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Effect of temperature on incubation period and hatching success of obscure puffer *Takifugu obscurus* (Abe) eggs

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Abstract

Artificially fertilized eggs of obscure puffer *Takifugu obscurus* were obtained by induced spawning of cultured broodstock and incubated at temperatures of 15, 19, 23, and 27 °C. The results showed that the optimal temperature for obscure puffer embryonic development ranged from 19 to 23 °C, based on total hatch rate, viability of newly hatched larvae 24 h post-hatch, and total mortality rate of eggs. At the given temperature range, the times taken for 50% embryos to hatch were 11.3, 6.6, 5.0, and 4.2 days, respectively. There was significant difference in time to 50% hatch among the temperatures used in this experiment. The power law model, quadratic equation, exponential equation, and effective degree-day model all provided good fits for the relationship between incubation temperature and time to 50% hatch, with r^2 values greater than 0.90. The formulae for these were $y=1031.7T^{-1.6885}$, $y=44.721-3.1574T+0.0615T^2$, $y=34.663e^{-0.0813T}$ and $y=78.905/(T-7.6033)$, respectively, where y is time to 50% hatch in days, and T is incubation temperature in degrees Celsius. The effective degree-day model was determined to be the best model because of efficient computation, good fit to the experimental data, and most importantly, the derived parameters, k (the sum of effective degree-days) and t_0 (the temperature of biological zero), have important biological meaning. Based on the effective degree-day model, the t_0 and k values were calculated as 7.6033 °C and 78.905 degree-days, respectively.

Keywords: Obscure puffer; *Takifugu obscurus*; Temperature; Embryonic development; Time to 50% hatch; Hatching success

1. Introduction

There are approximately 100 different species of puffer fish in the world. Some species, such as bullseye puffer *Sphoeroides annulatus* (Duncan et

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al., 2003; Komar et al., 2004), tiger puffer *Takifugu rubripes* (Miyaki et al., 1992; Chuda et al., 1997; Matsuyama et al., 1997), purple puffer *Takifugu porphyreus* (Fujita and Abe, 1992), brown puffer *Takifugu exascurus* (Fujita and Honma, 1991), and obscure puffer *Takifugu obscurus* (Yang and Chen, 2004, in press; Yang and Yang, 2004), have been studied with regard to seeding production because they appear to be promising aquaculture species. Most species inhabit marine or coastal waters throughout their life cycle, and only a few species, such as the obscure puffer *T. obscurus*, are anadromous. They migrate to freshwater rivers for reproduction during the spawning season of February to May. The newly hatched larvae remain in freshwater for several months before they emigrate to sea. Most feeding and growth of subadults take place at sea over several years. Approaching maturity, the adults return to freshwaters to spawn (Yuan and Xie, 1986). Obscure puffer is a fish with considerable commercial importance in China owning to its high-quality meat, but overexploitation and environmental degradation are diminishing its natural populations (Yang and Chen, 2003). Although we have successfully developed the technique of induction ovulation in both wild and cultured obscure puffer in order to protect and increase natural populations and meet the increasing demand for consumption (Yang and Chen, 2004, in press), little is known about the biological and ecological requirements of this species as a basis for fishery management, commercial cultivation, and replenishing natural populations.

An essential step in the successful culture of any species is to understand the optimal environmental conditions for egg incubation. Temperature is one of the most decisive environmental variables affecting embryonic development in fish eggs (Brännäs, 1987; Beacham and Murray, 1990; Baynes and Howell, 1996; Bermudes and Ritar, 1999; Kamler, 2002). Therefore, determination of the optimal temperature for obscure puffer egg incubation is necessary to maximize the seeding production. The present investigation is a part of a larger study of effects of extrinsic factors on the hatching success and survival of obscure puffer eggs. The result of this study will be useful in improving the production in hatcheries.

2. Materials and methods

2.1. Fertilized eggs collection

Artificially fertilized eggs of obscure puffer were obtained by induced spawning of cultured broodstock maintained in 20 m^2 concrete tanks. Twenty individuals of 3-year-old females (above 0.65 kg body weight) were injected intraperitoneally with [D-Ala^6-Pro^9-Net]-luteinising hormone releasing hormone analogue (LHRH-a) at a dose of 30 μg kg^{-1} body weight (Yang and Chen, in press). Multiple injections were given and the interval between injections was 36 h. Beginning 2 days after initiation of hormonal treatment, abdominal palpation was performed every day to check expansion and hardening of the abdomen due to the hydration of oocytes which indicates completion of final oocyte maturation. Most females ovulated after the fourth LHRH-a injections (Yang and Chen, in press). The eggs were stripped manually and artificially fertilized. At 15 h post-fertilization, when fertilized eggs were at the stage of middle blastula, dead and physically damaged eggs were removed using a wide-mouth pipette and only developing fertilized eggs were placed into experimental units.

2.2. Conditions of incubation

Experiments were conducted in water baths equipped with thermoregulators and immersion heaters or coolers. Experimental temperatures of incubation were 15, 19, 23, and 27 °C. There were three replicates for each of the 4 treatments. Positions of temperature treatment replicates were randomized within the water baths. Eggs were transferred and counted using a wide-mouth pipette. Experimental incubation units consisted of 100-ml glass beakers filled with 50 ml sterilized freshwater. Eggs were stocked at a number of 50 eggs per beaker. All temperature gradients were adjusted at an appropriate rate from initial temperature of 19 °C to their final temperatures within 3 hours. Eggs were incubated statically in the beakers under natural light and photoperiod. Fifty percent of the incubation water in each beaker was replaced daily with new sterilized freshwater.