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A new classification of pre-ovulatory oocyte maturation stages in pikeperch, *Sander lucioperca* (L.), and its application during artificial reproduction

Daniel Żarski[1], Dariusz Kucharczyk[1], Katarzyna Targońska[1], Katarzyna Palińska[2], Krzysztof Kupren[1], Pascal Fontaine[3], Patrick Kestemont[4]

[1]Department of Lake and River Fisheries, University of Warmia and Mazury, Olsztyn, Poland

[2]Department of Zoology, University of Warmia and Mazury, Olsztyn, Poland.

[3]Laboratoire de Sciences Animales, INPL-UHP Nancy, Nancy, France

[4]Unit of Research in Organismal Biology, University of Namur (FUNDP), Namur, Belgium

Correspondence: D Żarski, Department of Lake and River Fisheries, University of Warmia and Mazury, ul. Oczapowskiego 5, pok. 325, 10-957 Olsztyn, Poland. E-mail: danielzarski@interia.pl

Abstract

This study aimed at improving the reproduction effectiveness and synchronization of ovulation in the pikeperch, *Sander lucioperca* (L.), during induced spawning, which is one of the main bottlenecks in the aquaculture of this species. For this purpose, a new categorization of maturation stages in pre-ovulatory oocytes was applied. It is generally based on two morphological indicators: germinal vesicle migration or its breakdown (GVBD) and different oil droplet coalescence rates. This categorization covered seven stages (from I to VII) – from the end of vitellogenesis to ovulation. The categorization was verified by controlled reproduction with the use of hormonal stimulation (500 IU of hCG per kg of female body weight) and low spawning temperature (12 °C), which extended the latency time. In addition, some morphological indicators (pseudo-gonadosomatic index, Fulton's condition coefficient) of females were calculated in order to determine their usability in determining the maturation stage. However, these indicators proved to be ineffective for this purpose, further highlighting the need to determine the maturational stages in pre-ovulatory oocytes to synchronize ovulation in pikeperch. During the experiment, ovulation seemed to be synchronized among the experimental treatments. Statistical differences were found in terms of latency time between experimental groups at different maturity stages (II – 78–98 h; III – 57–78 h; IV – 48–58 h; V – 32–49 h; VI – 5–30 h) according to the proposed classification. This classification and the results presented in the study significantly improved the synchronization of ovulation, which may positively affect the effectiveness of pikeperch production under controlled conditions.

Keywords: oocyte maturation stage, pikeperch, percid culture, reproduction, hormonal treatment

Introduction

Pikeperch *Sander lucioperca* (L.) is a highly valued species with great potential in European freshwater aquaculture (Kestemont & Mélard 2000; Philipsen 2008; Wang, Xu & Kestemont 2009). One of the most problematic stages of production is its artificial reproduction (Kucharczyk, Kestemont & Mamcarz 2007; Muller-Belecke & Zienert 2008; Zakęś & Demska-Zakęś 2009; Wang, Mandiki, Henrotte, Bouyahia, Mairesse, Rougeot, Mélard & Kestemont 2009), which directly affects the profitability of intensive culture in recirculating aquaculture systems (Philipsen 2008; Hakuć-Błażowska, Kupren, Turkowski, Targońska, Jamróz, Krejszeff, Kwiatkowski, Żarski & Kucharczyk 2009; Hakuć-Błażowska, Kupren, Turkowski, Targońska, Żarski & Kucharczyk 2010).

Ovulation in species such as pikeperch or Eurasian perch, *Perca fluviatilis* L., can be obtained without hormonal stimulation (Demska-Zakęś & Zakęś 2002; Muller-Bellecke & Zienert 2008; Ronyai & Lengyel 2010). However, its application significantly affects ovulation rate and synchronization of ovulation in wild spawners, which have similar maturation stages (Kucharczyk, Kujawa, Mamcarz, Skrzypczak &

Wyszomirska 1996, 1998; Demska-Zakęś & Zakęś 2002; Kucharczyk *et al.* 2007). When females have different maturation stages, it is impossible to synchronize ovulation (Demska-Zakęś & Zakęś 2002; Kucharczyk, Targońska, Krejszeff, Szkudlarek, Szczerbowski & £uczyński 2008). Asynchronous reproduction has a negative impact on production as it may lead to differentiation in the size of reared larvae and intensification of cannibalism (Baras & Jobling 2002; Baras, Kestemont & Melard 2003). Egg deposits in the tank are very frequent in artificial spawning in the pikeperch and the Eurasian perch. This prevents effective breeding programmes for genetically valuable breeding lines, carrying out genome manipulations or egg fertilization with cryopreserved sperm, which require manipulation on eggs before contact with water (Foresti 2000; Bokor, Muller, Bercsenyi, Horvath, Urbanyi & Horvath 2007; Bokor, Horvath, Horvath & Urbanyi 2008). Moreover, incubation of eggs obtained in spontaneous reproduction creates numerous problems and negatively affects embryo survival and the number of larvae produced (Kucharczyk *et al.* 2007). Hence, a precise system determining the oocyte maturity and the latency time between hormonal stimulation and ovulation is very important in aquaculture.

The moment of ovulation following the administration of hormonal agents depends closely on the oocyte maturity, which was determined *in vivo* on the basis of a classification system, which, in turn, was based on the position of the germinal vesicle (GV) in pre-ovulatory oocytes (Kucharczyk *et al.* 1996, 2008; Zakęś & Demska-Zakęś 2009). The system developed for cyprinids covered four stages:

Stage I – GV in the oocyte centre
Stage II – early stage of a migrating GV (less than half the oocyte diameter)
Stage III – late stage of a migrating GV (more than half the oocyte diameter)
Stage IV – peripheral GV.

This classification was first given in its simplest form (Brzuska & Bieniarz 1977) and was subsequently improved in later years. In its current form, it is based on the results of histological and histochemical investigation of oocytes during final oocyte maturation (FOM) (Brzuska 1979). Next, these stages were improved during *in vivo* experiments in common carp, *Cyprinus carpio* L. (Brzuska 1988). This system was applied in controlled reproduction of pikeperch (i.e. Zakęś & Demska-Zakęś 2009). For many fish species, including pikeperch and Eurasian perch, this classification is insufficient to describe the changes that occur during the FOM (Migaud, Mandiki, Gardeur, Fostier, Kestemont & Fontaine 2003; Migaud, Fontaine, Kestemont, Wang & Brun-Bellut 2004; Kucharczyk *et al.* 2007). Thus, Kucharczyk *et al.* (2007) in a description of the maturational stages in pikeperch included information on the importance of oil droplet morphology in pre-ovulatory oocytes as an important indicator. However, on the basis of published data, some imprecision regarding stage IV can be noted. Kucharczyk *et al.* (2007) included germinal vesicle breakdown (GVBD) in the fourth stage, whereas Zakęś and Demska-Zakęś (2009) classified oocytes after GVBD as a stage IV. Despite such ambiguity in the classification, a relationship between latency time and stage of oocyte maturity in the pikeperch has been reported by Kucharczyk *et al.* (2007, 2008) and Demska-Zakęś and Zakęś (2002). However, considerable differences in ovulation occurrence, variability and low latency time predictability are reported, although the reproduction procedures are similar (Zakęś & Demska-Zakęś 2009). Therefore, it is justified to classify FOM stages for pikeperch in a more precise manner in order to increase the predictability of the latency time.

A new method of pre-ovulatory oocyte classification has been applied in this study in controlled reproduction of a wild pikeperch population in order to improve reproduction effectiveness and synchronization.

Materials and methods

Broodstock handling

Pikeperch spawners, selected by size to achieve homogeneity of female age [total length (TL) of about 50 cm, 14 males and 33 females], were caught in 2010 with gillnets in Lake Dadaj (north-eastern Poland) at the beginning of April. The maximum water temperature in the lake did not exceed 12 °C. Immediately after being caught, the fish were transported separately in bags with oxygen supply to the hatchery of the Department of Lake and River Fisheries at the University of Warmia and Mazury in Olsztyn. On arrival, all fish were anaesthetized with an MS-222 solution (150 mg L^{-1}) (Argent, Redmond, WA, USA), tagged and weighed (± 0.1 g). Females were measured for TL (± 0.1 cm) and standard length (SL) and an oocyte sample was taken from each female using a catheter (with 2 mm external and 1.2 mm internal diameter) as described by Kucharczyk *et al.* (2007). The oocyte samples were immersed in Serra's clarification solution (70% ethanol, 40% formaldehyde and 99.5% glacial acetic acid in the ratio of 6:3:1), which is commonly used for determination of the maturational stage of oocytes in

pikeperch (e.g. Kucharczyk *et al.* 2007, 2008). Following cytoplasm clarification, the oocytes were photographed under a stereoscopic microscope (Leica MZ 12.5, Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) with the PROGRES® CAPTURE PRO 2.5 image acquisition software pack (Jenoptic, Germany). The images were used later in measuring oocyte diameters. Moreover, 30 randomly selected oocytes were classified on the basis of the classification developed by D. Żarski, Z. Bokor, L. Kotrik, B. Urbanyi, A. Horvath, K. Targońska and D. Kucharczyk (unpublished) for the Eurasian perch, which was based on detailed microscopic analysis throughout the FOM period, taking into account the GV position and the morphology of oil droplets. The pikeperch modified classification included seven stages, from the final stage of vitellogenesis to ovulation:

- Stage I – the GV is situated in the oocyte centre, and oil droplets are poorly visible (Fig. 1a);
- Stage II – the beginning of GV migration (GV in majority is located very close to the centre of oocyte) and the beginning of coalescence of oil droplets, which are very well visible (Fig. 1b), are observed;
- Stage III – migrating GV (reached half the oocyte diameter) and oil droplets are clearly visible (Fig. 1c);
- Stage IV – the GV is located above half the oocyte and a large oil droplet is clearly visible (the droplet diameter is greater than the GV diameter and it reaches the size of about 1/3 of the oocyte diameter) with visible smaller droplets (Fig. 1d);
- Stage V – the GV is located above half the oocyte, and one large (size of about half the oocyte diameter) oil droplet is clearly visible (Fig. 1e);
- Stage VI – oocyte samples taken for analysis are macroscopically transparent; no visible GV after they were placed in Serra's solution (following GVBD) and oocytes at the pre-ovulation stage (Fig. 1f) are observed;
- Stage VII – ovulation is observed.

This modification of the classification, in comparison with that described by D. Żarski, Z. Bokor, L. Kotrik, B. Urbanyi, A. Horvath, K. Targońska and D. Kucharczyk (unpublished), regarded only the position of the GV. In Eurasian perch, the GV in stages IV and V was clearly located at the edge of the oocyte, whereas the position of the GV in pikeperch in these stages was closer to the oocyte centre (as presented above).

Experimental design

The fish were divided by maturity stage. The percentage of oocytes was taken into account when classifying each female. The main variable used for this classification was the majority of oocytes (over 50%) representing a specific stage. The following groups were thus isolated: E1, E2, E3, E4, E5 and E6, which represented the maturity stages I, II, III, IV, V and VI respectively. The number of females is presented in Table 1. Each female in each group was injected with hCG (Biomed, Poland) at 500 IU kg^{-1} of body weight. After the injections, the fish were placed in 1000 L tanks with controlled water temperature and photoperiod (Kujawa, Kucharczyk & Mamcarz 1999). The water temperature in the tanks was 12 °C throughout the experiment. Such thermal conditions were applied to maximize the latency period and to avoid the effect of thermal stimulation on the oocyte maturation rate and the moment of ovulation. Moreover, females were separated from males to avoid pheromonal stimulation between males and females. The fish were not fed while they were kept inside the hatchery. All procedures (checking the oocyte maturation, hormonal stimulation) were conducted on same day that the fish were caught.

Data collection

Ovulation control started 20, 40, 50, 60 and 80 h after the injection in groups E5, E4, E3, E2 and E1 respectively. The females were then subsequently generally checked (by gentle massage of the abdomen) to determine if the females were ready to spawn, every 5 h until the first female ovulated. Next, the females were then checked every 3–4 h. The ovulation control in group E6 was started 5 h after injection and was carried out every 2–3 h. During these manipulations, fish were anaesthetized in an MS-222 solution (150 mg L^{-1}) and checked for any egg deposition by delicately massaging their abdomen. If ovulation occurred, eggs were collected in dry plastic containers by stripping. Latency time was recorded during the examination as well as the egg weight and the post-spawn female weight. When females were found to deposit eggs in the tank, the female was localized, anaesthetized, the remaining eggs were collected, if possible, and the post-spawn weight was recorded. Latency time for females that ovulated in the tank is regarded as the moment when such female was localized. After spawning, the females were transferred to a separate tank. Female mortality rate was recorded during the examination in each group until day 5 after spawning.

Gamete management and embryo incubation

Eggs collected from every female fish under controlled conditions were mixed with sperm collected

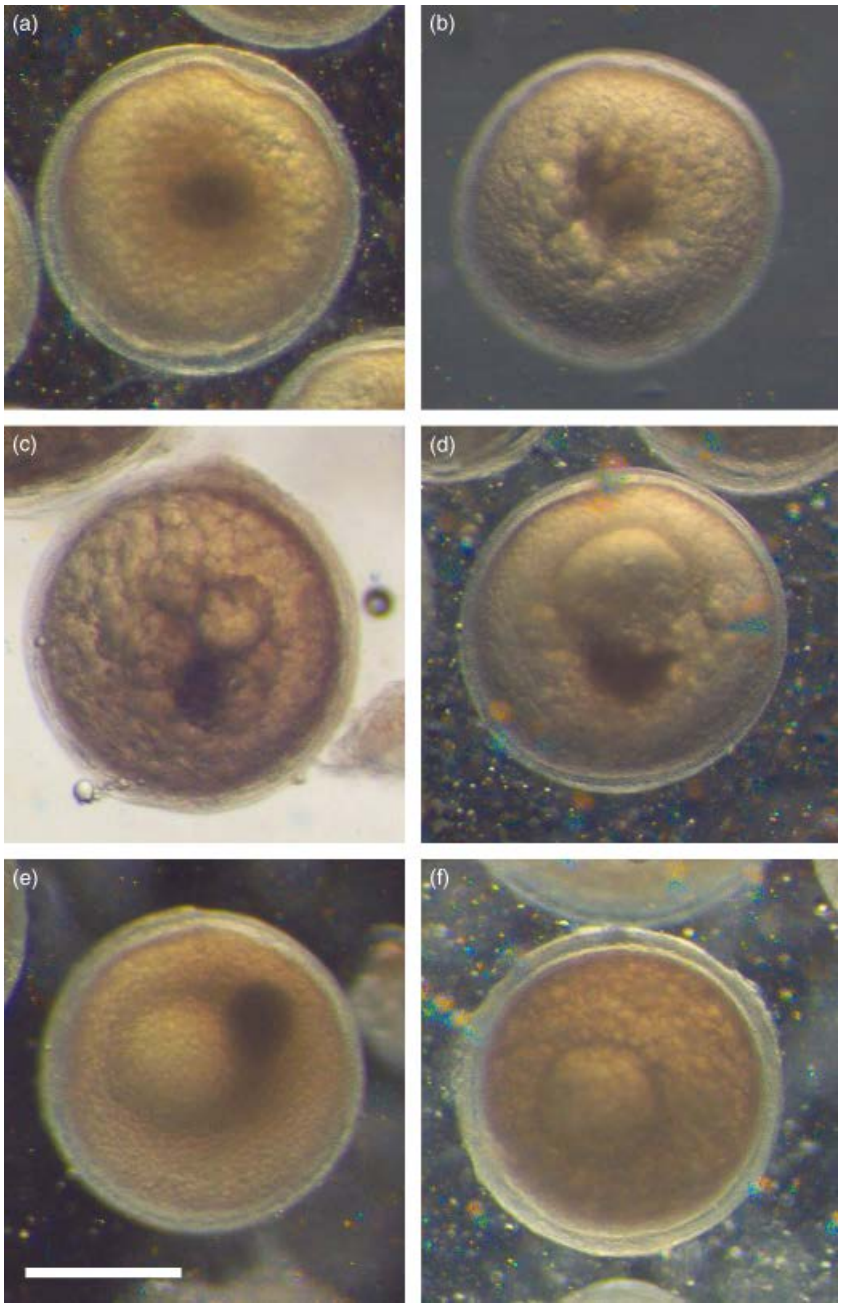

Figure 1 Preovulatory oocyte maturation stages in pikeperch, *Sander lucioperca* (L.) after cytoplasm clarification: (a) stage I, (b) stage II, (c) stage III, (d) stage IV, (e) stage V and (f) stage VI. Oocytes were cathetered shortly (on the same day) after the fish were caught. Bar represents 0.5 mm.

from at least three males and fertilized with clean 'hatchery water'. The sperm was collected with calibrated (± 0.1 mL) sterile syringes and 1 mL of the sperm mixture was used per every 100 g of eggs. Subsequently, 5 min after fertilization, the egg unsticking operation was carried out by a 5 min bath in tannin solution (0.7 g L^{-1}), using the method described by Kucharczyk *et al.* (2007). Eggs from each female were incubated in separate Weiss jars in closed water circulation at a constant temperature of 14 °C. The percentage of live embryos was determined 72 h after fertilization on the basis of 100 randomly selected embryos (in three replications). This incubation time was sufficient to evaluate the reproductive

Table 1 Initial characteristic of pikeperch females classified into six different maturational stages (groups E1 to E6)

		TL (cm)		SL (cm)		Weight (g)	
Group	*n*	Arithmetical mean	SD	Arithmetical mean	SD	Arithmetical mean	SD
E1*	2	52.2	—	45.9	—	1357	—
E2	8	53.2	5.9	46.6	5.8	1438	478
E3	7	50.8	2.7	44.5	2.5	1312	203
E4	5	53.8	6.9	46.9	6.5	1399	511
E5	6	51.5	2.0	44.4	2.2	1239	173
E6	5	52.7	5.1	46.4	4.6	1523	492

TL, total length; SL, standard length. No statistical differences were found between groups ($P > 0.05$).
*This group was excluded from the statistical analysis owing to the insufficient number of females.

effectiveness in Eurasian perch (e.g. Migaud, Wang, Gardeur & Fontaine 2006). In the case of pikeperch, it also allowed for embryo survival evaluation before the dead embryos were removed from the incubation jar.

Data analysis and statistics

The initial and final female weights made it possible to calculate the pseudo-gonadosomatic index (PGSI) for each stage, this latter representing the percentage of egg weight versus female body weight upon the fish being caught. The index was calculated from the formula: $(BW_1 - BW_0) \times 100 \times BW_1^{-1}$, where BW_1 and BW_0 represent the initial and final (post-spawn) body weight (g) of females. In addition, the relative fecundity of females was calculated, which was expressed as egg weight per kg of female body weight ($g\,kg^{-1}$) according to the formula: $EW \times (BW_0)^{-1}$, where EW represents the eggs weight (g) and BW_0 – post-spawn female body weight (kg). Fulton's female condition index was calculated from the formula $K = 100 \times W \times L^{-3}$, where W is the mean body weight (g) and L is the length (cm). The condition index was calculated for TL (K_{TL}) and SL (K_{SL}). Moreover, the diameters of 30 randomly selected oocytes, sampled from each female, were measured (± 0.01 mm) under the stereoscopic microscope, after clarification of the cytoplasm in Serra's solution, as described above.

All of the data (expressed in percentages) were subjected to arcsine transformation before statistical analysis. The data for the condition indices (K_{TL} and K_{SL}) and relative fecundity were analysed by a Kruskal–Wallis non-parametric test ($\alpha = 0.05$). The other data (weight and length of females, ovulation rate, female and embryo survival, latency time, PGSI and oocyte diameter) were analysed by ANOVA. Where the analysis revealed significant differences, an *post hoc* Duncan test ($\alpha = 0.05$) was carried out. The regression analysis between oocyte diameter and maturity stage (linear regression), between PGSI and maturity stage (second-degree curvilinear analysis of regression) and between maturation stage and latency time (linear regression) was conducted. The statistical analysis was carried out with STATISTICA 9.0 (StatSoft Inc., Tulsa, OK, USA) as well as MS Excel 2007 for Windows.

Results

Female fish of similar sizes were found to be at different maturity stages. The highest numbers of females were recognized at stages II and III (eight and seven fish respectively) of the proposed classification. Only two fish were classed as stage I (Table 1). No statistical differences ($P > 0.05$) were found between experimental groups for TL (50.8–53.8 cm), SL (44.4–46.9 cm) or female body weight (1239–1523 g) (Table 1). A positive correlation was found between the proposed stage of maturity and oocyte diameter. However, no significant differences were found between groups ($P > 0.05$) (Fig. 2). A positive correlation between PGSI and maturity was found only until stage IV–V. This was followed by a decrease in PGSI at stage VI (Fig. 3). Significant differences for PGSI were found between female fish only in E1 (12.87% of BW) and E5 (16.46% of BW) ($P < 0.05$). However, no differences were found with respect to K_{SL} (mean: 0.94–1.48) or K_{TL} (mean: 0.62–1.00) (Table 2).

No female fish from group E1 survived after being kept in the hatchery. Therefore, no additional data were obtained for this group. No significant differences were recorded in the other groups with respect to ovulation percentage (75–100%), female survival

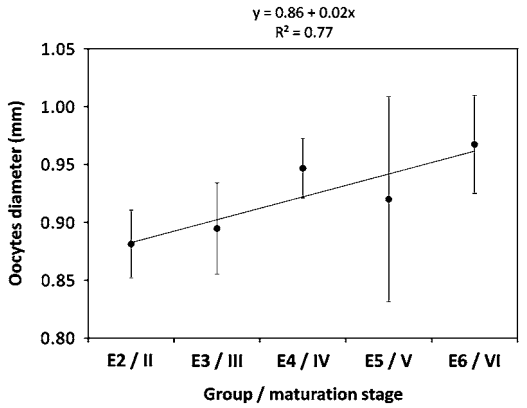

Figure 2 The relationship between oocyte diameter and maturation stage of pre-ovulatory oocytes classified into six different stages (groups from E2 to E6) in pikeperch, *Sander lucioperca* (L.). Oocytes were cathetered shortly (on the same day) after the fish were caught. No statistical differences were found between the groups ($P > 0.05$). Black dots (closed circles) represent mean and the vertical lines represent SD. Mean calculated on the basis of the 30 randomly chosen oocytes from eight, seven, five, six and five females from groups E2, E3, E4, E5 and E6 respectively.

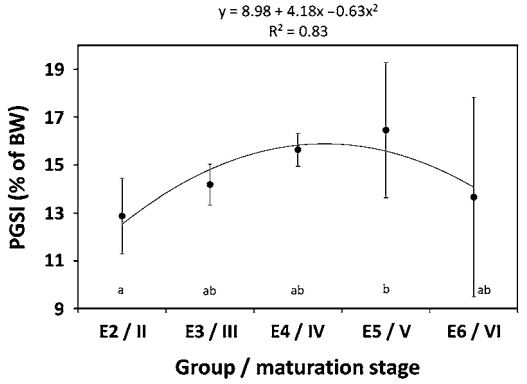

Figure 3 The relationship between pseudo-gonadosomatic index (PGSI) and maturation stage of females determined on the basis of different maturational stages of pre-ovulatory oocytes (groups from E2 to E6) in pikeperch, *Sander lucioperca* (L.). Data marked with different letters are statistically different ($P < 0.05$). Black dots (closed circles) represent mean ($n = 6$, $n = 7$, $n = 5$, $n = 5$ and $n = 4$ for groups E2, E3, E4, E5 and E6respectively) and the vertical lines represent SD.

rate (75–100%), embryo survival rate (71.25–79.50%) or relative fecundity (130.87–149.87 g kg^{-1}).

Significant differences between experimental groups ($P < 0.05$) (Fig. 4) were found in the latency time from injection to ovulation. The greatest range was found in group E6 (25 h) and the smallest was in E4 (10 h). The latency period overlapped for 1 h in groups E4 and E5, and E3 and E4. Regression analysis revealed a significant relationship between maturation stage and latency time ($R = 0.99$) (Fig. 4).

Discussion

Final oocyte maturation in fish is a process that usually starts immediately after completed vitellogenesis. During FOM, the first meiotic division is completed and the second division proceeds up to metaphase II. Moreover, GV migrates to the animal pole and GVBD and ovulation then occur (Rinchard & Kestemont 1996; Nagahama & Yamashita 2008). In addition, female body weight was observed to increase during FOM, probably because of the hydration of ovaries (Milla, Jalabert, Rime, Prunet & Bobe 2006; D. Żarski, Z. Bokor, L. Kotrik, B. Urbanyi, A. Horvath, K. Targońska and D. Kucharczyk unpublished), which causes the gonadosomatic index (GSI) to increase up to 22% of body weight in pikeperch (Schlumberger & Proteau 1991). In this paper, the process is reflected in the positive correlation between oocyte maturation stage (according to our classification) and oocyte diameter. The weight increase in the Eurasian perch (during FOM alone) can reach 11% (Migaud *et al.* 2003; D. Żarski, Z. Bokor, L. Kotrik, B. Urbanyi, A. Horvath, K. Targońska and D. Kucharczyk unpublished). Significant differences in GSI have been reported earlier between females of different classes of size and age (Lappalainen, Dorner & Wysujack 2003); hence, this experiment was carried out with females of similar size from one population. As the changes may be perceived subjectively, K_{TL} and K_{SL} were calculated to objectively characterize the degree of ovary hydration (by correlation between mass increase and length) as a potential oocyte maturation indicator. Moreover, PGSI was calculated, which indirectly characterizes gonad weight at various stages. However, the results show that a positive correlation can only be found until stage V of the proposed classification, followed by a small decrease in PGSI. However, the high variability eliminates the usability of this indicator in determining the maturity stage. A high variability of K prevents clear determination of the maturity stage on the basis of the indices of females of similar sizes. This indicates the absolute necessity to determine the female maturity stage on the basis of a pre-ovulatory classification of oocytes.

The reproduction results achieved in this study corroborate earlier reports on the percentage of

Table 2 Results obtained after induced spawning of wild pikeperch females under controlled conditions

Group	K_{TL}		K_{SL}		Ovulation rate (%)	Number of females depositing eggs in the tank	Females' survival (%)	Embryos survival at 72 h after fertilization (%)		Relative fecundity (g kg^{-1})	
	Mean	SD	Mean	SD				Mean	SD	Mean	SD
E1*	1.38	—	0.9	—	—	—	0	—	—	—	—
E2	1.21	0.50	0.81	0.33	75	3	75	71.25	13.35	143.67	38.87
E3	1.48	0.07	1.00	0.06	100	3	86	73.25	11.35	149.87	41.56
E4	0.94	0.82	0.62	0.54	100	2	80	77.00	15.87	133.65	43.65
E5	1.07	0.67	0.69	0.43	83	2	100	76.50	15.61	139.39	33.12
E6	1.19	0.73	0.74	0.50	80	1	80	79.50	9.71	130.87	40.76

Fish were treated with hCG (500 IU kg^{-1}) at six different maturation stages (E1–E6). No statistical differences were found between groups ($P > 0.05$).

*This group was excluded from the statistical analysis owing to the insufficient number of females.

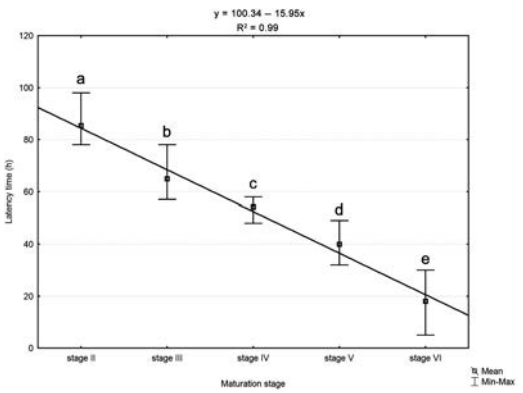

Figure 4 The relationship between maturation stage and latency time during induced spawning of pikeperch, *Sander lucioperca* (L.). Fish were treated with hCG (500 IU kg^{-1}) at six different maturation stages ($n = 6$, $n = 7$, $n = 5$, $n = 5$ and $n = 4$ for groups E2, E3, E4, E5 and E6 respectively). Data marked with different letters are statistically different ($P < 0.05$).

ovulation (Zakęś & Demska-Zakęś 2009), survival of embryos and spawners (Muller-Belecke & Zienert 2008; Zakęś & Demska-Zakęś 2009; Wang, Mandiki *et al.* 2009) and relative fecundity (Steffens, Geldhauser, Gerstner & Hilge 1996). The only recorded difference relates to the latency time following hormonal stimulation, which was probably caused by the thermal regime applied (2–4 °C lower than reported by other authors). Thus, the recorded latency time cannot be compared with other studies. According to the set of data published in a review by Zakęś and Demska-Zakęś (2009), the latency time in a female pikeperch during the reproductive season ranged from 10 to 70 h following an hCG injection. In out-of-season spawning, ovulation was observed within a range of 66 to 101 h following hCG injection, which was caused by a less advanced stage of oocyte maturity. Therefore, the data published to date indicate great variability in the latency period, despite applying similar reproduction procedures (including a thermal regime) as reported by other authors (Zakęś & Demska-Zakęś 2009).

The application of the 'old' system (developed for cyprinids) to the fish in this study would have extended the latency period in the fish groups. Moreover, stage I according to the 'old' system would have included fish from groups E1 and E2 of the present classification. This would have caused a lack of ovulation in some of the females (as in group E1). In addition, groups E3 and E4 would be regarded as a one group, where ovulation would be determined between 48 and 78 h following injection. As well, fish in group E5 in the 'old' system were frequently classified together with groups E3 and E4, as shown in the description provided by Zakęś and Demska-Zakęś (2009). Such ambiguity in the 'old' classification of maturation stages in pikeperch affected latency time variability. In the study conducted by Demska-Zakęś and Zakęś (2002), no differences were found between I and II stage, whereas differences were found between II and III stage of the 'old' classification. Therefore, if the old classification had been applied in the present study, this would have given similar results to those described earlier, i.e. the time of ovulation would have been extremely difficult to predict.

Kucharczyk *et al.* (2007) and Zakęś and Demska-Zakęś (2009) reported that a temperature ranging

from 14 to 16 °C is usually applied in pikeperch reproduction. It has been previously reported that a temperature that is lower by several degrees than the optimum value does not affect the ovulation rate, but extends the latency period and causes desynchronization of ovulation (Żarski, Kucharczyk, Sasinowski, Targońska & Mamcarz 2010). This study confirms these reports. This procedure enabled extending the latency period in each stage, providing statistical confirmation of the high usability of the new classification of the pikeperch pre-ovulatory maturity in reproduction. This is very important in view of the fact that fish caught at short intervals in the same lake showed great variability in their maturity stage. Such great variability may have caused a broad range of latency times in the studies published to date. Therefore, it may be suggested that immediately after being caught, female fish should be classified according to the proposed maturity stage and subsequently, after the appropriate thermal, light and endocrinological manipulations (Kucharczyk *et al.* 1996, 1998; Migaud *et al.* 2004; Zakęś & Demska-Zakęś 2009; Mylonas, Fostier & Zanuy 2010), the reproduction procedure should be carried out at a convenient time. Such actions may significantly reduce the amount of work and stress for fish associated with the manipulations.

The proposed system of pre-ovulatory oocyte maturity is based on the nucleus position in the oocyte and on the coalescence of oil droplets. The latter feature, which is typical of percids (Sulistyo, Rinchard, Fontaine, Gardeur, Capdeville & Kestemont 1998; Migaud *et al.* 2003; D. Żarski, Z. Bokor, L. Kotrik, B. Urbanyi, A. Horvath, K. Targońska and D. Kucharczyk unpublished), has proven to be an important indicator in pikeperch. The system creates the possibility of greater precision in determining the moment of ovulation, which may positively affect pikeperch reproduction effectiveness in controlled conditions. However, precise prediction of ovulation time with the proposed classification system must be preceded by proper verification in local conditions. However, it should be noted that the latency time during controlled reproduction is directly affected by differences between populations (Krejszeff, Targońska, Żarski & Kucharczyk 2010), water temperature (Targońska, Kucharczyk, Kujawa, Mamcarz & Żarski 2010; Żarski *et al.* 2010), type and dose of hormone (Żarski, Kucharczyk, Targońska, Jamróz, Krejszeff & Mamcarz 2009) as well as the level of domestication (Muller-Belecke & Zienert 2008; Krejszeff, Targońska, Żarski & Kucharczyk 2009; Kujawa, Kucharczyk, Mamcarz, Żarski & Targońska 2011), but more work is needed to include the effects of these variables.

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