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Assessment of fish health status in the Pechora River: Effects of contamination

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ABSTRACT

The present study aimed to assess the ecological situation in the Pechora River Basin (east part of Sub-Arctic Russia) using histopathologies of fish and to relate fish health to environmental quality. This paper reports histopathological alterations of fish kidney, liver, and gills and their association with chemical contamination of the Pechora River. A variety of histopathological changes was found. Differences between studied species and sites of the Pechora River with regard to the type, prevalence, and severity of lesions were studied. The types of the lesions indicated that fish respond to both direct toxicant effects of contaminated water and sediment, and secondary stress effects caused by factors such as parasitism. The structural modifications found in this study are a result of acute damage associated with short-term exposure as much as chronic response due to long-term pollution.

1. Introduction

The Pechora River is the biggest river of the Arctic Ocean drainage basin in the east of the European Sub-Arctic region. It originates from the western slope of the Northern Urals, extending approximately 1809 km to the inland and its basin covers 2/3 of the territory of the Komi Republic and a considerable part of a Nenets Autonomous District of the Archangelsk region. The Pechora River is very important for both terrestrial and aquatic biodiversity in the region, and for the reproduction of the most valuable fish species of the European North (36 fish species from 13 families inhabit the river).

Threatening this biodiversity, the Pechora River Basin has a long history of multifactor pollution, including chronic oil spills. The river and its tributaries are passed by many oil pipelines and oil pollution is one of the urgent problems of the Pechora's ecosystem. The Pechora ecosystem has been under stress for many decades with the prospect of worsening due to new oil developments. In 1994 a large-scale accident at the oil pipeline occurred. This oil spill enormously worsened the ecological situation in the Pechora River Basin and became the largest oil spill in the modern history of Russia. The amount of oil that spilled out was about 100,000 ton (Muljak and Ivanov, 2004).

So the increase in range of pollutants and the multiple mechanisms of toxicity are the current issues we face today. It is well known that most of the organic pollutants, including polycyclic aromatic hydrocarbon (PAH), polychlorinated biphenyl (PCB), and persistent organic pollutant (POP), cause adverse consequences on fish health and population growth (Monosson, 1997; Myers et al., 1998; Stehr et al., 2003; Avci et al., 2005). Establishing cause-effect relationships in field situations under the complex anthropogenic impact with the presence of multiple stressors can be complicated. In this context, the importance of mixture effects for biological responses on various biological levels has to be emphasized.

The present study, as part of a larger investigation, aimed to assess the ecological situation in the Pechora River Basin using histopathologies of fish and to relate fish health to environmental quality. The oil production intensification in the Arctic Region and a need for the creation of a unique complex system for the objective assessment of pollution level for the purpose of water ecosystems protection and preservation intensify this work's urgency. The paper focuses on histopathological investigations which have been proved to be a sensitive tool to detect direct toxic effects of chemical compounds within fish organs in laboratory experiments (Brand et al., 2001; Ortiz et al., 2003; Thophon et al., 2003; Nero et al., 2006) and in field studies (Schrank et al., 1997; Schwaiger et al., 1997; Myers et al., 1998; Gernhofer et al., 2001) and are increasingly being used as indicators of environmental stress (Stentiford et al., 2003). In addition to assessing the possibility of gills, liver, and kidney histopathologies' usage for estimation of the effect of the different level of complex anthropogenic contamination, a further objective of our investigation was to identify the main pathological manifestations of chronic intoxication in the fish of the Pechora River, to study the suitability of different fish species as sentinel organisms and to determine current oil pollution state of the Pechora River Basin using total oil product concentrations in water and sediments. Two areas – upstream (US) and

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downstream (DS) of the Pechora River – that differ from each other not only with regard to the level of anthropogenic impact (chemical pollution), but also characterized by different physicochemical parameters, were studied. And two species of fish, ide (*Leuciscus idus*) and whitefish (*Coregonus lavuretus*) were the objects of our investigation.

2. Materials and methods

2.1. Study area

The Pechora River rises in the Ural Mountains in the south-eastern part of the Komi Republic and flows into the Barents Sea. Two sampling sites along the river were investigated in 2007–2009 (Fig. 1): the US site, located close to Usinsk, which is the center of a booming oil and transportation industry; and the DS site, situated at the Pechora Delta, is a gas production area. These areas were selected because they are located far apart and represent a range of regional water sources and potential pollutant exposure conditions. The US of the Pechora River receives discharges from the oil pipelines located close to water courses. The DS is known to be under threat from upstream pollution sources, gas industries, and may also have small inputs from local urban and suburban discharges.

2.2. Histological procedures and assessment

The study was carried out according to the recommendations of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS .123), Council Directive 86/609/EEC on the approximation of laws, regulations, and administrative provisions of the Member States, regarding the protection of animals used for experimental and other scientific purposes, and "The rules of work with experimental animals" (USSR Ministry of Health order . 755 from 12.08.1977). In the autumn of 2008, a similar number of whitefish and ide individuals from each site were caught by gill nets. Macrodiagnostics to determine fish health were carried out under field conditions. Sex, standard length, and weight of all fish were recorded. Only fish of comparable size (25 cm) and weight (250–300 g) were selected, randomly from the sample at each site for further analysis. The clinical and pathological anatomical signs of intoxication and any abnormalities were documented on the basis of visual examination of the fish during the first hour after fishing. In the process of visual examination, special attention was paid to the intensity of color; the state of pigment; the total amount of mucus on the fish body; the state of squama and oral cavity; the cases of hyperemia, hemorrhages, sores, or hydremia of the body; the deformation of skull and skeleton bones; and the state of eye crystalline lens and cornea. Branchiae were examined for color, presence and amount of mucus, the state of branchial petals. After the abdominal cavity was dissected directly in the field, the state of fish muscles was studied, as well as the presence of exudate in the abdominal cavity, the amount of cavitary fat, its color and density. The dimensions, color, density, edges, hemorrhages and zones of necrosis of viscera (liver, kidneys, gonads, spleen, heart, stomach and intestines) were studied. For histological analysis only freshly killed fish were used and the target organs (liver, kidney and gills) were quickly removed. Histological techniques were performed according to Bucke (1994). After fixation in Bouin's solution for 1 week at room temperature, tissues were dehydrated and routinely processed for paraffin embedding. Then, 5–7 μm thick sections were made in a rotary microtome and stained with hematoxylin and eosin. Tissues were screened for a variety of histopathological features and lesions, which were qualitatively described and some of them were evaluated semi-quantitatively ranking the severity of the lesions: 0—none; 1—minimal; 2—low; 3—moderate; and 4—severe.

2.3. Water and sediment chemistry

For the evaluation of oil pollution, sediment and water samples were taken from the same sites of the river. At each site three samples were taken. Quantitative identification of petroleum products in these samples was made using Fourier-transform IR spectroscopy (FTIR-8201PC, Shimadzu, Japan) according to approved procedures GD 52.24.476-95 (1995) and GD 52.18.575-96 (1999). According to applied procedures, the evaluation of the total oil product concentration in water and sediments can be done within the range of 0.04–2.00 mg/l and 25–950 mg/kg, correspondingly.

2.4. Statistics

Statistical analysis was performed using the Statgrafics program version 2.0 for Windows. Data are presented as means $\pm$ standard deviation (SD). Due to the limited sample size, non-parametric Mann–Whitney U tests were used to compare mean values between the sites and the species.

3. Results

3.1. Water and sediment chemistry

The results for total oil product concentration in water and sediment samples collected from the US and the DS of the Pechora River are presented in Table 1. The excess of the permissible level of pollution, judged by the total oil products concentration, was marked only in the US water samples. The analysis of the total oil products concentration in sediments collected from the studied sites did not reveal excess of the permissible level of pollution. However it also showed that the US of the Pechora River was affected less severely than the DS in relation to sediment contamination.

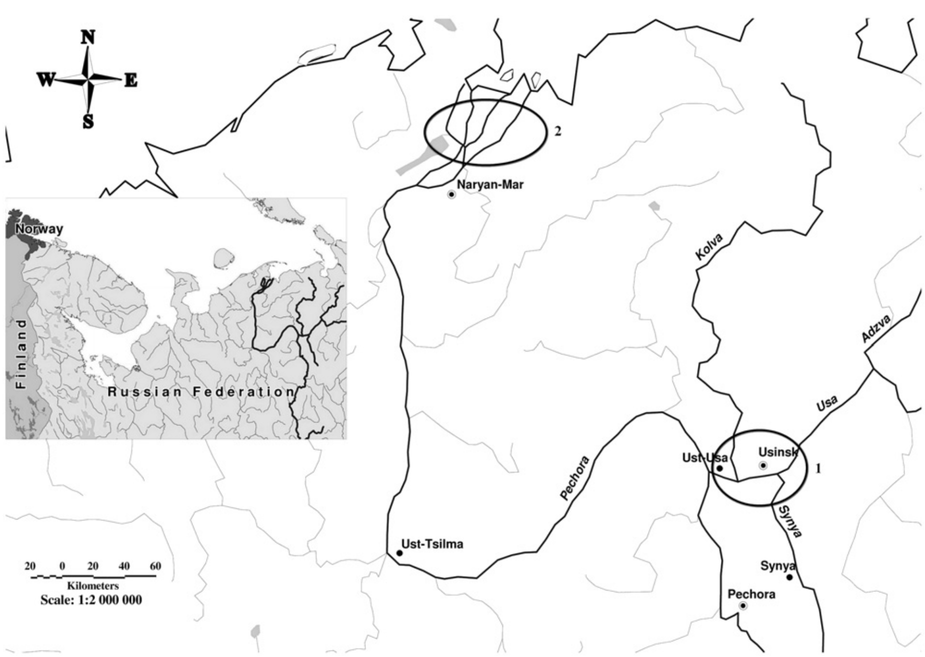

Fig. 1. A map of the Pechora River Basin (east part of Sub-Arctic Russia) showing the location of sampling sites for the current study: 1—US; 2—DS.

Table 1
Total oil product content in water (mg/L) and sediment (mg/kg d.w.) of the US and the DS of the Pechora River.

	Water samples			Sediment samples		
	Total oil products content	SD	Permissible level[a]	Total oil products content	SD	Permissible level[b]
US	0.32	0.15	0.3	136.67	21.85	1500
DS	0.28	0.07		376.67	169.04	

[a] According to Krotov (2003).
[b] According to Standard pollution level and the criteria for the sediment pollution evaluation in bodies of water (1996).

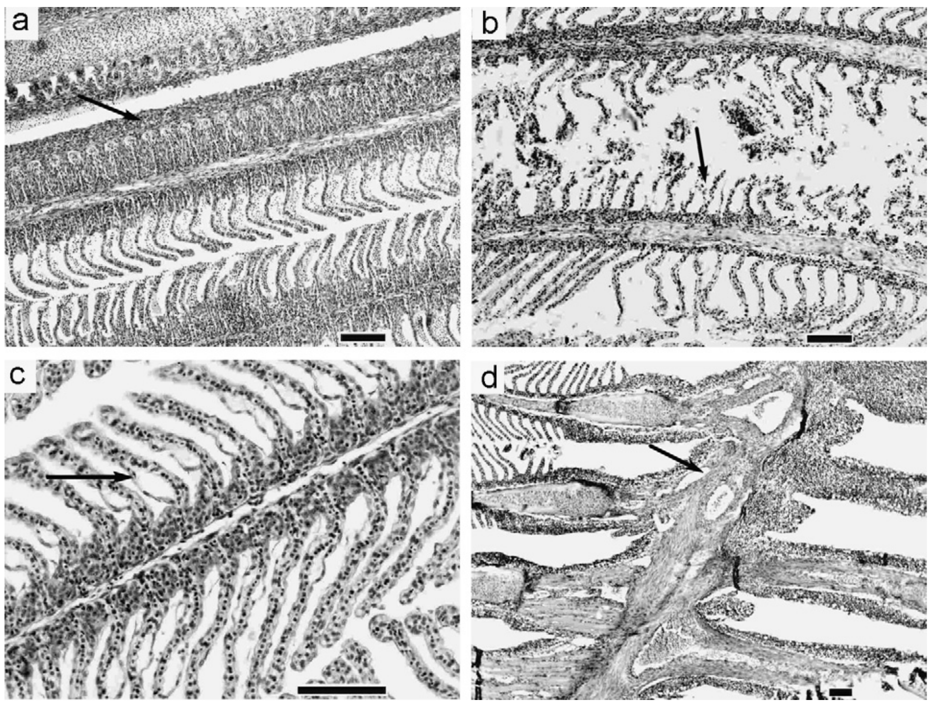

Fig. 2. Gill histological sections of fish from the Pechora River. (a) Cellular proliferation between secondary lamellae leading to a complete fusion of the secondary lamellae. (b) Necrosis of the secondary lamellae. (c) Epithelial lifting of the secondary lamellae. (d) Structural changes in gill leading to destruction of filaments and secondary lamellae. Scale bar = 100 μm.

3.2. Histopathological responses

In whitefish and ide, a variety of histopathological changes were found in the organs examined. Most of the lesions were observed at both studied sites but the prevalence and severity differed between the US and the DS of the Pechora River.

Histopathological analysis of fish gills showed signs of progressive alterations, which were characterized by epithelial hyperplasia. In some cases, the severity of proliferative alterations resulted in complete and partial fusion of secondary lamellae or formation of a membrane-like structure covering the tips of the gills and enclosing the filament (Fig. 2a). Hyperplasia of the epithelial cells was diagnosed in 100% of whitefish and ide, caught from the studied parts of the Pechora River (Table 2). Analysis of the site-specific differences of the lesion severity did not reveal any significant changes; however, species-specific comparison showed that ide from the US and the DS of the river had significantly greater severity of the lesion than whitefish (Fig. 3).

Over the range of degenerative alterations, necrosis and breakdown of erythrocytes were the most remarkable pathologies. Focal necrosis of respiratory epithelium often associated with full destruction of secondary lamellae (Fig. 2b) was present in over 90% of ide and whitefish sampled from the Pechora River (Table 2). Concerning necrosis severity, the US fish tended to have lower values compared to the DS ones, but the differences were significant only in ide. An assessment of species-specific differences showed that the gills of ide from the DS Pechora were affected more severe than those of whitefish. But this tendency was not observed in the US Pechora.

Circulatory disturbances consisted mainly of hemorrhage with the rupture of secondary lamella epithelium, aneurysms and stasis. In whitefish, the occurrence of hemorrhage was more pronounced in the DS fish, while ide from the DS of the river had lower prevalence of the lesion (Table 2), which resulted in significant differences between whitefish and ide sampled from the US of the Pechora River. Spatial comparison of the lesion severity did not indicate any statistical differences between the studied sites (Fig. 3). While between-species study revealed that at both sites ide tended to more pronounced alteration compared to whitefish, and at the US of the Pechora River the differences were significant. The prevalence of aneurysms in the specimens examined was less than 40%, being equal at both samplings of whitefish and slightly varied in ide from the different parts of the Pechora (Table 2).

Inflammatory alterations contained mainly epithelial lifting (Fig. 2c). Low-to-moderate edema was detected in almost all whitefish caught in the river (Table 2), and there were no significant

Table 2
The prevalence (%) of several gill pathological alterations in whitefish and ide from the two studied sites of the Pechora River.

Location	Sample number	Hyperplasia of epithelial cells	Hemorrhage with rupture of epithelium	Lifting of epithelial cells	Necrosis	Fibrosis	Lamellar aneurysm
Whitefish							
US	20	100	22.2	88.9	100	33.3	33.3
DS	20	100	46.7	100	93.3	53.3	33.3
Ide							
US	20	100	77.8[a]	55.6	100	11.1	22.2
DS	20	100	60.0	80.0	100	60.0	40.0

[a] Significant differences between whitefish and ide from the US of the Pechora River.

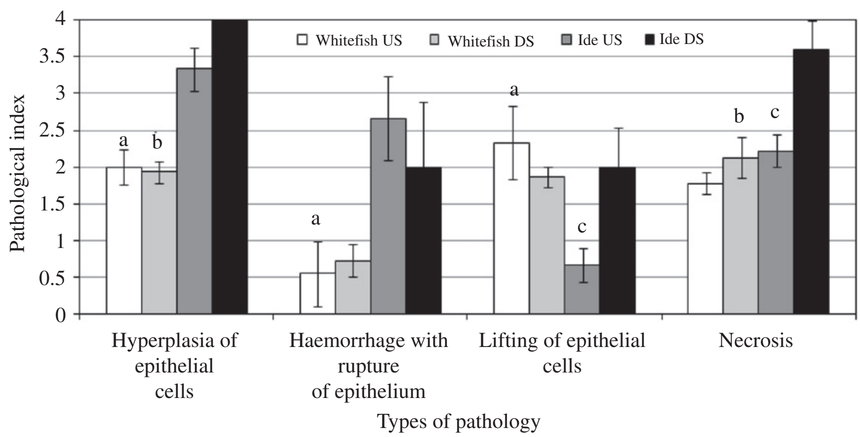

Fig. 3. Severity of gill pathologies (mean $\pm$ SD) in whitefish and ide from the US and the DS of the Pechora River. a - represent significant differences between whitefish and ide from the US of the Pechora River; b - represent significant differences between whitefish and ide from the DS of the Pechora River; c - represent significant differences between ide sampled from the different sites.

differences in severity of this lesion among studied sites (Fig. 3). In ide this lesion was present in 55% of the US fish and in 80% of the DS ones and the study revealed significant site-specific differences in severity of the alteration. An assessment of species-specific differences showed that the gills of whitefish from the Pechora's US were affected more severely than those of ide. But this tendency was not observed in the Pechora's DS.

Fibrosis of the gills (Fig. 2d) that resulted in structural disturbances of filaments was present in all studied samplings, with an increase in incidence in the downstream direction; this was especially apparent in ide (Table 2). But site- and species-specific comparison of the data did not reveal any significant changes in the lesion frequency.

In addition to pathological abnormalities mentioned above, several species of endo- and ecto-parasites infesting fish were observed; unspecified ameba and ciliates were among them. The incidence of ameba invasion was characterized by hyperplastic lesions resulting in fusion of gill lamellae and the formation of cavities, while ciliates did not elicit any apparent host response.

Liver tissue of the Pechora fish was affected with several types of histopathological alterations. In both whitefish and ide such degenerative lesions as breakdown of erythrocytes, vacuolization (Fig. 4a), megalocitic hepatosis, karyopiknosis, necrosis and cystic degeneration were diagnosed. Necrosis was revealed in whitefish from all studied sites, with a significant downstream decrease in occurrence and severity (Table 3; Fig. 5); vacuolization and cystic degeneration (Table 3; Fig. 5) showed the same tendency with one exception; these abnormalities were evident only in fish, caught from the US of the Pechora, and were totally absent in the DS of the river. As for ide, the DS fish tended to have lower prevalence of degenerative alterations compared to the US fish, except for necrosis, which showed slight increase downstream (Table 3). It should be noted that there were no significant differences in the severity of necrosis between ide from two studied sites; however statistical differences in vacuolization severity were observed for the US fish compared to the DS ones (Fig. 5). Site-specific comparisons revealed that ide from both studied sites suffer from more severe vacuolization compared to whitefish, but significant difference was revealed only in the US of the Pechora (Fig. 5). Mild karyopiknosis was diagnosed in 22% of the US whitefish and 64% of the DS ones. As for ide, the abnormality was observed only in fish caught at the US of the river. These changes in the lesion occurrence of fish from DS Pechora resulted in significant species-specific differences. Analysis of the site-specific differences in the lesion prevalence did not reveal any significant differences (Table 3).

Inflammatory alterations of liver consisted of focal lymphocytic infiltration and macrophage aggregates (MAs). Mild MAs were one of the predominant liver alterations in studied fish species (Table 3). In whitefish MAs were diagnosed in 78% of the DS fish and were even more frequent in fish caught at the US of the river. Similarly changes in the lesion occurrences were found for ide; however ide had a greater decrease in incidence the DS compared to whitefish. Moreover, in contrast to whitefish, significant differences between ide from the two Pechora sites were evident in severity of the lesion (Fig. 5). A number of site- and species-specific differences in focal lymphocytic infiltration prevalence and severity were observed (Table 3, Fig. 5). In whitefish, the occurrence of the abnormality was more frequent in the DS fish than the the US ones, while ide from the DS of the river had lower prevalence of the lesion than the fish from the US Pechora. These changes resulted in significant difference between whitefish and ide sampled from the

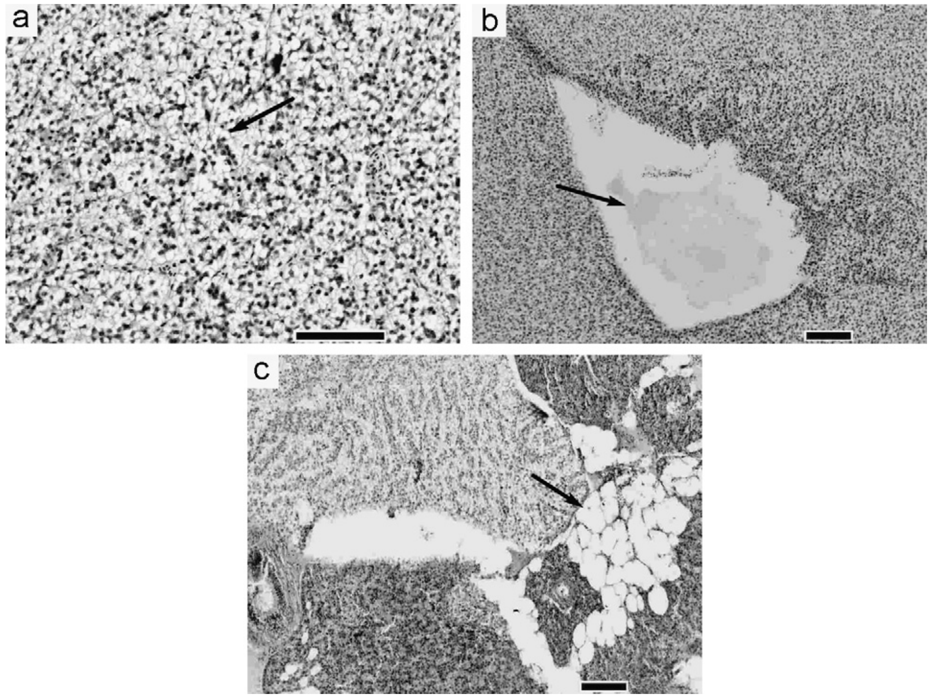

Fig. 4. Liver histological sections of fish from the Pechora River. (a) Abundant lipid-type hepatocellular vacuolization. (b) Thrombus in blood vessel. (c) Ito-cells in the liver. Scale bar = 100 μm.

Table 3
The prevalence (%) of several liver pathological alterations in whitefish and ide from the two studied sites of the Pechora River.

Location	Sample number	Bile duct fibrosis	Bile duct proliferation	Hemorrhage	Karyopiknosis	Necrosis	MAs	Ito-cells	Vacuolization	Cystic degeneration	Foci of lymphocytic infiltration
Whitefish											
US	20	44.4[a]	11.1	44.4	22.2	100[a]	88.9	0.0	55.6[a]	44.4[a]	77.8
DS	20	7.1[b]	7.1	28.6	64.3[b]	57.1[b]	78.6	0.0	0.0	0.0	100[b]
Ide											
US	20	77.8	22.2	55.6	11.1	77.8	88.9	11.1	88.9	33.3	77.8
DS	20	80.0	40.0	60.0	0.0	100	40.0	0.0	40.0	20.0	40.0

[a] Significant differences between whitefish from the different sites.
[b] Significant differences between whitefish and ide from the DS of the Pechora River.

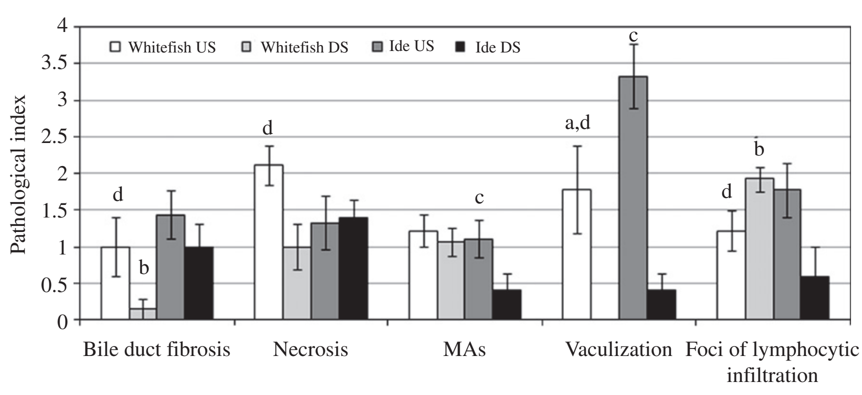

Fig. 5. Severity of liver pathologies (mean ± SD) in whitefish and ide from the US and the DS of the Pechora River. (a) Significant differences between whitefish and ide from the US of the Pechora River; (b) significant differences between whitefish and ide from the DS of the Pechora River; (c) significant differences between ide sampled from the different sites; and (d) significant differences between whitefish sampled from the different sites.

DS of the Pechora River. Between-species study also revealed statistical differences of the lesion severity at the DS of the Pechora River. Spatial comparison of the lesion severity in whitefish also indicated statistical differences between the studied sites.

Similarly to gills, in liver sections of whitefish and ide, several circulatory disturbances were present. This category of alteration consisted of hemorrhage, stasis and thrombus (Fig. 4b). The occurrence of hemorrhage in whitefish was more pronounced in the US fish compared to the DS ones, while in ide the lesion was more abundant at the DS of the river (Table 3). However, site- and species-specific comparison of the alteration prevalence did not indicate any statistical differences.

Histological analysis of ide and whitefish liver also revealed non-neoplastic proliferative lesions such as wall vessel proliferation and bile duct hyperplasia and fibrosis. A fibrosis surrounding bile ducts was evident in more that 70% of ide sampled from the different parts of the studied river. The prevalence and severity of the lesion in whitefish from both studied sites was lower than in ide; however significant differences were observed only in fish caught from the DS of the river. It should be noted that in whitefish the frequency and severity of the alteration demonstrated a significant downstream decrease. Similarly, two studied species demonstrated multidirectional tendency in relation to bile duct proliferation: whitefish had a downstream decrease in the incidence of the lesion, whereas ide had a downstream increase in the abnormality occurrence, but all the differences were not significant (Table 3). Between-species study also did not detect any statistical differences in the pathology prevalence in either of the studied sites in the Pechora.

In several cases, fish liver also demonstrated presence of Ito-cells (Fig. 4c). Such alteration was rare and species-specific; it was observed only in 11% of ide sampled from the US of the Pechora River (Table 3).

Histopathological analysis of fish kidney showed signs of proliferative alterations, characterized mainly by proliferation of wall vessels, fibrosis around tubules and increased number of newly developing nephrons. Developing nephrons were present in whitefish from both studied sites with a significant downstream decrease in incidence (Table 4). Ide kidney exhibited the opposite tendency; the abnormality was present in 100% of fish caught from the DS and was totally absent in the US. The species-specific comparison showed that there were significant differences of the pathology frequency in both sites of the Pechora River.

Inflammatory alterations of the kidney consisted of lymphocytic infiltration foci (Fig. 6), MAs, granulomas, dilation of glomerular capillaries and glomerulonephritis, characterized by thickening of Bowman's capsule, shrunken glomerular tuft and edema in glomerular space. Moreover most of the cyst of unspecified parasites, observed in the kidney tissues, also showed surrounding inflammatory reaction of differing severities. Granuloma was observed in whitefish from all studied sites with slight downstream increase in occurrence, while in ide the abnormality was evident only in fish, caught from the US of the Pechora, and was totally absent in the DS of the river (Table 4). Glomerulonephritis in combination with mild MAs were present in all studied whitefish irrespective of the sampling site. And analysis of between species differences revealed that whitefish from both studied sites were more affected by glomerulonephritis than ide (Fig. 7).

Histological analysis of ide and whitefish kidney also revealed some signs of degenerative alteration such as breakdown of erythrocytes, necrosis of interstitial tissues (Fig. 6b) and the features of the toxic form of tubular epithelial necrosis (swelling of tubular epithelium, detachment of the tubular epithelial cells from the underlying tubular basement membrane and deposits of desquamating tubular cells and casts in the lumina of the tubuli), cystic and lipoid degeneration. The most abundant degenerative feature in the kidney of fish from both studied sites was low-to-moderate necrosis of interstitial tissue (Table 4). An analysis of site and species-specific differences did not reveal any significant changes in the lesion prevalence. Concerning swelling of tubular epithelium, detachment of the tubular epithelial cells from the underlying tubular basement membrane and cystic degeneration, fish from the DS of the Pechora tended to have lower prevalence of the alterations than the fish caught from the US, but the differences were not significant (Table 4). Nevertheless a number of significant differences of the lesion prevalence were observed between ide and

Table 4
The prevalence (%) of several kidney pathological alterations in whitefish and ide from the two studied sites of the Pechora River.

Location	Sample number	Hemorrhage	Necrosis	Granuloma	Newly developing nephrons	MA	Lipoid degeneration	Glomerulonephritis	Myxosporean infection	Detachment of the tubular epithelial cells from the underlying tubular basement membrane	Swelling of tubular epithelium	Fibrosis	Cystic degeneration
Whitefish													
US	20	88.9	100	11.1	55.6[a,c]	100	11.1	100	55.6[c]	22.2[c]	88.9	88.9[a]	11.1[c]
DS	20	92.3	100	38.5	7.7[d]	100	0,0[d]	100	84.6[d]	0.0[d]	84.6	46.2	0.0[d]
Ide													
US	20	100	100	33.3	0,0[b]	88.9	55.6	77.8	0.0	88.8	77.8	44.4	88.9
DS	20	100	100	0.0	100	100	60.0	100	0.0	100	40,0	80.0	80.0

[a] Significant differences between whitefish from the different sites.
[b] Significant differences between ide from the different sites.
[c] Significant differences between whitefish and ide from the US of the Pechora River.
[d] Significant differences between whitefish and ide from the DS of the Pechora River.

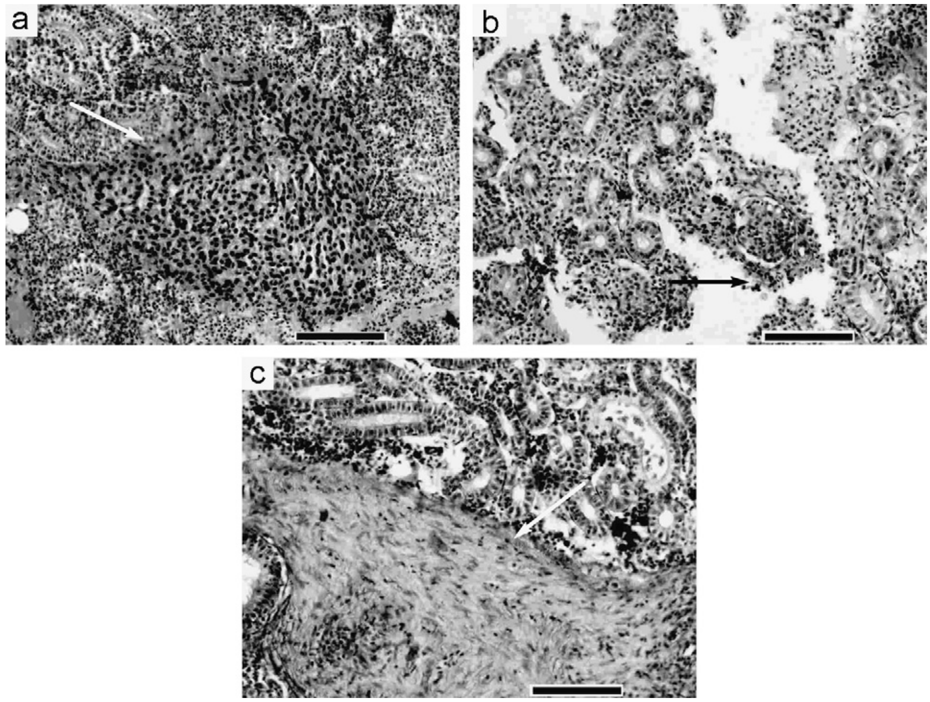

Fig. 6. Kidney histological sections of fish from the Pechora River. (a) Interstitial inflammatory response, mainly lymphocytes, (b) necrosis of haemopoietic tissue and (c) fibrosis in the kidney. Scale bar = 100 μm.

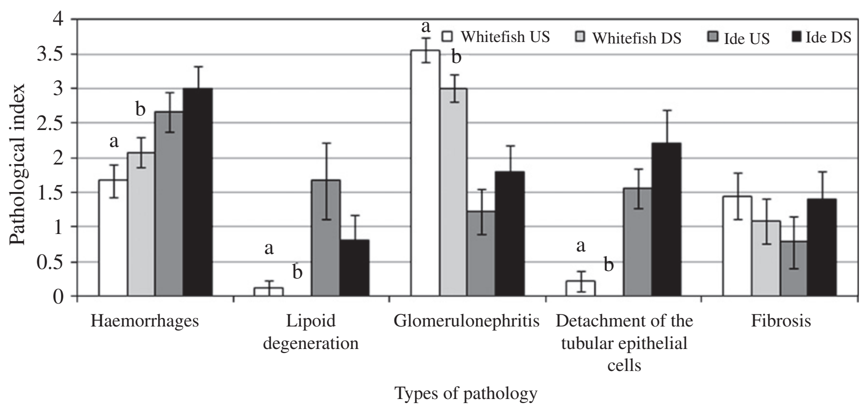

Fig. 7. Severity of kidney pathologies (mean ± SD) in whitefish and ide from the US and the DS of the Pechora River. (a) Significant differences between whitefish and ide from the US of the Pechora River; and (b) Significant differences between whitefish and ide from the DS of the Pechora River.

whitefish caught in the same sites of the river. As for severity of the pathological alteration, such as detachment of the tubular epithelial cells from the underlying tubular basement membrane, there were considerable differences between studied fish species at both sites of the Pechora River (Fig. 7). Such degenerative abnormality as lipoid degeneration was more abundant in ide compared to whitefish in both studied sites; however significant differences were observed only for the fish caught at the DS of the Pechora. Statistical differences between whitefish and ide at the both studied sites were found in the severity of the lesion.

Kidney circulatory disturbances consisted of blood congestion and hemorrhage. In whitefish, occurrence of kidney hemorrhage was more pronounced in the DS fish, while ide from both studied sites revealed equal abundance of the lesion (Table 4). Site- and species-specific analysis did not show significant differences in relation to the injury prevalence; however statistical differences were observed between whitefish and ide in lesion severity (Fig. 7).

Also in the studied kidneys of both whitefish and ide such abnormality as fibrosis was diagnosed (Fig. 6c). Studied species demonstrated multidirectional tendency: in whitefish the lesion was more frequent in the US, whereas in ide the lesion was more frequent in the DS of the river, but the differences were significant only for fibrosis occurrence in whitefish (Table 4). Between-species study did not detect any statistical differences in the pathology prevalence in both studied sites of the Pechora. Concerning the severity of the lesion, comparison analysis did not reveal any significant site- and species-specific differences (Fig. 7).

It is important to note that most of whitefish kidney examined were severely infected by unspecified myxosporea; the prevalence of myxosporean infection in renal collecting ducts of fish increased from 55% to 84% in DS Pechora (Table 3). This lesion was species-specific, as far as it was absolutely absent in ide sampled at the same sites.

4. Discussion

This paper presents the investigation results for the concentration of the total oil products in water and sediments of the Pechora

River Basin, the estimation of which allows us to determine current pollution state. Revealed high levels of petroleum hydrocarbons in water samples confirm the fact of permanent oil products pollution of the Pechora River and its tributaries. The excess of the permissible level of pollution, judged by the total oil product concentration in the US water samples, is also evidence of the influence of local pollution sources, such as oil pipelines located there. The differences between water and sediment level in the total oil product concentration at the same sites can be explained by non-uniform distribution of the pollutants connected with differences in water temperature, river flow rate and topographic features of the bottom. As bottom sediments of the US Pechora are mostly represented by fine and silty sand and flow velocity reaches 0.5 m/s, oil and its derivatives are drifted downstream at long distance. Oil products accumulate in more calm, stagnant zones, which can be found at river estuary and deltaic parts. In those parts of the river flow velocity decreases to a few cm/s, only wind induced flow is observed and bottom sediments are represented by fine silts, which serve as a good adsorbent of pollutants. Thus revealed higher concentration of the total oil products in sediments in the DS compared to the US is bound up with the drift and accumulation of oil products in stagnant waters of the Pechora Delta.

As part of a comprehensive research program, this study emphasizes the use of histopathological investigation for assessing the effect of the different levels of complex anthropogenic contamination on the health of the two fish species. The histopathological responses of fish from the Pechora River were shown to be both site- and species-specific.

It is a well known fact that the suitability of different fish species as sentinel organisms varies. The ide and the whitefish were the objects of our investigation as they are widespread in the water bodies of the country and can be considered as promising test-organisms in Russia. To date, there are few papers concerning the assessment of ecological consequences of pollution, where special attention was paid to whitefish pathology. In the investigations of Moiseenko and Kudryavtseva (2001) and Moiseenko et al. (2006) frequent kidney (nephrocalcitosis and fibroelastosis), liver (lipoid degeneration, signs of necrosis and connective tissue expansion) and gills (hyperemia and necrosis of epithelial cells) pathologies of whitefish affected by metals were described. There is also some information on ecotoxicological studies on lake whitefish (*Coregonus clupeaformis*), which is closely related to whitefish species. Thus, Cooley et al. (2000) pointed out that histopathological lesions in liver (necrosis, abnormal architecture, clear foci of liver parenchyma and alterations of the bile ductule epithelium) and kidney (tubular necrosis, inflammation, hemorrhaging, depletion of haematopoietic tissues, alterations of distal tubules and collecting ducts, tubule dilation, pigmented macrophage proliferation and glomerular lesions) of lake whitefish fed U-contaminated diets were sensitive indicators of exposure. Mikaelian et al. (1998), in addition to such pathologies as MAs, hepatocyte vacuolation, anisokaryosis, nuclear pleomorphism, bile duct hyperplasia, lymphocytic proliferation and foci of coagulative necrosis, reported on a series of hepatic tumors in a wild lake whitefish. As for ide, we failed to find any published information concerning ide organs histological structure and pathologies, except for the research devoted to morphofunctional analysis of hepatocellular adaption to extreme nutritional conditions (Segner and Braunbeck, 1988). However cyprinids are often used for histological investigations, and such fish species as European chub can be considered as an equivalent species for ide. Such histopathological reactions as epithelial lifting, hyperplasia, hypertrophy and necrosis of cells in gills and lymphocytic infiltrations, MAs and single cell necrosis in liver were revealed in chub from river, which were shown to be polluted by metals (Triebskorn et al., 2008). Paolini et al. (2005) revealed a wide spectrum of pathologies (including epithelial lifting and aneurisms in gills; necrosis and leukocyte infiltration in liver; vacuolar degeneration in renal tubule epithelial cells; and lymphocytes infiltration in kidney) in chub from two Italian rivers that were probably affected by uncontrolled sewage waste-water discharge.

Differences among whitefish and ide in lesion types and severity often observed in the studied organs might be due to differences in life histories, diet, contaminant exposure history, migratory behavior or sensitivity to chemicals. According to our study, ide in most cases demonstrated more severe pathological alterations than whitefish. But this tendency was not so obvious in relation to the lesion occurrence rate. Moreover, considerable differences between individuals from two studied sites in lesions frequency were diagnosed in whitefish rather than ide. So, based on the findings it appears that ide might not be as sensitive indicator species for water contamination as whitefish. It can be explained by the enhanced adaptive mechanism and resistance to toxins in ide compared to coregonids. Schwaiger et al. (1997) and Norey et al. (1990) revealed that another cyprinid fish species (loach) is less susceptible to toxic effects of heavy metals and in general can better tolerate water contamination compared to other fish species (Schwaiger et al., 1997; Norey et al., 1990). And it is also confirmed by ide population growth observed in the Pechora River and its tributaries in recent years (Tumanov and Martynov, 2010).

On the other hand, increased sensitivity of whitefish to toxic effects of pollutants may be a function of compromised immune system and stress due to parasite load. Histopathological analysis indicate that Pechora's whitefish respond to both direct toxicant effects of contaminated water and sediment, and secondary stress effects caused by factors such as parasitism. But it also cannot be excluded that stress due to poor water quality increase the susceptibility of fish to parasite invasion.

Our findings demonstrate strongly that fish of the Pechora River were under long-term chronic pollution as much as accidental exposure. Such pathologies as aneurysm, hepatocellular vacuolization, new developing nephrons and swelling of tubular epithelium are known to be an acute response of organism. So, developing nephron observed in the study has been suggested to be an early toxic response that usually starts from 2 to 4 weeks after exposure to the stressor (Reimschuessel, 2001) and could even take 2 months to be completed (Gernhofer et al., 2001). Taking into consideration the high level of oil products in water samples, we can suggest that there are still local accidental oil spills, which could be a cause of such pathologies. At the same time, the presence of epithelial hyperplasia and fibrosis in the gills; fibrosis and tubule degeneration, coupled with necrosis in the kidney; and fibrosis of bile duct, necrosis, vacuolization and cystic degeneration in the liver indicates that Pechora's fish are exposed to long-term chronic pollution.

Most of the histopathologial alterations observed in the present study (e.g. hyperplasia and lifting of epithelial cells in gills; hepatocellular necrosis, vacuolization and cystic degeneration in liver; glomerulonephritis, haemorrages and necrosis in kidney, etc.) could be interpreted as a nonspecific response to stress and are described in fish exposed to a wide spectrum of pollutants. The gill structural modifications found in this study are similar to those often reported for many unrelated toxicants such as heavy metals (Coutinho and Gokhale, 2000; Oliveira Ribeiro et al., 2000; Cerqueira and Fernandes, 2002), pesticides (Ortiz et al., 2003), crude oil (Brand et al., 2001), oil sands process-affected water (Nero et al., 2006a); excessive ammonia (Wedemeyer et al., 1976) or protozoan ecto-parasites (Rodgers and Gaines, 1975). Hepatic degeneration and necrosis of fish liver may be due to oxygen deficiency as a result of the gill degeneration and/or to vascular dilation and intravascular hemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed, 2001) or natural toxins produced by viral infections (Carls et al., 1998). A number of investigations have showed that focal, multifocal and diffuse vacuolar degeneration of hepatocytes can be a result of fish exposure to a variety of different carcinogenic agents and hepatotoxic insults, such as dieldrin, lindane, Aroclor 1248, Aroclor 1254 (Couch, 1975; Mathur, 1975; Nestel and Budd, 1975) or reduced food intake due to stress (Khan and Kiceniuk, 1988). Cholangiofibrosis in fish can have inflammatory or toxic causes (Wolf and Wolfe, 2005). In the kidney, tubule and glomerulus degenerations were described in fish exposed to heavy metals (Handy and Penrice, 1993; Thophon et al., 2003), to organic contaminants (Veiga et al., 2002) and mixed environmental contaminants (Schwaiger et al., 1997; Pacheco and Santos, 2002). Proliferation of MAs has been associated with age, starvation and tissue catabolism (Agius, 1979; Agius and Roberts, 1981; Wolke et al., 1985; Herraez and Zapata, 1986), infectious diseases and parasite infestations (Roberts, 1975; Agius, 1979; Vogelbein et al., 1987), toxicant-induced hemolytic anemias (Herraez and Zapata, 1986), heat stress (Blazer et al., 1987) and sediment contamination (Wolke, 1992). Developing nephrons are known to be induced in fish by various compounds such as hexachlorobutadiene, gentamicin, mercuric chloride, tetrachloroethylene and PCBs (Reimschuessel et al., 1990, 1991, 1994; Reimschuessel and Gonzalez, 1992; Cormier et al., 1995) or can be a consequence of kidney disease or infection.

Nonetheless, the simultaneous presence of many tissue alterations and the fact that all of them can be result of toxic exposure can provide increase in evidence that observed changes are the result of toxicity induced by the specific pollution of the river. It is well known that such alteration as focal hepatocyte degeneration, cytoplasmic vacuolation, fatty change, sinusoidal congestion, infiltration of lymphocytes in liver and degeneration of renal tubules and desquamation of the epithelial cells in kidney tissue are among the effects of Aroclor 1254 (Couch, 1975; Nestel and Budd, 1975; Sivarajah et al., 1978). And according to Zhakovskaya et al. (2010), this technological mix is one of the predominant source of the PCBs entry into the Pechora River. Moreover, Zhakovskaya et al. (2010) also pointed out that primary source of sediments' PAHs pollution is oil and its derivatives. A number of investigations show that such lesions as cellular vacuolation of hepatocytes and especially lipid accumulation, respiratory epithelial hyperplasia, lifting and necrosis, vascular congestion and fibrotic changes in liver and kidney, and new nephron development can be induced by exposure to petroleum compounds (Waluga, 1966; DiMichele and Taylor, 1978; McCain et al., 1978; Engelhardt et al., 1981; Rousseaux et al., 1995; Schrank et al., 1997; Brand et al., 2001; Shailaja and D'Silva, 2003). Our assumption that alterations revealed in Pechora's fish were likely due to hydrocarbon exposure were also based on results of field studies devoted to investigation of oil spill consequences. Thus research devoted to Exxon Valdez oil spill in 1989 registered such pathologies as hepatic lipid and megalocytosis (Marty et al., 2003), increase in MAs amount (Khan and Nag, 1993) and hepatic necrosis (Marty et al., 1999). Hepatic megalocytosis was also observed in fish laboratory exposed to sublethal concentrations of the water-soluble fraction from Alaska North Slope crude oil (Brand et al., 2001). Changes in gill histology such as epithelial lifting, vascular constriction, hyperplasia and fusion Brand et al. (2001) also relate to hydrocarbon exposure.

The data of the present study on frequency and severity of histopathological alterations in organs examined were controversial. Analysis revealed a number of significant differences in the severity of some pathologies between fish from two different parts of the river. And according to the results of the gill histopathology, health status of fish from the DS of the Pechora River impaired more seriously compared to the US ones in relation to the severity of the lesions. And on the contrary to the gills, higher prevalence and severity of several lesions observed in the liver and kidney of both studied fish species indicate that the US of the Pechora River was more polluted compared to the DS. The fact that fish health from the DS affected less seriously compared to the US fish corresponds to the results of total oil product concentration in water samples. On the other hand, the results of the gill histopathological analysis partly can be explained through the fact that sediment samples of the DS Pechora contain twice as much oil products as the US ones. Probably, polluted sediments can contribute to the secondary oil products pollution of bottom water layer and therefore bottom-dwelling fish. And the fact that the gills of the DS fish were affected severely compared to the US ones can be associated with the presence of multiple contaminants that were not measured herein but could mask real cause of diagnosed pathologies. Moreover the absence of clear tendency in alteration occurrence and severity rate along the river can be explained by the revealed non-uniform distribution of the pollutants. However, it should be noted that the fact that liver and kidney tissues were less severely affected in the DS fish may be a function of the observed changes in the gill surface area that may potentially reduce water flow over the gills and gill perfusion and decreased binding and entry of contamination into the vascular system and transport to the other organs (Nero et al., 2006).

5. Conclusion

The types of histopathological lesions observed in this study indicate that Pechora's fish respond to both direct toxicant effects of contaminated water and sediments and secondary stress effects caused by factors such as parasitism. Our findings demonstrate that observed alterations are associated with acute damage due to short-term exposure as much as chronic response due to long-term pollution. The results of the present study showed that both fish species from each studied site responded to the poor water environment and probably fish from the US of the river were affected severely than those of the DS. Revealed high concentrations of the oil products in the US water samples also explain the elevated levels of some pathologies, which is known to be an acute response, in the US fish compared to the DS ones. The absence of clear tendency in alterations occurrence and severity rate along the river can be explained by the revealed non-uniform distribution of the pollutants.

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