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Brief Communication

Parental Background Predisposes Baltic Salmon Fry to M74 Syndrome

By *P. Koski*

National Veterinary and Food Research Institute, Oulu Regional Unit, Oulu, Finland.

M74 syndrome is a thiamine responsive disease of Baltic salmon (the Baltic group of *Salmo salar* L.) leading to the death of nearly all yolk-sac fry of certain females (see e.g. *Koski et al.* (1999)). *Börjeson et al.* (1995) reported that M74 syndrome in Baltic salmon in Swedish rivers was bound to the females, while milt from wiggling males – fish showing symptoms of a thiamine related-illness (*Larsson & Haux* 1996, *Amcoff et al.* 1999) – produced healthy offspring. In Finnish salmon farming, farmed broodfish produce the bulk of the eggs needed in restocking programmes. Both wild (fish which have returned from a feeding migration to the Baltic Sea proper) and farmed salmon eggs and milt were available for this experiment. The experiment was performed to determine whether M74 syndrome is associated only with wild salmon and not with farmed fish, and also whether mortality is associated with the eggs or the sperm or both.

A cross-fertilization experiment was performed at Lautiosaari State Fish Hatchery, Keminmaa, Finland in 1993-94. The wild fish were caught from the Bothnian Bay at the mouth of the River Simo in summer 1993 and kept under similar conditions to the broodfish described in *Koski et al.* (1999). Farmed broodfish of the River Simo strain of the Baltic salmon were held at Taivalkoski Game and Fisheries Research (males) and River Simo State Fish Farm (females) and were fed on standard commercial dry salmon pellets. On 5 October 1993, male and female gametes from these 2 fish farms were brought to the hatchery of Lautiosaari for fertilization together with gametes from the wild salmon. The eggs of 10 farmed and 10 wild females were fertilized with the milt of 10 farmed and 10 wild males. The milt of each individual male was only used to fertilize eggs from one farmed and one wild female. After fertilization there were thus 40 batches of eggs. The water-hardened eggs were disinfected with an iodophor (100 ppm free iodine for 10-15 min) and the eggs from each crossing were incubated separately. The development of yolk-sac fry was followed as described in *Koski et al.* (1999). The eggs of three crossings died during incubation; all other groups of eggs originating from the same broodfish were then excluded. Consequently, final experiment comprised 32 groups of yolk-sac fry. The cumulative mortality of the yolk-sac fry was followed from hatching to the exhaustion of the yolk sac. Mortality was statistically analysed by pairwise comparison of outcome for a particular broodfish mating with a wild fish and a farmed counterpart.

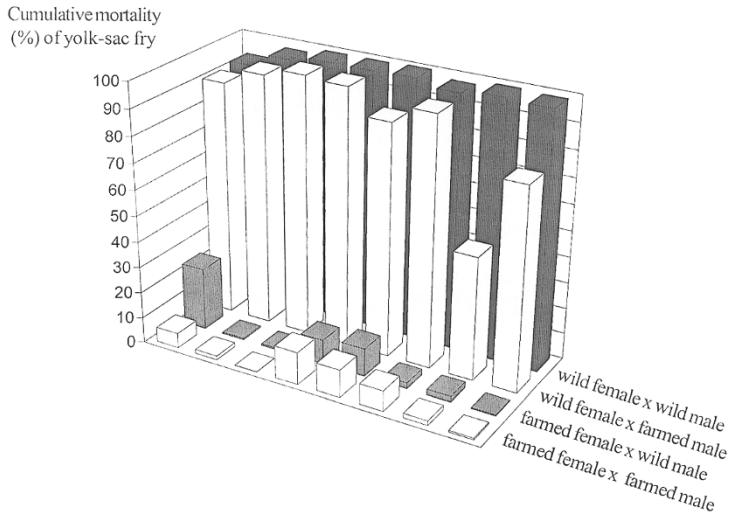

Fig. 1. Cumulative yolk-sac mortality following experimental cross-fertilization of wild and farmed Baltic salmon. Each bar represents the cumulative mortality of the yolk-sac fry of a certain female x male combination. Totally 4x8=32 cross-fertilization combinations.

The results of the experiment are presented in Fig. 1. Mortality varied greatly between the 4 types of cross-fertilization. The effect of the background of the female was especially pronounced. Almost all yolk-sac fry derived from wild females died (median 97%, range 48%-100%, Fig. 1), but the cumulative mortality of the offspring of farmed females varied between 0% and 24% (median 2%). On the basis of the Wilcoxon signed rank test ($p<0.001$), this difference in yolk-sac fry mortalities is statistically significant. Symptoms associated with M74 (light colour, loss of flight reaction, atactic swimming and a progressive loss of the ability to swim, exophthalmia, haemorrhaging, oedema and white precipitates in the yolk-sac, see e.g. *Börjeson et al.* 1995) were seen in the fry of the both cross-fertilization groups originating from wild females, but not in those from farmed females. The mortality of fry from the wild females was rapid, the whole progeny usually dying within a week. This is also typical for M74 (see e.g. *Koski et al.* (1999)). The cumulative yolk-sac mortality of the offspring of the farmed females can be regarded as typical for farmed salmon at the Lautiosaari State Fish Hatchery (unpublished statistics of the farm).

Besides the marked effect of the background of the female fish on the appearance of M74, there was also a minor effect of male background on the level of yolk-sac mortality of M74 fry (Fig. 1). Crossings of wild females with wild males resulted in greater mortalities (median 99%, range 92%-100%) than with farmed males (median 97%, range 48%-100%, Wilcoxon signed rank test $p<0.05$). These findings contradict those of *Fisher et al.* (1995) concerning M74-like mortality of landlocked Atlantic salmon (Cayuga syndrome) in certain lakes of New York State. The present experiments were performed before the key role of thiamine in the M74 syndrome was known and no thiamine measurements were taken from the eggs or fry. My later unpublished results of the total thiamine concentrations in the newly stripped milt of the Simo River wild salmon (autumn 1995 spawners) are about 5 times as high as those in the eggs: mean ± SD concentrations in the milt were 1.61 ± 0.61 mg kg^{-1} ($n = 8$) and respective concentrations in the eggs 0.38 ± 0.15 mg kg^{-1}

(n = 13; published in *Koski et al.* 2001, 2-sample t-test, $p<0.001$). This is further accentuated by the fact that only ca. 1/5 of the milt of the Baltic salmon is made up of the male gametes (*Piironen* 1995). It appears, however, unlikely that the observed male effect on the mortality of the yolk-sac fry in the present study would result from even a disparity of this magnitude. The diameter of the salmon egg is about 6 mm (*Aulstad & Gjedrem* 1973). According to *Billard* (1983) the longest axis of the brown trout and rainbow trout sperm head is only about 2.5 μm and only 1.5-2 μm in diameter. Thus only a minute proportion of the volume of the zygote originates from the sperm cytoplasm. Furthermore, the bulk of the tissue stores of thiamine are located in mitochondria (*McGormick & Greene* 1994), which are situated in the middle piece of the sperm cell (*Billard* 1983). The middle piece does not penetrate into the egg cytoplasm in the fertilization. It is improbable that the minute volume of the zygote originating from the sperm cell head would have a very high thiamine concentration.

The farmed male fish were held at a different fish farm from the wild broodfish. According to *Campbell et al.* (1992, 1994), acute or chronic stress in the salmonid broodfish can result in reduced progeny survival. The wild male fish caught for this study could have been more stressed than the farmed ones. The highest mortality of fry in the study of *Campbell et al.* (1992) occurred during hatching, but among the progeny of the stressed broodfish there was also greater mortality – although not statistically significant – from hatching to swim-up.

The possibility of a genotype-dependent susceptibility to the M74 was studied by *Nævdal & Skaala* (1999), but they did not find any obvious connection between the mortality of the fry and allozyme genotype or individual heterozygosity of the parent fish. In this study the broodfish were all of the same strain, which makes a genetic aetiology even more unlikely than in the larger material of *Nævdal & Skaala* (1999). *Koski et al.* (1999) found that injection of female broodfish with astaxanthine caused a proportionally greater decrease in thiamine concentration during the development of their offspring from newly fertilized eggs to yolk-sac fry. There might be also other effects of this kind on thiamine metabolism, some of which could be mediated by the male. The definition of such possible effects would, however, require further research.

It can be concluded that the factors associated with the M74 mortality are mostly carried in the egg of the wild female. It is unlikely that the hypothetical genetic background of M74 syndrome would be expressed only in the offspring of females returning from the feeding migration. The observed small effect of male background on the degree of yolk-sac fry mortality from M74 syndrome is more likely to be mediated via the quality of the sperm cells than to result from the direct contribution of the sperm cell to the thiamine concentration of the zygote.

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Reprints may be obtained from: P. Koski, National Veterinary and Food Research Institute, Oulu Regional Unit, P.O. Box 517, FIN-90101 Oulu, Finland. E-mail: perttu.koski@eela.fi, tel: +358 8 5622642, fax: +358 8 5544977.