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A closed recirculating aquaculture system for artificial seed production of the European eel (*Anguilla anguilla*): Technology development for spontaneous spawning and eggs incubation

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ABSTRACT

The objective of the present study was to obtain spontaneous reproduction in captivity of the European eel (*Anguilla anguilla*) by using a new closed recirculating aquaculture system provided with spawning and incubation chambers. The influence of two levels of water-flow rates (Low-Flow: 0.8 ± 0.05 L/s and High-Flow: 2.4 ± 0.05 L/s) on the spawning, fecundity and egg quality was also investigated. For this purpose 12 silver eel females were induced with increasing doses of carp pituitary extract (10, 20, 30 and 40 mg CPE/kg BW). Twenty-four hours after the last CPE injection, each female ovulation was induced by injecting a DHP-solution and then transferring them to a new closed recirculating aquaculture system, where they were maintained for 16 h with spermiating males (sex ratio 4/1) in order to obtain spontaneous reproduction. The reproduction was tested with 6 females in Low-Flow rate conditions and 6 females in High-Flow rate conditions.

The results showed that the designed closed-loop system made it possible to carry out a more spontaneous reproduction for more than 80% of the females that underwent standardized gonadotropic treatment and favored the automatic and complete transfer of the eggs to the hatchery. The results also point out that high or low water current conditions in the tank do not hinder the mating and the emission of gametes by the breeders, but the High-Flow rate in the two incubation chambers showed unsuitable hydrodynamic conditions for embryonic development resulting in a constant loss of viable eggs which reached a mortality of 100% among females with the highest incubation density.

1. Introduction

The eel is one of the species with the highest market for freshwater aquaculture in the world. To date, all the seedlings for cultivation are wild glass eels or elvers collected in the estuarine waters. However, over the last several decades, natural stocks of eels, especially the commercially valuable temperate species, European eel (*Anguilla anguilla*), American eel (*Anguilla rostrata*) and Japanese eel (*Anguilla japonica*), have decreased markedly (Casselman, 2003; Dekker, 2003; Tatsukawa, 2003) due to overfishing, environmental destruction, oceanographic/climatic changes and other as yet unknown factors (EELREP, 2006; van Ginneken and Maes, 2005).

The European eel was recently included in the Red List of the IUCN, as a Critically Endangered Species; as a consequence a short fishing season, a minimum capture size, larvae protection and an implemented trade regulation have been imposed to protect this species. Unfortunately the application of the measures has not reduced the risk of extinction (Mordenti et al., 2012a).

One effective solution to the issue would be to set artificial reproduction techniques for the production of seedlings for aquaculture so that to reduce the demand of wild glass eels.

At present, the only way to obtain sexually mature eels is to artificially induce sexual maturation in silver eel females using repeated injection of carp (CPE) or salmon (SPE) pituitary extract and a final injection of 17,20β-dihydroxy-4-pregnen-3-one (DHP) while the males are induced following standard protocol with hCG injection (Ohta et al., 1996; Palstra et al., 2005; Burgerhout et al., 2011). Males and females are then hand stripped and milt and eggs are separately collected (Burgerhout et al., 2011).

Concerning European eels, studies have focused on the successful protocols based on hormone injection dose and timing and on the definition of optimal environmental parameters (water temperature, water salinity, and photoperiod) (Durif et al., 2006; Mordenti et al., 2012a) in order to obtain gametes by stripping for artificial fertilization; while laboratory experiments that have shown spontaneous spawning of artificially matured European eels in captivity remain elusive.

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The objective of the present study was to obtain spontaneous reproduction in captivity of the European eel (*A. anguilla*) by using wild female silver eels. For this purpose a new closed recirculating aquaculture system provided with spawning and incubation chambers was tested.

In this study the influence of two levels of water-flow rates on the spawning, fecundity and egg quality was also investigated.

2. Materials and methods

2.1. Animal

Wild female eels were caught using traditional "*lavoriero*" (downstream trap) in the brackish water lagoon near the sluices of the North Adriatic Sea (*Valli di Comacchio*, Emilia-Romagna, Italy).

Larger female eels (>500 g body weight – BW) were selected (n 23) at the catch and then transported to the laboratory where they were measured and sampled to obtain an external indicator of their maturation state (silver index – SI) (Durif et al., 2005; Di Biase et al., 2012). Only 12 eels with a maximum level in SI (V) were selected and used for reproduction. The animals were marked individually by inserting fish-tags (FLOY TAG Mod Floy T-Bar Anchor) and maintained in starvation for the entire duration of the trial.

At the same time, cultivated male eels (n = 25 fish, 104–212 g in BW) reared in freshwater were purchased from a commercial eel supplier and they were gradually acclimated to sea water over 7 days.

All the subjects were kept in two 700 L tanks (one with females and one with males) connected to a recirculation system and maintained completely in dark conditions (-0.04×10^3 lx at the bottom of the tank without water) (Mordenti et al., 2012a) and seawater condition (salinity 32‰), up to a complete gonadal maturation. A seawater controlled temperature system was set at 15.5 ± 0.5 °C.

The females received intramuscular injections once a week with carp pituitary extracts (CPE) at a dosage of 10 mg/kg BW (1st–3rd week), 20 mg/kg BW (4th–6th week), 30 mg/kg BW (7th–9th week) and 40 mg/kg BW (10th–final maturation) (Mordenti et al., 2012a).

Males were induced following standard protocols (Ohta et al., 1997; Palstra et al., 2005) and started spermiation after a 5-week treatment. Just before fertilization, the males received a booster hCG injection to reactivate spermiation (Burgerhout et al., 2011).

2.2. Induction of maturation

Twenty-four hours after the last CPE injection (increase in female BW around 120%) (Mordenti et al., 2012b), ovulation was induced by injecting a DHP-solution (2 mg/kg BW dissolved in 95% ethanol and diluted with buffered saline solution) (Palstra et al., 2005) in 10 different areas of the ovary.

After the DHP injection, each female was weighted then transferred to a new closed recirculating aquaculture system, where the seawater temperature was raised to 20 ± 0.5 °C (Dou et al., 2008), and maintained for 16 h with spermiating males (*sex ratio* 4/1) in order to obtain spontaneous reproduction. Sperm motility was checked and only males with at least 50% sperm motility (continuous activity of >50% of spermatozoa) were used for the reproduction (Burgerhout et al., 2011).

2.3. System description

A new closed recirculating system is shown in Figs. 1 and 2. This system consisted in two fish-rearing tanks (0.47 m^3/tank; water volume 0.43 m^3/tank; water surface area 0.93 m^2/tank), a foam separation tank (0.05 m^3) (protein skimmer) and an biological filter (0.21 m^3) contained plastic porous balls (0.15 m^3) (Fig. 1). The total amount of water in this system was 1.12 m^3 and was transported by a circulating pump (1.1 kW; max delivery 16,000 L/h). The rearing water reached the biological filter and the protein skimmer with an up-flow style (natural falling water) and the treated rearing water was returned to the rearing tank by a circulating pump. The system was also provided with a thermal regulation system (compact cooling equipment, 1.4 kW) to adjust the rearing water conditions (20 ± 0.5 °C), a UV-sterilizer lamp (max delivery 500 L/h, 36 W), an ozonizer (250 mg O_3/h) and an aerator (electromagnetic air compressor; delivery 170 L/min, pressure 34 kPa, 150 W) (Fig. 1).

The core of the system was the reproduction tank, which was made of four components: one spawning chamber (240 L), two incubation chambers (52 L/cad) and one outlet chamber (90 L).

The spawning chamber is connected to the incubation chambers through two 5-mm lengthwise splits located on the top side of the dividing panel. Two pipes allow the water to enter from the base of the spawning chamber (inlet tubes 1) in order to guarantee the water exchange and promote, once the gametes are released, the entrance of the eggs into the incubation chambers (Fig. 2). The water exchange rate is regulated by a valve (valve 1) (Fig. 2).

The incubation chamber has a cylindrical base and a tube on the top (inlet tube 2) and it is provided with inlet jets that produce a circular revolving current: an outlet mesh screen (200 mm × 200 mm; 300 μm diameter and exchangeable) is located on the dividing panel between the incubation and the outlet chambers; inlet jets push water across this mesh screen (Fig. 2). The water flow and the current speed in the incubation chamber are calibrated by the inlet tube valve of the spawning chamber (valve 1) and by the hatchery water pipe valve (valve 2) (Fig. 2).

The system described has a vertical configuration and is inspired by the one originally developed by Greve (1975) (called "planktonkreisel") for the maintenance of planktonic animals, later modified by various researchers for larval *Palinurus japonicas* culture (Matsuda and Takenouchi, 2007) and for rearing eel leptocephali (Okamura et al., 2009).

The overall tank water level is determined in the outlet chamber by adjusting the outlet tube height; at this point the water is discharged into the filtration system therefore adjusting the environmental parameters (Fig. 2). The water outflow takes place by overflowing, thanks to a collecting tray (440 mm × 100 mm) positioned at the top of the outlet tube, whirls in the outlet chamber were so avoided.

Finally, 3 covers positioned on the tank had the purpose of maintaining the conditions of darkness and trapping the breeders inside the tank.

2.4. Reproduction

During the reproduction trial, two levels of fixed water-flow rate, 0.8 ± 0.05 L/s (Low-Flow) and 2.4 ± 0.05 L/s (High-Flow), were achieved in incubation chamber by adjusting the valve 1 (0.5 ± 0.05 L/s in Low-Flow and 1.5 ± 0.05 L/s in High-Flow) and valve 2 (0.3 ± 0.05 L/s in Low-Flow and 0.90 ± 0.05 L/s in High-Flow). The reproduction was tested with 6 females in Low-Flow rate conditions and 6 females in High-Flow rate conditions.

The water-flow upper limit rate (2.4 ± 0.05 L/s) is the common rate practiced in two 700-L tanks used during the hormonal treatment and the lower one (0.8 ± 0.05 L/s) is an estimated minimum flow rate to minimize the current while maintaining system minimal functionality.

After 16 h all the breeders were removed from the spawning chamber.