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First Evidence of Pheromonal Stimulation of Maturation in Eurasian Perch, *Perca fluviatilis* L., Females

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Abstract

The effect of pheromonal stimulation on the progress of maturation in wild Eurasian perch, *Perca fluviatilis*, females was determined. The females were kept in two separate tanks in a flow through system (12°C). (experimental group) males were placed together with females into one of the tanks. A second tank was without males. The maturation stage of females was determined *in vivo* every 10 days up to day 30. The results obtained indicated that pheromonal stimulation induced uneven maturation and significant diversification of maturation stages (from I to V) in females that were kept with males. In females kept without males 90% of fish were still in stage I. The results suggest that spawners of both sexes should be kept together if it is necessary to stimulate reproduction, whereas they should be kept separately in order to synchronize or delay the spawning.

Keywords: Artificial spawning, synchronization of ovulation, oocyte maturation, percids culture, aquaculture.

Introduction

Reproduction is one of the most important stages in aquaculture production. Its efficiency affect the quality of larvae and fry and, consequently, the success of the further stages of production (Zohar and Mylonas, 2001; Mylonas *et al*., 2010). It regards also Eurasian perch, *Perca fluviatilis* (L.), which is a very prospective species for diversification of freshwater aquaculture (Kucharczyk *et al*., 1998; Migaud *et al*., 2002; Szczerbowski *et al*., 2009).

In the case of perch artificial reproduction, wild fish are usually obtained during the reproductive season (Kucharczyk *et al*., 1996; 1998; Ronyai and Lengyel, 2010; Żarski *et al*., 2011). However, the stage of maturity of females is then very diversified (Kucharczyk *et al*., 1998) which hinders the possibility of ovulation synchronization. One of the methods of obtaining perch females with a similar stage of maturity is to collect fish before the reproductive season (Żarski *et al*., 2011), i.e. in January/February. The stimulation of ovulation out-of the reproductive season usually results in low quality of eggs and larvae (Migaud *et al*., 2004; Szczerbowski *et al*., 2009; Żarski *et al*., 2011). A potential solution to this problem is to obtain the spawners before the reproductive season and to keep fish for a few weeks and then to start artificial spawning when oocyte maturation progresses. The conditions in which wild perch females should be kept during this period have not yet been determined.

Pheromones were described as a substances which are excreted to the "outside" by one specimen and received by other specimen which is able to react in a specific way (for particular pheromone) (Karlson and Luscher, 1959). These substances were investigated in variety of animals and were proven to interact not only between the individuals of the same species (Karlson and Luscher, 1959; Abbott, 1987; van derWalt *et al*., 2001, Heinze and Oberstadt, 2003; Sorensen and Stacey, 2004). In the case of fishes, pheromones were described to play a role as an alarm substances (e.g., anti-predation cues), a substances regulating social interactions of a stock of fish (non-reproductive aggregation, migration) as well as a substances regulating reproductive behavior and maturation (extensively revised by Sorensen and Stacey, 2004). In stimulation of maturation steroids and prostaglandins were reported be have strong pheromonal effect (Stacey and Sorensen, 2002).

Pheromonal stimulation is one of the less known methods for stimulation of maturation in fish under controlled conditions, since females are very often kept separated from the males (Kucharczyk *et al*., 2005; Targońska *et al*., 2010; Żarski *et al*., 2011; 2012b). It has been proven that female pheromones

may induce spermation in male of common carp, *Cyprinus carpio* (L.) (Billard *et al*., 1989) as well as spermation and hormonal response in crucian carp, *Carassius carassius* (L.) (Olsen *et al*., 2006). Female pheromones have also influenced the volume of ejaculate in goldfish, *Carassius auratus* (L.) (Hoysak and Stacey 2008). Male pheromones stimulated the reproduction of females in zebrafish, *Danio rerio* (Hamilton) (Chen and Martinich 1975), and pacific herring, *Clupea harengus pallasi* Valenciennes (Carolsfeld *et al*., 1997). However, there has not been any data on the impact of pheromonal stimulation in Eurasian perch.

The aim of this study was to determine the impact of male presence (potential pheromonal stimulation) on the maturation of wild Eurasian perch females in controlled conditions before the spawning season.

Materials and Methods

The wild perch spawners (112 females and 32 males) were collected in Lake Sasek Wielki during under the ice catching with drag-nets on February 15, between 8.00 am and 12.00 o'clock. Immediately after catching, the fish were transported to the laboratory of the Department of Lake and River Fisheries in Olsztyn where they were placed in 1000 l tanks in which the temperature (±0.1°C) and photoperiod (±10 min) were controlled (as described by Kujawa *et al*., 1999). After 5-day acclimatization from 4 to 10°C (at the photoperiod of 6h [6L:18D]), the fish were segregated according to sex. Such thermal regime was tested earlier for out-of-season reproduction of Eurasian perch (Żarski *et al*., 2012c). The determination of sex was carried out *in vivo* with catheterization. The females were placed in two separated (1000 l) tanks (61 female in each one). The tanks operated in a constant flow through system (6 L min^{-1}) with tap dechlorinated water. All (n=32) males (mean weight 42.5±12.8 g) were then placed with the females (58.9±19.2 g) in one of the tanks (experimental group [E]). In the second tank (control group [C]), the females (60.1±16.8 g) were kept alone. Fish were not fed during the experiment. The biomass of the fish in the tanks was 3.67 and 4.95 kg m^{-3} in group C and E, respectively.

At the beginning (20th February, day 0) of the 30 day long experiment, the samples of oocytes were taken with a catheter (with 2 mm external and 1.2 mm internal diameter) from all females and the stage of oocyte maturation was determined. The oocyte sample was placed on a Petri dish and immersed in clarifying Serra's solution (70% ethanol, 40% formaldehyde and 99.5% glacial acetic acid in 6:3:1 ratio). After the clarification of cytoplasm, the analysis of oocyte maturation was carried out according to the classification for perch described by Żarski *et al*. (2011). Next, the determination of oocyte maturation stage was conducted on day 10, 20 and 30 of the experiment. Each time, the oocytes were sampled from 30 randomly-selected females from group C and E. The oocyte samples were then clarified with Serra's solution and the maturation stage of each sample was determined (based on 30 oocytes which were randomly photographed). The samples were assigned to a given stage based on the majority (above 50%) of oocytes representing the respective stage as described by Żarski *et al*. (2011). If an equal number of oocytes represented two adjacent stages, the female was classified in the less advanced stage.

The same thermal (10°C) and light conditions (14L:10D) both, in group C and E were maintained in the tanks throughout the experiment. Before each handling, the spawners were anesthetized in MS-222 solution (150 mg L^{-1}) (according to Żarski *et al*., 2011).

The data on female maturation stage was analyzed with on-way analysis of variance ANOVA and then if the analysis of variance revealed statistical differences, a post-hoc Duncan test with 5% significance level was applied (α=0.05). The data, expressed in percentage values, were subjected to arc-sine transformation before statistical analyses. For the changes of mean maturational stage in females in both treatment groups linear regression analysis was performed with the use of MS Excell for Windows.

Results

At the beginning of the experiment (day 0) the oocytes in all females were classified to stage I. During the consecutive 20 days in all females in group C the oocytes remained in stage I. In this group on day 30, stage II of the oocytes was reported in only 10% of females (Figure 1). In group E, a gradual increase in the average maturation stage of females (R^2>0.9) was observed during the whole experiment (Figure 1). During the observations, a considerable diversification in maturation stages was noticed. On day 10, the majority (60%) of females ($P<0.05$) still remained at stage I whereas in 26% of females, oocytes in stage II were observed. In less than 7% stages III and IV were recorded. On day 20, the majority of females in group E had reached stages I and II and they significantly outnumbered ($P<0.05$) the females in stages III and IV. On day 30, the majority of females had reached stage III (over 53%) whereas stages I, II, IV and V were represented by a comparable ($P>0.05$) number of females (13.3, 16.7, 10.0 and 6.7% for stages I, II, IV and V, respectively) (Figure 2).

Discussion

The results of the presented study, for the first time, clearly indicated potential pheromonal stimulation in perch females. The stimulation induced rapid and uneven maturation of females and, in the

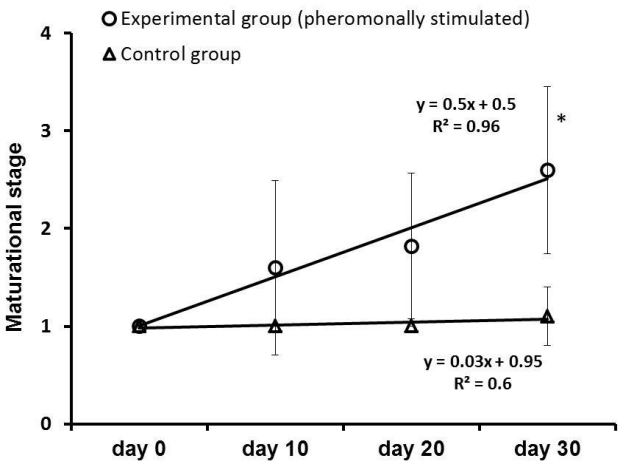

Figure 1. Changes of maturation stage in females of Eurasian perch kept with males (pheromonally stimulated experimental group) and without males (control group) during 30 days experiment. Data marked with asterisk were statistically different (P<0.05) at a respective day.

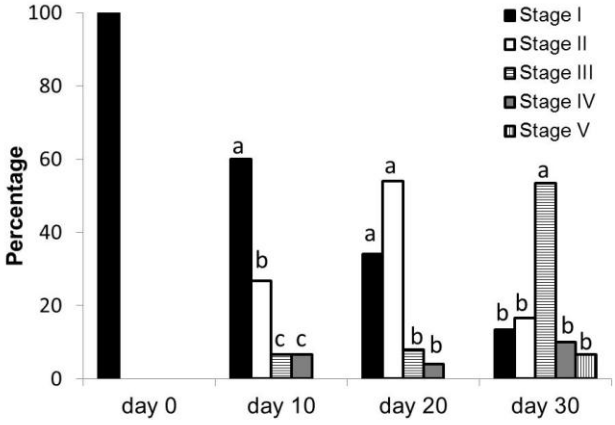

Figure 2. Chaneges of percentage share (n=30) of maturation stages in pheromonally stimulated (kept with males) Eurasian perch females during 30 day observation. Data at a respective day marked with different letter were statistically different (P<0.05).

end, significant diversification of maturation stages (on average stage III, ranging from I to V) in the females that were kept with males. It was particularly evident in comparison with the females from the control group in which the maturation of oocytes had not been observed for the first 20 days (stage I).

The technique of fish reproduction aims at obtaining eggs and sperm when required by a farmer. In the case of artificial reproduction of perch, different hormonal preparations have been tested in order to synchronize ovulation during the reproductive season (Kucharczyk *et al*., 1996; 1998; Kouril *et al*., 1997) and to induce ovulation out-of the reproductive season (Migaud *et al*., 2004; Szczerbowski *et al*., 2009). The data presented in this paper indicates that the farmers should also consider another factor, i.e. pheromones which provoked rapid development of gonads in comparison with the females from the control group (which were kept without males). However, at the end of experiment, the maturation stage of the females that were pheromonally stimulated was extremely diversified (from stage I to V). This effect may cause considerable diversification of ovulation time. If the method of determination of oocyte maturation described by Żarski *et al*. (2011) is followed, fish can be divided according to the stage of maturity and, therefore, the moment of ovulation in a given group can be precisely determined. It should be emphasised that the development of oocytes in the control group was not observed until the day 30 of the experiment. Żarski *et al*. (2011) also reported that the stage of oocyte maturation did not progress in the females which were kept separately, not stimulated hormonally and collected before the natural spawning season. However, the slight progress of maturation in some of the females at the end of experiment may suggest that longer duration of fish keeping may have

cause further maturation process. But it has to be more closely studied where also photo-thermal manipulation should be considered as a potential stimuli.

The observations of pheromonal stimulation in fish have indicated that pheromones positively influenced the synchronization of maturation in females and their spawning readiness (Chen and Martinich, 1975; Dulka *et al*., 1987). However, the results of this study indicate that pheromonal stimulation induces the maturation of perch females, but this process is not synchronized in a group of fish which were initially homogenous in maturation stage. One of the reasons could be the stress caused by captivity and handling. It is well known that stress stimulates or restricts the maturation of oocytes and ovulation (Carolsfeld *et al*., 1997; Schreck *et al*., 2001; Schreck 2010). In the case of wild perch, stress is a factor that negatively influences maturation and reproduction (Wang *et al*., 2006; Żarski *et al*., unpublished). The obtained results suggest that in perch, the reaction to stress may have an individual character where individuals which were more resistant to stress reacted more intensively to pheromonal stimulation (at the end of the experiment, their maturation stages were more advanced). In perch caught in the natural environment during reproductive season it has been very often observed that the maturation stage of females collected at the same moment from the same lake was very diversified (Żarski *et al*., 2011). This phenomenon is similar in the case of other freshwater percid which is pikeperch, *Sander lucioperca* (L.) (Żarski *et al*., 2012a, 2012b). Therefore, the diversification of maturation stage of percids, e.g. in perch, remains unclear and it may be hypothesized that desynchronization of maturity observed in captivity (as reported in the present study) may result from species-specific biology of reproduction or different reaction to stress in individual females. Further research is needed to include the potential impact of both factors.

It was already reported in goldfish, that steroids (including 17*α*, 20*β*-dihydroxy-4-pregnen-3-one [DHP]) are the main reproductive pheromones (Zheng and Stacey, 1997, Poling *et al*., 2001, Stacey, 2003). However, most studies aimed at investigation of pheromones originating from females and affecting maturation and spermiation in males. Stimulation of maturation in females were reported mainly in the final stages of maturation and ovulation (Chen and Martinich 1975, Carolsfeld *et al*., 1997). Fontaine *et al*. (2003) suggested, that DHP could be an important pheromone involved in ovulation and synchronization of spawning in Eurasian perch females. At such early maturation stage of females, as in the present study (end of the vitellogenesis and before the final oocyte maturation process, according to Żarski *et al*., 2012c), DHP is not synthesized in female organism (Mylonas *et al*., 2010), thus no effect in control group was observed (without males). That is why, the effect observed in the present study could stem only from the hormones originating from males. During the observation males were already spermiated, since the spermiation in Eurasian perch may occur from November to April (Alavi *et al*., 2010). Because in early spermiation process the most important role plays 11-ketotestosterone instead of DHP (Schulz *et al*., 1994; Mylonas *et al*., 1997; Alavi *et al*., 2010), it may be suggested that this hormone may act as a pheromone affecting progress of maturation in females at such early stages of maturation, as in the present study. However, this phenomenon need to be more closely studied. Especially, when ethological (e.g., pre-spawning behavior) character of stimulation of maturation (when fish of both sexes are kept together) may not be totally excluded.

The results of this study show very strong impact of pheromonal stimulation on the perch females kept together with males before the reproductive season. This data proves for the first time that the procedure of keeping perch spawners in controlled conditions before artificial spawning causes considerable variability in the maturity stages of females. However, further studies are needed to assess the impact of individual variables (e.g., different maturation stages of females at the beginning of observations, temperature, photoperiod) that would allow complete control of pace and synchronization of female maturity and, consequently, of ovulation.

At the present state of knowledge it is recommended to keep females, caught before the reproductive season, together with males since, in perch, the hormonal stimulation before maturational stage III results in lower egg quality (Żarski *et al*., 2011; Żarski *et al*., 2012c), and keeping females without males does not stimulate maturation and may lead to ovarian atresia (Migaud *et al*., 2002). When the majority of females represent stage III, fish should be divided according to the stage of maturity and hormonal stimulation can then be performed to females representing maturity stage III or later. Pheromonal stimulation may become a useful tool in hastening spawning maturity in females and consequently precisely plan hatching time. However, more work is still needed. The results of this study also confirm the necessity to determine the stage of oocyte maturation in perch females in order to synchronize ovulation in artificial reproduction (Żarski *et al*., 2011).

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