and its natural population has collapsed. These recordings show the need of preserving this species in our waters. An alternative is trying to cultivate them for commercial and restocking purposes. To do this, it is fundamental to know and manage diverse stages of this species´ life cycle, feeding habits, cultivation conditions, technostructures, and handle in an adecquate way the reproductive processes like spawn, fecundation, and artifitial incubation.

Some aspects of this species have been studied in Chile. Amongst these we find: the life cycle, karyology and migrations (Campos 1970, 1972, 1973), the ovary microstructure and seasonal gonadal activity (Peredo and Sobarzo 1993, 1994), its adaptation to salinity (Vega et al., 1993) and the artificial feeding of larvae (Dantagnan et al., 1995). There are not records on the biology of the sperm of this specie (and of other fish with commercial value). The only research papers on this topic were made by Pérez (1978) and Almendras (1993) who studied the rainbow trout´s milt and by Valdebenito et al (1995) who studied the quantitative and cualitative aspects of puye's milt without evaluating its fertility. For this reason, this research´s aim is to determine the fertility of *G. maculacus* sperm diluted in freshwater.

## MATERIALS AND METHODS

On September 1993, 500 specimens of wild *G. maculatus* adults were captured in the Cautín river near Temuco city, IX region, Chile. The conditioning was done in 100 l capacity, circular, open flow tanks and fed with trout comercial pellets which had to have a lipid adaptation done to them. Once they reached their second sexual maturity (September 1994) a sample of twenty male adults of a total longitude average of 7.33 $\pm$ 0.61cm and 1.37 $\pm$ 0.27g average weight, was taken. They were selected because of their abundant milt produccion. They were then anaesthetized with MS-222 and the maximum milt was extractes by stripping.

Different amounts of the sample were used to determine the sperm density (number of spermatozoa per ml, determined by a recount in Neubauer´s camera), the sperm motility in freshwater (according to Sanchez-Rodríguez and Billard, 1977 in scale of 0 to 5)(Table 1) and fertility (percentage of embrions observed after 5 hours of incubation). This last parameter was evaluated in a pool of eggs extracted by stripping 18 adult females (total longitude of 7.99 $\pm$ 0.31cm and 2.22 $\pm$ 0.20g of total weight) taken from the same tank as the males. Each male´s milt (aprox. 0.01 ml) was diluted in a petri dish containing 5 ml of freshwater, after that, a sample of aproximately 500 eggs taken from the same pool of eggs, was added to the petri dish. To facilitate the fertilization process, the mixture was stirred for aproximately 30 seconds, then, it was left to settle. After 5hs of incubation the percentage of fecundation was determined under a a stereomicroscope taking a sample of 50 embryos aproximately. The embryos which had 2 or more cells were considered fertilized . The incubation temperature was 12 $\pm$ 1°C.

## RESULTS

*G. Maculatus* milt was whitish and thick, frequently the small volume extracted adhered to its skin, making its extraction and handling more difficult. For this reason, milt was diluted in river water immediately after its extraction.

The quantitative analysis showed an average sperm density of 61.68 $\pm$ 10.3 x $10^9$ sperm/ml (maximum 88.8 x $10^9$ and a minimum of 50.3 x $10^9$ sperm/ml). Motility observed in all the individuals was low and only a small vibration which, according to the Sanchez-Rodríguez and Billard´s scale corrresponded to grade 1. This motility was prolonged for more than a day at an temperature of 10° C. Although it had a low motility, the spermatozoa was fertile, having an average fecundation percentage of 92.56 $\pm$ 9.25%, evaluating aproximately five hours after the fertilization. The sperm density and fertilization percentage ratio was determined as r = 0.177 (Fig 1).

## DISCUSSION

The low flagellar activity observed in all the specimens´ spermatozoa studied, is a characteristic which was already observed by Valdebenito et al., (1995). He attributes this to an unknown reproductive strategy of the species or to a condition produced by the stress of captivity, he tried to trigger off the flagellar activity diluting the semen in various solutions, reaching the highest activity rates in a carmabide solution. The low flagellar activity is a characteristic observed in other fish. Ginzburg (1972) reported that the Pacific Herring spermatozoa has a low motility, its beats are aproximately equal to the width of the spermatozoa´s head., an energetic movement starts when it is near the egg. Strüssmann et al., (1994) showed that sperm of "pejerrey" (*Odontesthes bonariensis*) remain only feeble vibrations after dilution with ovarias fluid and complete lack of movement has been reported in the fresh milt of *Alosa volgensis,* brook trout,

lake trout and sturgeons (Ginzburg, 1972). He does not report if it is a normal or a pathological characteristic in that species.

The sperm density of 61.68 x $10^9$ sperm/ml determined in this research is similar to the one given by Valdebenito et al., (1995). Both results are higher than those given for the rainbow trout (Pérez, 1978), coho salmon, (Bouck & Jacobson, 1976) and Atlantic salmon (Aas et al., 1991), also being a bit higher than the value determined by Grinzburg (1972) for *Perca fluviatilis*, a species that presents a sperm desity of 52 x $10^9$ sperm/ml. The high sperm density of the puye may be a biological adaptation to the water currents in which the species lives, Ginzburg (1972) said that species living in strong current waters need a greater number of spermatozoa to fertilize than the species in still water habitats.

Valdebenito et al., (1995), did not evaluate milt´s fertility due to the low milt volume produced by each male, but he reported to have used similar milt to the one studied in other experiments, and it was fertile. This observation was proved, in this research, when the good fertility of puye´s milt was established, registrating an average fertility percentage of 95.56 $\pm$ 6.46%, a superior value than the one observed by Billard (1990) in trout´s spermatozoa. It reached 80% of fertilization when it was diluted in a saline solution and upto 60% when it was diluted in freshwater, higher than the 89.9% of fertility observed by Suquet et al., (1995) en Turbot (*Scophthalmus maximus)*.

The high fertility observed in puye´s sperm may be helped by the high sperm density used in this investigation. 0.01ml of milt for aproximately 500 eggs was used, this means a total of 1 x $10^6$ sperm/egg. This sperm density is quite higher than the one recommended by Billard (1990) for the artificial fertilization of salmonids. He says that the necessary number of spermatozoa needed to fertilize an egg is between 10.000 and 300.000. Suquet et al., (1995) informed that in Turbot, 6 x $10^3$ sperm/egg were needed to obtain high fertility percentages. These records were proved by Ginzburg (1972), who points out that a direct relation exists between fertility and sperm density in sturgeons. The high sperm density added to the smaller size of puye´s egg (aproximatel 1 mm of diameter after being hidrated) would be the factors that make spermatozoa´s fertility better, adding to this, good quality gametes obtained from the females, in which their ovulation time can be detected adequately due to their captivity.

In most salmonides, the spermatozoa´s flagellar activity only reaches aproximately 30 sec. when it is diluted in river water (Billsard & Cosson 1988, 1989; Billard 1990 and Perchec et al., 1993), instead, in puye, it is possible to observe a small beat upto 24 hs after it has been diluted in water, after this time the spermatozoa still is viable (Valdebenito, 1996 unpublished). This characteristic is similar to the observations done by Galkina (1957) (in Ginsburg, 1972) who points out that in the *Clupea harengus* the spermatozoa lives 4-5 days in sea water and its fertility reaches 62% after 48 hs of being diluted (Yanagimachi, 1957 in Ginzburg, 1972). The prolonged "flagellar activity" of *G. maculatus* is contrary to Ginzburg´s reports (1972) who states that the sperm motility is greater in salty water than in landlocked waters, which could explain why the populatios of *G. maculatus* in landlocked waters still mantain their own milt characteristics that are proper of diadromous populations.

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**Table 1. Index for sperm motility (Adaptade from Sanchez-Rodríguez & Billard, 1977)**

| Index | Motility characteristics |
|---|---|
| 5 | Most spermatozoa display rapid movement; impossible to track the course of any sperm. |
| 4 | Most spermatozoa move rapidly while some move slowly. |
| 3 | Three classes of spermatozoa can be found; spermatozoa moving rapidly, slowly or vibrating, and those immotile. |
| 2 | Most spermatozoa are vibrating or immotile while some present forward movement. |
| 1 | Most spermatozoa are immotile and some present lateral vibration |
| 0 | All spermatozoa are immotile |

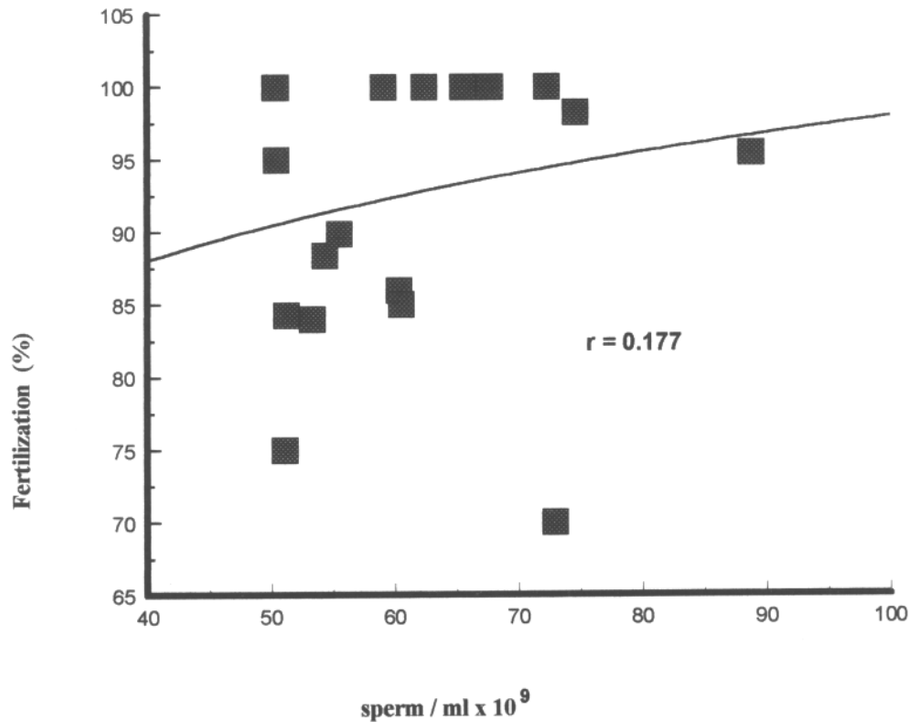


**Fig. 1. Relationship between sperm concentration and fertilization (%) in *G. maculatus*.**