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Development, and effect of water temperature on development rate, of pikeperch *Sander lucioperca* embryos

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ABSTRACT

Knowledge of embryo development is essential to the application of reproductive biotechnology in aquaculture, including for pikeperch *Sander lucioperca*. We describe pikeperch embryo development and demonstrated effects of temperature on the duration of embryogenesis. Developmental stages in embryos incubated at 15 °C were identified as zygote, 0–1.5 h post-fertilization (hpf); cleavage, 2.5–7.5 hpf; blastula, 9–18.75 hpf; gastrula, 21–39, hpf; segmentation, 45–105 hpf; and hatching, 125–197 hpf. Additional groups of eggs were fertilized and incubated at 10, 15, 20, and 25 °C to document stages of development, development rate, and survival. The optimal fertilization and incubation temperature was shown to be 15 °C, with the highest fertilization, survival, and hatching rates. Embryo development was slower at 10 °C, with 45% of fertilized embryos surviving to hatching. Development was accelerated at 20 °C, and resulted in a 56% survival rate of fertilized embryos. At 25 °C, embryos did not develop to the blastula stage. Pikeperch could be a valuable percid model for research in which flexible incubation temperatures is required.

1. Introduction

Fish embryo development is a critical aspect of developmental biology, and knowledge of its characteristics is essential to improving reproduction techniques for commercially valuable and endangered species. The developmental stages of many species have been described in detail. Currently, zebrafish *Danio rerio* [1], medaka *Oryzias latipes* [2,3], ice goby *Leucopsarion petersii* [4], and goldfish *Carassius auratus* [5] are the preferred model fish species in studies of developmental biology. Developmental stages have also been described in summer flounder *Paralichthys dentatus* [6], Pangas catfish *Pangasius pangasius* [7], and striped snakehead *Channa striatus* [8], which are cultured species with increasing demand. Commercially valuable, as well as endangered, species including tench *Tinca tinca* [9], loach *Misgurnus anguillicaudatus* [10–12], and Siberian sturgeon *Acipenser baerii* [13] are attracting interest with respect to advanced biotechnologies such as generation of chimeric fish and clonal production [14,15].

Pikeperch *Sander lucioperca* is a freshwater perciform teleost highly valued in aquaculture. In Western Europe, pressure for the conservation of natural pikeperch stock has led to its intensive production in recirculating aquaculture systems (RAS) [16]. To improve broodstock management in RAS, whether for scientific investigation or commercial hatchery production, studies have focused primarily on egg and larva quality [17–20]. Pikeperch reproductive features, including production of large numbers of eggs (200,000 kg^{-1} body weight) and accessible, transparent, robust embryos; a relatively short incubation period [65–110° days (d°)] [16,21]; and its tolerance to micromanipulation, makes pikeperch a good potential candidate as a surrogate host or donor of perciforms [22]. However, micromanipulation is successful for only a limited time during a specific period in embryogenesis [22–24]. Knowledge of the developmental rate of pikeperch embryos relative to water temperature may allow retardation of development for more successful micromanipulation.

Temperature is among the most influential of environmental factors on development of percid embryos [25], especially during fertilization and incubation of embryos, and is species-specific [26–32]. Knowledge of the temperature range at which normal development occurs can limit mortality related to temperature fluctuations that exceed safe limits. The optimal temperatures for pikeperch spawning (8–16 °C), fertilization (12–16 °C), and incubation (11.5–20 °C) are reported to be among the highest in

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investigated percids [21,25]. Oprea et al. [33] studied effects of two temperature ranges on the duration of pikeperch embryogenesis in eggs incubated under hatchery conditions. Duration of development was 9 days at 9–13 °C and 6 days at 13–16 °C with high rates of fertilization, survival and hatching [33].

Development of embryos has been documented in several percids [26–32], but development stages of pikeperch embryos are not. The goal of this study was to characterize the stages of pikeperch embryo development to first feeding at 15 °C, to investigate effects of temperature on development rate, and to determine incubation temperatures that retard development while minimizing negative effects on survival and growth.

2. Material and methods

2.1. Ethics

All experimental procedures followed the principles of laboratory animal care and national law 246/1992 Animal Welfare on the protection of animals, and the criteria of the EU Harmonized Animal Welfare Act of the Czech Republic.

2.2. Fish source

Three female and three male *Sander lucioperca* broodfish were maintained in an RAS at the University of South Bohemia, Faculty of Fisheries and Protection of Waters, during the spawning season from March to April 2016. The fish were anesthetized with an aqueous solution of 30 mg L^{-1} clove oil (Dr Kulich Pharma, s. r. o., Czech Republic). Human chorionic gonadotropin (Chorulon) was injected intramuscularly at 500 IU kg^{-1} body weight to induce ovulation and spermiation [34]. Eggs and sperm were collected at ~87 d° post-injection.

2.3. Preparation of embryos for characterization of embryonic stages at 15 °C

Eggs were fertilized in carbon-filtered and aerated tap water at 15 °C. A group of embryos was prepared for monitoring, photographing, and documenting development by removing the chorion.

To remove the chorion, two minutes post-activation, embryos were treated with 0.2% trypsin and 0.4% urea in Ringer's solution buffered by 10 mM TAPS to pH 8.5. After 10 min embryos were transferred into 1.6% albumen in Ringer's solution buffered with 10 mM HEPES to pH 7.5 to stop the enzymatic reaction. The stickiness was removed, and the softened chorion was removed using fine forceps as previously described due to the low surface tension of embryos [22]. The dechorionated embryos were divided into five groups of 50, placed in 1% agar-coated Petri dishes, and incubated in 1.6% albumen in Ringer's solution buffered with 10 mM HEPES to pH 7.5 until somitogenesis [22]. The Ringer's solution was replaced every 2 h. After completion of epiboly, to avoid the bacterial contamination, the dechorionated embryos were transferred into 0.01% penicillin and 0.01% streptomycin in Ringer's solution and incubated.

2.4. Stage definition

Developmental stages were defined morphologically by counting numbers of cells, blastomeres, and somites under a stereomicroscope (Leica M165FC, Germany) and photographed (Leica DFC425C, Germany).

2.5. Temperature trial

To describe normal pikeperch embryo development, a control group of 250 embryos with intact chorion were incubated at 15 °C for documentation of timing of embryonic stages as well as fertilization, survival, and hatching rates. Immediately after fertilization, embryos were treated for 2 min with 1.5 ml L^{-1} alcalase to eliminate stickiness, divided into five groups of 50 embryos, rinsed four times in carbon-filtered and aerated tap water, and incubated.

Two experimental groups were created to assess temperature effects. Group A: ~750 eggs were divided into three sub-groups and fertilized, enzyme-treated (as above), and incubated at 10 (Group A1), 20 (Group A2), or 25 °C (Group A3) in carbon-filtered and aerated tap water. Group B: After insemination and enzyme treatment at 15 °C, ~750 eggs were divided into three sub-groups designated B1, B2, and B3 and incubated at 10, 20, or 25 °C, respectively, in carbon-filtered and aerated tap water. Each subgroup was distributed among five 95 mm Petri dishes (n = 50 per dish).

Fertilization rate was calculated at the cleavage stage. Survival rate was determined by the number of fertilized eggs reaching the hatching stage. Hatching rate was calculated over a 72-h hatching period, determined as the number of surviving embryos.

2.6. Statistical analysis

Differences among control and temperature-treated groups in fertilization rate, survival rate, and hatching rate was analysed by the ANOVA and Tukey's HSD test. Differences were considered significant at P-value <0.05. Data are presented as mean ± standard deviation (SD). Analyses were performed using Statistica 13.

3. Results and discussion

3.1. Embryo development in pikeperch at 15 °C

Characterization of the development of the pikeperch embryo was based on morphological features as compared to developmental stages of zebrafish [1], loach *Misgurnus anguillicaudatus* [11], summer flounder *Paralichthys dentatus* [6], ice goby *Leucopsarion petersii* [4], medaka *Oryzias latipes* [2,3], goldfish *Carassius auratus* [5], and percid species including the rainbow darter *Etheostoma caeruleum* [29], yellow pope *Gymnocephalus schraetser* [27,35], yellow perch *Perca flavescens* [26], logperch *Percina caprodes* [30,31], walleye *Sander vitreum* [32], Danube streber *Zingel streber* [28]. Stages were categorized as zygote, cleavage, blastula and gastrula, segmentation, hatching, the free embryo period, initial oral feeding, and larva.

A major feature of sticky eggs, as in pikeperch, is a central lipid drop within the yolk. After egg activation by contact with freshwater, the chorion lifted away and formed a perivitelline space. Pikeperch has the smallest fertilized egg diameter (0.9–1.0 mm) [22] among studied percid species [36]. Fertilization rate was 75% (n = ~5000). Survival rate of fertilized eggs to hatching was 97.2%. Hatching rate of survivors was 92.9% at 15 °C.

Neither cell cleavage nor duration of gastrulation showed similarity with other percid species. Mean time from fertilization to first feeding was 10.2 days. Hatching time was 5.2 days post-fertilization (dpf) at 15 °C, with 5 days post-hatching (dph) to mouth opening, Blastodisc formation was observed at 1 hpf, and the one-cell stage was observed at 1.5 hpf. The duration of cleavage was 2.5–9 hpf, of blastula 9–21 hpf, of gastrula 21–42 hpf, and of segmentation 42–125 hpf. Hatching began at 125 hpf, and duration was three days. Time of free embryo to first oral feeding was five days. Each period was subdivided into several stages, from