488. Five years of optimizing the assisted reproduction protocol for European eel: what worked and what didn't?

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Abstract

The production cycle of the European eel needs to be closed in order to supply aquaculture with juvenile glass eels and to alleviate fishery pressure on the natural population, thereby contributing to its recovery. Currently, we are able to produce larvae batches three times per week. Over the past five years we have executed experiments aiming to condition glass eels into high quality brood stock females and optimize the artificial reproduction protocol. Feminization of young juveniles contributes to shortening the generation time at least 5-fold. Simulated migration induces early sexual maturation. Steroid implants containing 17 methyltestosterone (17MT) and 17β estradiol (E2) induce the more advanced maturation stages and shorten the stressful period of weekly carp pituitary extract (CPE) injections to fully mature females. Instead of using CPE, stable eel-specific recombinant gonadotropins have been successfully applied to produce eel larvae.

Introduction

The life cycle of the European eel is fascinating. Eels can reside in the continental fresh waters for decades. Then, at a largely unspecified moment in autumn, they stop feeding, start swimming and only then does puberty commence. Still in pre-pubertal condition, the eel disappears into the ocean, and swims for up to 6,000 km. When arriving at the spawning grounds in the Sargasso Sea, each female eel consists of approximately 50% of ovarian tissue and is ready to spawn several million eggs. It is generally assumed that parents die shortly after spawning. However, most of the available knowledge comes from the laboratory (e.g. Palstra *et al.*, 2020), as there are no records of maturing or spawning European eels in the ocean, nor are there tracks of their carcasses. Since the eel cannot currently be propagated and raised to the juvenile glass eel stage, each large European eel, including those from the farm, once hatched in the Sargasso Sea.

In 2016, the Eel Reproduction Innovation Centre (EELRIC) was launched, as a collaborative initiative of Wageningen Livestock Research with the Dutch eel sector, united in the sustainable eel foundation DUPAN. The major aim of EELRIC is to close the production cycle of the European eel in order to supply eel aquaculture with juvenile glass eels and to alleviate fishery pressure on the natural population, thereby contributing to its recovery. Now, 5 years later, we are able to produce larvae batches from different females three times per week although larval quality is still often poor as evidenced by high mortalities during the first week and occurrence of deformities. Over the past five years we have executed many experiments aimed to condition glass eels into high quality brood stock females and optimize the artificial reproduction protocol. An overview is presented of the methods that worked and those that didn't.

Materials & methods

Feminization was applied as developed for Japanese eel by Chai *et al.* (2010) and involved feeding with E2 coated pellets for 5-7 months during the early elver stage. The simulated migration procedure was originally described by Mes *et al.* (2016; Figure 1). Eels were subjected to ~3,000 km simulated migration, and in combination with a single CPE injection (20 mg/kg), or a dopamine antagonist implant of eticlopride (Jolly *et al.*, 2016). In contrast to the routine protocol of weekly human chorionic gonadotropin (hCG) injections,

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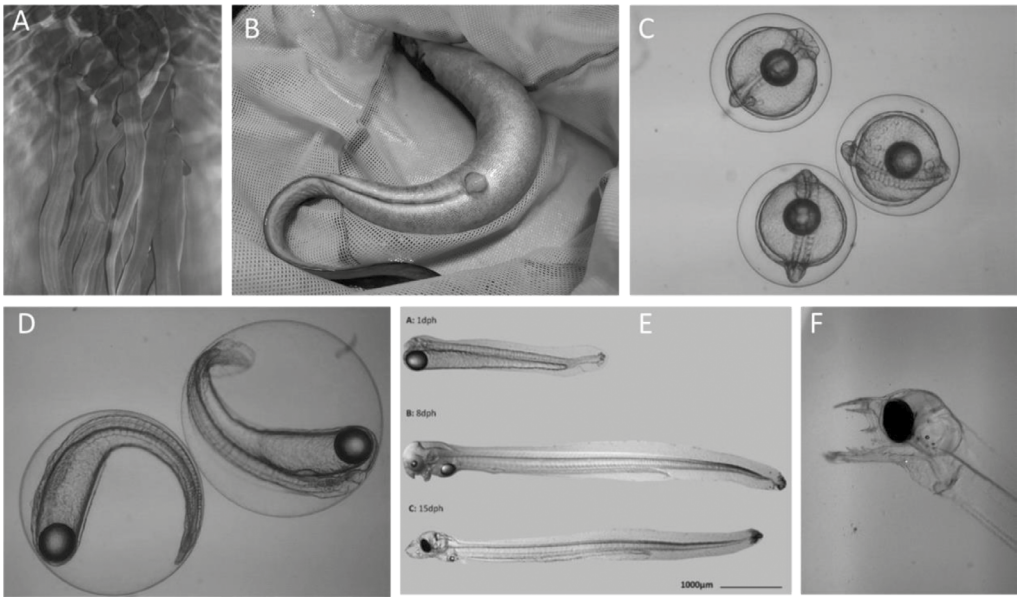

Figure 1. Photo panel showing (A) eels swimming during simulated migration; (B) mature eel with extruding egg bulb; (C) early eel embryos; (D) late eel embryos just before hatching; (E) eel larvae at 1-, 8- and 15-days post hatching (Jéhannet *et al.*, 2021); (F) eel larva at 15 days post hatching ready to initiate exogenous feeding.

males were matured by a single hCG injection (1000 IU; Kahn *et al.*, 1987) followed by a 2nd injection 24 h before stripping. Females were matured by weekly CPE injections (20 mg/kg) followed by a CPE booster at ~10% body weight increase and a single 17α,20β-dihydroxy-4-pregnen-3-one (DHP; 2 mg/kg) injection at ~20% body weight increase (Palstra *et al.*, 2005). The 17MT (5 mg), E2 (2 mg) and the combined 17MT+E2 implants were tested as pre-treatments for the weekly CPE injections (Thomson-Laing *et al.*, 2019). Three experiments have been executed testing weekly injections with recombinant FSH (recFSH: 6 or 12 µg) and LH (recLH: 10 or 20 µg) replacing CPE treatment.

Results

Feminization provides 99% females of which 90% reach 300 g in 12-30 months and can then be used as brood stock.

Simulated migration makes these feminized eels silver (larger eyes reflecting the oceanic phenotype) and induces early sexual maturation (increased Gonadosomatic Index – GSI). Simulated migration in combination with a single CPE injection increased the GSI further but not up to values indicating the onset of vitellogenesis (yolk deposition in the oocytes). Eticlopride was expected to lift the dopaminergic inhibition of gonadotropin production and release but did not show any additional effect on sexual maturation to simulated migration.

Both 17MT and E2 implants significantly increased GSI and oocyte diameter and decreased the number of weekly CPE injections required to mature the eels. The combined implants worked synergistically in advancing vitellogenesis (GSI of 6.6).

Eels that were treated with recFSH followed by recLH matured after 15-22 weeks, similar to eels that were treated with recFSH followed by CPE treatment, but different from eels treated with CPE of which most matured after 7-9 weeks. Larvae were produced from eels of all treatments (Figure 1).

Discussion

The feminization procedure also works for European eel and generation time is shortened by at least 5-fold. Simulated migration can be applied for the natural triggering of early sexual maturation but does not stimulate the more advanced maturation stages, even in combination with a single CPE injection or dopamine antagonist implant. 17MT+E2 implants do stimulate development to more advanced, vitellogenic stages of oocyte development. These implants can be considered as a pre-treatment to reduce the period of stressful weekly hormonal injections although 17MT implant treatment, followed in time by E2 treatment, may result in a more natural progression of steroid-mediated effects. For the first time, European eel larvae were produced with recombinant gonadotropins, but dose and durations still need optimization.

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