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Ontogeny and growth of early life stages of captive-bred European eel

Sune Riis Sørensen [a,*], Jonna Tomkiewicz [a], Peter Munk [a], Ian A.E. Butts [a], Anders Nielsen [a], Peter Lauesen [b], Christian Graver [c]

[a] National Institute of Aquatic Resources, Technical University of Denmark, Charlottenlund, Denmark
[b] Billund Aquaculture Service Ltd, Billund, Denmark
[c] Danish Aquaculture Organisation, Silkeborg, Denmark

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ABSTRACT

Captive breeding of European eel, *Anguilla anguilla* is challenged by the complex hormonal control of Anguillid eel reproduction and the distinctive ontogeny of the leptocephalus larvae that are unique to the Elopomorph superorder. Recent experimental research has succeeded in the production of viable eggs and larvae of European eel, providing the basis for studies on early life stages of this species in captivity. In this study, we describe and illustrate morphological characteristics of eggs, embryos, and larvae from fertilization to termination of the yolk sac stage and provide a comparison with additional commercially important eel species. Furthermore, we model growth during the critical first phase in larval ontogeny, i.e. the yolk sac stage, and test for maternal effects. The eggs of *A. anguilla* typically have numerous oil droplets that coalesce into a single large oil droplet, while the zygote forms a large perivitelline space, reaching an egg diameter of 1.45 ± 0.12 mm at 3.0 to 3.5 h post fertilization. Embryonic development from fertilization to larval hatch lasted ~46–48 h at 20 °C with the larvae emerging in a relatively undeveloped stage with a protuberant yolk sac. During the period of yolk and oil absorption, the larvae undertook significant changes in head and body morphology. At the completion of yolk sac absorption, the largely transparent larvae had a set of protruding teeth, pigmented eyes and tail, and a simple alimentary tract. Larvae appeared capable of feeding at ~12 days post hatch at 20 °C, and were able to survive another ~10 days without feeding. Larval length approached an asymptotic maximum of 6.8 mm around day 10 in non-fed larvae. Larval batches from different maternal origins varied in yolk sac size and the extent of yolk sac resources influenced larval size at the end of the yolk sac stage. The ontogenetic description presented here fills a gap in knowledge about the yet undiscovered early life stages of native European eel, which can provide a framework of reference for the development of hatchery technology. Such progress is urgently needed for a self-sustained aquaculture of this high-value and critically endangered species.

Statement of relevance: European eel is a high-value species in aquaculture, however, production is presently hampered by reliance on wild caught fry. Captive production of glass eels will reopen markets in Europe and Asia, benefiting European eel producers. The results presented here document recent progress within assisted reproduction and larval culture of this species in aquaculture and aid establishing hatchery technology of this species.

1. Introduction

Production of Anguillid eels for aquaculture requires a supply of wild-caught glass eels as the life cycle for this high-value commercial species has still not been closed in captivity. The establishment of eel hatchery practise faces a number of obstacles, as little is known about their natural reproductive processes and early life history stages. Firstly, Anguillid eels do not reproduce naturally in captivity. This is caused by a pre-pubertal neuroendocrine blockage, where dopamine exerts an inhibitory control on gonadotropin release (Vidal et al., 2004; Dufour et al., 2010). Therefore, fundamental treatment schemes to induce maturation in eel involve repeated hormonal injections (Dufour et al., 2003; Rousseau et al., 2013), which may compromise gamete quality and limit production. Secondly, culture techniques and technology for eels are still in their infancy as it is a challenge to sustain development and ongrowing of the distinct leptocephalus larvae, unique to the Elopomorph superorder that comprises the Anguillid eels (Greenwood et al., 1966).

In order to reproduce, the European eel (*Anguilla anguilla*) crosses the Atlantic Ocean, swimming up to 6000 km to reach the Sargasso Sea area (Schmidt, 1922). Until now no spawning eels have been caught in the Sargasso Sea, hence, the spawning sites have solely been identified by the distribution of newly hatched larvae in this area during March–April (Munk et al., 2010). While the ovaries and testes are undeveloped at the onset of eel migration, the process of natural

* Corresponding author.
E-mail address: srs@aqua.dtu.dk (S.R. Sørensen).

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maturation is unknown, and information on gametogenesis and the pre-leptocephalus stages of European eel relies exclusively on observations during the captive propagation experiments.

Among Anguillid species, reproduction and early stage characteristics are most intensively studied for Japanese eel, *Anguilla japonica*. Assisted reproduction protocols were established for this species in the 1970s (Yamamoto and Yamauchi, 1974; Yamauchi et al., 1976; Ohta et al., 1996, 1997; Tanaka et al., 2001), which led to the first production of glass eels (Tanaka et al., 2003; Kagawa et al., 2005) as well as second-generation captive hatchings (Okamura et al., 2014; Tanaka, 2015). In addition, experimental propagation of American eel, *Anguilla rostrata*, as well as two species of eel from New Zealand, *Anguilla diffenbachii* and *Anguilla australis*, has led to viable eggs and hatched larvae (Lokman and Young, 2000; Oliveira and Hable, 2010).

In the 1970s, experiments using assisted reproduction of European eel first produced fertilized eggs (Boëtius and Boëtius, 1980) and two decades later, hatched larvae that survived up to 3.5 days post-hatch (DPH) (Bezdenezhnykh et al., 1983; Prokhorchik, 1986; Prokhorchik et al., 1987). Fertilized eggs and hatched larvae in limited numbers were produced by Pedersen (2004), adopting the Japanese protocol (Ohta et al., 1997). Recently, advances in assisted reproduction technology and offspring culturing techniques, have enabled repeated production of large batches of viable eggs and larvae that reach the first-feeding stage (Tomkiewicz, 2012; Tomkiewicz et al., 2013; Mordenti et al., 2013). This progress has been aligned with the development of techniques for artificial fertilization (Peñaranda et al., 2010; Sørensen et al., 2013; Gallego et al., 2013; Butts et al., 2014), embryonic incubation (Sørensen et al., 2014, 2015), and early larval rearing (Politis et al., 2014), enhancing offspring survival.

Eel eggs share characteristics of marine pelagic spawners, such as a large oil droplet, which in Anguillids has an unusually high amount of lipids (Heinsbroek et al., 2013). Additionally, eel eggs have a large perivitelline space, which is sealed by a smooth distended chorion (Sørensen et al., 2015). Together, these characteristics result in a relatively large egg, as compared to other marine pelagic teleosts (Ahlstrom and Moser, 1980). While embryonic development under controlled conditions has been described for Japanese eel (Yamamoto, 1981; Okamura et al., 2007; Ahn et al., 2012) information is more fragmented for the European eel (Prokhorchik et al., 1987; Pedersen, 2004). Thus, there is a need for a comprehensive description of embryonic development into the yolk sac stage.

Anguilliform larvae at hatch feature a yolk sac that extends ventrally along the entire abdomen and an underdeveloped head. The head differentiates during the yolk sac stage, as illustrated for the Japanese eel (Miller, 2009) and European eel (Bouilliart et al., 2015). Unique to the leptochephalus larvae of the superorder Elopomorpha, is their distinctive morphology having a high surface to body ratio formed by its laterally compressed shape (Inoue et al., 2004; Miller, 2009). Their body is mainly composed of a transparent gelatinous matrix rich in glycosaminoglycans (GAGs) that provide support for the unique body structure (Pfeiler, 1999; Pfeiler et al., 2002). Based on available information, European eel larval ontogeny appears to correspond to the larval development of Japanese eel, especially during the first 3 to 4 DPH considering culture temperature differences. However, no description is available for European eel larvae beyond ~4 DPH (Palstra and van den Thillart, 2009) until the stage after yolk sac absorption when information is available from eel larvae caught in the Sargasso Sea (e.g. Munk et al., 2010). The leptocephalus larvae are found across a wide area of the Atlantic Ocean when they drift towards the European coasts from the spawning areas in the Sargasso Sea (Schmidt, 1922). Until now spawning eels have never been observed in the Sargasso Sea, but the long spawning migration of approximately 6000 km is feasible (Van Ginneken et al., 2007) and spawning appears to take place here while newly hatched larvae emerge in the area during March–April (Munk et al., 2010).

In order to develop hatchery techniques and technology for European eel, knowledge about ontogenetic development is needed for this species, especially for the "critical" early life history stages from egg to first feeding. With aforementioned advances in assisted reproduction, it is now possible to acquire this knowledge. Here, we document, for the first time, ontogenetic development and growth in terms of body length increments of European eel under controlled conditions from fertilization through to first feeding. Specifically, we describe key morphological characteristics, fit growth models to length increase and analyses growth patterns as well as investigate potential maternal effects on larval characteristics and development. The overall goal is to provide a framework for benchmarking European eel breeding and offspring culture protocols.

2. Materials and methods

2.1. Broodstock origin and experimental production of offspring

Eggs originated from farmed and wild-caught female broodstock, and milt from farmed male broodstock. The farmed broodstock were raised at a commercial eel farm: Stensgård Eel Farm A/S in Jutland, Denmark. Mean (±SD) total length, ±0.1 cm (L_T) and body weight, ±1 g, (W_B) of the female broodstock were 75.1 ± 3.7 cm and 878.1 ± 120.1 g, respectively ($n = 35$), while length and body weight of the males were 36.9 ± 2.2 cm and 100.1 ± 13.3 g, respectively ($n = 60$). Wild-caught female broodstock were obtained from Lake Vandet, a freshwater lake in northern Jutland, Denmark. Mean (±SD) L_T and W_B of the wild-caught females were 68.2 ± 6.9 cm and 662.6 ± 227.5 g, respectively ($n = 9$).

The broodstock eels were transferred to an experimental facility of the Technical University of Denmark, where they were kept in 300 L tanks equipped with a closed recirculation system at a maximum density of 10 females per tank and ~30 males per tank. Here, broodstock were kept under a continuous saltwater flow rate of 95 to 100 L min^{-1}. The saltwater was local freshwater adjusted to ~36 psu using Tropic Marin® Sea Salt (Dr. Biener Aquarientechnik, Wartenberg, Germany). While in captivity, water temperature was maintained at ~20 °C. At the onset of experiments, broodstock fishes were anaesthetized (ethyl p-aminobenzoate, 20 mg L^{-1}; Sigma-Aldrich Chemie, Steinheim, Germany), tagged with a passive integrated transponder, and L_T and W_B were recorded.

2.2. Induction of gametogenesis and final maturation

Females were matured by weekly injections of salmon pituitary extract (SPE; Argent Chemical Laboratories, USA; variable dose 12.5–25 or constant dose 18.75 mg kg^{-1} body weight) and males by weekly injection of human chorionic gonadotropin (hCG, Sigma–Aldrich Chemie, Steinheim, Germany; 150 IU per male) according to Tomkiewicz (2012). Final follicular maturation was induced using the maturation inducing steroid, 17α,20β-dihydroxy-4-pregnen-3-one (DHP crystalline, Sigma–Aldrich Chemie, Steinheim, Germany) (Tomkiewicz, 2012). Female broodstock were euthanized after stripping and male broodstock at the end of the experiment.

2.3. Rearing water, gamete extraction, fertilization, and incubation

Natural North Sea seawater (~32.5 psu) was filtered using a 0.8 μm cartridge filter (CUNO 3M®, St. Paul, MN, USA) and salinity adjusted to 36 psu (hereafter referred to as FSW). The FSW was kept at 20 ± 0.5 °C and used for egg activation, fertilization, incubation, and larval culture.

For each female, milt was collected from three to four males 2 h prior to fertilization by applying gentle abdominal pressure. Milt was diluted in sterile-filtered (0.2 μm, Nalgene® vacuum filtration system, Thermo Fisher Scientific Inc., Waltham, MA, USA) artificial seminal plasma