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SHORT PAPER

Influence of Temperature on Successful European Eel Female Maturation under Controlled Conditions

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Abstract

This study determined the optimum thermal conditions to induce gonad maturity in female The aim of the study was to verify which of the three studied water temperatures: 15ºC, 18ºC or 21°C is the most suitable for inducing gonad maturation and reaching maturity by the female European eel. The thermal regime during the period of female eel maturation should be established nearly individually, depending on the degree of maturation of spawners and it should be lower than that proposed until recently. The study found that 15°C is the best temperature to bring females of European eel to maturity. Excessively high temperatures were found to cause accelerated gonad maturation and to result in a lower oocyte quality. Moreover, the fish kept at higher temperatures (18ºC, 21°C) consume their energy resources (for example, for formation of gonads) faster than those kept in water at 15°C. The highest quality was noted for eggs obtained from females kept at lowest tested temperature.

Keywords: Temperature, Anguilla anguilla, BWI, diseases, maturation.

Introduction

In general, fish grow, reproduce and function best within a specific temperature range (Elliot 1981). For every species, there is a strictly-defined optimum temperature range in which life processes run with the greatest effectiveness and outside of which they are disrupted (they can act as a stressor, which disrupts the physiological and behavioural activity), leading to death in extreme conditions (lethal temperature) (Fry *et al.*, 1942; Kamler 1992; Kucharczyk *et al.*, 1997; 1998; Kujawa *et al.*, 1997; Kupren *et al.*, 2011). In many fish species, the optimal thermal regimes for any life period are known. But for some species, like European eel *Anguilla anguilla* (L.), data on thermal regimes for migration and spawning are still lacking.

European eel is a species which is susceptible to degradation caused by climatic changes. According to Bonhommeau *et al.*, (2008), of great importance is the effect of decreased recruitment in eel populations (not only in those of European eel, but also in those of American eel *Anguilla rostrata* and Japanese eel *Anguilla japonica*), which is a result of changes in the primary production in oceans caused by climatic changes, for example, an increase in the average water temperatures in spawning grounds. Changes in the water temperature in the north Atlantic and possible loss of strength of the Gulf Stream, caused by melting Arctic ice, may also be a problem for eel (Wirth and Bernatchez, 2003).

The information about the thermal requirements during eel spawning migration is only residual, especially during the beginning and middle part of it. No adult fish (spawners) were found in the North Atlantic before, during or after spawning and there is no data on European eel eggs found in nature (Van Ginneken and Maes, 2005). Initially, the females swim at a mean temperature between 10°C (day) to 11°C (night) (Aarestrup *et al.*, 2009). Under controlled conditions during European eel maturation usually high temperature of about 20 °C was applied (e.g. Pedersen, 2003; 2004; Palstra *et al.* 2005) but last research showed that lower temperature might be much more useful (Perez *et al.*, 2011; Nowosad *et al.* 2014a, 2015).

The aim of the study was to verify which of the three water temperatures under study (15ºC, 18ºC, 21°C), at which females were kept during the gonad maturation process, is the most suitable for inducing gonad maturation and reaching maturity by the female European eel.

Materials and Methods

Fish

Eel brooders were caught during commercial fishing activities in freshwater in the Mazury region (north-eastern Poland) as fish which start their spawn migration. After being caught, the fish were transported to the laboratory of the Department of Lake and River fisheries, University of Warmia and Mazury, Olsztyn, where they were put into a 1 m^3 tank, operating in closed water circulation (Kujawa *et al.,* 1999). The initial water temperature was 12° ± 0.2°C. The photoperiod applied was natural and photo-cell-controlled. The oxygen level in the water was at least 6 ppm. The fish stayed in the freshwater tank until the start of the experiment.

Handling the Fish

All fish handling was conducted in a state of anaesthesia (MS-222 (300 ppt), Finquel, USA). After being anesthetized, the fish were measured (TL, eye diameter), weighed and labelled with individual PIT labels (Biomark, USA). The fish handling was conducted in a slightly-dimmed room. The fish, with a mean body weight (BW) of 0.5 - 1.2 kg, were transferred to 1m^3 tanks operating in closed water circulation. The circulation had controllable physico-chemical conditions (temperature, photoperiod) and the system was also equipped with a polygeser filter and UV lamps (2 x 36 W each). The initial water temperature was set at 12°C and the photoperiod was 0 L: 24 D. The initial water temperature was set at 12°C and the photoperiod was 0 L: 24 D. Subsequently, the water was salted (Aqua Nova, Australia) over 7 days to the level of 33-35‰ and the water was heated to the level of 15ºC, 18 ºC and 21ºC (±0.1ºC). The fish were not fed throughout the experiment.

Hormonal Stimulation

The hormonal stimulation was started after the desired water salinity level was achieved. After being taken out of the tanks, the fish were put in a container with an anaesthetic. After being anesthetized, the fish were weighed and had their eye diameter measured. Subsequently, they had an intraperitoneal injection of carp pituitary homogenate (CPH, Agrent, USA) at 18 mg kg^{-1} (Nowosad *et al.,* 2014a, b) per injection. The hormones were not given to the fish in the control group. The fish handling was conducted at 5-day intervals, for up to 17 weeks.

Spontaneous Ovulation

Females which body weight index (BWI) increased after 30% were moved to additional tank. The water temperature was slowly changed to the 18°C. They were checked daily for possible ovulation. Also, in the water out-flow from the tank, the basket from small mesh (200 µm) was installed, and the presence of the eggs in the basket were verify. The percentage of ovulated females, and eggs quality was noted.

Data Analysis and Statistics

The data were analyzed by means of a one-way analysis of variance (ANOVA) and subsequently with Duncan's post-hoc test (α=0.05). The statistical analysis was conducted with Microsoft Excel and Statistica v.10 (StatSoft Inc.2011, USA).

Results

It was confirmed that the temperature has a strong effect on the maturation process of female European eel gametes. Although maturation was the fastest in the females which were kept at 21ºC (Figure 1.), the obtained gametes indicated that the process of maturation at this temperature was too fast – the oocytes obtained in it, with a very large diameter (over 2 mm), were clearly over-matured. Individual females spontaneously secreted small amounts of spawn, frequently with a very large diameter, which looked as if they were swollen (Table 1).

The body weight of the fish in the control group was found to decrease during the experiment (Figure 2). The decrease depended on the temperature and it was proportional to the increasing metabolism rate – it was the fastest at 21ºC; the females in this group lost about 15% of their initial weight by the end of the experiment (after 25 weeks). The decrease was considerably slower in the group of fish kept at 15ºC – the females lost a little more than 5% of their initial body weight during the same time.

The rate of the body weight increase in the females stimulated hormonally was proportionate to the temperature – it was the fastest in the females in the group kept in water at 21ºC (Figure 3). The body weight of the fish in that group was the lowest at the end of the experiment. The slowest body weight increase was recorded in the group kept at 15ºC, but their final weight increase was the highest. The differences in the body weight increase (Figure 4) indicate temperature dependence.

Temperature have a strong impact for possibility to spontaneous ovulation and simple parameters described the eel egg quality (Table 1). The ovulation rate of females kept at the lowest tested temperature was the highest.

Discussion

Fish, as poikilotherms, are animals whose entire life is greatly affected by the temperature of the water (Herzig and Winkler 1986; Kucharczyk *et al.,* 1997; 1998; Davies and Bromage, 2002; Anguis and

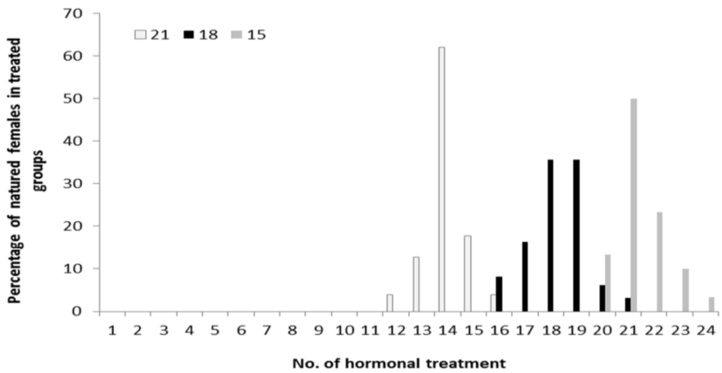

Figure 1. The rate of achieving sexual maturity (understood here to mean the achieving of BWI of over 15%) by female eels kept in water at different temperatures.

Table 1. The results (mean ± SD) of spontaneous spawning of European eel females kept in different temperatures

Group/temperature	15°C	18°C	21°C
Spontaneous ovulation rate (%)	78	44	22
Transparent eggs (%)	97.8 ± 1.4[a]	98.0 ± 2.1[a]	64.5 ± 3.5[b]
Floating eggs (%)	92.1 ± 2.3[a]	86.1 ± 2.7[b]	56.1 ± 3.5[c]
Number of oil droplets in oocytes	4.8 ± 4.0[a]	6.5 ± 3.3[ab]	10.2 ± 2.5[b]

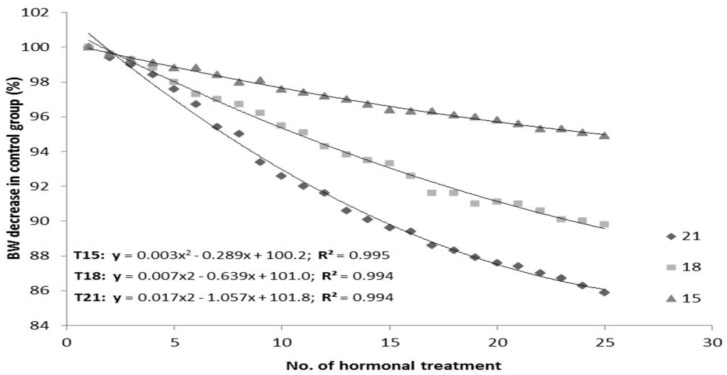

Figure 2. Changes in the body weight of female European eel in the control group (without hormonal stimulation) kept in water at different temperatures (T) (15ºC, 18ºC and 21oC).

Cañavate, 2005). Temperature is one of the factors with the strongest effects on metabolism. It affects fish nutrition and, consequently, their growth (growth rate and, indirectly, the size of fish) very strongly. Reproduction is another part of a fish's life which is strongly affected by temperature. Both the temperatures at which fish live before spawning (and their changes), as well as the water temperature in which spawn is incubated, may affect whole populations and fish species on a global scale. Hence, the weather changes observed in recent years (and perhaps climate changes in future) are potentially one of the elements with the greatest effect on the biodiversity of aquatic ecosystems (including fish) (Targońska *et al.*, 2014).

Failure to develop an effective method of eel reproduction under controlled conditions may be the result of an inadequate thermal protocol. Haro (1991) described that American eel (*Anguilla rostrata*) in saltwater preferred the temperature of 17.5 °C for both: matured and unmatured breeders. The most frequently used temperatures in experiments with Japanese eel are between 20ºC and 23°C (Ohta *et al.*, 1995; Kagawa *et al.*, 2003; Abe *et al.*, 2010). Similarly, the water temperature for the stimulation of gamete maturation in European eel was close to 20°C (Pedersen, 2003, 2004; Palstra *et al.*, 2005; van Ginneken *et al.*, 2005; Pérez *et al.*, 2011). However, such temperatures deviate greatly from those established for the beginning of a European eel's journey under normal conditions (8 – 13°C) (Aarestrup *et al.*, 2009). In a study conducted by Pérez *et al.*, (2011) in two thermal variants, the effects of reproduction of fish kept at lower (and variable)

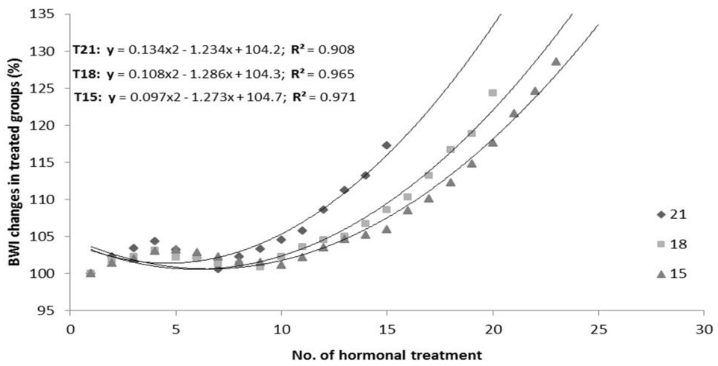

Figure 3. Changes in the body weight of female eel in the study group (with hormonal stimulation) kept in water at different temperatures (T) (15ºC, 18ºC and 21ºC).

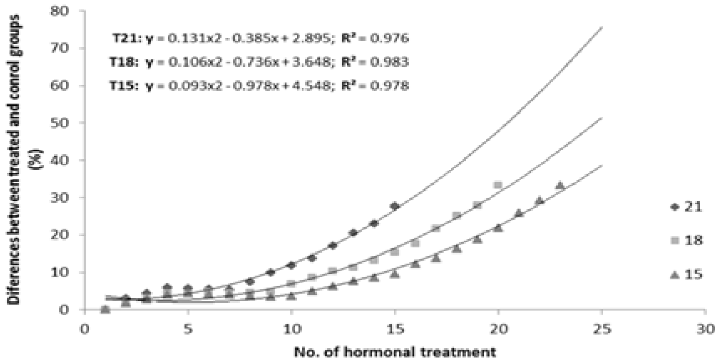

Figure 4. Relative body weight increase (BWI) in female eels at different water temperatures (T).

temperatures were much better than those in fish kept at a constant temperature of 20°C. The authors suggest that temperatures should be maintained below 15°C at the initial stage of stimulation of female European eel (until week 8). An increase in GSI in the females kept at 15°C is larger than in those kept at 20°C. After week 12, a much larger GSI increase was observed in females kept at higher temperatures. Moreover, Mordenti *et al.,* (2013) showed that temperatures below 20°C are more suitable for the stimulation of eel females. The findings of this study are similar. Although fish matured the fastest at 21°C, this temperature proved too high for normal gonad development. At the same time, weight loss in the fish in the control group was the greatest at this temperature. Of the two temperatures applied in the experiment, 15°C seems the most suitable if the thermal protocol of reproduction is to maintain a constant temperature. However, it seems that during the final gamete maturation, water temperature should be raised by 2 – 3°C (Pérez *et al.*, 2011; Mordenti *et al.,* 2013). Also, the simple markers for oocyte quality, such as percentage of transparent eggs or percentage of floating eggs showed that the lowest tested temperature (15°C) resulted the highest egg quality. Recent research has shown that the thermal protocols applied in the reproduction of Japanese eel should also be lower than those applied to date. Sudo *et al.,* (2011) have shown that a high water temperature at the beginning of the female maturation period inhibits the development of ovaries and oocytes. On the other hand, Unuma *et al.,* (2011) proposed a different thermal protocol: 15°C, and raising the water temperature to 20°C when oocytes reach a size exceeding 0.6 mm and the body weight increase reaches at least 5%. Yoshikawa (2012) claimed that if the final gamete maturation took place at 18°C instead of 20°C, then the quality of eggs was better (survival rate above 45% versus 17%). 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. However, other factors should also be taken into account when developing a reproductive protocol for eel which takes temperature into consideration. Okamura *et al.,* (2008) found that the body weight increase in Japanese eel at 20°C depends on the degree of maturity. The yellow form of eels lost weight when subjected to hormonal stimulation, whereas the maturation (body weight increase) of silver eel largely depended on the oocyte maturation. There is still a lack of knowledge of the spawning temperature of the European eel. Van Ginneken and Maes (2005) reported full sexual maturation in European eel male takes about 20 days at 25°C and three times longer at 15°C. At lower temperatures, gonadal development does not progress. On the other hand, Bast and Klinkhardt (1988) reported that a migrating maturing female eel has been caught at a depth of 500 m close to the Azores. This eel had a GSI of 10 and gonads containing advanced oocytes. Gallego *et al.,* (2012) and Baeza *et al.,* (2014) suggest that the temperature of the probable spawning area in the Sargasso Sea is about 20°C. However, because the European eel migration takes several months, it seems likely that gonadal development happens during the journey, at low temperatures, whereas the spawning probably takes place at high temperatures.

Conclusions

In conclusion, the thermal regime during the period of female eel maturation should be established nearly individually, depending on the degree of maturation of spawners and it should be lower than that proposed until recently. It seems that the temperature during the initial phase of stimulation can range from 10ºC to 15°C and it can be raised slightly during the final gamete maturation (Pérez *et al.,* 2011, Mordenti *et al.,* 2013, this paper). Excessively high temperatures result in faster gonad maturation, but also probably in lower quality oocytes. It is also important that fish kept at higher temperatures (18ºC, 21°C) consume their energy resources faster than those kept in water at 15°C, which manifests itself in greater weight loss in the former. An excessively high temperature may favour the development of parasitic (Horvath *et al.,* 2011) and bacterial (Pérez *et al.,* 2011) infections, which may result in high brooder mortality.

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