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Annual Developmental Cycle of Gonads of European Perch Females (*Perca fluviatilis* L.) from Natural Sites and a Canal Carrying Post-cooling Water from the Dolna Odra Power Plant (NW Poland)

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The European perch is a species endowed with high adaptation capabilities as regards different environmental conditions. The aim of the study was to analyse the annual developmental cycle of ovaries of the European perch from the Oder river, Lake Dąbie and a drainage canal (Warm Canal) carrying post-cooling water from the Dolna Odra power plant (annual average water temperature in the canal is higher by 6-8°C than the water of the other sampling sites). Most of the female perch caught in the canal carrying post-cooling water had immature stage 2 gonads (delayed development of the gonads) and were smaller than the fish from the other sites. No traces of spawning in the form of deposed egg strings were found in the drainage canal. Adult individuals avoid high temperatures found in the Warm Canal. In April, in perch from all sites, ovaries with post-spawning oocytes were observed. The spawning season of the females lasted from the beginning of April until May. Stage 4 of gonad development, with oocytes in advanced vitellogenesis, was the longest and ranged from September through February.

Key words: European perch, *Perca fluviatilis*, annual cycle; gonads; females; cooling water.

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The European perch is a common fish species inhabiting both standing and flowing water, as well as brackish water (TERLECKI 2000). Characterization of gonad generation and the stages of gonad development constitutes a major subject of studies on this species (SANDSTRÖM *et al.* 1995; MIGAUD *et al.* 2003). Investigations of gonad development, hormonal regulation and fertility have been conducted in many parts of the distribution of this species (SULISTYO *et al.* 1998; MIGAUD *et al.* 2002, 2003). The European perch is a species endowed with high adaptation capabilities (HEIBO 2003). Female gonads in this species are capable of developing normally even at pH 4.0-5.0 (TALIKINA *et. al.* 1996). However, water pollution causes morphological disorders in perch (ØSTBYE *et al.* 1997) and induces hormonal changes in the sexual cycle of females (NOAKSSON *et al.* 2005), oocyte degeneration (VIRBICKAS & LUKŠJENĖ 1993), problems with spawning (LUKŠJENĖ *et al.* 2000) and decreased fertility (SANDSTRÖM *et al.* 1995). Therefore it is crucial to monitor the development of this species in waters subjected to moderate anthropogenic pressure. To this end, an analysis of sexual cycle and gonad development status was conducted in female perch from the mouth of the Oder river, found at sites carrying water of good quality (WIOŚ 2014): the Oder river, Lake Dąbie and a canal carrying post-cooling water (Warm Canal). Sample collection sites at the Oder river and Dąbie Lake were similar regarding temperature, while water temperature in the Warm Canal was higher. The aim of the study was achieved by analysis of histological samples of gonads, determination of the stages of gonad development, analysis of gonad maturity rate, measurements of oocytes in previtellogenesis and at each stage of vitellogenesis, as well as identification of oocytes undergoing degeneration. The conducted study will elucidate the course of the sexual cycle of female perch from the three aforementioned sites.

Material and Methods

Study areas

The fish were caught at three sites (1) the eastern Oder River, up to 20 km above the Dolna Odra power plant; (2) an effluent canal (Warm Canal) from the power station at 200 m from the point of discharge of cooling water (it is an open canal and fish can freely move in and out of it), Nowe Czarnowo, near Gryfino, NW Poland (53°11'N, 14°29'E), (3) Lake Dąbie, 20 km below the Dolna Odra power plant. Lake Dąbie is an eutrophic lake positioned in the catchment area of the Oder and P³oñ rivers (53°27'49"N 14°40'29"E) within the administrative border of the city of Szczecin, in NW Poland, at about 20 km below the Dolna Odra power plant. The area of the lake is 52 km^2, its maximum depth is 6.8 m, while the mean depth is 2.8 m.

The mean water temperature in the Oder river and Lake Dąbie in the season of study was 11.8°C, in April, May and June it was 11.7, 16.0 and 22.3°C, respectively, while in the Warm Canal it was 20.0, 23.1 and 28.0°C, respectively; the average annual temperature of the water in the Warm Canal is higher by 6-8°C in relation to the other analyzed waters (DOMAGAŁA, unpublished data). The O_2 content in the water of the Oder upstream of the canal varied between 7.9 mg/dm^3 (December) and 14.9 mg/dm^3 (July), while in Lake Dąbie it varied between 6.2 mg/dm^3 (August) and 12.0 mg/dm^3 (March), (DOMAGAŁA, unpublished data). Temperature and dissolved oxygen concentration (DO) at the sites were measured using a DO and pH meter (CX-401, Elmetron, http://www.elmetron.com.pl/). Details of the methods can be found in DOMAGAŁA and PILECKA-RAPACZ (2007).

Sampling and preparation

The perch were caught for analysis at the three sites between June 2009 and May 2010, and from each site, 1-4 specimens per month were taken. Altogether, 470 females were caught (Table 1). The fish were bycatch in commercial fishing. The fish were aged 2+ to 9+; the age was determined based on an analysis of rings on collected scales. After the fish were caught, the body length (total length TL) of the fish was measured to the accuracy of 0.1 mm. They were weighed on an electronic scale to the accuracy of 0.1 g. Then, the gonads were prepared and fixed in Bouin fluid, and weighed to the accuracy of 0.1 mg. Histological analysis was performed on all female gonads. According to the standard paraffin technique, histological specimens with a thickness of 5 mm were made from the middle section of the gonad and stained with Heidenhain ferruginous haematoxylin. From 50 to 100 snips were observed from one female gonad. The preparations were evaluated under a Nikon Eclipse 80i microscope which has a maximum magnification of 1000x.

The aims of the histological evaluation of the gonads were to (1) determine the gonad maturity stage. In this case, a universal VI-stage scale by SAKUN and BUTSKAYA (1968) as modified by DOMAGAŁA *et al.* (2013) was used, and the stage of gonad maturity was classified on the basis of the most developed oocytes (WEST 1990). (2) Describe the diameters of oocytes at each development stage and in each month; (3) identify oocytes undergoing degeneration. The following measurements were taken: (1) the most developed oocytes at the highest gonad development stage of a given month (5 females per site, 30 diameters per 1 gonad); (2) oocytes at each development stage from each site – oocytes in vitellogenesis at the stage of vacuolization, yolk accumulation and termination of vitellogenesis (10 gonads per stage, 30 measurements per 1 gonad). The measurements were taken to the accuracy of 0.01 mm using the NIS Elements BR 3.0 system of digital image analyser. Oocyte diameters were calculated from measurements of the longest and shortest oocytes with a visible nucleus (HUNTER & GOLDBERG 1980). Photographs of the gonad preparations were taken with a Nikon digital camera.

Table 1

Number of fish used in the study

Fishing location	Months												Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
	number of fish												
Oder River	23	3	21	13	40	13	4	10	28	8	12	3	178
Dąbie Lake	13	7	9	25	12	11	17	12	10	18	7	4	145
Warm Canal	6	9	12	10	12	15	15	15	12	13	12	6	137
													470

Table 2

Characteristics of the examined fish

Fishing location	Total length (cm)	Fish weight (g)	C_F	Gonad weight (g)	GSI
	mean±SD range				
Oder River	21.70±5.68[c] 15.40-35.30	151.05±137.56[c] 36.70-617.00	1.27±0.23[c] 0.91-2.40	13.97±24.83[c] 0.18-114.60	6.92±6.85[c] 0.26-22.40
Dąbie Lake	18.79±4.27[b] 15.20-36.30	104.54±129.27[b] 31.20-774.10	1.20±0.50[b] 0.80-1.81	9.56±26.10[b] 0.07-205.50	5.02±7.24[b] 0.04-26.55
Warm Canal	15.34±3.85[a] 11.80-30.50	57.02±71.69[a] 14.20-430.00	1.19±0.17[a] 0.79-1.59	0.46±2.08[a] 0.04-16.30	0.76±2.23[a] 0.23-15.55

Values marked with different letters (a, b, c) are significantly different ($P<0.05$; Kruskall-Wallis test).

Statistics

Statistical analysis was done using the program STATISTICA (data analysis software system), version 9.1. www.statsoft.com., StatSoft, Inc. (2010). The nonparametric Kruskal–Wallis test was used to analyse the following characteristics of the perch from three sites: fish length and mass, condition coefficient (C_F), gonad mass, gonadosomatic index (GSI) and oocyte size. The nonparametric Mann-Whitney U test was used to evaluate oocyte size in perch from two sites.

Results

General characteristics

The length of female perch from the Oder river and Lake Dąbie was between 15.2 and 36.3 cm, while fish mass ranged between 31.2 and 774.1 g. Mean length and mass was the highest in the fish caught in the Oder river, however, the perch from each site differed significantly (Table 2). Most of the caught fish (90% from Oder River and 88% from Dąbie Lake) were adult, aged 2 and above, and the abundance of perch from the Oder river and Lake Dąbie was sufficient for the analysis of the annual cycle of gonad development. Conversely, most of the female perch (68%) caught in the Warm Canal were young and had immature gonads. Despite multiple catches, also in the preceding seasons, only a few females with post-spawning gonads were caught in the Warm Canal between March and June.

Fish from all sites were in good condition and their mean C_F value ranged between 1.24 and 1.27, exhibiting similar annual dynamics (Fig. 1a).

Gonad mass, GSI

Gonad mass in the female perch from the Oder river and Lake Dąbie was the lowest in July and August. In the following months gonad mass increased gradually, reaching a peak value for perch from the Oder river and Lake Dąbie in March. In these months, gonad mass did not differ significantly in perch from those sites (Man-Whitney U test, $P>0.05$), Fig. 1b. Gonad mass in females from the Warm Canal was significantly lower than that of females from other sites.

The GSI value in female perch was the lowest in the summer months and in July averaged 0.20 and 0.41 in fish from the Oder river and Lake Dąbie, respectively. In the following months GSI gradually increased and a pronounced increase in its value occurred in perch from both sites between January and March (Fig. 1c). The average GSI values in the group of females from the Oder river and Dąbie Lake with pre-spawning gonads in the period March-May were 13.4 and 12.5, respectively, while in the post-spawning group, the values were 2.8 and 3.2, respectively. After the spawning season the GSI value decreased rapidly and was below 1 in perch from both sites in May. The GSI value in the females from the Warm Canal was the highest in April.

Annual gonad developmental cycle

The Oder river and Lake Dąbie

Between June and August female perch from both sites were at stage II and III of gonad development (Fig. 3a, b). Oocytes from stage 2 gonads found in fish caught in July contained few lipid droplets, while oocytes obtained in August had multiple lipid droplets (Fig. 2a). In September, 66 and 70% of female perch from the Oder river and Lake Dąbie, respectively, already had stage 4 gonads in vitellogenesis with oocytes containing

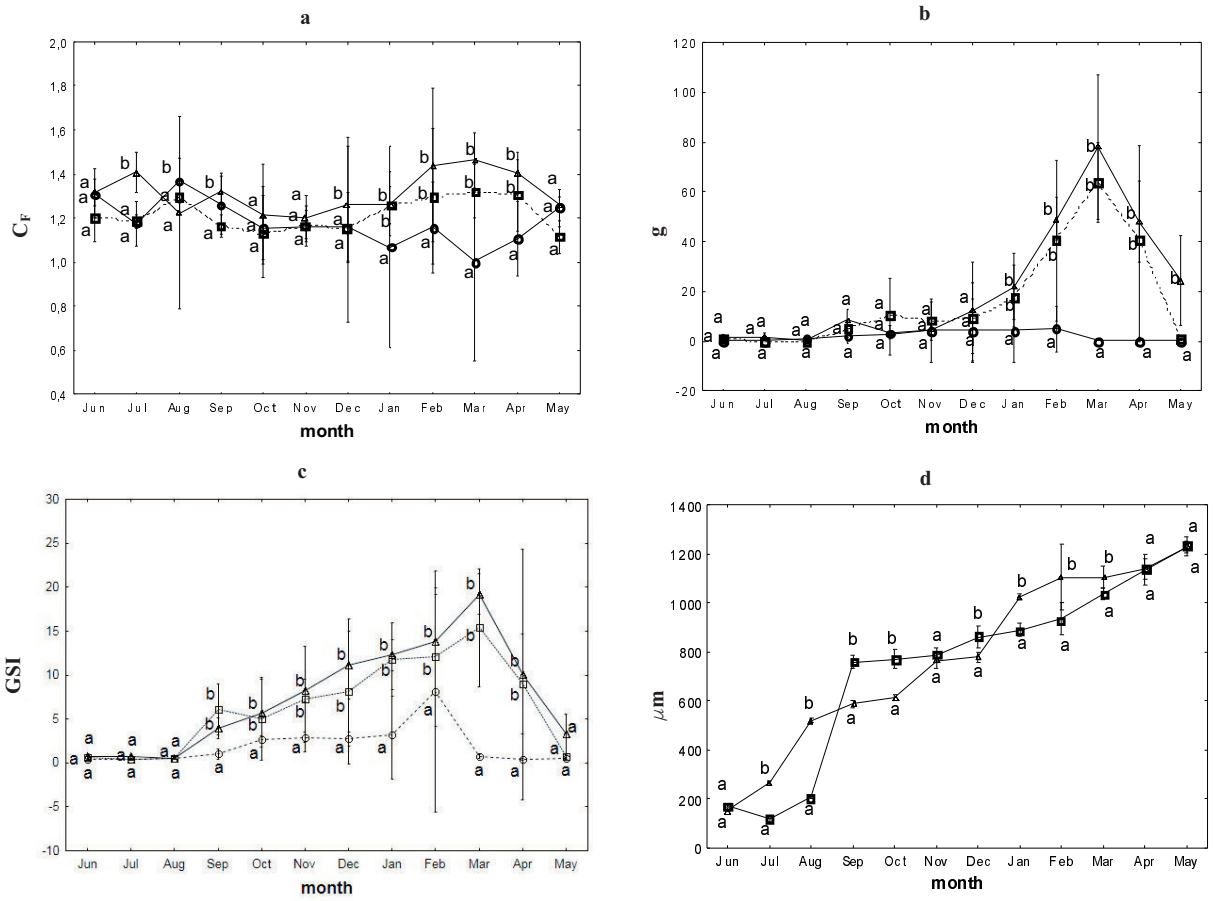

Fig. 1. Gonad weight (a); GSI (b); CF (c); the diameter of the most developed oocytes (d) analyzed female perch of the study areas: ▵ Oder River; ○ Warm Canal; □ Dąbie Lake.
Values marked with different letters (a, b) are significantly different (P<0.05; Kruskal-Wallis test).

multiple lipid droplets and yolk (Fig. 2b). Among the female perch caught in the Oder river in October, 30% still had stage III gonads, while the gonads of the remaining fish were in advanced vitellogenesis (Fig. 2c). All individuals from Lake Dąbie (since October) and the Oder river (since November) until February had stage IV gonads with oocytes at peak size (Fig. 1d).

In late March/early April, in perch from both sites, fusion of yolk granules and oocyte nucleus migration occurred in gonads, along with spawn (stage V). In April, some perch from both sites had already completed spawning with the postovulatory follicles of unreleased oocytes (stage VI) and oocytes in previtellogenesis (Fig. 2d). Other perch in that period finalized vitellogenesis or were about to start spawning. In May, the gonads of most perch from the Oder river and all perch from Lake Dąbie were recovering after spawning at stage II and III. In the first decade of May, a few individuals from the Oder river still had their gonads in the pre-spawning state and in the post-spawning state with few oocytes unreleased during spawning (Fig. 2e). Therefore, the spawning season of perch in Lake Dąbie and in the Oder river occurred in April and May. In the gonads of perch caught in May at both sites, degenerating oocytes were also observed (Fig. 2f).

The increase in oocyte diameter in perch from the Oder river was gradual (Fig. 1d). The largest oocytes finalizing vitellogenesis (1227.7 μm in diameter) occurred in females in early May. A leap in oocyte size in late August / early September was observed in perch from Lake Dąbie, which was caused by intense vacuolization at the beginning of vitellogenesis. The largest oocytes (1133.7 μm) in these perch were found in April. Oocyte sizes in perch from both sites were similar during vacuolization and yolk accumulation. Significant differences in oocyte size occurred at the end of vitellogenesis (Mann-Whitney U test, P<0.05), (Table 3).

The annual gonad developmental cycle in the female perch from the Oder river and Lake Dąbie was very similar. Based on the histological analysis of ovaries, the spawning season started at the beginning of April and ended in May.

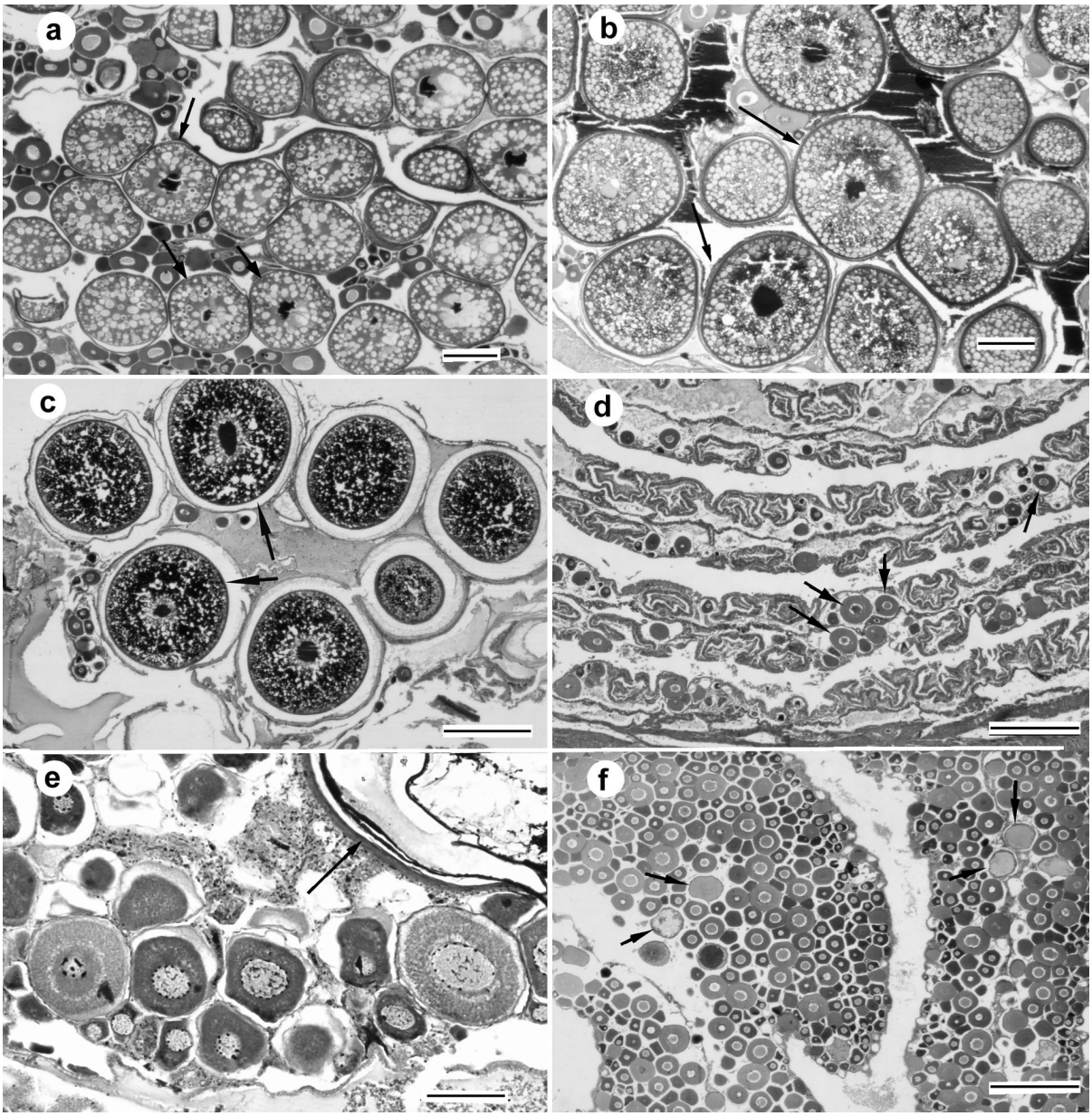

Fig. 2. Gonad of female perch with oocytes in early vitellogenesis (arrow), August, bar = 200 μm (a); with oocytes in rapid growth in vitellogenesis (arrow), September, bar = 200 μm (b); in advanced vitellogenesis (arrow), early January, bar = 500 μm (c); after spawning, the oocytes at the beginning of the next generation of previtellogenesis (arrow), April, bar = 100 μm (d); with numerous degenerated oocytes (arrow), May, bar = 100 μm (e); the absorbed individual oocytes (arrow), May, bar =100 μm (f).

Warm Canal

Most female perch (68%) caught at this site had immature gonads. In June, all individuals had stage II gonads. In July, few individuals had stage III gonads, while the remaining perch still had stage II gonads. Between August and November the gonads were at stage II and III. Between November and March, a few individuals had stage III gonads, while the remaining perch still had stage II and III gonads. In mid April and early May, 20% of females with post-spawning gonads were caught. They had the typical appearance of post-spawning gonads of perch from the other sites. Based on the material collected from the Warm Canal, it may be presumed that spawning, if it occurred in this reservoir, took place in April. However, no traces of spawning in the form of deposited egg strings were found in the water.

Discussion

The European perch is a common species occurring in Poland and most European countries, as

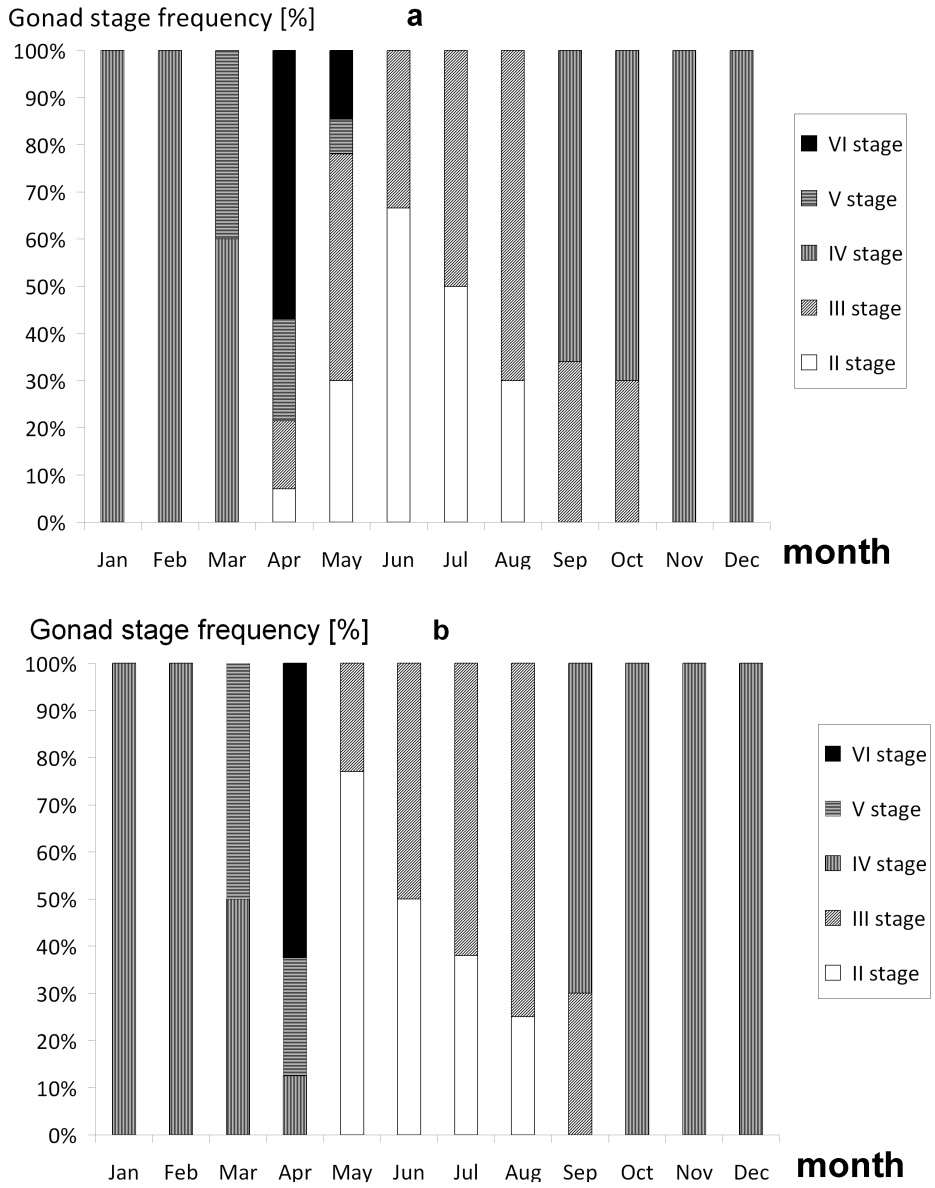

Fig. 3. Gonad maturity stages of females of perch from Oder River (a), Dąbie Lake (b).

Table 3

Sizes oocytes (μm) at different stages of perch vitellogenesis of two positions

Fishing location	Size oocytes		
	beginning of vacuolisation	first occurrence of the yolk	completed vitellogenesis
	mean±SD range		
Oder River	205.81±29.20[a] 108.30-254.58	518.74±19.06[a] 487.14-546.71	1139.05±82.73[a] 981.15-1267.81
Dąbie Lake	210.73±6.81[a] 192.64-225.14	586.46±41.81[a] 500.10-659.25	1227.42±36.99[b] 1187.71-1288.55

Values marked with different letters (a, b) are significantly different ($P<0.05$; Man Whitney U test).

well as North America and Asia. It is a species endowed with high adaptation capabilities as regards different environmental conditions (TALIKINA *et al.* 1996; EPLER *et al.* 2005; LINLØKKEN & HESTHAGEN 2011). The conditions in both Lake Dąbie and the Oder river are good for perch with respect to temperature and oxygen content (DOMAGAŁA & PILECKA-RAPACZ 2007). The sizes of the analysed fish were similar to the sizes of perch from Lake Geneva, ranging between 15 and 35 cm (DUBOIS *et al*. 1996), and were larger than perch from the Rożnów reservoir (15.9-27.42 cm, fish mass 49.3-319.2 g; EPLER *et al.* 2005). The few analysed individuals obtained their peak condition coefficient (C_F) just before spawning, in April, whereas perch from Lake Aydat obtained their peak C_F in March, two months prior to spawning (JAMET & DESMOLLES 1994). However, the highest mean C_F values in the course of the annual cycle were obtained in March, which coincided with the GSI peak observed in perch from other sites (JAMET & DESMOLLES 1994). The smallest females with post-spawning gonads caught in the Warm Canal were 12 cm in length, while the smallest perch that started spawning were 14.1 cm in length (CIEPIELEWSKI & HORNATKIEWICZ-ŻBIK 2006). The highest GSI values were obtained immediately before the spawning season, while the lowest values were obtained in June and July, after spawning, which is in accordance with TREASURER and HOLLIDAY (1981). The GSI values in females from the Oder river and Lake Dąbie were comparable to those reported by LECREN (1951) in Lake Windermere, English Lake District, by JAMET and DESMOLLES (1994) in Lake Aydat, France, by SULISTYO *et al.* (1998) in Lindre pond, France, and by NOAKSSON (2005) in Lake Isunda, Sweden.

Female perch start spawning for the first time at the age of 3-4 years (BIENIARZ & EPLER 1991; JAMET & DESMOLLES 1994). According to TERLECKI (2000), the spawning season of perch in Poland lasts from the second half of April until the end of May, while in France spawning lasts 6-7 weeks, from March to April (FONTAINE *et al.* 2004). The perch from the Rożnów reservoir started spawning in April (EPLER *et al.* 2005), while those from Lake Geneva in May (GILLET *et al.* 1995). In the Oder river and Lake Dąbie, spawning took place throughout April and May. The presence of female perch caught in the Warm Canal with post-spawning gonads in early May may indicate that spawning took place in April and early May. It is also possible that the females which spawned at another site might have moved into the Warm Canal where they were caught. During this period the temperature in the Warm Canal was 20°C in April and 22°C in May (DOMAGAŁA, unpublished data) and might have been too high for proper spawning to occur. The European perch is a flexible species endowed with high adaptation capabilities, also as regards the spawning temperature (HOKANSON 1977). According to TERLECKI (2000), perch spawning occurs at 6-22°C, while the optimum range is 12-16°C. As was demonstrated by KOPIEJEWSKA (2005), a temperature of 23 °C causes oocyte atrophy in previtellogenetic perch gonads. According to HELLAWELL (1986), perch spawning takes place in water at 5-19° C and the critical upper temperature is 36°C.

Gonad recovery started in June, while vitellogenesis growth started in July, similarly to perch from the Rożnów reservoir (EPLER *et al.* 2005). The ovaries of all perch caught between November and February contained oocytes with completed vitellogenesis, which is in accordance with the characteristics of spring-spawning fish (BIENIARZ & EPLER 1991). Gonads recovering after spawning (in previtellogenesis) and those in early and late vitellogenesis, found in perch from both sites, had a similar histological image to those found in perch from other sites characterized in the literature (TALIKINA *et al.* 1996). In the gonads of fish caught in May, degenerating oocytes that had not been laid in the spawning season (post-spawning gonads) and smaller degenerating oocytes in previtellogenesis (stage II gonads) were observed. Numerous oocyte resorption events induced by high temperature and shortened photoperiod were observed by SANDSTRÖM *et al.* (1995). In the spring, when the perch with degenerating oocytes were caught, water temperature in the Oder river and Lake Dabie did not exceed 20°C. In our climate, water temperature in these reservoirs probably did not affect gonad degeneration in perch caught in May. The optimum temperature for the development of perch is 25°C (HOKANSON 1977). However, the effect of increased temperature on accelerated gonad development in perch with partially degenerated oocytes in previtellogenesis cannot be excluded. The fish could previously remain in the Warm Canal at the Dolna Odra power plant (where water temperature in the warmest months, as in other years, reached 30°C (DOMAGAŁA, unpublished data), and then moved downstream into the Oder river.

Data on oocyte size in perch during vitellogenesis are scarce due to variation in descriptions of gonad developmental stages and distinguishing between intermediate stages. As reported by TALIKINA *et al.* (1996), the sizes of the most developed oocytes in July, September and February were larger than oocyte sizes in perch from the Oder river and Lake Dąbie (the smallest difference was observed in July). This may indicate that the fish from the Russian lakes Dubovskoye and Ut-

eshkovo (TALIKINA *et al.* 1996) initiate their gonad recovery earlier after spawning. However, further comparison indicates that with another spawning season approaching, oocyte sizes in the perch from the study by TALIKINA *et al.* (1996) become closer to the oocyte sizes reported here. In contrast, in the study by TREASURER and HOLLIDAY (1981) oocytes derived from ovaries of perch caught in August were smaller than those obtained in our study. This may be due to the effect of photoperiod on gonadal development of perch from different latitudes (SULISTYO *et al*. 2000).

The annual cycle of ovary development in perch from the Oder river and Lake Dąbie had a similar course, the spawning season started at the beginning of April and lasted until May, while oocytes presented normal morphology. Gonads of the few female perch caught in the Warm Canal also reached consecutive stages of development, similarly to the fish from the remaining study sites. However, no traces of spawning in the form of laid spawn strings were found in the Warm Canal. Sexual cycle analysis, as well as the size of the fish caught, demonstrate that the Oder river and Lake Dąbie are a good environment for the development of the fish. The exclusive presence of young individuals caught in the Warm Canal in the spawning season may possibly indicate that adult individuals avoid high temperatures found in the Warm Canal. The predicted climatic changes leading to increased temperatures in early spring and high temperatures in summer (VAN KLEEF *et al*. 2008) may affect the distribution and population structure of the species. As a result, such changes can have a significant impact on biodiversity of lakes and rivers.

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