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Effects of salinity on the development of fresh-water and brackish-water ruffe *Gymnocephalus cernuus* (L.) embryos

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Eggs of ruffe inhabiting a fresh-water (FW) lake and a brackish-water (BW) Baltic bay were inseminated and reared in sea water of different salinity. Normal prelarvae hatched at salinities up to 9 ppt in the BW ruffe and 6 ppt in the FW ruffe. The duration of incubation until hatching in the BW ruffe remained constant at 0–8 ppt but increased in the FW ruffe at salinity > 2 ppt. The maximum length of prelarvae in the BW ruffe was observed at higher salinity than in the FW ruffe. These differences indicate an adaptive increase in embryonic salinity tolerance of ruffe inhabiting brackish water.

1. Introduction

Deviations from the optimal water salinity have various lethal and sublethal effects on fish gametes and embryos (e.g. Ginsburg 1968, Holliday 1969, Khlebovich 1977, Zhukinskii 1986). Effects of water salinity on fish embryogenesis have been well studied in several marine species; data for freshwater fish are mostly limited to sperm motility and egg fertilizability. Moderate salinity (usually up to 6 ppt) enhances both the duration of sperm motility and the fertilization rate of eggs of freshwater fish while higher salinity (usually about 7–8 ppt) suppresses both of them (Khlebovich 1977, Zhukinskii 1986).

Brackish coastal waters of the eastern Baltic are inhabited by various freshwater fish; among Estonian species, in general, only rheophils are mostly lacking here. Some of these species (e.g. cyprinids) spawn in rivers or river estuaries at low salinity while others e.g. perch *Perca fluviatilis* L. and ruffe *Gymnocephalus cernuus* (L.) can spawn in the sea where water salinity reaches 6–8 ppt which are critical values for gametes and embryos of several freshwater fish.

The aim of this study was to investigate the effects of water salinity on fertilization, embryonic development, hatching and prelarvae length of embryos of ruffe originating from a freshwater lake population and a brackish-water population from the Baltic Sea. Evidence is presented that suggests increased salinity tolerance of ruffe embryos from a brackish water population.

2. Materials and methods

Experiments were carried out in May and June 1994. Mature fish with "running" gametes were collected on the spawning grounds from a freshwater (FW, salinity < 1 ppt) lake (Vôrtsjärv, SE Estonia; 58°10′N, 26°05′E) and a brackish-water

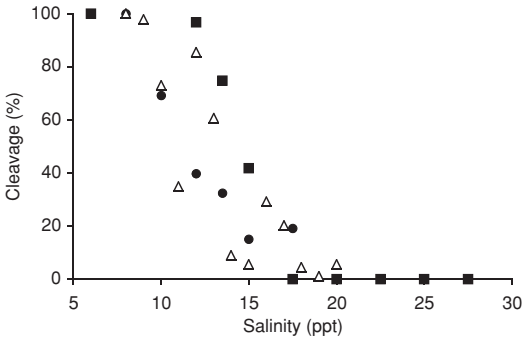

Fig. 1. Number (%) of normally cleaving eggs of the BW ruffe at different salinities. 100% equals to the cleavage rate at 6 ppt salinity (68, 89, and 91% in three females).

(BW, salinity 3–6 ppt) bay (Pärnu Bay, W Estonia; 58°21′N, 24°28′E) using various active and passive gear. The total length (*TL*) of FW ruffe was less than 10 cm while *TL* of spawning fish in the BW was about 15 cm. Three females of both the FW and BW ruffe were represented in each experiment; altogether eggs of three FW and eight BW ruffe were used.

Live fish were transported to the laboratory, where they were stripped and the eggs of each female distributed into several Petri dishes (100–150 eggs per dish). Eggs and sperm were mixed in sea water of different salinity (0–27.5 ppt). In each series, sperm from two males were used. Sea water of various salinity was obtained by dissolving sea salt in distilled water and was exchanged daily. Eggs were maintained at a constant salinity during the whole experiment. Experiments were carried out at a constant temperature of 12 ± 0.2°C (FW) or 14 ± 0.2°C (BW). These temperatures lie in the range of optimal temperatures for ruffe embryos (Saat & Veersalu 1996). Dynamics of embryo development were expressed in relative time units, τ_0 (Dettlaff & Dettlaff 1961). The value of τ_0 for ruffe embryos is 60 minutes at 14°C, and 76 minutes at 12°C (Saat & Veersalu 1996). The number of fertilized eggs was determined by counting the number of normally cleaving embryos with 2 or 4 blastomeres. Dead embryos were counted and removed twice a day. Altogether 15 developmental stages were distinguished in this investigation (Vetemaa, unpubl.). Stage 1 is the beginning of gastrulation, stage 3 — the end of gastrulation (complete epiboly); further stages until the beginning of hatching (stage 15) were distinguished according to morphological and physiological (e.g. heart beat) changes of embryos. The dynamics of ovulation were also followed at various salinities. The *TL* of 10–20 (less in some cases at higher salinity) normal prelarvae (yolk-sac larvae) was measured at each salinity (at a magnification 10×) after their immobilization in 1% paraformaldehyde solution.

All the data are expressed as mean ± *S.D.* Descriptive statistics and the coefficients of regression lines were calculated using the EXCEL 5.0 program package. Probit-analysis was according to Zaicev (1984).

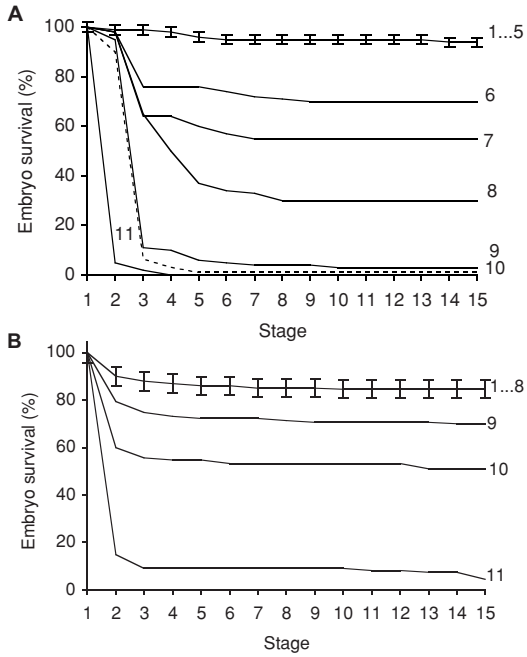

Fig. 2. Effect of salinity on embryonic development of the FW (A) and BW (B) ruffe: percentage of living embryos at different stages of development. Number of live embryos at stage 1 (100%) is 80–120, except for the FW ruffe at 10 and 11 ppt salinity (n = 36 and 40, respectively).

3. Results

3.1. Fertilization

The percentage of normally cleaving eggs was used as a measure for egg fertilizability. The "critical" salinity decreasing the cleavage rate by 50% was 12.3, 13.5 and 14.1 ppt for three females of the BW ruffe (probit-analysis; Zaicev 1984). The percentage of cleaving eggs decreased sharply at salinities > 10 ppt; however, the normal cleavage of the BW ruffe eggs was observed at salinities up to 17.5–20 ppt (Fig. 1). The normal cleavage of the FW ruffe embryos decreased at > 6–8 ppt but it was observed at at all salinities tested (up to 11 ppt).

3.2. Embryo mortality

Embryos of the FW ruffe developed well at salinities up to 5 ppt. A negligible (< 5%) mortality was observed during gastrulation (stages 2–3, 20–40 τ_0 from insemination). Mortality during

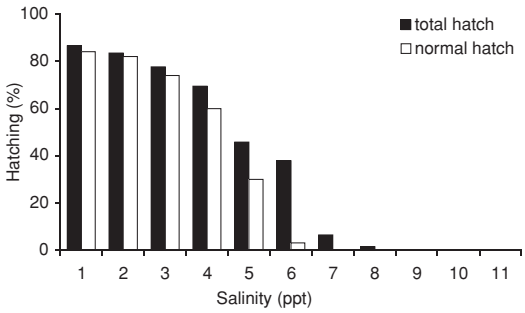

Fig. 3. Number (%) of hatched prelarvae at different salinities in the FW ruffe.

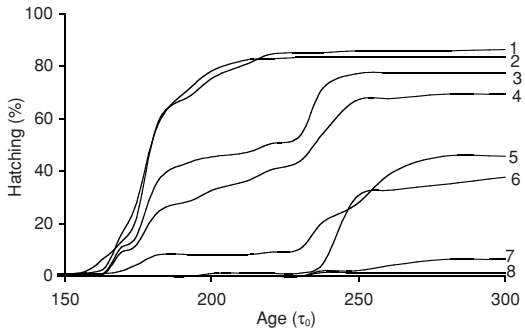

Fig. 4. Hatching dynamics (τ_0) of the FW ruffe at different salinities (1–8 ppt).

gastrulation increased at 6–8 ppt salinity, and most embryos died by the end of gastrulation at 9–11 ppt (Fig. 2A). Normal prelarvae were observed at salinity up to 6 ppt (Fig. 3).

As in the case of the FW ruffe, most of the BW ruffe embryos surviving gastrulation could develop until stage 15 (beginning of hatching) (Fig. 2B). Embryos reared at salinity up to 8 ppt exhibited moderate (15%) mortality by the end of gastrulation. The mortality during gastrulation was about 25% at 9 ppt, 45% at 10 ppt, and 90% at 11 ppt (Fig. 2B). Normal prelarvae were observed at salinity up to 9 ppt. However, over 50% of late embryos and prelarvae at this salinity and all embryos and prelarvae at higher salinity were abnormal, usually with shortened and/or curved tail region and very slow heart beat.

No differences in the chronology of embryo development between the FW and the BW ruffe or at different salinities were observed, except for delayed hatching at high salinity of the FW ruffe embryos (see 3.3).

3.3. Age at hatching

Hatching in the BW ruffe began at the age of 160–170 τ_0 and the time when 50% of the prelarvae were hatched (final hatch = 100%) or H_{50} remained relatively constant at salinity 0–8 ppt (174–190 τ_0, mean $\pm$ *S.D.* 183 $\pm$ 7 τ_0). Hatching in the FW ruffe reared at salinity 1–5 ppt began at the same age (160–170 τ_0) but at higher salinities it began significantly later (Fig. 4). H_{50} was 182–183 τ_0 at 1–2 ppt salinity and it increased to approximately 200–205 τ_0 at 3–4 ppt, 220 τ_0 at 5 ppt, and 250 τ_0 at 6 ppt.

3.4. Length of prelarvae

The *TL* of normal prelarvae was measured at the age of 250 τ_0. Prelarvae of the BW ruffe had maximum *TL* at salinity 2–6 ppt. The *TL* of prelarvae of the FW ruffe decreased at salinities over 3 ppt (Fig. 5).

4. Discussion

Ruffe is one of the most widely distributed freshwater fish in Estonian lakes and it is also common in the coastal waters of the Baltic Sea at salinities up to at least 6–8 ppt. The spawning grounds are usually located in bays receiving considerable fresh water input (salinity usually less than 3–4 ppt) (e.g. Pärnu, Matsalu), but they also spawn elsewhere, in Väinameri (Moonsund), where salinity reaches 6–8 ppt.

A salinity of approximately 8 ppt has been shown to be critical for fertilization of several freshwater fish species as their spermatozoa can not be activated at a higher salinity (Ginsburg 1968, Khlebovich 1977, Zhukinskii 1986). The salinity tolerance of ruffe gametes is apparently higher. Klinkhardt and Winkler (1989) detected high percentage of fertilized eggs of BW ruffe at all salinities tested (up to 8.3 ppt), and we observed the normal cleavage even at > 10 ppt salinity.

The salinity range for further embryonic development of ruffe, as well as perch, pikeperch and roach embryos (Klinkhardt & Winkler 1989) was remarkably narrower than for fertilization. Klinkhardt and Winkler (1989) investigated three developmental stages: fertilization, morula/blastula and "eye spot" stage. They observed increased embryo mortality

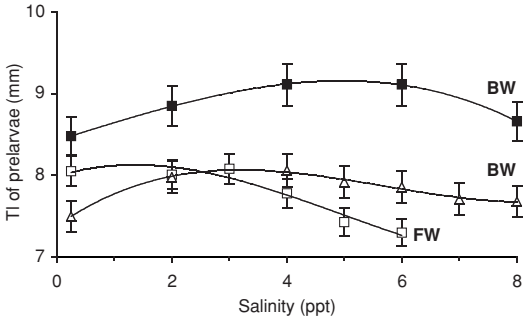

Fig. 5. Mean *TL* (± *S.D.*) of ruffe prelarvae at different salinities (data for three females have been presented).

by the "eye spot" stage at salinity below 1, and over 7 ppt. Our data suggest that the highest embryonic mortality at increased salinity (as well as in optimal conditions) occurs during gastrulation. Low mortality at later stages is probably associated with the beginning of true osmoregulation in teleost embryos shortly following gastrulation (Alderdice 1988). However, embryo development at high salinity is often atypical and most of such embryos fail to hatch.

There exist only limited data on intraspecific variability in salinity tolerance of freshwater fish embryos. It has been shown that embryos of wild carp, *Cyprinus carpio* L., and cultured mirror carp and their hybrids differ in this respect (Abdurahmanova & Kasimov 1979). Further evidence on the genetic polymorphism of salinity tolerance has been obtained for embryos of euryhaline fish (threespined stickleback *Gasterosteus aculeatus* (L.) (Heuts 1947) and killifish *Fundulus heteroclitus* L. (Bush & Weis 1983)). Significant differences in embryonic salinity tolerance have been detected for marine fish (spring-spawning and summer-spawning Baltic sprat, *Sprattus sprattus balticus* (Schneider) and the White Sea and the Baltic Sea cod, *Gadus morhua* L.; see Zhukinskii 1986 and Nissling 1995 for references).

We observed increased mortality during gastrulation in the BW ruffe at salinities over 8 ppt which is slightly more than in a German population (7 ppt; Klinkhardt & Winkler 1989). However, both these values are higher than for the FW ruffe (6 ppt). Hatching of normal prelarvae in our experiments was observed at 9 ppt in the BW ruffe and 6 ppt in the FW ruffe. Hatching in the FW ruffe was retarded at high salinity, and the maximum *TL* of their prelarvae was observed at lower salinity than in the BW ruffe. These data suggest an adaptive increase in salinity tolerance of BW ruffe embryos. However, any possible genetic basis for these differences remains to be evaluated.

The main factor determining the rate of embryonic development in ruffe, as well as in other fish, is temperature (Saat & Veersalu 1995). The chronology of embryonic development in ruffe did not depend on salinity. Alderdice & Forrester (1968, 1971ab) observed small but real effects of salinity on the duration of the period from fertilization to hatching in several marine fish species. They also noticed an increased duration of the hatching period at suboptimal (low) salinity (Alderdice & Forrester 1971b). The only process where temporal pattern was obviously affected by salinity in our experiments was also hatching. Intraspecific variation in hatching age of fish embryos is high depending on various environmental factors (Yamagami 1988). However, salinity has not been proven among the main factors affecting the hatching glands (Yamagami 1988). Most probably the delayed hatching at high salinity in ruffe is not associated with the direct effect of salinity on the hatching glands but indirectly, caused by morphological and physiological abnormalities of embryos.

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