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Effect of incubation temperature on eggs and larvae of lumpfish (*Cyclopterus lumpus*)

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ABSTRACT

Two batches of lumpfish eggs were incubated at three temperature regimes; 1-Ambient seawater 4–6 °C (cold), 2-Ambient seawater for 10 days followed by a gradual increase to 10 °C (gradient), 3-Constant 10 °C seawater (warm). The eggs incubated in cold water had the highest egg mortality (38.5% ± 15.7) and lowest hatching success (46.1% ± 7.2), while the gradient group regime showed highest hatching success (74.9% ± 4.2). Larvae from the gradient regime showed the most synchronized hatching as hatching started at 280 dd (35 days post fertilization (DPF)) and reached the hatching peak the same day with almost 80% of all larvae hatching. Hatching started at 279 dd (28 DPF) in the warm regime, reached a hatching peak (50% of total hatching) at 3 days post hatch (DPH), and ended at 9 DPH. In the cold temperature group hatcing started at 285 dd (63 DPF) and the hatching peak was reached at 3 DPH. Hatching lasted until 13 DPH. Hatched larvae from the cold regime were longest (6.11 mm) and heaviest (5.55 mg), followed by larvae from the gradient (5.71 mm, 4.88 mg) and warm (5.33 mm, 4.37 mg) regimes respectively. Newly hatched larvae from the warm group had the highest occurrence (34.7%) of body deformities compared to 8.9 and 7.6% in the gradient and cold water groups. Studies of organ and tissue histomorphology of hatched and two weeks old larvae did not reveal obvious developmental differences between the groups at these timepoints.

1. Introduction

Cleaner fish, like wrasses (Labridae) and lumpfish (*Cyclopterus lumpus* L.), may represent sustainable solutions for reducing the lice problem in the salmon industry (Treasurer, 2002; Imsland et al., 2014a, 2014b, 2014c, 2015a, 2015b). Due to the wrasses' southern distribution and the fact that their appetite is reduced at low temperature, use of wrasses in the northern parts of Norway is a challenge (Durif, 2015). The lumpfish has a more widespread natural distribution, and is found further north than the northernmost species of wrasses (Davenport, 1985; Durif, 2015). Consequently, the common lumpfish has been suggested as a cold-water cleaner-fish, and initial results are very promising with up to 93–97% less lice infection (adult female lice) in seapens with lumpfish (Imsland et al., 2014a, 2014b, 2014c, 2015a, 2015b).

In nature, from February and onwards in the spring, sexually mature lumpfish return from open waters to spawn at shallow localities in coastal areas (Davenport, 1985; Moen and Svensen, 2004; Durif, 2015). Females spawn in several batches and have relatively high fecundity, laying between 100.000 and 400.000 demersal eggs in total (Brown et al., 1992; Moen and Svensen, 2004). Males guard the egg clutches, each of which can be from several females. The eggs are 1.8–2.6 mm in diameter and can have a variety of colours; pink, orange, yellow, green, brown and red and stick to each other after exposure to saltwater (Davenport, 1985; Davenport and Thorsteinsson, 1989; Moen and Svensen, 2004). The larvae hatch after 40 days at 5 °C (200 day-degrees (dd), Davenport, 1983) and 25 days at 9.8 °C (245 dd, Collins, 1978). Recent data from the lumpfish industry have found egg development time to be nearer 290 to 300 dd.

Several studies show that temperature as a physiological factor has an effect on development and survival of fish eggs and larvae (see e.g. Hansen and Falk-Petersen, 2001; Sund and Falk-Petersen, 2005; Geffen

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et al., 2006; Jonsson and Jonsson, 2014). Fish from temperate zones appear to be more sensitive to temperature changes during early life than juveniles and adults (Rombough, 1997). Furthermore, marine fish embryos are suggested to have four periods particularly sensitive to temperature during development: cleavage, early gastrula, embryo appearance and blastopore closure (Kazuyuki et al., 1988). Few scientific studies exist on temperature tolerances of early life stages of lumpfish. Collins (1978) found that lumpfish eggs incubated at average temperatures of 6.4 °C and 9.8 °C hatched after 31 and 25 days respectively, and eggs incubated at an average temperature of 3.8 °C did not hatch at all. Initial production protocols at one of the pioneer production facilities of lumpfish juveniles in northern Norway (Research and Innovation Centre Kraknes, Tromsø), included egg incubation at both ca. 4 °C and 10 °C. Both temperatures resulted in relatively high hatching percentages, but larvae from eggs incubated at 4 °C hatched less synchronously and the eggs were also suffered from higher loads of microorganisms on the surfaces (Thor Arne Hangstad, Research and Innovation Centre Kraknes, pers. comm.). Studies of effects of incubation temperature are important in order to optimize rearing conditions for successful cultivation of good quality juveniles.

The objective of this study was to investigate how different incubation temperature regimes influenced egg development, mortality, hatching success and early larvae size, deformities and histomorphology of lumpfish larvae.

2. Materials and methods

2.1. Experimental fish and set-up

Sexually mature wild lumpfish were caught by gill nets in Sandnessundet outside Kraknes, Troms County, Norway during late autumn and winter of 2014. Lumpfish eggs were collected from two wild caught females (hereafter called, batch 1 and batch 2) and put in two separate plastic containers. Milt from two wild caught males was then added to both egg batches. One ml of eggs was subtracted from each batch using a syringe and placed on two petri-dishes and the eggs counted to estimate the number of eggs ml^{-1}. Two ml of eggs were then distributed in each experimental incubator (2 l), after having been carefully separated and seawater slowly added. The experimental system used was on flow through water. Each of the incubators in the experimental apparatus was made using 2 l plastic bottles turned upside down and stuck in a styrofoam plate. The bottom of the bottles was removed and a hole drilled in the bottle-cap. A plastic plate with 1.5 mm mesh holes made up the bottom of the incubator. The incubators were set up in three rows of 10, with each row representing a different temperature exposure groups. Two batches of eggs were incubated at three temperature regimes, in five replicates (30 incubators in total, ca. 200 eggs in each):

1. Ambient seawater temperature 4–6 °C (Cold, C), average 4.7 °C;
2. Ambient seawater temperature for the first 10 days followed by gradual increase of ~1.3 °C/day over 4 days to reach 10 °C (Gradient, G); and
3. Constant 10 °C seawater (Warm, W).

Eggs from two replicates from each temperature regime and batch were sampled during the incubation period, while three replicates from each regime and batch were left undisturbed until hatching. From the unsampled incubators, 50 larvae were kept alive and fed with 0.1–0.2 mm pellets (AgloNorse Extra, Tromsø Fiskeindustri AS, Tromsø, Norway) for 2 weeks after hatching to study possible late effects on larval quality. The larvae were expected to hatch around 280 day-degrees (dd); at 260 day-degrees a cap with a 0.5 mm mesh was put on the water outlet of the incubators to avoid larval escape. The hatched larvae were kept in containers similar to the incubators, but with switched water inlet and outlet. The water temperature in the larval containers was 10 °C. Oxygen saturation was stable both during incubation and after hatching. During incubation, the average oxygen saturation was 109.2% in the warm groups 108.3% in the gradient groups and 103.1% in the cold groups. After hatching, it was 108.8%, 110.1% and 105.5% in the respective groups. Salinity was stable at 33.5 ppt during the experimental period.

Temperature and oxygen saturation levels were recorded daily using an OxyGuard Handy Alpha (Sterner Aquatech, Ski, Norway). The temperature was measured in one incubator from every temperature treatment. Originally, the water flow in each incubator was set to approximately 2 l min^{-1}. Light was on during working hours, from 08:00 h to 16:00 h every day. Cleaning of the incubators was done if the accumulation of debris became visible. The eggs were removed using a plastic spoon and a plastic pipette and cautiously tranfered to a bucket with seawater at the respective temperature regime.

The experiment was carried out at Kraknes Research Station (Tromsø, Norway) between 11 March and 30 May 2015. Larval measurements and histological preparations and analyses were carried out in the laboratory at the Department of Arctic and Marine Biology, University of Tromsø.

The experiment described has been approved by the local specialist responsible for laboratory animal science, under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority. The experiment has thus been conducted in accordance with the laws and regulations controlling experiments on live animals in Norway, i.e. the Animal Protection Act of 20 December 1974, No. 73, chapter VI sections 20–22 and the Animal Protection Ordinance concerning Biological Experiments in Animals of 15 January 1996.

2.2. Sampling of eggs

Fertilization percentage and average egg diameters were calculated under a stereomicroscope (Leica WILD M10, Wetzlar and Mannheim, Germany) by taking 15 eggs from each sampling-incubator. To study the development, abnormalities and mortality of the eggs incubated at different temperatures, samples (minimum $N = 20$ from each temperature group) were taken throughout the incubation period from the sample incubators. In the first two days, egg samples were taken twice a day. From day three and onwards, sampling was done every second or third day until hatching occurred. A minimum of five eggs were taken from each sampling incubator (i.e. 10 eggs from each batch, and thus 20 from each temperature regime). The egg samples were sampled using a plastic spoon, lifting the eggs to the surface and then carefully separating them. The eggs were then were put into glass vials with water from the incubator until they were studied under the stereomicroscope. The eggs were photographed through the ocular of the stereomicroscope using a mobile phone camera (iPhone 4 and iPhone 6, Apple Inc., CA., USA) and then stored on 4% buffered formaldehyde in case additional examinations were needed. Number of abnormal and dead embryos were estimated from each sample.

2.3. Sampling of larvae

At hatching 30–50 larvae from each of the triplicate incubators of both batches were moved to feeding containers to be kept alive for 2 weeks after the hatching peak. Larvae stuck on the water outlet or swimming in consecutive circles were excluded. All other larvae, except the 50 larvae transferred to the feeding containers, were killed with an overdose of anaesthetics (™[18]Finquel, 150 mg l^{-1}) and stored on 4% buffered formaldehyde to be examined later.

The larvae were hand feed during working hours at approximately 08.00, 10.00, 13.00 and 15.00 h. They were given approximately 1 cl of pellets (AgloNorse Extra, Tromsø Fiskeindustri AS, Tromsø, Norway) each time. Half an hour after the last feeding, the excess feed accumulated on the bottom and bacterial growth was rinsed away. Two weeks after peak hatching, the larvae kept in the containers were taken