

# Pilot Study of Selected Bioindicators of Fish Health in Northern Pike *Esox lucius* Linnaeus, 1758 from Northeastern Poland

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## Abstract

A pilot study of bioindicators of environmental health was performed in a non-industrial region of Poland in April 2008. Twenty wild-caught northern pike *Esox lucius* Linnaeus, 1758 were used. Established biomarkers of environmental stress were studied in these fish, including condition factor, macroscopic lesions, morphometry of hepatic melanomacrophage cells, follicular atresia, and histopathology of liver and gonads. Body weight, total body length, and gender were recorded; livers, gonads, and organs with macroscopic lesions were collected for histopathology. H&E-stained paraffin-embedded sections were studied by light microscopy. All fish appeared in good health and had no macroscopic lesions except for two fish with gut trematodes. Microscopic lesions were minimal and included peribiliary fibrosis and cholangitis, focal hepatic necrosis and vasculitis, biliary myxozoanosis, intestinal metazoan parasites, and post-spawning peritonitis. Very low levels of hepatic MMC and ovarian follicular atresia were found. The data constitute the first report of biomarkers of environmental stress in northern pike from northeastern Poland and can be used as a reference for future monitoring of fish health and aquatic pollution in this region.

**Keywords:** bioindicators, *Esox lucius*, fish health, melanomacrophages, northern pike

## Introduction

Natural and anthropogenic stressors can impact aquatic organisms and produce sublethal or lethal effects [1]. Biomarkers are functional measurements of exposure to such stressors, and they can be expressed at any level of biological organization [2]. Biomarkers have been used to study the health and robustness of aquatic organisms, but also, indirectly, the health of the ecosystem itself. Accordingly, biomarkers can differentiate among polluted and non-polluted ecosystems and can be applied to monitor changes in their water quality [3]. Data in this study were

collected at two levels of biological organization: organismal (condition factor, macroscopic lesions) and cellular/tissue (histopathology). Condition factor CF is employed regularly in fish studies and is based on length/weight ratio. It assumes that heavier fish are in better condition than lighter fish of the same length. A variety of environmental stressors can impact overall fish health and condition; therefore, CF can be employed as an integrative biomarker. The natural fluctuations or differences in CF of fish due to species, sex, and season (prey quantity/quality) have to be considered while interpreting its values [3, 4].

Histopathological biomarkers represent a cumulative effect of endogenous and exogenous stressors [1, 5, 6]; while some of them can be linked to exposure to specific

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stress factors, others are less specific but sensitive markers of poor environmental quality [6]. In the latter group, the melanomacrophage cells MMC and hepatic and gonadal histopathology are commonly used as biomarkers in fish [7-11].

In fish, MMC appear after the first feeding episode and they consist of accumulations of pigmented or not, presumably phagocytic cells that associate with sinusoids and/or lymphoid centers within the spleen, liver, and head kidney [7]. The pigments in MMC can include melanin, ceroid/lipofuscin, and hemosiderin but MMC are occasionally formed by non-pigmented, oval cells [7]. The postulated principal reason for the aggregation of MMC is their ability to form intercellular connections and network in trapping and processing of antigens and endogenous products of cellular degradation [12].

The numbers and morphologic characteristics of MMC can be influenced by a range of physiological parameters and pathological conditions including species and gender, age, nutritional status and degree of tissue catabolism thermal stress, and necrosis, infections and hemolysis [7, 13, 14]. MMC have been used as bioindicators of exposure to polychlorinated biphenyls PCBs immunosuppressive toxins like polycyclic aromatic hydrocarbons PAHs, paper mill effluents and generally polluted environments [15]. Historically, MMC have been considered as reliable indicators of exposure to sediments contaminated with organics or low dissolved oxygen and are reportedly more numerous in fish from contaminated sites [16-21].

It has been widely accepted that many environmental contaminants can have a direct or indirect negative impact on reproduction [22-24]. Accordingly, gonadal histology has been used to detect the morphologic effects of such chemicals in the aquatic environment. The gonadal biomarkers of environmental stressors include the level of ovarian follicular atresia, ovotestes, and testicular degeneration, all of which can also occur as part of normal physiological stages in fish [22].

The high sensitivity, coupled with lack of specificity in the response of biomarkers to environmental toxins necessitates the validation of their use in biomonitoring by establishing background data on their “normal” characteristics in a particular species at a specified life history moment [7]. The goal of this study was to collect preliminary data on levels of commonly used biomarkers in fish from a historically non-industrial region of Warmia and Mazury of in northeastern Poland near the town of Elk (Fig. 1) [26-28].

## Materials and Methods

Twenty free-ranging, northern pike *Esox lucius* Linnaeus, 1758 were randomly collected on April 16, 2008. The fish were part of regular commercial harvest by fisherman from the Aquaculture Cooperative Sp. Z.O.O in Elk, Poland (Fig. 1). The fish were sacrificed immediately following collection by a blunt head trauma and immediately examined for macroscopic lesions. The gender of each fish was determined, and total length TL (to the nearest 10 mm) and wet body weight BW (to the nearest 10 g) were recorded and used to calculate condition factor CF based on the formula applied by Drevnick et al. [29]:

$$CF = [100,000 \times BW \text{ g}] / [TL \text{ mm}]^3$$

Representative samples from organs with macroscopic lesions, and from liver (two sections: middle of left lobe and right lobe with gallbladder), testes (one transverse mid-organ section) and ovary (three representative sections with the largest follicles) were collected into 10% buffered formalin. These samples were processed routinely for paraffin embedding, sectioned at 4-5  $\mu\text{m}$ , stained with hematoxylin and eosin H&E, permanently mounted on glass slides using standard histological techniques [30], and examined by bright-field microscopy. Gonadal staging was done according to Goodbred et al. [31], and atretic follicles were count-

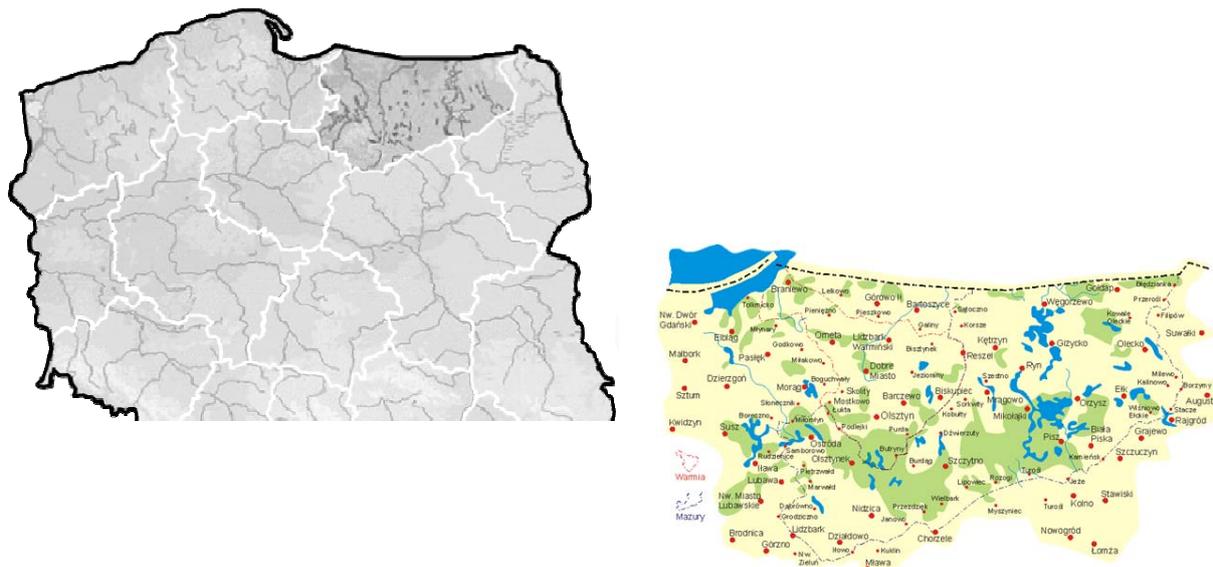


Fig. 1. Map of Poland with the marked north-east region of Warmia and Mazury and detailed map of the region around the town of Elk where the fish were sampled from small lakes.

ed in all three ovarian sections. Average number of atretic follicles per surface area at 4× objective equal to 2 mm<sup>2</sup> in each fish was calculated by dividing the total number of atretic follicles found by the number of 4× objective fields examined.

Morphometric analysis of hepatic MMC was performed on 35 arbitrarily selected high-power fields HPF (40× objective) from the two liver sections to obtain 35 independent measurements for each fish. Based on these, the mean number of MMC in one HPF, the mean surface area of one MMC, and the mean percent area occupied by MMC in one HPF were calculated for each fish. The measurements were done with a digital measuring system (Spot Insight TM Diagnostic Instruments Inc., Sterling Heights MI, USA) mounted on an Olympus BX41 microscope (Olympus America, Melville, NY, USA). Prussian blue staining was used on select sections of liver to identify the pigments within the MMC [30].

## Results

Twenty pike, including three males and 17 females, were examined. The BW ranged from 1,100-8,000 gm for female, and 600-900 gm for male fish. The TL ranged from 530-990 mm for females and 400-520 for males (Table 1). All fish were sexually mature (both by TL and histological staging of gonads), appeared in good body condition, and the only macroscopic findings consisted of gut trematodes in fish Nos. 1 and 14. Condition factor ranged from 0.64 to 0.94 (Table 1).

Microscopic findings were minimal, their prevalence in % is provided below in parentheses, and their summary is shown in Table 1. The gut had gastric ulceration associated with trematodes in fish No. 1 (5%). Intestinal trematodiasis, cestodiasis, and nematodiasis all without mucosal lesions were found in fish No. 14 (5%). The liver had focal hepatocellular necrosis (10%), peribiliary fibrosis (60%), chronic inflammation of the bile ducts/cholangitis (45%), chronic inflammation of hepatic blood vessels/granulomatous vasculitis (30%), and organisms compatible with coelozoic myxozoa within bile ducts (25%) (Figs. 2 a, b). There was one case of post-spawning granulomatous peritonitis in fish No. 12 (6% out of 17 female fish). No lesions were found in the gonads.

The total number of atretic ovarian follicles was generally low and ranged from 0 to 57 in all 3 ovarian sections examined, with an average number of atretic follicles per 4× field (2 mm<sup>2</sup>) ranging from 0 to 6.3 (Table 1).

The MMC in the liver were rare and seen as singular cells or small clusters of perivascular or peribiliary “curvilinear” aggregates (Figs. 1 a, b). Their tinctorial characteristic varied from pale yellow granular to dark brown/granular, with the latter less common and found predominantly around bile ducts. Prussian blue staining to detect ferric iron in hemosiderin was negative both on MMC and hepatocytes, which suggests that the majority of the curvilinear aggregates contained predominantly lipofuscin, while the singular MMC had a mixture of melanin and

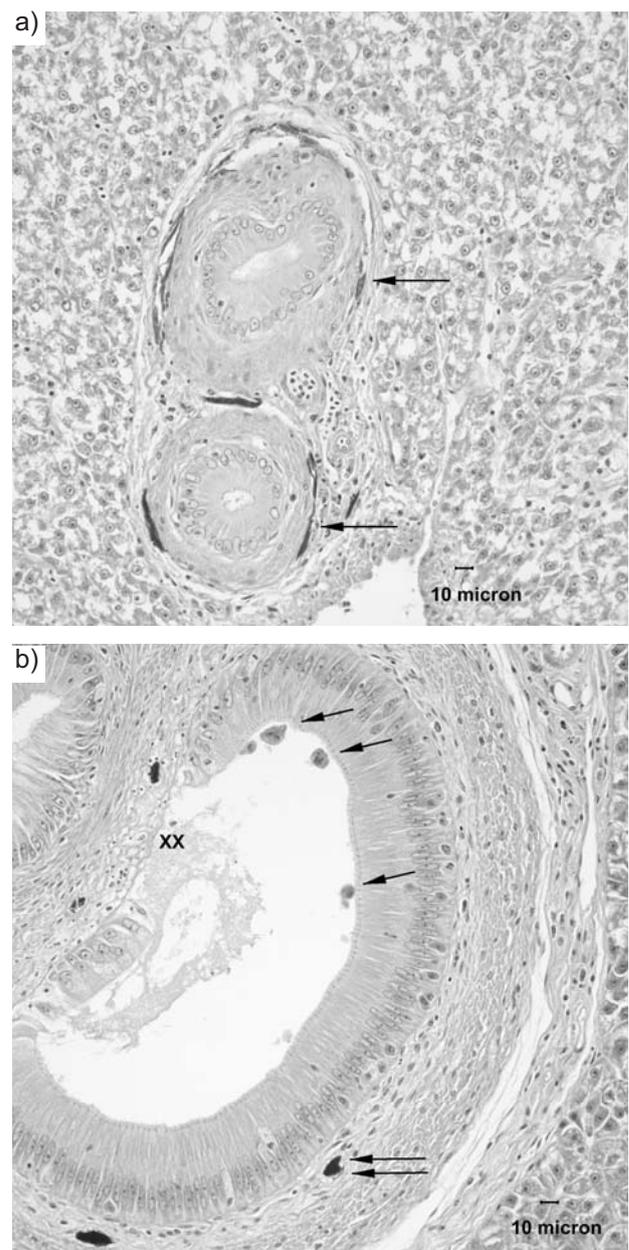


Fig. 2. a) Peribiliary fibrosis and curvilinear MMC aggregates surrounding bile ducts in the liver of pike; arrows indicate MMC with golden brown to dark brown granular cytoplasmic pigments (H&E, Bar = 10  $\mu$ m). b) Several forms of myxozoan organisms (single arrows) in the lumen of a bile duct with focal mucosal ulceration XX and peribiliary MMC with black cytoplasmic pigment, melanin (double arrow) (H&E, Bar = 10  $\mu$ m).

lipofuscin. The morphometric analysis of MMC revealed the average number of MMC per one HPF to range from 0-0.83, average surface area of one MMC to range from 0.0001-0.0015  $\mu$ m<sup>2</sup>, and the average % of liver HPF occupied by MMC to range from 0 to 0.66% (Table 2).

## Discussion

Based on the limited sample size and unequal gender distribution, our results should be considered a pilot data set

Table 1. Body weight, total body length, condition factor CF, gender with gonadal stages, atretic follicles, and macro- and microscopic lesions in the 20 studied pike.

Fish No.	BW [g]	TL [mm]	CF	Sex	Gonadal stage	Macroscopic lesions	Total atretic follicles	Average atretic follicles per [2 mm <sup>2</sup> ]	Macroscopic lesions
1	8000	950	0.93	F	late vitellogenic	gut trematodes	3	0.1	1. peribiliary fibrosis, 2. cholangitis, 3. vasculitis/liver, 4. ulcerative gastritis/ trematodiasis
2	7150	990	0.74	F	postovulatory	nsl4	14	0.8	nsl
3	4800	880	0.70	F	postovulatory	nsl	8	0.6	1. cholangitis, 2. peribiliary fibrosis
4	4400	850	0.72	F	postovulatory	nsl	8	0.7	1. biliary myxozoanosis
5	3750	800	0.73	F	postovulatory	nsl	25	3.6	1. cholangitis
6	3550	780	0.75	F	postovulatory	nsl	3	0.3	nsl
7	1400	530	0.68	F	postovulatory	nsl	2	0.2	1. cholangitis, 2. peribiliary fibrosis
8	4150	800	0.81	F	postovulatory	nsl	57	6.3	1. biliary myxozoanosis, 2. peribiliary fibrosis, 3. vasculitis/liver
9	1800	630	0.72	F	postovulatory	nsl	9	1.5	nsl
10	1650	630	0.66	F	late ovulatory	nsl	23	2.9	1. biliary myxozoanosis, 2. Cholangitis, 3. peribiliary fibrosis, 4. vasculitis/liver
11	1700	580	0.68	F	ovulatory	nsl	1	0.2	1. cholangitis, 2. peribiliary fibrosis
12	1750	590	0.94	F	postovulatory	nsl	13	1.6	1. postspawning peritonitis, 2. focal hepatic necrosis, 3. peribiliary fibrosis, 4. vasculitis/liver
13	1100	530	0.74	F	postovulatory	nsl	17	1.4	1. cholangitis, 2. biliary myxozoanosis, 3. vasculitis/liver
14	1400	590	0.94	F	postovulatory	Gut trematodes	0	0	1. peribiliary fibrosis, 2. Gut nematodes, 3. Gut cestodes, 4. Gut trematodes
15	1700	630	0.87	F	postovulatory	nsl	3	1.3	1. cholangitis, 2. peribiliary fibrosis
16	1750	590	0.85	F	postovulatory	nsl	2	0.3	nsl
17	1750	570	0.85	F	postovulatory	nsl	16	1.6	1. peribiliary fibrosis
18	800	470	0.77	M	Late spermatogenic	nsl	-	-	1. peribiliary fibrosis
19	900	520	0.64	M	Late spermatogenic	nsl	-	-	1. cholangitis
20	600	400	0.94		Late spermatogenic	nsl	-	-	1. peribiliary fibrosis

BW – body mass to the nearest 10 g

TL – total body length to the nearest 10 mm

CF – condition factor

nsl – no significant lesions

Table 2. Data on MMC in livers presented in ascending order of body weight in the 20 pike studied.

Fish No.	BW (g)	Average MMC per HPF (0.1 mm <sup>2</sup> )	Average surface area of one MMC (μ <sup>2</sup> )	% surface occupied by MMC
20	600	0.54	0.0005	0.41
18	800	0	0	0
19	900	0.1	0.0005	0.14
13	1100	0.09	0.0003	0.03
14	1400	0.05	0.0003	0.04
7	1400	0.26	0.0001	0.06
10	1650	0.06	0.0001	0.01
15	1700	0.22	0.0002	0.09
11	1700	0.09	0.0001	0.02
17	1750	0.03	0.0003	0.03
16	1750	0	0	0
12	1750	0.09	0.0003	0.04
9	1800	0.13	0.0002	0.01
6	3550	0.83	0.0004	0.45
5	3750	0.19	0.0002	0.09
8	4150	0.11	0.0003	0.06
4	4400	0.19	0.0002	0.12
3	4800	0.27	0.0001	0.07
2	7150	0.06	0.0015	0.01
1	8000	0.54	0.0006	0.66

BW – body weight to the nearest 10 g

MMC – melanomacrophages

HPF – high power field equal to 0.1 mm<sup>2</sup>

for *E. lucius* collected in April in northeastern Poland. Northern pike spawn in the spring and are sexually mature at 25-63 cm TL, which marks the 3-4 and 2-3 year of life for females and males, respectively [32]. All fish in this study were sexually mature based on total body length TL; gonad histology revealed that most females were postovulatory, and all three males had stage 3/late spermatogenesis testes. Values for the CF for pike were reported in two North-American studies previously. In the first, the CF ranged from 0.05-0.68 and was calculated for pike shorter than ours (TL between 498-639 mm) that were collected in Alaska from waters contaminated predominantly by mining operations [14]. In the second, the CF ranged from 0.4 to 0.85, and was calculated for pike impacted by methyl-mercury pollution; no body size was provided [29]. A recent study from Poland [4] reported a mean CF value of 1.03 for 85 northern pike collected between 1997-2000 in lake Miedwie, currently classified as having poor water quality. The same study cited a CF values range of 0.82-1.01 for

pike from various lakes of the former USSR from 1947, and a mean CF value of 1.16 from lake Węgorzewo in 1961; no specifics on TL and time of the year sampled was provided in the three last studies. The range of CF for pike in this study 0.64-0.94 is higher than for the North-American fish, but lower than for the previous European collections. Due to differences in, or lack of TL data, and unknown time of collection, i.e. life history moment, a direct comparison of CF among all the cited studies is impossible. It is interesting to note though that the European pike had a higher CF in the past, possibly indicating shifts in age of population or deterioration of environmental quality. Further studies are needed to validate this observation.

The prevalence and severity of hepatic lesions was very low and did not include specific toxicopathic effects associated with environmental hepatotoxins, including carcinogens [6]. We also encountered a low parasitic burden as compared to previously reported from pike in Poland collected from lake Miedwie [33], a polluted lake in north-western Poland [28]. Biliary Myxozoa, have been reported in pike from Alaska [14] but to the best of our knowledge this is the first report of these parasites from the biliary system in Poland, although renal, ovarian, and gill myxozoa [33] have been recorded in Polish pike. The hepatic lesions of cholangitis, peribiliary fibrosis, and granulomatous vasculitis found in our study could all be associated with the biliary myxozoanosis.

There was no apparent association of levels of follicular atresia with the gonadal stage, BW, TL, and thus age of fish. Due to differences in time of collection (April in our study, May-October in Hinck et al. [14]), we cannot directly compare our data to those previously reported from pike, but the level of follicular atresia in the present study appears low. It is important to note that our fish were all late ovulatory or postspawning females, and no such data are provided in Hinck et al. [14]. No histological lesions were present in the gonads.

Hinck et al. [14] reported the percentage of liver area occupied by MMC in pike to range between 0.24- 0.75 with a linear regression between MMC percentage and total levels of polychlorinated biphenyl PCBs. The above range is higher than our range of 0.0-0.66%. In our study there was no apparent correlation with the MMC values and size of fish, although the highest value was present in the largest fish collected (Table 2). There was no apparent correlation of MMC values with the minimal histopathological lesions in our fish.

## Conclusions

This report provides preliminary data on a select number of biomarkers of fish health and environmental quality from a historically non-polluted region from northeastern Poland. The lack of significant macroscopic lesions, low prevalence of gut helminths, and insignificant histopathological lesions coupled with low levels of hepatic MMC and ovarian atresia are consistent with the current ecologi-

cal assessment of this region as non-polluted [26-28]. Our data could serve as a reference for future monitoring of environmental conditions in this region of Poland.

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