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Ovarian alterations in wild northern pike *Esox lucius* females

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ABSTRACT: The aim of the present study was to analyse the occurrence of macroscopically visible ovary alterations in 2 populations of northern pike *Esox lucius* L. originating from lakes in the Mazurian Lake District (NE Poland). The alterations were characterised by ovary tissue that was morphologically malformed, in part or in whole, and contained immature oocytes, i.e. trophoplastic or previtellogenic oocytes instead of vitellogenic oocytes. These alterations were found only in the ovaries, and no morphological alterations of the testes were noted. Macroscopic and histological analyses were carried out in order to classify the observed alterations in the ovaries. Three types of alterations were identified in which morphological malformations as well as histological investigation of the ovaries were considered. An analysis of the size and age of the fish in relation to the occurrence of alterations as well as of the macroscopic and histological nature of the alteration types was made. The data obtained revealed no lake or age dependency of the observed alterations. Based on the results obtained, we suggest that the presence of endocrine disruptors in the environment or/and genetic factors could be responsible for these kinds of gonad anomalies. However, our results did not allow us to determine the aetiology of the alterations.

KEY WORDS: Northern pike · Ovary alterations · Endocrine disruptors · Reproduction · Recruitment success

INTRODUCTION

The northern pike *Esox lucius* L. is a predator situated at the top of the freshwater food web (Craig 2008). Its distribution area covers the Holarctic ecozone in Eurasia and North America (Crossman 1996). It is a species with a broad capability for adaptation to a wide variety of different environments. It has been found in both small and in large rivers, in lakes of various sizes and trophic levels and in brackish waters (Maitland & Campbell 1992, Jepsen et al. 2001, Craig 2008).

The northern pike is a fish of great ecological and economic importance. As a top-level predator, it directly affects the ichthyofauna structure (Casselman & Lewis 1996, Findlay et al. 2005). Controlling the size and age structure of its population may effectively help in biomanipulations aimed at counteracting negative effects of eutrophication of inland waters (Prejs et al. 1994, Berg et al. 1997, Findlay et al. 2005). Pike is one of the most important fish species for anglers and commercial fisheries (Habekovic & Pazur 1998, Paukert et al. 2001, Balik et al. 2006, Lappalainen et al. 2008). Hence, stocking programmes and scientific activities are carried out in many countries, aimed at supporting natural recruitment (Skov et al. 2002, Launey et al. 2003, Margenau et al. 2008). However, the size of many populations of

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northern pike has decreased (Lorenzoni et al. 2002, Launey et al. 2003, Balik et al. 2006, Wang et al. 2011). This has led to the development of techniques of artificial reproduction and rearing of larvae and broodstock under controlled conditions (Babiak et al. 1995, Szabo 2003, Hulak et al. 2008, Szczepkowski 2009) as well as population gene pool management programmes (Launey et al. 2003, Wang et al. 2011).

One of the most important factors which affect the population size is population fecundity, which also determines recruitment success (especially in exploited populations) (Rickman et al. 2000, Lambert 2008). In this context, any anomalies associated with reproduction or population fecundity are highly dangerous and should be explained, especially when such an ecologically and commercially important fish species is involved.

Within the present study, 2 populations of northern pike from 2 different lakes were investigated. Both of the lakes are situated at a distance of 18 km from each other in the Olsztyn Lake District, which is part of the Mazurian Lake District, the largest lake complex in Poland and one of the most extensive ones in Europe. Lake Mosąg (53° 51′ 20″ N, 20° 23′ 50″ E) is a typical flow-through eutrophic lake, situated on the biggest river in the region, Łyna River. The area of the lake is ~50 ha, and the maximum depth is 12 m (Choiński 1991). The structure of ichthyofauna in Lake Mosąg is typical of large lowland rivers (where the dominating species are rheophilic cyprinids including ide *Leuciscus idus*, asp *Aspius aspius*, and chub *Leuciscus cephalus*) and of eutrophic lakes (with northern pike, pikeperch *Sander lucioperca*, perch *Perca fluviatilis*, common tench *Tinca tinca*, bream *Abramis brama* and roach *Rutilus rutilus* dominating). Located 6.1 km above the lake is a wastewater treatment plant serving the city of Olsztyn. Lake Klebarskie (53° 44′ 30″ N, 20° 35′ 52″ E) is a typical eutrophic lake, with a maximum depth of 10 m and an area of ~190 ha. It is connected with Lake Silickie (with an area of ~73 ha). The lake is surrounded by agricultural land and meadows, and the village of Klebark Wielki (Choiński 1991) is located on the lake as well. Lake Klebarskie contains species typical of eutrophic lakes (for example, roach, rudd *Scardinius erythrophthalmus* L., perch, common tench and northern pike). The only possible source of pollution is the surrounding agriculture.

Anomalies in ovarian morphology, not previously reported for this species, were observed during commercial fishing in some females which originated from 2 separate lakes in the Mazurian Lake District (NE Poland). The alterations were found only in the ovaries, and no morphological alterations of the testis were noted. Therefore, a comparative analysis of some selected biological parameters and a histological analysis of the ovaries regarded either as morphologically ‘normal’ or ‘abnormal’ (with alterations of different natures and intensity) were carried out.

MATERIALS AND METHODS

Females of northern pike were caught with gill nets at 2 locations, Lake Mosąg (n = 60) and Lake Klebarskie (n = 77), in October and November 2010, at least 4 mo before the natural spawning season. The analyses were conducted during standard commercial processing of the fish (by a commercial processing company). The body length (longitudo caudalis, LC) was measured, and the age of the fish was determined based on the scales and the first ray of the dorsal fin. Subsequently, their abdomens were opened, and the ovaries were gently excised, then weighed and analysed for any alterations (morphological anomalies). Then, fragments of gonads (those regarded as ‘normal’ ovaries and those regarded as changed, i.e. ‘abnormal’) were cut out for histological analyses. After the internal organs were removed, the fish were weighed (without the entrails), and these weigths were used to calculate the gonadosomatic index (GSI). This eliminated the potentially variable weights of the other organs (because of, among other reasons, different levels of filling in the alimentary tracts). GSI was calculated according to the following formula: GSI = $(100 \times W_g)/W_0$, where W_g represents gonad weight, and W_0 represents the weight of the body together with the gonads but without the entrails. Additionally, the presence of the prey in the stomachs was noted.

All the tissues taken for histological analysis were fixed in Bouin’s solution (a saturated solution of 15 ml picric acid, 5 ml 40% formaldehyde and 1 ml glacial acetic acid) according to Zawistowski (1986). Tissues for analysis were dehydrated in an ethanol series of increasing concentration (from 50 to 96%), sealed in paraffin and cut on a microtome into 5 µm thick sections. The cross-sections were transferred onto microscope slides, de-paraffinised in xylene and hydrated in a series of alcohols. After staining the preparations with haematoxylin and eosin (Zawistowski 1986), they were dehydrated again, cleared in xylene and sealed in Canada balsam prior to analysis. Histological evaluation of the preparations was performed under a stereoscopic microscope (Leica MZ 16).

The data were presented as the mean values and the standard deviations (SD). They were used to analyse the percentage of fish ovaries with alterations found in each lake, the age of the fish in which alterations were observed and the GSI values. The different types of alterations were also classified, taking into account macroscopic morphology data, GSI values and histological analysis. GSI values were analysed with ANOVA, and then (after statistical differences were found), Tukey's post-hoc test was performed at the significance level of $\alpha = 0.05$.

RESULTS

The fish age ranged from 3+ to 6+. All the specimens investigated had prey in their stomachs. The largest age group was 4+ (25 and 32 fish from Lake Mosąg and Lake Klebarskie, respectively), whereas only 1 fish was caught at the age of 6+. The average weight of fish of the same age group in Lake Mosąg was higher than that of fish caught in Lake Klebarskie. The same relationship was recorded for the LC (Table 1). It is noteworthy that fish aged 4+ from Lake Mosąg were longer than fish aged 5+, but their average weight was lower. In contrast, fish aged 3+ from Lake Klebarskie had a higher average weight and a lower average length compared to fish aged 4+ (Table 1).

Characteristics of the ovaries observed in the analyses were classified into 4 categories on the basis of 2 criteria: (1) the portion of the gonads affected by the alteration (ovary tissue was often smaller than in normal ovaries and/or shrank and had no oocytes in the ovary tissue or contained oocytes at the previtellogenic or trophoplasmatic stage) and (2) the GSI. Alterations were distinguished as follows:

Table 1. *Esox lucius*. Characteristics of northern pike females caught in the late autumn from 2 different lakes (n: number of specimens; LC: longitudo caudalis)

Lake	Age	n	Weight of the body without the entrails (g)		LC (cm)	
			Mean	SD	Mean	SD
Mosąg	3+	21	1553	226	57.1	2.7
	4+	25	3024	824	73.4	6.6
	5+	13	3210	788	67.5	16.8
	6+	1	3093	–	74.5	–
Klebarskie	3+	18	997	316	49.5	5.7
	4+	32	1152	338	50.8	6.6
	5+	27	2096	360	65.0	4.4

(1) No alteration (regarded as 'normal' gonads): Both gonads were filled with macroscopically visible oocytes with diameters of 1.2 ± 0.3 mm (Fig. $1a_1$, a_2); the GSI value (>10%) was the highest of the 4 categories (Fig. 2); the histological image (Fig. 3a) shows oocytes in the final phase of vitellogenesis.

(2) Type I alteration: The alterations covered part of one or both ovaries (Fig. $1b_1$,b_2) and were characterised either by the absence of oocytes or by the presence of immature oocytes (previtellogenic or early trophoplastic, as shown on Fig. 3b,c); GSI was >8.5% on average and was comparable with the GSI of the fish without altered gonads (Fig. 2).

(3) Type II alteration: The alteration involved at least one entire ovary (one of the 'ovarian strand') or there were no vitellogenic oocytes in either ovary (Fig. $1c_1$,c_2); the histological appearance of altered ovary tissue was the same as for the Type I alteration (Fig. 3b,c); the GSI ranged from 4 to 10% (Fig. 2).

(4) Type III alteration: The alteration involved at least one whole gonad (Fig. $1d_1$,d_2); there were no oocytes in the histological image of altered ovary tissue (Fig. 3d); the GSI was <6% and was the lowest values of all those observed ($p < 0.05$) (Fig. 2).

Alterations were found in the all age groups (including only 1 fish at age 6+) in both populations. Fish with altered ovaries accounted for 30 and 32% of the populations of Lake Mosąg and Lake Klebarskie, respectively. The proportions of each type of alteration were similar in both populations. The highest percentage of fish with altered gonads had Type I alterations, whereas those with Type III alterations accounted for the lowest percentage (Fig. 4). No relationship was found between age group and the percentage of alterations of a certain type (Fig. 5).

DISCUSSION

A number of studies have documented morphological gonad alterations in fish (e.g. Jobling et al. 1998, 2002, Kavanagh et al. 2004, Metcalfe et al. 2010). However, the alterations were usually caused by laboratory research associated with the toxic properties of heavy metals or other chemical compounds causing endocrine mechanism disorders (e.g. Nath & Kumar 1990, Crump & Trudeau 2009, Segner 2011) or with sex reversal resulting from hormone therapies (e.g. Kipfer et al. 2009). Gonad alterations in fish caught under natural conditions have been reported only sporadically, and the observed changes were associated mainly with a decrease in fish fecundity and histopathological changes in male and female

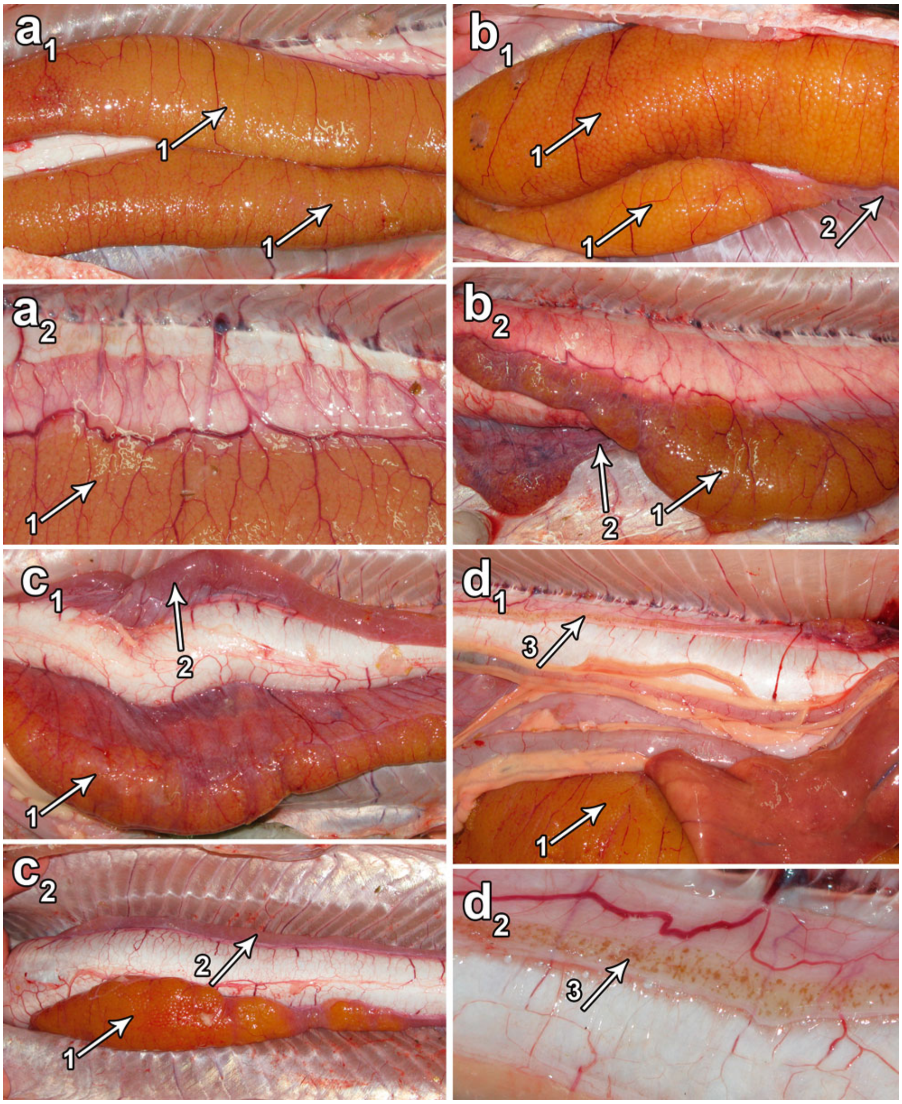


Fig. 1. *Esox lucius*. Ovaries of northern pike (2 examples for each gonadal type): (a_1,a_2) gonads regarded as 'normal'; (b_1,b_2) Type I alteration where both ovaries contained a small number of vitellogenic oocytes; (c_1,c_2) Type II alteration where only one ovary contained a small amount of vitellogenic oocytes; (d_1,d_2) Type III alteration where at least one ovary has undeveloped structure (there were no oocyte cells). 1: ovary or part of ovary containing vitellogenic oocytes; 2: ovary or part of ovary which contain previtellogenic or early trophoplasmatic oocytes (as shown in Fig. 3b,c); 3: ovaries with no oocytes (as shown in Fig. 3d). Samples were n = 60 and n = 77 in Lake Mosąg and Lake Klebarskie, respectively

(and intersex) gonad tissues (Jobling et al. 1998, van Aerle et al. 2001, Noaksson et al. 2001, Kavanagh et al. 2004, Hanson 2009). Hermaphroditic histological changes in the ovaries have also been reported in northern pike (Dominguez et al. 1989, Vine et al. 2005). However, the results presented in the present paper are the first data about extreme alterations of ovaries in northern pike, the top predator in freshwater food webs. The morphological characteristics of some alterations (i.e. gonadal atrophy) observed in northern pike are similar to those found in whitefish (Bernet et al. 2004), except for the total absence of oocytes in the ovaries (Fig. 3d). It is noteworthy that the percentage of pike in which gonad alterations were found is similar (~30%) to that observed for the whitefish population in Lake Thun (Bernet et al. 2004, 2008). Interestingly, a detailed investigation of male pike gonads from the 2 lakes studied (n = 27 from Mosąg and n = 32 from Klebarskie) showed no alterations in the testis (J. Rechulicz et al. unpubl. data). This suggests that the occurrence of the alterations affects only females.

The absence of oocytes or the presence of immature oocytes (previtellogenic and early stage of trophoplasmatic growth) in the ovaries during the period when female gonads should contain oocytes in the late vitellogenesis stage (as in the gonads regarded as 'normal') (Fig. 3a) may suggest the following: that the species or population spawns every few years, like some sturgeon species (e.g. Schueller & Hayes 2010), or that some individuals skip one or more reproduction seasons, as has been described in the burbot *Lota lota* (Pulliainen & Korhonen 1993). However, these hypotheses should be rejected

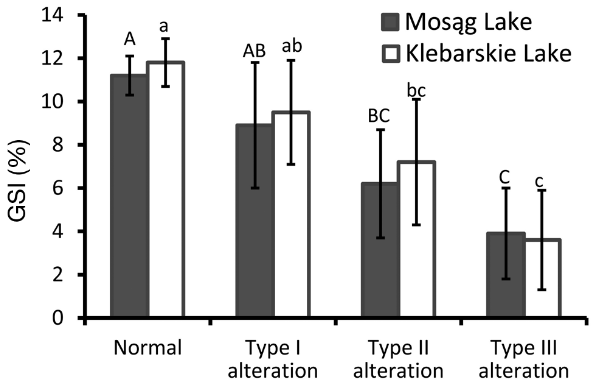


Fig. 2. *Esox lucius*. Gonadosomatic index (GSI) of northern pike females with ovaries regarded as 'normal' or with a different gonad morphology (Type I, II and III alterations). Data marked with different letters (capital letters for Lake Mosąg and small letters for Lake Klebarskie) were statistically different ($p < 0.05$)

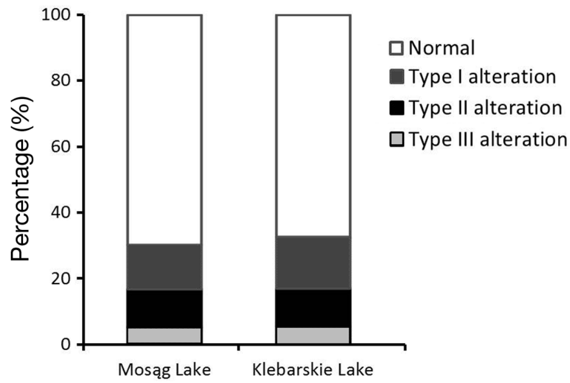
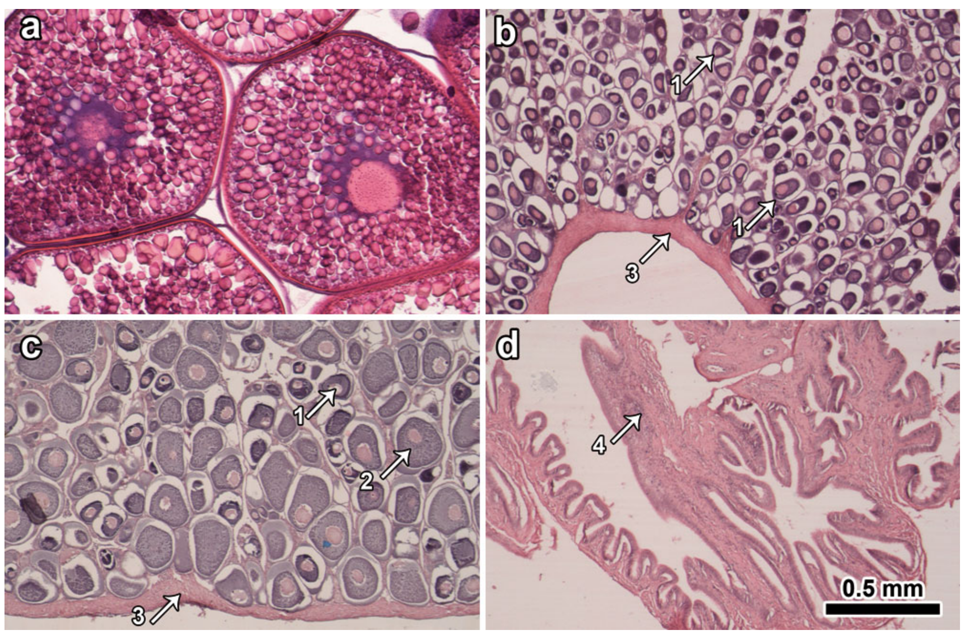


Fig. 4. *Esox lucius*. Percentage of northern pike females with normal or different types of ovary alterations

because this has never been observed in northern pike, and all the data on the species clearly indicate that pike spawn only once a year (e.g. Balik et al. 2006, Craig 2008, Lappalainen et al. 2008). June (1970) observed that fluctuations in water temperature had disrupted spawning and caused atresia in northern pike. However, there are no data that gonadal alterations occurred in these fish in the following spawning seasons. An initial analysis of 2 other lakes located in the same catchment as the 2 lakes studied in the present paper indicates that there are populations of northern pike without gonadal alterations where almost the same thermal conditions had occurred (J. Rechulicz et al. unpubl. data). Moreover, our results indicated that in many cases of pike gonad alterations, oocytes were produced only in some parts of the ovaries. Therefore, this kind of alteration may be considered as abnormal and caused by external factors other than those mentioned above.

Gonad alterations may be caused by endocrine disruptors (ED) (Jobling & Tyler 2003) originating from wastewater and sludge treatment processes, which may cause disturbances in aquatic ecosystems (Birkett & Lester 2003). EDs have been identified as the direct cause of reproductive abnormalities in fishes (van Aerle et al. 2001, Noaksson et al. 2001, Bernet et al. 2004, 2008, Kirby et al. 2004, Hliwa et al. 2011). ED are usually chemical compounds which directly

Fig. 3. *Esox lucius*. Histological cross-section of different kinds of ovaries in northern pike: (a) ovary regarded as 'normal' containing vitellogenic oocytes; (b) ovary alteration Type I where previtellogenic oocytes were observed; (c) ovary alteration Type I where different size classes of oocytes were observed at different levels of development (previtellogenic and early trophoplasmatic); (d) ovary alteration Type II where no oocytes were present. 1: previtellogenic oocytes; 2: early trophoplasmatic oocytes; 3: ovary wall; 4: tissue of ovary Type II alteration



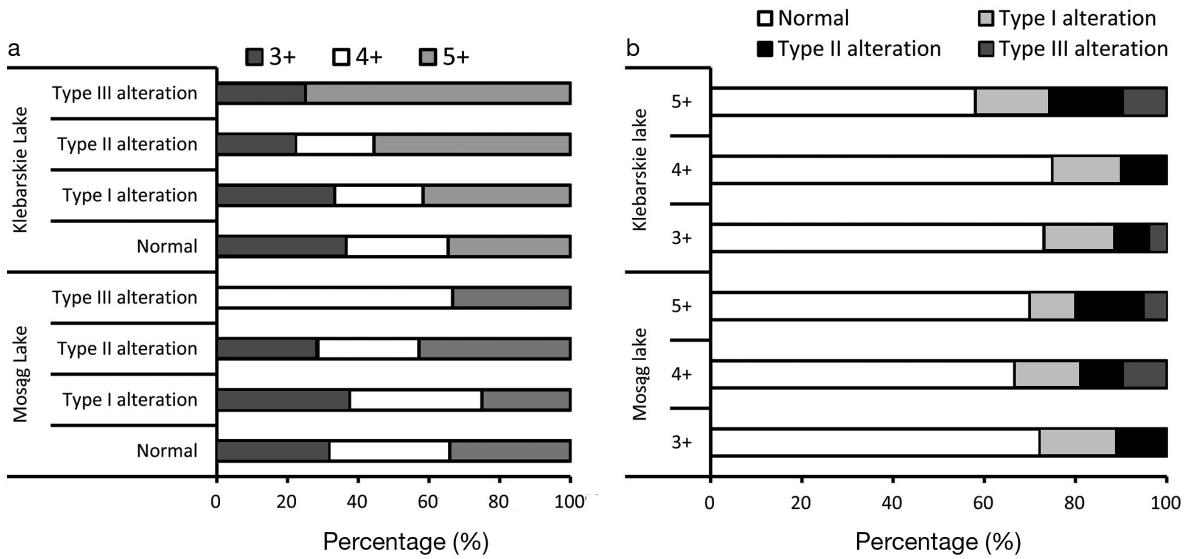


Fig. 5. *Esox lucius*. Percentage of northern pike females of different age classes with different types of ovarian alterations (Type I, II and III) as well as gonadal morphology regarded as 'normal' ordered by (a) ovarian morphology or (b) age class. Fish aged 6+ were omitted due to an insufficient number of specimens

affect the mechanisms of endocrine mediation (for details, see Vos et al. 2000, Jobling & Tyler 2003, Balch et al. 2004, Scholz & Klüver 2009). The effects of ED frequently include changes which result in the formation of intersexual individuals or changes in ovary tissue toward male gonads (Jobling et al. 1998, van Aerle et al. 2001, Noaksson et al. 2001, Bernet et al. 2004, Hanson 2009). No gonads with a male character were found in our study in either macroscopic observations or in the histological examination. Therefore, the alterations we observed may be regarded as exceptional and not previously described.

The analysed populations of pike are used for commercial purposes in accordance with Polish legal regulations. Commercial exploitation involves annual stocking with material produced in a hatchery. Spawners are caught in spring, and larvae are produced by artificial spawning. The offspring is not reared but released soon after it can swim. The approximate number of restocked fish was 1000 swimming larvae per hectare annually. To date, there is no evidence (based on the fisheries capture data) that pike populations in the 2 lakes are declining (B. Wyszyński pers. comm., manager of the 2 lakes studied). Due to the character of the stocking policy implemented in the lakes, one cannot exclude the possibility that the alterations are caused by genetic factors resulting from an improper artificial breeding practice (inbreeding, an effect of disadvantageous crossbreeding). Such negative effects of mass-scale hatchery activity have been reported in coregonids by Demska-Zakes & Mamcarz (1998) and by Luczynski et al. (1999). Improperly organised captive breeding might be a risk for the establishment of protected fish stocks and populations (Philippart 1995). Recent findings of a study conducted in Lake Thun indicate that genetic factors may play a significant role in producing gonad alterations (Bittner et al. 2011). This is a possible aetiology of this phenomenon for northern pike as well, particularly given that 2 different environments were considered, and only one was subjected to industrial pollution (from the wastewater treatment plant). Also, Vine et al. (2005) reported that the pollution originating from the treatment plant did not cause gonadal malformation in northern pike. However, to support the hypothesis that improper hatchery management can cause genetic changes resulting in gonadal alterations, a detailed evaluation is needed of the protocols in hatcheries where the restocked fry was produced as well as an evaluation of spawner origin and condition.

The data presented emphasise a serious problem, which largely affects reproductive ability and, in consequence, recruitment success, which results in a considerably lower GSI value in pike (Fig. 2). Our analysis does not provide the basis for determining the aetiology of this phenomenon. The relationship between the age and length of the fish in our study indicates that their growth rate is comparable to or greater than that of other populations (e.g. Clark & Steinbach 1959, Balik et al. 2006), which shows that both food and the physicochemical conditions in the lakes create favourable living conditions for the species. Also, the presence of food in the stomachs of all caught specimens suggests that the fish were in good condition and that food was available. Moreover, an analysis of the alterations in different age groups (Fig. 5) suggests the absence of any clear biological factor directly related with growth rate or age of the fish involved in their occurrence. Therefore, the aetiology of the alterations probably involves a factor which acts only as an ED or one with a different (for example, genetic) effect. However, this factor will have to be more closely studied.

A number of studies have been conducted with whitefish which aimed at identifying the potential factors that could produce such alterations, but the results are inconclusive and are only conjectures (Bernet et al. 2004, 2008, Bittner et al. 2009, 2011, Bogdal et al. 2009). Therefore, it is very important to conduct comprehensive studies aimed at finding a potential factor which causes alterations. Only in this way can freshwater ecosystems be protected efficiently. Such studies should include both abiotic and biological factors, including an in-depth analysis of different trophic levels. It is also important to determine the scale of the phenomenon. This will be even more difficult due to the fact that no male gonad alterations were observed. This aspect is extremely relevant for understanding the possible causes of the alteration. Our results indicate a high probability that the analysis covered 2 separate populations. This is also shown by the age and size of fish from both lakes. Therefore, it is likely that the problem will be encountered in many populations and aquatic ecosystems since the populations under analysis live in aquatic ecosystems which are representative (in terms of the trophic level, composition of ichthyofauna and exposure to pollution) of the Mazurian Lake District.

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