Fisheries Research 164 (2015) 50–58

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Ultrasonography as a non-invasive tool for sex determination and maturation monitoring in silver eels

Sarah Bureau du Colombier*, Louis Jacobs, Charline Gesset, Pierre Elie, Patrick Lambert

Irstea UR EPBX Ecosystèmes estuariens et poissons migrateurs amphihalins, 50 avenue de Verdun, F-33612 Cestas Cedex, France

ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form 4 October 2014
Accepted 16 October 2014
Handling Editor B. Morales-Nin

Keywords:
Anguilla anguilla
Early monitoring
Gonadosomatic index
Sex determination
Ultrasound

ABSTRACT

In the context of the severe decrease in temperate eel abundance, understanding and control of eel maturation has strong interest for scientific and commercial purposes. Possible use of ultrasonography for improvement of sex determination and maturation monitoring in silver eel was investigated. Gonads of 96 *Anguilla anguilla* silver eels were observed using portable equipment associated to a 6–15 MHz probe, and sex determination was tried before artificial induction of maturation. To estimate gonad mass and monitor individual gonadosomatic index (GSI) in females, cross-sectional images were captured at different times of maturation and gonad length was measured at scanning. Two methods were tried for ovary mass estimation using ultrasonography: one based on a linear model and another on calculating ovary volume from a representation of gonad shape. Ultrasonography resulted in 100% success in sex determination. Ovary mass estimated by ultrasonography was strongly correlated to true ovary mass ($R^2 = 0.97$). The use of a linear model for gonad mass and then GSI estimation seemed more appropriate than the use of a representation of gonad shape. Evolution of GSI estimates during maturation supports possible detection of early inter-individual differences in maturation using ultrasonography in female silver eels. This non-invasive tool can then obviously be exploited to improve sex determination in silver eels caught in the wild and to monitor maturation at the individual level. Ultrasonography thus has great potential for use in eel both for conservation and aquaculture. To our knowledge, this is the first report on the use of ultrasonography on eels or any anguillid species.

1. Introduction

Scientific and commercial interest in eels is clearly established. The current state of temperate eel populations due to severe decrease in abundance through the last three decades of the 20th century (Dekker et al., 2003) discourages exploitation of natural stock. Because spawning areas are located offshore in the open ocean, eel reproduction is not fully understood. A close monitoring of maturation is required to appreciate factors impacting genitor quality, and to make progress in eel breeding for self-sustained aquaculture.

The European eel (*Anguilla Anguilla*) is one of the most endangered temperate species and is currently subjected to conservation measures (CITES, 2013; IUCN, 2013). When European eels start the reproductive migration to the Sargasso Sea they are at the silver stage. At this stage, males and females present morphological differences generally used to determine sex. The main one is body length as, over the distribution area of European eel, mean total length lies within 50–61 cm in females and 35–46 cm in males. Nevertheless, extreme values vary from 38 to more than 100 cm in females and from 29 to 54 cm in males, and the size range 40–50 cm is usually the most unpredictable (Bertin, 1951; Tesch, 2003). Sex determination is not necessarily improved by the use of additional biometric values such as body mass, pectoral fin length and eye diameter (Durif et al., 2009). Thus, no external criteria ensure correct sex determination in silver eels of intermediate size.

Silver eels are in a prepubertal stage when they start reproductive migration and in aquaria they never reach sexual maturity without hormonal treatment (Dufour, 1994). Previous studies showed high variability in response to hormonal treatment between individuals reared in similar conditions. For example, some authors have reported coefficient of variation relative to gonad mass ranging from 4 to 59% in silver eels having received similar hormonal treatment (Durif et al., 2006). The origin of such variability is not easy to define accurately as many factors can be involved, including age (Palstra et al., 2007), stage, length,

* Corresponding author. Tel.: +33 557 890 998.
E-mail addresses: bureau_sarah@yahoo.fr, Sarah.Bureau-du-Colo@irstea.fr (S. Bureau du Colombier), Louis.Jacobs@irstea.fr (L. Jacobs), Charline.Gesset@irstea.fr (C. Gesset), pirelie@hotmail.fr (P. Elie), Patrick.Lambert@irstea.fr (P. Lambert).

http://dx.doi.org/10.1016/j.fishres.2014.10.015
0165-7836/

initial energy stores (Durif et al., 2006; Müller et al., 2004), previous exposure to contaminants (van Ginneken et al., 2009) and probably others. In other words, reproductive potential in silver eels is difficult to predict without hormonal treatment. Furthermore, there is currently no report of a method to assess variability in individual response in the early stages of maturation (i.e. at the start of hormonal injections) in this species. Understanding of early response would imply better understanding of the impact of the different factors on maturation variability and thus reproduction.

Biopsy and germ cell analyse can be used to assess gonad maturity in fish but this method is not non-invasive. Sexual maturation in eels often is evaluated using the gonadosomatic index (GSI) (Durif, 2003), which corresponds to the ratio of gonad mass to total body mass. However, this indicator is never used in individual monitoring because it requires fish sacrifice for gonad mass measurement. A recent study involving a small number of females reported the use of computed tomography to estimate eel GSI (Müller et al., 2004). This method is accurate but restrictive because it requires fish transport to scanning facilities, anaesthesia and significant preparation and manipulation time.

Ultrasonography is another non-invasive method sometimes used for gonad visualisation in fish, mainly for sex determination and maturation monitoring (Novelo and Tiersch, 2012). Considerable advantages of this method are the existence of portable equipment, short examination time and optional use of anaesthesia in some species. To our knowledge, the fish documented for now are sturgeon (Bryan et al., 2007; Chebanov and Galich, 2009; Colombo et al., 2004; Masoudifard et al., 2011; Moghim et al., 2002; Petochi et al., 2011; Wildhaber et al., 2005, 2007), cod (Davie et al., 2003; Karlsen and Holm, 1994; McEvoy et al., 2009; Newman et al., 2008), striped bass (Blythe et al., 1994; Jennings et al., 2005; Will et al., 2002), salmon (Martin et al., 1983; Mattson, 1991; Reimers et al., 1987, 1993), rainbow trout (Evans et al., 2004a,b; Hliwa et al., 2014), catfish (Bosworth et al., 2001; Bryan et al., 2005; Guitreau et al., 2012), halibut (Martin-Robichaud and Rommens, 2001; Martin-Robichaud et al., 1998; Shields et al., 1993), haddock (Martin-Robichaud and Berlinsky, 2004; Martin-Robichaud and Rommens, 2001; Martin-Robichaud et al., 1998), flounder (Martin-Robichaud and Rommens, 2001; Martin-Robichaud et al., 1998; Matsubara et al., 1999), red hind (Whiteman et al., 2005), hapuku wreckfish (Kohn et al., 2013), pacific herring (Bonar et al., 1989), shark (Carrier et al., 2003; Daly et al., 2007; Whittamore et al., 2010) and thornback ray (Whittamore et al., 2010), but ultrasonography on eels or any anguillid species has never been documented. The use of this method could actually improve early genitor selection and monitoring of maturation for scientific and commercial purposes in eel.

Studies on ultrasonography in fish display differences in approach, equipment, fish handling, instrument setting and image analysis (Novelo and Tiersch, 2012), partly correlated to the purpose of the study and the species. For sex identification, the ease of reaching the correct decision varies between fish species and maturation stage, but is generally based on differences in texture, grey colour and visibility between ovaries and testes on ultrasound images (e.g. Karlsen and Holm, 1994; Martin-Robichaud and Rommens, 2001; Mattson, 1991). On the other hand, monitoring maturation in fish generally depends on gonad diameter or estimation of gonad volume, but estimation of gonad mass for GSI calculation is not documented. Two main kinds of method are used to estimate gonad volume but they are applied the one or the other without any justification. Total gonad length and/or cross-sectional gonad area at different locations are generally estimated and then used for prediction, either using linear models (e.g. Jennings et al., 2005; Whiteman et al., 2005; Will et al., 2002) or by associating these measurements to a gonad shape representation (e.g. Bryan et al., 2005; Bryan et al., 2007; Chebanov and Galich, 2009). Because of differences in the approach currently used, and because of the eel's particular body shape, the method and best measurements to estimate gonad mass in this fish warrant investigation.

The aims of this study were: (1) to determine whether ultrasonography could improve sex determination in silver eels; (2) to evaluate the possibility of assessing Gonadosomatic Index on live, unanaesthetised eels; (3) to define the best measurements and methods to estimate female gonad mass, and (4) to provide a tool to monitor and compare individual trajectories from early maturation stages.

2. Materials and methods

2.1. Experimental fish

Ninety-six silver eels from the Gironde basin and coastal marsh of the 'Domaine de Certes' (Southwest France) were tagged individually. They were placed in circular tanks and gradually acclimatised to rearing conditions. Eel sex was determined using two methods separately for comparison: body length alone and gonad observation at ultrasonography.

2.2. Induction of maturation and rearing conditions

The usual protocol for hormonal induction of maturation in *A. anguilla* silver eels consists of weekly perivisceral injections of carp pituitary extracts (CPE) at a dose equivalent to 20 mg CPE powder kg^{-1} for about 20 weeks in females (Durif et al., 2006; Pierron et al., 2008), and perivisceral injections of human chorionic gonadotropin (hCG) at a dose equivalent to 1.5 IU g^{-1} for about 8 weeks in males (Peñaranda et al., 2010). As the present study focuses on early maturation, injections were interrupted after 11 weeks in females (T11) that is a little over half the mean total number of injections usually required to reach maturity using this protocol.

During maturation, fish were subjected to water current of 16 ± 1 cm s^{-1} for females, and lower than 1 cm s^{-1} for males. Water oxygen content was maintained above 9 mg l^{-1}, pH at about 7.5, temperature at 15 ± 1 °C for females and 22 ± 1 °C for males, and salinity above 30. Water was continuously aerated and reconditioned using mechanical and biological filters added to UV treatment system. As some females died or presented signs of external damage on arrival at the laboratory, all of them received antibiotic treatment. Migratory eels fast naturally so both females and males were kept unfed throughout the experiment. The fish were visited daily. Twenty three females and one male died during the experiment. Dead individuals were removed and immediately frozen at −20 °C until further analyses.

2.3. Fish handling and scanning procedure

A landing net was used to remove swimming eels from tanks for weighing. Individuals were carefully manipulated in wet cloths and placed on a table for total length and ultrasound measurements.

Unanaesthetised eels out of the water were quickly calmed by covering their head with wet cloths. Fish were held ventral side up (i.e. in dorsal recumbency). They were scanned all along the abdominal surface, in the transverse plane, from head to tail, with the ultrasound probe perpendicular to body cavity. The probe surface was covered with acoustic gel and a plastic sheath to optimise transmission.

Eel sex was checked at the time of scanning by three of the authors. In females, gonad total length (GL_E) was estimated by detecting organ ends, and measured from outside the fish using a ruler. Three cross-sectional images were digitally captured and recorded (JPEG format) in the ultrasound memory: at the anterior