Changes in body weight and eye size in female European eel kept in fresh and salt water

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Abstract

The eel is a catadromous fish which spends most of its life in freshwater and adults swim to the Sargasso Sea region to spawn. While preparing for the reproductive process, eels undergo a metamorphosis to become what is called silver eel; a process involving changes in the colour and weight of the body and an increase in the eye size. These are indicators of fish maturity and they facilitate the selection of fish for reproduction under controlled conditions. During this study, changes in the body weight (BW) and eye size in female European eel were observed while being given weekly hormonal injections of 20 mg kg⁻¹ carp pituitary homogenate and kept in 15°C freshwater and in 15°C saltwater with a salinity of 32-33‰. Fish kept in saltwater but not subjected to hormonal stimulation were used as a control group. Furthermore, after the experiment was finished, females in the control group were kept for next 5 months, with the same environmental parameters maintained. Differences between the treated groups were observed as early as week 4 of the experiment. An increase in fish BW was observed in fish kept both in salt and freshwater which were subjected to hormonal stimulation. On the other hand, changes in eye size were observed in the fish kept in saltwater, both in those hormonally stimulated and otherwise. The eye diameter in the fish kept in fresh after hormonal stimulation did not change significantly.

Introduction

The European eel (Anguilla anguilla, L.) is one of the least-known fish species. There are still several aspects of its life (i.e. biology, migration and artificial reproduction) which have not been properly elucidated (Dekker, 2003; van Ginneken and Maes, 2005). Although the European eel is an object of scientific interest, the species is also significant for the economy, recreation and aquaculture (Ide et al., 2011). However, due to excessive exploitation of its population and the lack of any repeatable reproduction or larvae rearing procedures, the population size of the European eel in its European distribution area has been reduced by 99% in recent decades (Pierron et al., 2009; Bevacqua et al., 2011). Such a huge reduction in the population has been attributed mainly to climatic change (Friedland et al., 2007; Belpaire et al., 2009), the deteriorating condition of the natural environment (Palstra et al., 2005b), infections by a parasitic nematode Anguillicoloides crassus (Fazio et al., 2012; Lorin-Nebel et al., 2013), as well as excessive fishing, which, according to estimates, accounts for up to 25 thousand tonnes of eel annually (van Ginneken and Maes, 2005; Haenens et al., 2010). All of these factors have resulted in the European eel being placed on the Red List of critically endangered species (CITES, 2007; Belpaire et al., 2009; Freyhof and Kottelat, 2010). The European eel is a catadromous species which spends most of its life in fresh waters and migrates to spawn in the Sargasso Sea region, covering a distance of thousands of kilometres (Durif et al., 2005; van Ginneken and Maes, 2005; Cleveestam et al., 2011; Burgerhout et al., 2013). In the course of preparation for spawning, with a consequent change in the environment from fresh to salt water, eels turn into silver eel, with numerous accompanying physiological and morphological changes (Durif et al., 2005; Cleveestam et al., 2011). The changed features include body colour and weight, head shape and an increase in eye size. These are, therefore, indicators of progressive fish maturation, which facilitate the choice of the individuals for reproduction under controlled conditions. However, there is still a lack of information about the biology of European eel reproduction, including the transformation of their bodies during the process of sexual maturation and achieving readiness for reproduction. Such information is necessary to develop a protocol of artificial reproduction of this endangered species. The aim of this study was to determine the changes that take place in the female European eel, regarding eye diameter, eye index (EI) and body weight index (BWI) during their hormonal stimulation, which induces the maturation of gonads, in both fresh- and salt-water.

Materials and methods

Origin of the fish

The eel brooders (27 females were used for main experiment) were caught in an inland lake in the Mazury region (north-east of Poland) near Szczyno during the beginning of their spawning migration. After being caught, the fish were transported to the hatchery, where they were put into three 1000 L tanks with fresh water with controllable environment parameters (Kujuwa et al., 1999). The initial water temperature was set at 12°C.

Handling of the fish

All the fish were marked individually with PIT marks (Biomark, Boise, ID, USA). After 15 days, the water temperature was raised from 12 to 15°C (±0.1°C) and maintained at this level until the end of the experiment. Subsequently, the water in one of the closed-water unit was salted to the level of 32-33‰ with salt produced for marine aquaculture (Aqua Nova, Geebung, Australia). Apart from salinity, the females kept in freshwater had the...
same conditions as those kept in saltwater. All tanks had a blackout capability and the fish (except for the control group) were given hormonal stimulation once a week. Before any manipulations were conducted, the fish were anaesthetised in a solution of MS-222 (300 ppt) (Finquel©; Argent, Redmond, WA, USA). The fish were not given any food throughout the experiment.

Three groups (9 fish each) were identified for the experiment: i) control (C), the fish were kept in saltwater, but they were not subjected to hormonal stimulation; ii) freshwater (F), stimulated hormonally; iii) and saltwater (S), stimulated hormonally.

The fish from groups F and S obtained the hormones at the same time. The start time of the experiment varied from 9 to 11 weeks after the first injection was done. The average initial BW was 1026±111.2, 1078±120.4 and 1056±116.7 g in Group C, F and S, respectively. The initial gonadosomatic index (GSI) (data from 5 females), was between 1.5 and 1.7%. Initially, the fish density did not exceed 10 kg m⁻³. Groups F and S were subjected to stimulation with carp pituitary homogenate at 20 mg kg⁻¹ (Palstra et al., 2005a, 2010) every 7 days during 14 weeks. The hormones were homogenised in sterile physiological saline (0.9% NaCl). All the injections were intraperitoneal into the abdominal part of the body near the anal hole.

In order to confirm whether keeping fish in suboptimal conditions (darkness, salinity of 32-33‰, water temperature 15°C, pH 7.8 to 8.3, dissolved oxygen over 80% of saturation, ammonia and nitrite at level below 0.1 and 0.02 ppm respectively) has any impact on female maturation, 5 eel females from group C were kept for the subsequent 5 months in saltwater. Initially, the fish were handled in the same way as the fish in the other groups and were subsequently left for 5 months with no handling. After that period, samples from the fish group were taken in order to determine the fish BW compared to the beginning of the experiment, as well as the change in the eye size and GSI.

**Observations**

The fish individual BW, horizontal and vertical eye diameters were recorded using the electronic calliper (±0.01 mm) during the weekly fish handling from groups C, S and F. The fish body length was measured at the beginning and at the end of the experiment. The EI (Mordenti et al., 2013) and the GSI were calculated from the data obtained in the experiment:

\[
EI=100 \left[ \left( \frac{D_h+D_v}{2} \right) 0.25 \right] (10 \text{ L}_0)^{-1}
\]

where \(D_h=\text{horizontal eye diameter (mm)}, D_v=\text{vertical eye diameter (mm)}, L_0=\text{body length (cm)}\).

\[
\text{GSI}=\left( \frac{\text{GW}}{\text{BW}} \right) 100
\]

where \(\text{GW}=\text{gonad weight (g)}\) and \(\text{BW}=\text{body weight (g)}\).

**Statistical analysis**

The statistical analysis was performed with a one-way ANalysis Of VAriance (ANOVA) followed by Duncan’s post-hoc test and the differences were regarded as statistically significant (\(P<0.05\)).

**Results and discussion**

Achieving reproductive maturity of European eel females is a long process, which takes usually from 20 to 30 weeks under controlled conditions (Palstra et al., 2005a, 2005b; Mordenti et al., 2013). An increase in BW and eye size has been adopted as determinants of the sexual maturity in females of this species (Duril et al., 2005, 2006). Under natural conditions, European eels swim to the spawning ground in saltwater after a long period spent in freshwater. All the changes in the body shape, weight and eye size are slow (Colombo et al., 1984; Duril et al., 2005). The time after which the final gamete maturation is achieved during the reproductive process under controlled conditions is reduced rapidly. In extreme situations, it can be as short as 38 days (Epler and Bieniarz, 1978; Epler et al., 1981).

In experiments which involved bringing European eel to maturity, its brooders were kept either in freshwater (Müller et al., 2005, 2012; Horvath et al., 2011; Prigge et al., 2012) or in saltwater (Epler and Bieniarz, 1978; Mordenti et al., 2013). Water salinity under controlled conditions can be changed very quickly – much more so than in the natural environment. Therefore, it is much easier to observe any changes that take place in the process of artificial reproduction under controlled conditions than in nature. This study is the first to compare the results of keeping European eel spawners in freshwater and saltwater conditions during the process of stimulating sexual maturation. Moreover, the results were compared to the C group (without hormonal stimulation) kept in saltwater. The fish survival rate at the end of the experiment was 89% in groups C and S, and 78% in group F. Mortality was noted between 2 and 4 weeks of the experiment in all groups. Differences in the increase in BW between the hormonally stimulated fish and those in C were observed from the second week of the experiment (Figure 1). The BW of females in the treated groups (S and F) was found to increase, while it decreased in C. This tendency was maintained until the end of the experiment, whereas the body length did not change throughout the experiment. The differences between the treated groups were observed from fourth...
week of the experiment, with greater BW increases were observed in the fish kept in saltwater than in those kept in freshwater (P<0.05). The fish BW in S group increased by over 10%, which stood in contrast to the 5% decrease (BW=94.59±1.01%) in the control group. A nearly 3% increase in BW (BW=102.87±1.21%) was observed in fish stimulated hormonally but kept in freshwater. Horvath et al. (2011) showed that eel female and male can be stimulated to achieve sexual maturity in freshwater (Müller et al., 2005), but the process is much faster in saltwater. The increase in the BW of the eel kept in saltwater (110.44±1.25%) is satisfactory as compared to the results observed by other authors. Mordenti et al. (2013) kept two groups of European eel from Comacchio and Morano-Grado for 30 weeks (salinity 33‰, water temperature 15.5±0.5°C) and observed BW increases of 113.53±3.68 and 120.63%±4.16, respectively.

Different results compared to the change of the female BW were observed regarding the changes of the eye diameter (Figure 2 A and B). A much greater increase in the eye diameter was observed in the fish kept in saltwater, regardless of whether they had been given hormonal agents or not. A greater increase was recorded in the eye height than in its width. The values of EI in groups S and C (17.77±1.67 and 16.61±1.56, respectively) at the end of the experiment differed significantly (P<0.05) from the EI value in group F (12.02±1.81) (Figure 3). Statistically significant differences were also observed between groups S and C at the end of the experiment (P<0.05). The EI in group F did not differ significantly from the values at the beginning of the experiment (P>0.05). The EI values in groups C and S were not significantly different (P>0.05). The average value of EI (10.21±1.91) at the start of the experiment was close to that recorded by other authors: 10.8±1.7 (Durif et al., 2005), and 10.8±1.7 and 9.9±1.6 (Mordenti et al., 2013) for silver eel. According to Durif et al. (2005), the EI calculated in this study in the beginning of the experiment corresponds to stages IV-V of eel females silverying process.

In order to verify whether keeping the eel for a longer time under suboptimal conditions would stimulate the sexual maturation of female, five fish in the control group were kept in saltwater for another five months. The environmental parameters, such as temperature and salinity of water, did not change and it appears that keeping fish in saltwater longer did not produce any positive results (Table 1). The fish in that group lost 15.6% of weight on average, and their GSI was 0.90±0.27. The GSI,
in comparison to the beginning of the study (1.5 to 1.7%), also decreased. A similar level of GSI (1.03±0.26) was observed by Horváth et al. (2011) for the control group, but they kept the eels in freshwater for 12 weeks. On the other hand, the value of EI in the group increased until week 14 (7.31±1.26 to 11.24±1.02) (P<0.05), to decrease to 8.47±0.15 in week 40 of keeping the fish in saltwater (P<0.05). Finally, no differences were found between the beginning of the study and week 40. Despite the sub-optimal conditions (darkness, salinity of 32-33‰, water temperature 15°C), the process of maturation without hormonal treatment is very slow and it can last many months, maybe even years. Considering the cost of artificial reproduction of European eel, its stimulation by environmental conditions alone does not seem to be a good option. However, in the case of European eel the strict influence of environmental conditions on maturation is not so evident; Utoh et al. (2013) found that temperature manipulations in a species related to eel, Japanese conger (Conger myriaster), may bring about final gamete maturation without hormonal stimulation.

An analysis of the changes of the eye size raised several issues. Large eyes are typical of pelagic fish. According to Opuszycki (1983), the majority of saltwater fish with large eyes live in the water layer between 200 and 1000 m. The eel which migrate to the spawning ground swim at similar depths (120 to 700 m) (Aerstrup et al., 2009). Therefore, it may be supposed that an increase in the eye size may be linked to a change from life at the bottom (freshwater) to a pelagic (saltwater) environment and the need to see (e.g. perceive the shape of) potential predators. However, the decrease in the eye size in female fish kept in saltwater for a long time needs a separate analysis.

Conclusions

The results of the experiment indicate that the maturation of female European eel under controlled conditions is affected by both hormonal stimulation and the salinity of the water. Keeping fish in saltwater alone results in a considerable increase in the eye diameter without causing an increase in BW. Water salinity may be an important stimulator of eel maturation, but it is not the main gametogenic factor. In freshwater, BW of eel increased, but the process is much faster in saltwater. However, the impact of the environmental conditions alone is not sufficient to stimulate the fish maturation. This can be achieved with hormonal agents. The findings of this experiment have shown that hormonal agents cause the greatest increase in BW.

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