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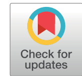
Oocyte and egg quality indicators in European eel: Lipid droplet coalescence and fatty acid composition



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ABSTRACT

During European eel assisted reproduction, timely administration of hormones that induce oocyte maturation and ovulation is a major factor influencing subsequent egg quality. This treatment commonly comprises one injection of fish pituitary extract (PE) as a primer followed by a 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) injection. In this context, the present study aimed at optimizing timing of the dual hormone administration by applying a lipid droplet-based oocyte maturation scale, previously developed for Japanese eel to determine the maturational status of each female. Using wild-caught female eels, the potential effect of female size, egg fatty acid composition and dry weight on egg quality was also analyzed. Larval survival at 3 days post hatch was used to differentiate High- and Low-quality egg batches. Results showed that lipid droplet diameter was significantly smaller in High-quality eggs than in Low-quality egg batches, indicating that females producing High-quality eggs received the PE primer and DHP generally at an earlier developmental stage than those producing Low-quality batches. These results confirm that oocyte lipid droplet diameter is a useful indicator of female maturational status for optimization of induction of oocyte maturation and ovulation in European eel. Additional parameters, including female size, egg fatty acid composition and dry weight, were similar between high and low quality egg batches. This insight regarding the fatty acid composition of eggs obtained from wild-caught female eels may help advancing the development of tailored diets for increased reproductive success of farmed broodstock.

1. Introduction

European eel (*Anguilla anguilla*) aquaculture presently relies on wild-caught juveniles. Therefore, the development of hatchery technology for aquaculture production is in progress with focus on producing large quantities of viable offspring. However, sexual maturation in eel does not occur spontaneously in captivity due to a strong dopaminergic inhibition (Dufour et al., 1988; Vidal et al., 2004) and low gonadotropin synthesis and release (Dufour et al., 1983). Gonadal development is therefore commonly induced using exogenous hormones adopting a protocol described by Ohta et al. (1996) for Japanese eel with some adaptations to European eel. Typically, hormonal treatments consist of repeated weekly injections of salmon or carp pituitary extracts (SPE or CPE) for females and human chorionic gonadotropin in males. Additionally, in female eels, oocyte maturation and ovulation is induced applying a priming dose of PE followed by provision of a maturation-inducing hormone (MIH), commonly 17α, 20β-dihydroxy-4-pregnen-3-one (DHP). Despite significant progress in assisted reproduction techniques (Butts et al., 2014; Di Biase et al., 2017; Mordenti et al., 2013; Palstra et al., 2005; Pedersen, 2003, 2004; Tomkiewicz, 2012), sub-optimal final maturation treatment of female eels frequently lead to unsuccessful ovulation, challenging the successful production of viable eggs.

Application of biomarkers as indicators of egg viability is a useful tool in aquaculture to optimize resources and to better understand the underlying mechanisms that determine high egg and larvae quality. In case of the Anguillid eels, the maturational status and developmental capacity of follicles in the ovary at induction of maturation and ovulation is an important factor determining the subsequent egg quality. To identify the timing for inducing oocyte maturation and ovulation, a common practice is to assess ovarian maturational status through the female weight increase followed by ovarian biopsies to assess oocyte

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maturational stage. In Japanese eel (*Anguilla japonica*), oocyte maturation and ovulation have been induced successfully based on body weight increase and oocyte diameter (Kagawa et al., 1995; Ohta et al., 1996). However, the same protocol applied to European female eels resulted in low reproductive success, with a few hatched larvae, embryonic malformation and high mortality (Pedersen, 2003, 2004). In species with ooplasm lipid formation, like the eel, a common feature of oocyte cytoplasmic maturation is the coalescence of numerous small lipid droplets that after ovulation form one or a few large lipid droplets (Kagawa et al., 2013; Lubzens et al., 2010; Lubzens et al., 2017). These cytological changes may serve as biomarkers for assessment of oocyte maturational status in relation to assisted reproduction procedures. For Japanese eel, a lipid droplet-based oocyte scale has been developed and proven useful as a quantitative measure to evaluate oocyte maturational status and determine the optimal timing of induction of oocyte maturation and ovulation (Unuma et al., 2011). In European eel, the determination of oocyte developmental stage is presently based on a seven-stage scale developed by Palstra et al. (2005), which focuses on the position of the migratory germinal vesicle, lipid droplet size and general appearance of the oocytes. However, high variability on the appearance of developing follicles and the lack of correlation with subsequent egg viability calls for validation of this methodology.

Another common factor influencing egg quality in fish is the nutritional status of the female, affecting among other, egg dry weight, total lipid and fatty acid composition (Izquierdo et al., 2001). In European eel, egg production and egg viability is generally higher in wild-caught female silver eels compared to cultured female eels (Tomkiewicz, 2012). By experiencing a natural feeding regime and naturally starting the spawning migration, wild-caught female silver eels should be capable to produce eggs containing the nutritional requirements for normal embryonic and yolk-sac larval development and survival. Therefore, differences in egg quality between wild and cultured broodstock may arise due to differences in female pre-spawning nutrition, in particular fatty acid composition, as it has been observed in e.g. black sea bass (*Centropristis striata*) (Seaborn et al., 2009) and Atlantic cod (*Gadus morhua*) (Lanes et al., 2012). In European eel, the effect of maternal dietary fatty acid composition on egg composition and quality has shown for farmed eels on different diets (Støttrup et al., 2013, 2016). Moreover, the fatty acid composition of broodstock diet and essential fatty acid content significantly influence ovarian development (da Silva et al., 2016) and egg quality in both Japanese and European eel (Furuita et al., 2003, 2006; Heinsbroek et al., 2013; Støttrup et al., 2016). Investigating the fatty acid composition of eggs from wild-caught female eels can add new and valuable information contributing to improve the reproductive success of farmed broodstock and assessment of egg quality validated through larval viability.

The aim of this study was to identify and evaluate egg quality indicators in European eel with focus on oocyte characteristics at the time of hormonal administration while considering influences of female weight and length as well as egg dry weight and fatty acid composition. As indicators of egg quality, we determined fertilization and hatching success, larval survival and larval longevity. Larval survival at 3 days post hatch (dph) was used as quality criterion for egg quality classification. This is an early estimate of larval viability reflecting the influence of hormonal treatments, while the effects due to rearing conditions are minimized (Kjørsvik et al., 1990).

2. Material and methods

2.1. Ethics statement

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC). Eel experimental breeding protocols were approved by the Animal Experiments Inspectorate (AEI), Danish Ministry of Food, Agriculture and Fisheries (permit number: 2010/561-1783). All efforts were made to minimize animal handling and stress.

2.2. Broodstock rearing conditions

European female silver eels (n = 16, body weight 748 ± 362 g; body length 71 ± 10 cm), were caught in the autumn of 2012 in a freshwater lake (Vandet Sø) in northern Jutland (Denmark) and transported to a research facility of the Technical University of Denmark located at Lyksvad Fish Farm (Vamdrup, Denmark). Eels were randomly distributed into 300 l tanks equipped with a recirculation system and were gradually acclimatized to salt water over a two week period, i.e. fresh water adjusted artificially to 36 psu salinity using Tropic Marin Sea Salt (Dr. Biener GmbH, Wartenberg, Germany). Male eels (n = 30, body weight 106 ± 13 g, body length 38 ± 2 cm), reared on DAN-EX 2848 (BioMar A/S, Brande, Denmark) were obtained at a commercial eel farm (Stensgård Eel Farm A/S, Randbøl, Denmark), transported to Lyksvad Fish Farm and kept in separate tanks under the same conditions as the female eels. At the onset of hormonal treatments, each fish was anaesthetized in an aqueous solution of benzocaine (ethyl p-aminobenzoate, 20 mg/l, Sigma-Aldrich, Germany), tagged with a passive integrated transponder (PIT tag) and body weight and length were measured. Throughout the experiment, all fish were maintained at ~36 psu (salinity), ~20 °C, and a natural local daily photoperiod was used. No feed was provided during experiments since eels in the migratory stage naturally cease feeding (Lokman et al., 2003).

2.3. Induction of gametogenesis and gamete extraction

Oogenesis was induced by weekly intramuscular injection of salmon pituitary extract (SPE) at a constant dosage of 18.75 mg/kg initial body weight (Kagawa et al., 2005; Ohta et al., 1996; Tomkiewicz, 2012). Pituitary extract was prepared using freeze-dried salmon pituitaries (Argent Chemical Laboratories, Washington, USA) that were grinded, diluted in NaCl 0.9 g/l and centrifuged (Ohta et al., 1996, 1997). Supernatants were stored at −20 °C until use. Females were weighed and inspected at the weekly injections to follow changes in body weight. At the onset of treatment, the females were weighed, PE dose determined and the first injection given concurrent with the tagging. Subsequently, each female was weighed and PE-treated weekly over a period of 16–20 weeks, depending on the female responsiveness. Induction of follicular maturation and ovulation was adapted to each female and initiated when the body weight at the weekly injection showed an increase of 10–15% compared to the initial body weight in combination with an increasing softness of the abdomen (Pedersen, 2003, 2004). Thereafter the female was transferred to a separate 300 l tank under the same conditions for individual care and the body weight observed the following day. In case of continued weight increase, oocyte stage was assessed, i.e., the female was anaesthetized in an aqueous solution of benzocaine, and an ovarian biopsy (~0.2 ml) was obtained, using a sterile disposable injection needle (16G × 1½”). The biopsy was taken at a standard location on the left side of the body, 5–10 cm anterior to the genital pore. The biopsy was inspected under the microscope and oocyte development graded on a scale from 1 to 7 according to Palstra et al. (2005). Progression of oocyte maturation varied in time and homogeneity, so each female was followed until the most developed oocytes exhibited characteristics of stage 4, i.e., transparent oocyte with a peripheral germinal vesicle. At this stage, an additional SPE injection as primer was given to females to boost follicular development (Kagawa et al., 2005; Pedersen, 2004). The timing of the primer treatment varied from 1 to 6 days after the standard weekly injection.

To complete follicular maturation and induce ovulation, the eel maturation inducing steroid, 17α, 20β-dihydroxy-4-pregnen-3-one (DHP crystalline, Sigma-Aldrich Chemie, Steinheim, Germany) was given ~24 h later at a dose of 2 mg/kg present body weight) (Ohta et al., 1996). Prior to DHP injection, a new biopsy (~0.2 ml) was obtained to evaluate the progression of oocyte development. DHP was

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