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Effects of salinity on the development of Peipsi whitefish *Coregonus lavaretus maraenoides* Poljakow embryos

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Eggs of Peipsi whitefish *Coregonus lavaretus maraenoides* Poljakow were artificially inseminated and reared in sea water of 0.2–6.2 ppt salinity. Egg fertilisation rate was 97%–99% at 0.2–3.3 ppt but declined to 75%–85% at 4.0–6.2 ppt. Survival until hatching remained high at 0.2–1.3 ppt. Normal embryos hatched at salinity ≤ 4.8 ppt. Salinity did not affect the timing of development except for hatching, which occurred earlier when salinity increased. The length of free embryos was highest at 2.1 ppt.

Introduction

Peipsi whitefish, *Coregonus lavaretus maraenoides* Poljakow, is an endemic whitefish form in Lake Peipsi. It appeared in the lake before the last glaciation when the pass of fish from the Baltic Sea into Lake Peipsi was blocked by the Narva River waterfall (Hang & Miidel 1999). There is no information on embryonic salinity tolerance of Peipsi whitefish or any other Coregonid species living exclusively in fresh waters. However, Jäger *et al*. (1981) studied salinity tolerance of the sea-living *Coregonus lavaretus* and *Coregonus albula* embryos.

Unfavourably high salinity may have different effects on reproduction of freshwater fish. Fertilisation success may fall as a result of reduced motility of spermatozoa. High salinity can cause increased embryo mortality and decreased length at hatching of freshwater fish (Klinkhart & Winkler 1989, Vetemaa & Saat 1996). Also, the duration of embryonic development can be affected by salinity (Depêche & Billard 1994).

There are several forms (species) of whitefish in the eastern Baltic, spawning either in the brackish water of the Baltic Sea or in the fresh water. Our aim was to reveal if changes in the breeding habits of a whitefish form living exclusively in fresh waters after the last glaciation have led to changes in embryo salinity tolerance. We studied the effects of salinity on Peipsi whitefish embryonic development (malformations, hatching rate and time, length of hatching embryos).

Materials and methods

The experiment was carried out from 12 November 2001 until 4 April 2002 for 144 days. Mature fish (3 females and 2 males) were caught on the spawning grounds in Lake Peipsi using trapnets. Live fish were transported to the laboratory, where they were stripped and the eggs of each female were distributed into 11 Petri dishes (approximately 200 eggs per dish). Sperm from

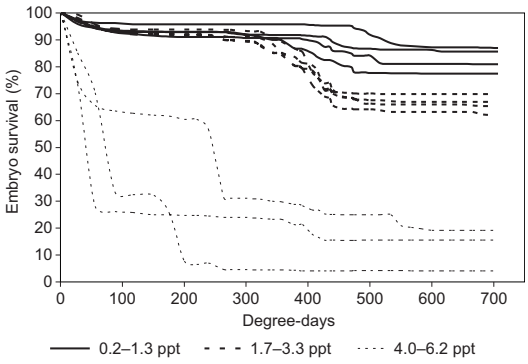

Fig. 1. The proportion of live embryos (malformed embryos included) at different salinities throughout the experiment, mean data for three females.

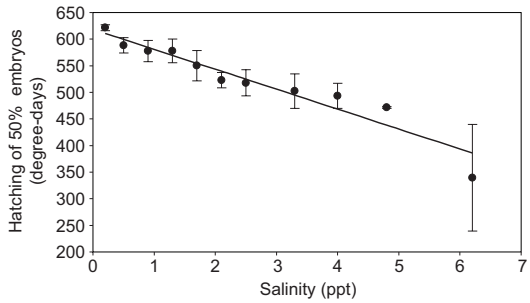

Fig. 2. Hatching time of 50% of embryos at different salinities (mean ± SE for three females).

two males was mixed and distributed into dishes with eggs. Then the water of 0.2, 0.5, 0.9, 1.3, 1.7, 2.1, 2.5, 3.3, 4.0, 4.8, 6.2 ppt salinity was added. So, three replicates (dishes) per each salinity were used. Additionally to the artificial seawater, embryos were incubated also in Lake Peipsi water. However, since the chemistry of the lake differs from the seawater and since survival in optimal salinities was good, the data on controls is not presented in the paper. Dishes contained approximately 60 ml of water and they were kept open during the whole experiment. Saline water was obtained by dissolving sea salt (Kesko OY, Finland) in distilled water. Salinities were checked with Conductivity Meter Mettler Toledo MC 126. The water was renewed every 7 days, and after the first embryos hatched, every 2 or 3 days. The salinity was checked before water renewals; its change due to evaporation appeared negligible. All dishes were kept under the same conditions at a natural photoperiod and natural light in a thermostated room. Water temperature was 4 °C in November–December, and then it increased gradually up to 6 °C in late March–April. These temperatures are in the range of optimal temperatures for *C. lavaretus* embryos (Tshernaev 1987, Saat & Veersalu 1996). Two samples from one of the series (1.7 ppt and 2.1 ppt) were lost during the experiment. Dead embryos were counted and removed before every water renewal. The number of living embryos was also counted every time. Free embryos (eleutheroembryos) with body malformations (curved tail etc.) or abnormal swimming pattern were classified as abnormal. At the end of the experiment (707 degree-days from fertilisation; all embryos were hatched or dead), embryos were immobilised in 70% ethanol. The total length of 30–35 (fewer in some cases at higher salinity, where the number of surviving embryos was smaller) normal free embryos was measured at each salinity in all the series using Image Tool software (UTHSCSA Image Tool for Windows version 2.00).

Results

Fertilisation and embryo survival

Egg fertilisation rate (determined as the proportion of normal embryos developing at 28 degree-days) was 97%–99% on average for the three females at 0.2–3.3 ppt and declined to 75%–85% on average at 4.0–6.2 ppt. Survival until hatching was high at 0.2–1.3 ppt (maximum 86% at 0.2 ppt), declined to 32%–56% at 1.7–3.3 ppt and 0%–9% at 4.0–6.2 ppt (Fig. 1). At 1.7–3.3 ppt, the highest mortality occurred shortly before hatching (~400 degree-days). At highest salinities, most of embryos died before or during gastrulation.

Hatching and abnormalities

The chronology of the development (e.g. reaching of the "eye-spot-stage") did not depend on salinity. Embryos reached the "eye-spot stage" at the age of 170–180 degree-days at all salinities tested.

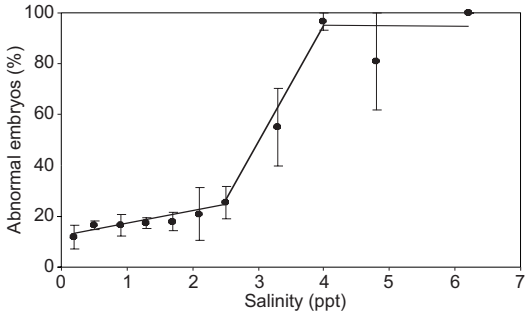

Fig. 3. Proportion of abnormal free embryos at different salinities (mean ± SE for three females).

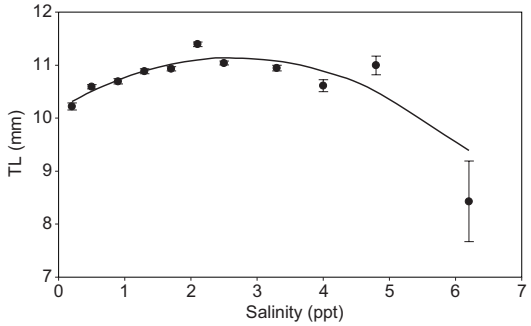

Fig. 4. The total length (TL) of normal free embryos at different salinities 707 degree-days after fertilisation at different salinity (mean ± SE for three females).

Hatching began at highest salinity (6.2 ppt) at 220 degree-days after the fertilisation. Time of hatching of 50% of larvae decreased from 616 degree-days at 0.2 ppt to 470 degree-days at 4.8 ppt and 239 degree-days at 6.2 ppt (Fig. 2). The trend of salinity (S) on hatching time (H) can be described by linear regression $H = 620.7 - 38.7S$; $r = 0.959$; two-tailed $p < 0.001$ ($t = 10.26$, d.f. = 9, 95% C.L of the slope 30.1 and 47.2). Percentage of abnormal free embryos was ~20 at 0.2–2.5 ppt but increased rapidly at higher salinities (Fig. 3).

Length of embryos at hatching

Polynomial model predicts salinity range of 1.9–3.5 ppt to produce the largest (> 11 mm TL) free embryos. The shortest embryos hatched at 6.2 ppt (Fig. 4).

Discussion

Coregonid fishes inhabit both fresh and brackish waters. In the Baltic Sea sympatric river-spawning and sea-spawning forms (often regarded as different species) of *Coregonus lavaretus* are known (Svärdson 1979).

Besides brackish waters of the Baltic Sea adult whitefish inhabit even saline waters of the North Sea. Juveniles of coregonids are, however, sensitive to saline water. A laboratory study by de March (1989) has demonstrated that salinity tolerance of *Coregonus nasus* increases with size: larvae (12–18 mm) died after 120 hours at salinity ≥ 12.5 ppt, juveniles (33–68 mm) survived this time at ≤ 15–20 ppt.

Whitefish *Coregonus lavaretus* from the Kiel area hatched at salinity up to 10.2 ppt (Jäger *et al*. 1981). In the present study normal embryos hatched only at ≤ 4.8 ppt. Obviously, Peipsi whitefish showed lower salinity tolerance in comparison to the Baltic Sea whitefish.

Embryonic salinity tolerance of several freshwater fishes inhabiting also brackish waters of the Baltic Sea has been studied: roach *Rutilus rutilus*, perch *Perca fluviatilis*, pikeperch *Sander lucioperca* (Klinkhardt & Winkler 1989), ruffe *Gymnocephalus cernuus* (Vetemaa & Saat 1996) and spined loach *Cobitis taenia* (Bohlen 1999). In the case of ruffe (Vetemaa & Saat 1996), higher embryonic salinity tolerance of the fish populations inhabiting brackish waters in comparison to freshwater populations has been demonstrated. Peipsi whitefish embryos have lower salinity tolerance than freshwater percids living in the Baltic (ruffe, perch, pikeperch), but higher tolerance than roach (Schoefer 1979, Klinkhardt & Winkler 1989, Vetemaa & Saat 1996).

According to Lebedeva (1981) Peipsi whitefish has TL at hatching of 10.8–12.4 mm. In the present study the maximum TL (> 11 mm) was observed at 1.9–3.5 ppt. At the salinity with the highest embryonic survival (0.5 ppt), average TL was 10.6 mm.

The accelerated embryonic growth of freshwater fish in water of moderate salinity is a well-known phenomenon. Brackish water reduces the osmotic and ionic gradients between the internal fluids and the external medium, and less energy

is wasted for osmotic regulation (Lam & Sharma 1985). In a freshwater population of ruffe, the longest embryos hatched at ~2 ppt (Vetemaa & Saat 1996). Also, larvae of common carp *Cyprinus carpio* are bigger at 1.5–3 ppt than in fresh water (Lam & Sharma 1985).

The main factor determining the rate of embryonic development of teleosts is temperature. As it has also been documented for ruffe (Vetemaa & Saat 1996), moderate salinity did not affect the timing of morphological development of embryos. However, teleost embryos can hatch at different stages of development depending on environmental conditions (Luczynski & Kolman 1987). In some cases, hatching is delayed at higher salinity (Vetemaa & Saat 1996). As shown in this study, in whitefish elevated salinity leads to precocious hatching. Furthermore, unfavourable salinity causes malformations and embryos' death.

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