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RESEARCH ARTICLE

UDN-like disease in spawners of salmonid fishes from the Rega, Parsęta, Wieprza, and Słupia rivers in 2009-2012

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Abstract. Since 2007 in Poland, skin lesions have been observed in salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) entering Pomeranian rivers to spawn. The clinical picture of affected fish resembled ulcerative dermal necrosis (UDN), a disease of unknown etiology affecting mainly the scalps of wild salmonids. The aim of the 2009-2012 study was to determine with microbiological tests the etiological agent of the skin lesions observed in salmonids entering the Pomeranian rivers. During the study, a total of 13 species of Gram-negative bacteria belonging mainly to the Yersiniaceae, Enterobacteriaceae, and Pseudomonadaceae families were isolated and identified from the skin and kidneys of diseased fish. The Gram-positive bacteria isolated were aerobic, hemolytic granules of the genus Streptococcus. Mycological examinations on damaged fish body surfaces revealed yeasts of the genus *Rhodotorula* and fungi of the family Saprolegniaceae. The results of our study did not permit us to identify unequivocally the direct cause of the fish diseases analyzed since all the bacteria isolated during the study are normally found in aquatic environments. Some of the isolated bacteria identified were assumed to be potentially pathogenic to fish. The fungal infections observed were probably secondary and only exacerbated ongoing disease processes.

Keywords: sea trout, ulcerative dermal necrosis, Pomeranian rivers

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Introduction

Since 2007 in Poland, skin lesions have been observed in salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) entering Pomeranian rivers to spawn. The clinical picture of the affected fish resembled ulcerative dermal necrosis (UDN) (Roberts 1993), a disease of unknown etiology affecting mainly the scalp of wild salmonids, mainly salmon, sea trout, and brown trout (*Salmo trutta* L.) (Bartel et al. 2009, Kurchalyuk et al. 2009, 2010, Kazuń et al. 2011, Grudniewska et al. 2012, Provotorov et al. 2023).

Ulcerative dermal necrosis (UDN) was reported as early as in the beginning of the twentieth century in salmon and sea trout from rivers in Ireland, England, and France. In the second half of the twentieth century, the incidence of the disease was found in salmonids in many European countries including in

Austria, Belgium, France, Luxembourg, Germany, Norway, Portugal, Switzerland, Sweden, the UK, and Canada (Brown and Collins 1966, Carbery and Strickland 1968, Ljungberg and Johansson 1977, Lounatmaa and Janatuinen 1978, Prost 1980, Eiras et al. 1988, Poppe 2016). UDN has also been reported in Denmark in cod (*Gadus morhua* L.) (Larsen and Jensen 1982). In Poland, UDN-like symptoms were described by Grawinski et al. (2009) in cod caught from the Baltic Sea in 2007–2008 and by Ciepliński et al. (2018) in sea trout caught in the Słupia River.

UDN is a chronic skin disease affecting mainly wild adult salmonids returning from seas to rivers to spawn. To date, it has not been possible to identify the primary etiological agent or direct cause of this disease (Henard et al. 2022). Factors favoring its development are water pollution and low water temperature (below 10°C) and poor fish condition caused by physiological changes related to gonad maturation.

Figure 1. Trout - initial stage of disease.

Figure 2. Trout - skin discoloration.

Undoubtedly, a secondary infection of the fungus *Saprolegnia parasitica* attacking the damaged skin of the fish has a great influence on the development of the disease. The aim of the study was to use microbiological tests to determine the etiological factors behind the skin lesions observed in salmonids entering Pomeranian rivers to spawn.

Materials and Methods

The study included an assessment of the health status of sea trout spawners caught in the estuary sections of the Słupia, Wieprza, Parsęta, and Rega rivers in 2009–2012 based on the results of ichthyopathological studies. In 2009 a total of 22 sea trout spawners were examined (Słupia 3 spec., Wieprza 5 spec., Parsęta 4 spec., Rega 10 spec.), in 2010 14 spawners (Słupia 4 spec., Wieprza 3 spec., Parsęta 4 spec., Rega 3 spec.), in 2011 20 spawners (Słupia 4 spec., Wieprza 5 spec., Parsęta 6 spec., Rega 5 spec.), in 2012 21 spawners (Słupia 6 spec., Wieprza 3 spec., Parsęta 6 spec., Rega 7 spec.). Clinical, microbiological, hematological, and immunological examinations were performed; the evaluation was carried out in fish that showed skin lesions of different intensity in clinical examination: discoloration, ulceration, and necrotic lesions covered with mold (Fig. 1–5).

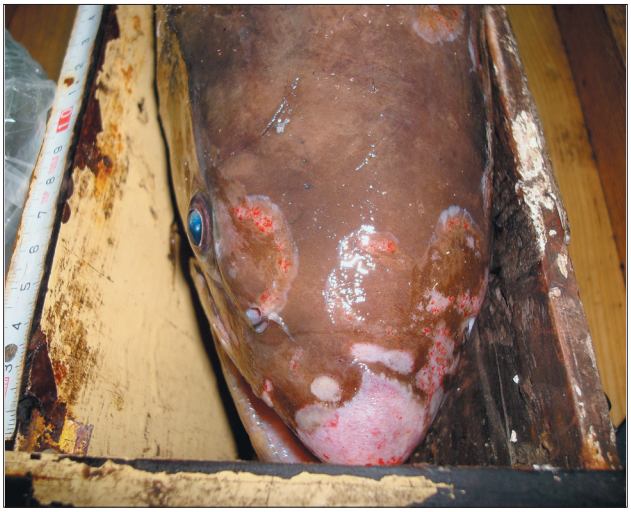

Figure 3. Skin lesions on the head of trout.

Figure 4. Trout - ulcers on the body surface.

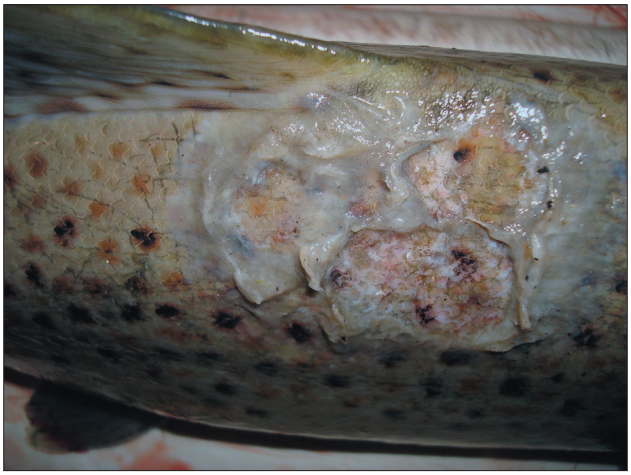

Figure 5. Skin lesions covered with mold.

Bacteriological and mycological tests

Bacteriological specimens were collected from clinically altered areas on fish skin and kidneys. Isolated bacteria were identified by culture and biochemical properties using selective-differentiating microbiological media: TSA agar, King B medium, Mac Conkey medium, Ryan agar (BTL), and API 20E and API Strep tests (bioMerieux). Mycological examinations included microscopic evaluation of direct preparations from clinical lesions and isolation of fungi by culture methods on Sabouraud medium with antibiotics (BTL). Fungal identification was based on the morphological evaluation of fungal colonies and biochemical properties using API 20C AUX and API ZYM (bioMerieux) tests. All tests were performed according to manufacturer recommendations. Test results were read out using apiweb identification software (bioMerieux).

Studies on selected biochemical parameters and non-specific humoral immune parameters

To determine the fitness and health status of sea trout spawners, selected hematological parameters and non-specific humoral immunity were tested in diseased and healthy fish. Blood for hematological and immunological tests was collected from the tail vein after the fish had been anesthetized with MS-222. The blood was centrifuged for 10 min at 5000 rpm. Serum was stored at -20°C until assays were performed.

Total protein levels were determined with the spectrophotometric method according to Lowry et al. (1951) as modified by Siwicki and Anderson (1993). Serum ceruloplasmin activity was determined with the spectrophotometric method according to Siwicki and Studnicka (1986). Lysozyme activity was determined with the turbidimetric method (Lowry et al. 1951) as modified by Siwicki and Anderson (1993) using a suspension of *Micrococcus lysodeikticus* bacteria (Sigma-Aldrich) and a chicken protein standard (Sigma-Aldrich). Gammaglobulin levels were determined using the method described by Siwicki and Anderson (1993). Polyethylene glycol 10000 kDa was used to precipitate the globulin fraction. The results were subjected to standard ANOVA analysis of variance ($P \leq 0.05$).

Results

Bacteriological and mycological tests

A total of 13 Gram-negative bacterial species was isolated and identified from the skin and kidneys of diseased fish during the study. In 2009, Gram-negative bacilli of the family Yersiniaceae

(*Serratia liquefaciens*) and Enterobacteriaceae (*Cedecea davisae*) predominated in the diseased tissues accounting for about 60% of the cultured bacteria, with the remaining bacteria identified belonging to the families Pseudomonadaceae and Shewanellaceae. Gram-positive bacteria of the genus *Streptococcus* were also found.

In other years, however, the proportions of bacteria grown in culture were reversed, with microorganisms from the family Pseudomonadaceae (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas luteola, Pseudomonas oryzihabitans*), which accounted for more than 60% of all Gram-negative bacteria, while the remaining isolated bacterial strains belonged to the families Yersiniaceae (*Serratia liquefaciens*) and Enterobacteriaceae (*Citrobacter freundi*). In addition, growth of bacteria classified into other families (*Aeromonas hydrophila*, *Shewanella putrefaciens*, *Ochrobactrum anthropi*, *Chryseobacterium indologenes*) was found. The Gram-positive bacteria isolated during these years belonged to the aerobic, hemolytic granules of the genus *Streptococcus* (Table 1). In the years analyzed, the presence of bacteria, mainly from the Pseudomonadaceae family (*P. aeruginosa*, *P. luteola, P. oryzihabitans*) was found in the kidneys of all the fish studied. Mycological examinations performed in individual years on the damaged fish body surface revealed yeasts of the genus *Rhodotorula* and fungi of the family Saprolegniaceae.

Studies on selected biochemical parameters and non-specific humoral immune parameters

The results of the analyses of biochemical and immunological parameters showed no statistically significant differences in the parameters studied among fish from the different rivers in each year (Fig. 6-9).

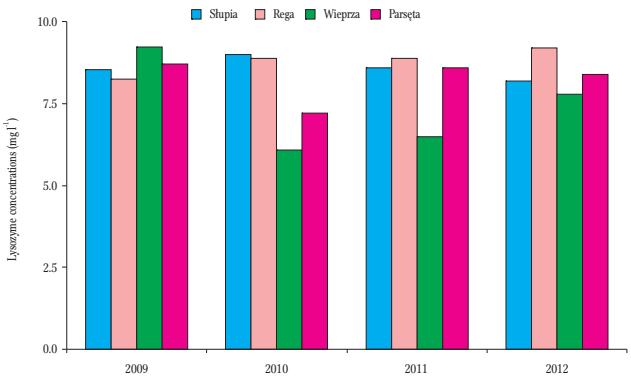

Figure 6. Lysozyme levels in serum of sea trout spawners caught in estuarine sections of Pomeranian rivers (Słupia, Wieprza, Rega, and Parsęta) in 2009–2012.

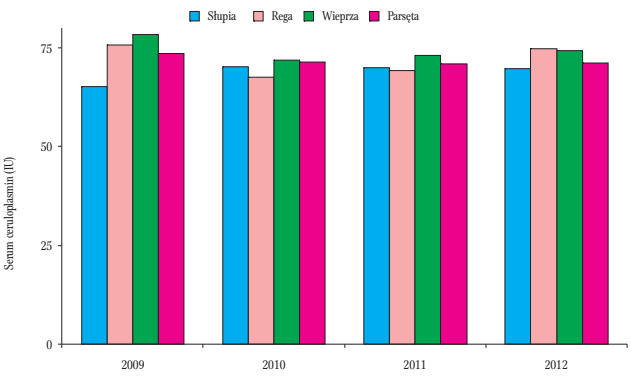

Figure 7. Serum ceruloplasmin levels in sea trout spawners caught in estuarine sections of Pomeranian rivers (Słupia, Wieprza, Rega, and Parsęta) in 2009–2012.

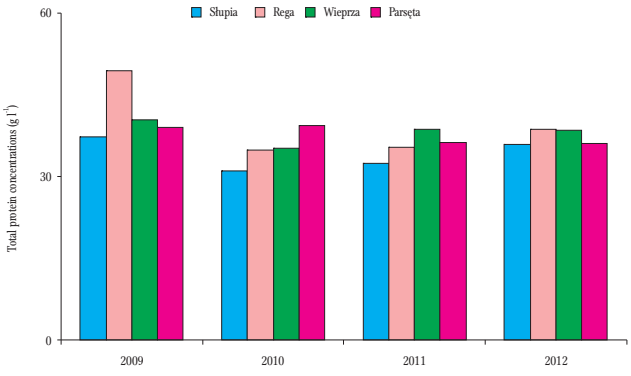

Figure 8. Total protein levels in serum of sea trout spawners caught in estuarine sections of Pomeranian rivers (Słupia, Wieprza, Rega, and Parsęta) in 2009–2012.

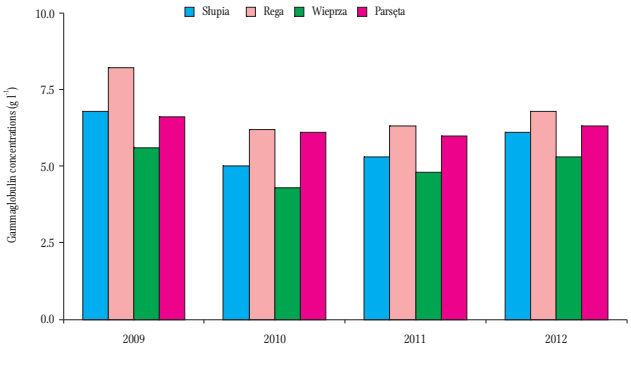

Figure 9. Gammaglobulin levels in serum of sea trout spawners caught in estuarine sections of Pomeranian rivers (Słupia, Wieprza, Rega, and Parsęta) in 2009–2012.

Table 1
Bacteria isolated from sea trout spawners from lesioned external tissues

Year	River			
	Słupia	Wieprza	Rega	Parsęta
2009	*Yersiniaceae*	*Yersiniaceae*	*Enterobacteriaceae*	*Yersiniaceae*
	S. liquefaciens	*S. liquefaciens*	*C. davisae*	*S. liquefaciens*
		Pseudomonadaceae	*Shewanellaceae*	*Shewanellaceae*
		P. aeruginosa	*S. putrefaciens*	*S. putrefaciens*
		Streptococcaceae	*Streptococcaceae*	*Streptococcaceae*
		Streptococcus sp.	*Streptococcus* sp.	*Streptococcus* sp.
2010	*Pseudomonadaceae*	*Pseudomonadaceae*	*Pseudomonadaceae*	*Pseudomonadaceae*
	P. aeruginosa	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
	P. fluorescens	*P. fluorescens*	*P. fluorescens*	*P. fluorescens*
	F. oryzihabitans	*P. oryzihabitans*	*P. oryzihabitans*	*P. oryzihabitans*
		P. luteola	*P. luteola*	
	Xanthomonadaceae	*Xanthomonadaceae*	*Xanthomonadaceae*	*Brucellaceae*
	S.maltophilia	*S. maltophilia*	*S. maltophilia*	*O. anthropi*
	Yersiniaceae	*Yersiniaceae*	*Yersiniaceae*	
	S. liquefaciens	*S. liquefaciens*	*S. liquefaciens*	
	Aeromonadaceae	*Enterobacteriaceae*	*Aeromonadaceae*	*Aeromonadaceae*
	A. hydrophila	*C. freundi*	*A. hydrophila*	*A. hydrophila*
	Streptococcaceae	*Streptococcaceae*	*Streptococcaceae*	
	Streptococcus sp.	*Streptococcus* sp.	*Streptococcus* sp.	
2011	*Pseudomonadaceae*	*Pseudomonadaceae*		*Pseudomonadaceae*
	P. aeruginosa	*P. aeruginosa*		*P. aeruginosa*
	P. fluorescens			*P. fluorescens*
	Aeromonadaceae		*Aeromonadaceae*	*Aeromonadaceae*
	A. hydrophila		*A. hydrophila*	*A. hydrophila*
	Streptococcaceae			*Streptococcaceae*
	Streptococcus sp.			*Streptococcus* sp.
2012	*Pseudomonadaceae*	*Pseudomonadaceae*	*Pseudomonadaceae*	*Pseudomonadaceae*
	P. aeruginosa	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
	P. fluorescens	*P. fluorescens*	*P. fluorescens*	*P. fluorescens*
	P. luteola	*Enterobacteriaceae*	*Enterobacteriaceae*	*Enterobacteriaceae*
		E. cloacae	*E. cloacae*	*E. cloacae*
	Aeromonadaceae	*Aeromonadaceae*	*Aeromonadaceae*	*Aeromonadaceae*
	A. hydrophila	*A. hydrophila*	*A. hydrophila*	*A. hydrophila*
	Brucellaceae	*Brucellaceae*	*Brucellaceae*	
	O. anthropi	*O. anthropi*	*O. anthropi*	
	Weeksellaceae			
	C. indologenes			
	Streptococcaceae	*Streptococcaceae*	*Streptococcaceae*	
	Streptococcus sp.	*Streptococcus* sp.	*Streptococcus* sp.	

Discussion

In the light of the research results obtained, it was not possible to identify unequivocally the direct cause of disease in wild sea trout and salmon after they entered coastal rivers, and, to date, this has not been established despite research undertaken by various scientific communities. Kurhalyuk et al. (2009, 2010, 2011) conducted studies on healthy and diseased fish caught in the Słupia River during the fall spawning period in 2007 and 2008 that revealed statistically significant differences in oxidative stress parameters between diseased and healthy fish. This would indicate the nature of the pathomechanism leading to damage to skin cell structures and to damage to blood cell structures and functions. These changes confirmed a reduced adaptive capacity in fish, which is crucial during the period in which fishes ascend from seas to rivers. Perhaps this is a natural selection process triggered by physical and chemical changes in the environment, such as increased UVB radiation, anthropogenic pollution, acid rain, or climate change (Stokowski et al. 2023). According to monitoring studies, Baltic fish might contain elevated levels of dioxins, organochlorine pesticides and PCBs, heavy metals, and other chemical residues (Niewiadomska et al. 2012). The presence of these substances in the environment and in the tissues of aquatic organisms might be important in regulating homeostasis. Secondary bacterial infection or circulatory failure stemming from ionic imbalance associated with damage to the skin barrier is also considered to be one of the causes of fish die-offs (Roberts 1993).

The results of our study did not permit us to determine unequivocally the direct cause of the fish health problems analyzed since all the bacteria isolated in the study are normally found in aquatic environments (in water and sediments). Some of the isolated bacteria identified are assumed to be potentially pathogenic to fish, e.g., *A. hydrophila*, *P. fluorescens*, *C. freundi*, *S. liquefaciens*, *S. putrefaciens*, and *C. indologens*, which, under favorable conditions, can cause disease symptoms, most often in immunocompromised fish (Raman et al. 2013).

In contrast, the fungal infections observed occurring on the skin surface were probably secondary and only exacerbated ongoing disease processes. Considered to be of low pathogenicity to fish, fungi of the genera *Saprolegnia* and *Achlya* are widespread in the aquatic environment and cause a disease of the outer skin of fish known as thrush. This disease usually occurs at low temperatures particularly in weakened fish the outer skin of which has been previously damaged mechanically or by pathogens and on which mycelial hyphae can penetrate deeply into muscle tissues (Willoughby and Wood 1986). Yeasts of the genus *Rhodotorula* are commonly found in soil and water, on plants and animals, and in the air and are mainly seen as spoilage or food-contaminating saprophytes (Lewicka et al. 2009). They are mostly mesophilic organisms with optimum growth temperatures in the 20–40°C range, but some also thrive at low temperatures (Krzyściak et al. 2007).

The results of lysozyme and ceruloplasmin activity and protein and gammaglobulin levels permit an overall assessment of the non-specific immunity potential and physiological condition of the fish tested. These parameters are also used to differentiate the degree of risk in immunotoxicological studies. Lysozyme and gammaglobulins play fundamental roles in the innate immune response, protecting the fish from various pathogens; however, since the study was conducted only on diseased specimens, it is difficult to assess whether the parameters of humoral non-specific immunity determined were or were not within the physiological norms for this species.

When searching for the cause of the occurrence of disease symptoms and deaths of sea trout entering the Pomeranian rivers, we can only assume that deteriorating environmental parameters result in conditionally pathogenic bacteria breaking through the defense barriers of the fish, causing infection. In this study of sea trout spawners, we were dealing with a mixed infection that was consistent with the thesis that the microflora of the aquatic environment is reflected on the skin of fish (Shewan 1977).

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