Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 12	Fasc. 1	5-12	2004

ATYPICAL BACTERIAL GILL DISEASE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*): INFLUENCE ON NON SPECIFIC HUMORAL DEFENCE MECHANISMS

Andrzej K. Siwicki,***, Francoise Pozet**, Marc Morand**, Krzysztof Kazuń*, Edward Głąbski*, Sylwia Trapkowska****

*The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland
**Laboratoire Departemental d'Analyses, Conseil General du Jura, France
***Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. Rainbow trout (10-20 g body weight) from a local fish farm were examined after low levels of mortality at a temperature of 10°C were observed. Dystrophic changes were observed only in the gills. Based on its unusual clinical presentation, this condition was termed atypical bacterial gill disease (ABGD). Only *Flavobacterium psychrophilum* was isolated from the gills. On the day the first symptoms of the disease appeared and on the third, seventh, and tenth days afterwards blood from 20 afflicted fish (diseased) and 20 unafflicted fish was drawn to examine the nonspecific humoral defense mechanism. In the present immunological studies a strong immunosuppression effect was observed in fish with symptoms of atypical bacterial gill disease. The results showed that serum lysozyme activity and Ig levels were statistically ($P < 0.05$) lower in fish with disease symptoms in comparison with control, disease-free fish. The activity of serum ceruloplasmine was very high in fish with disease symptoms in comparison with the control fish. The results indicated that the suppression occurred as atypical bacterial gill disease was developing. All immunological parameters were statistically significantly lower after three, seven, and ten days of disease symptoms in comparison with the disease-free, control fish. This preliminary study indicated that ABGD with *Flavobacterium psychrophilum* has a strong suppression effect on the nonspecific humoral defense mechanisms.

Key words: RAINBOW TROUT (*ONCORHYNCHUS MYKISS*), ATYPICAL BACTERIAL GILL DISEASE, *FLAVOBACTERIUM PSYCHROPHILUM*, NONSPECIFIC HUMORAL IMMUNITY

INTRODUCTION

Disease control in aquaculture has been and remains largely synonymous with eradication programs, hygienic precautions, and the systematic control of fish health by immunological techniques, active immunization, and the use of therapeutic agents. However, the field of nutritional immunology has recently become a more frequent

CORRESPONDING AUTHOR: Prof. dr hab. Andrzej K. Siwicki, Instytut Rybactwa Śródlądowego, Zakład Patologii i Immunologii Ryb, Żabieniec, ul. Główna 58, 05-500 Piaseczno, tel./fax: +48 22 7562490; e-mail: aksiw@infish.com.pl

subject of fish disease research. Surprisingly, the immune systems of fish and mammals have many similarities. A number of factors, some of which follow, suggest that fish rely on the nonspecific immune system to a greater degree than mammals do. The number of immune globulins in fish is limited in comparison with those in the mammalian humoral immune response. The correlation between synthesis and the level of circulating antibodies and protection against bacterial pathogens is poorly correlated. Lastly, the teleost lysozyme system has a stronger bactericidal effect than does its mammalian counterpart. As a direct consequence of this, a number of dietary factors have been evaluated for their general immune modulatory potential and for the particular effect they have on the fish humoral system. Experimental study suggested that the oxidized lipids in diets or modified proteins have a direct influence on nonspecific humoral defense mechanisms and protect against diseases, especially in intensive fish culture (Siwicki and Studnicka 1986, Siwicki and Anderson 1993).

Flavobacterium psychrophilum is an important bacterial pathogen that has been recognized since 1948 to cause bacterial coldwater disease (BCWD). It primarily affects juvenile salmonid fish and can cause losses in the infected fish population of up to 20%. Increased mortality rates to 50-80% in fry have been observed, and the disease has been referred to as Rainbow Trout Fry Syndrome (RTFS) by Lorenzen (1994). The symptoms and mortality of the disease depend on fish size. The pathogen can be cultivated from diseased tissue on low-nutrient media by incubating at around 15°C for 5-10 days. The pathogen is a gram-negative, yellow-pigmented bacterium that is strongly proteolytic and exhibits gliding motility, but is unable to metabolize carbohydrates. The organism is most virulent at water temperatures below 15°C. The pathogenesis of BCWD/RTFS has not been investigated to the same degree as the taxonomy of *F. psychrophilum*. A number of extracellular toxins and enzymes that are associated with the virulence of other fish pathogens have been described for *F. psychrophilum*. The exotoxins might contribute to the death of the fish or to the clinical symptoms of the disease, but presently their role in the pathogenesis of these diseases in fish is by no means clear. Limited information is available on the plasmid of *F. psychrophilum* isolates (Lorenzen 1994).

Many experimental studies suggest that *F. psychrophilum* have a varied pathogenecity that depends on fish size and water temperature. Speare (1999) examined two groups of Arctic char, *Salvelinus alpinus* (L.), and one of rainbow trout, *Oncorhynchus mykiss* (Walb.), from a commercial fish farm in eastern Canada. They

were found to have a mixed infection of the gills by the causative agents of *Flavobacterium psychrophilum*, which are responsible for bacterial gill disease (BGD), and amebae, which are similar to those responsible for nodular gill disease (NGD). Although the ameba species that cause NGD is unknown, it might belong to the family Cochliopodidae. The diagnoses were confirmed by immunofluorescence antibody testing and transmission electron macroscopy. The gill lesions were typical for a mixed BGD and NGD infection and the extensive, dramatic hyperplasia of filament epithelium was characteristic of NGD.

Ekman et al. (1999) examined Atlantic salmon, *Salmo salar* (L.), brood fish for the presence of *F. psychrophilum* in the kidney, spleen, brain and sexual products (ovarian fluid, unfertilized eggs and milt). Samples for bacteriology were taken at the time of capture when the fish were ascending their native river to spawn and after a period of captivity in indoor pools when they were stripped. During captivity, an abnormal wiggling behavior was recorded in some of the fish. Bacterial samples were taken to determine if *F. psychrophilum* had any role in the etiology of the condition. Furthermore, the presence of *F. psychrophilum* on egg surfaces during incubation was investigated. *F. psychrophilum* was isolated from internal organs and sexual products in 14% of the fish sampled at capture and 23% of the fish sampled at stripping. The Ekman study showed that the Atlantic salmon broods are carriers of *F. psychrophilum* during their spawning migration. The presence of the bacteria in sexual products from both females and males indicates that transmission from the brood fish to the offspring should be considered a significant route of infection.

Daskalov et al. (2000) reported that a diet containing oxidized lipids influenced the development of rainbow trout fry syndrome. Rainbow trout fry fed a diet containing high levels of oxidized lipids were challenged in three experiments with *F. psychrophilum*, the causative agent of RTFS. Following the challenge, *F. psychrophilum* was recovered as a pure culture from the gills and kidneys of dead fish more readily from fish that had received diets containing oxidized lipids than the control fish. Dystrophic changes were observed in the liver, kidney and muscles of fish fed high levels of oxidized lipids but no histopathological changes were observed in fish fed control diets. It was concluded that high levels of oxidized lipids in the diet are associated with the development of RTFS.

The present study examined the influence of the new atypical bacterial gill disease on the nonspecific humoral defense mechanisms in rainbow trout.

MATERIAL AND METHODS

Rainbow trout (10-20 g body weight) from a local fish farm were examined after low levels of mortality at a temperature of 10-12°C was observed. The fish had been fed with commercial pellets (45% protein, 24% lipids) at 1% of body weight. The mortality of the afflicted fish increased in only one of the four basins (B1). Either non-mortality or no disease symptoms were observed in the other three basins (B2, B3, B4). Dystrophic changes were observed only in the gills. Following a routine microbiological examination, only *Flavobacterium* sp. was identified on the gills by GRAM staining the slides. *Flavobacterium* sp. isolated from fish was stored in 15% glicerol at –80°C. Tryptose yeast extract salt agar (TYA) was used according to the method presented by Wakabayashi et al. (1994) to serotype *F. psychrophilum*. Due to its unusual clinical presentation, this condition was termed atypical bacterial gill disease (ABGD) in intensively reared rainbow trout. At the moment the first symptoms of the disease were noted (time 0) and then three, seven, and ten days afterwards blood from 20 afflicted fish (diseased) and 20 unafflicted fish was drawn to examine the nonspecific humoral defense mechanism. The fish were anaesthetized with Propiscin (IFI Olsztyn, Poland) and blood for the immunological assay was drawn from the caudal vein (Vacutainer system).

The serum lysozyme activity was measured in a turbidimetric assay developed for fish by Siwicki and Anderson (1993). The serum ceruloplasmine activity was determined according to Siwicki and Studnicka (1986) using a method that had been modified for micro-methods. The serum was incubated in microplates with 0.2% p-phenylenediamine (PPD, Sigma) and sodium azide (0.02%) was used to stop the reaction. The ceruloplasmine activity was measured at 540 nm on a microreader. The total protein level in the serum was measured by the colorimetric Lowry micro method (Sigma, Diagnostic Kits) and total immunoglobulin (Ig) level in the serum was also determined by the Lowry micro method modified for fish by Siwicki and Anderson (1993). Polyethylene glicol (10 000 Kda, Sigma) was used to precipitate immunoglobulin from the serum.

RESULTS AND DISCUSSION

Laboratory examination showed that lesions appeared only in the gills and were observed only in one of the basins. Fish from the other three basins were in good condition and lacked disease symptoms. A similar, gram-negative, yellow pigmented

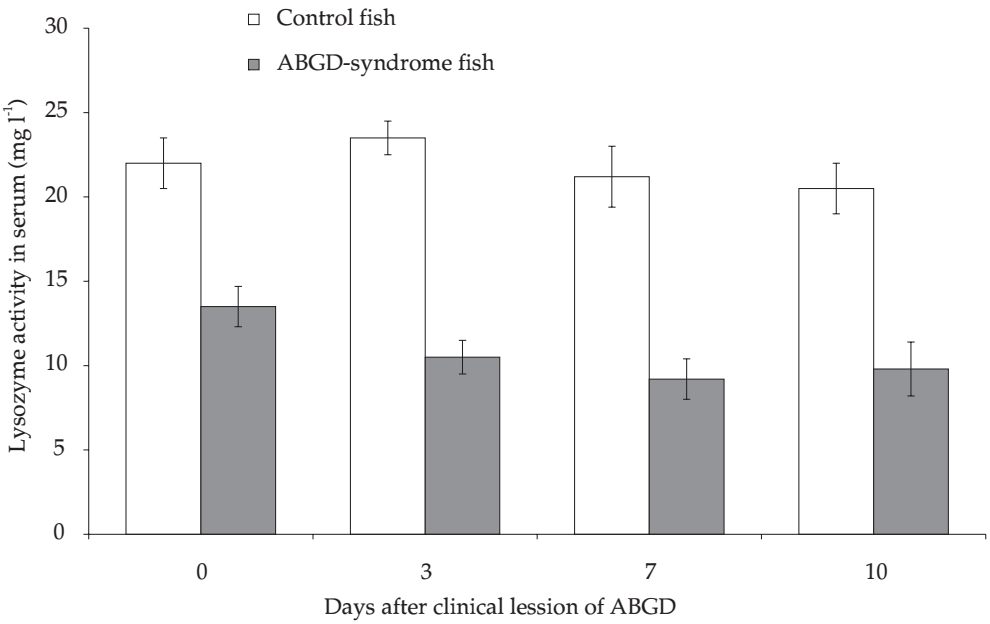

Fig. 1. Kinetics in lysozyme activity during the development of atypical bacterial gill disease in rainbow trout and in disease-free control fish (mean ± SD, n=20).

bacterium identified as *F. psychrophilum* was recovered only from the gills in the cultured salmonids from Canada (Ostland et al. 1999). Based on the unusual clinical presentation and the histopathological appearance of the gills, this condition was termed atypical bacterial gill disease.

In the current immunological study a strong immunosuppression effect was observed in fish with symptoms of atypical bacterial gill disease. The kinetics of the serum lysozyme activity are presented in Fig. 1. The results indicate that lysozyme activity was statistically ($P < 0.05$) lower in fish with disease symptoms than in the disease-free control fish. The serum ceruloplasmine activity determined in the fish with disease symptoms was highly statistically significant ($P < 0.05$) in comparison with that of the control fish (Fig. 2). Serum Ig levels in the fish with disease symptoms was statistically significantly lower ($P < 0.05$) in the fish with disease symptoms than in the disease-free control fish (Fig. 3). However, there were no statistically significant distinctions with regard to serum protein levels between the fish presenting symptoms of ABGD and the control fish (Fig. 4). The results of the present study indicated that the suppression effect developed along

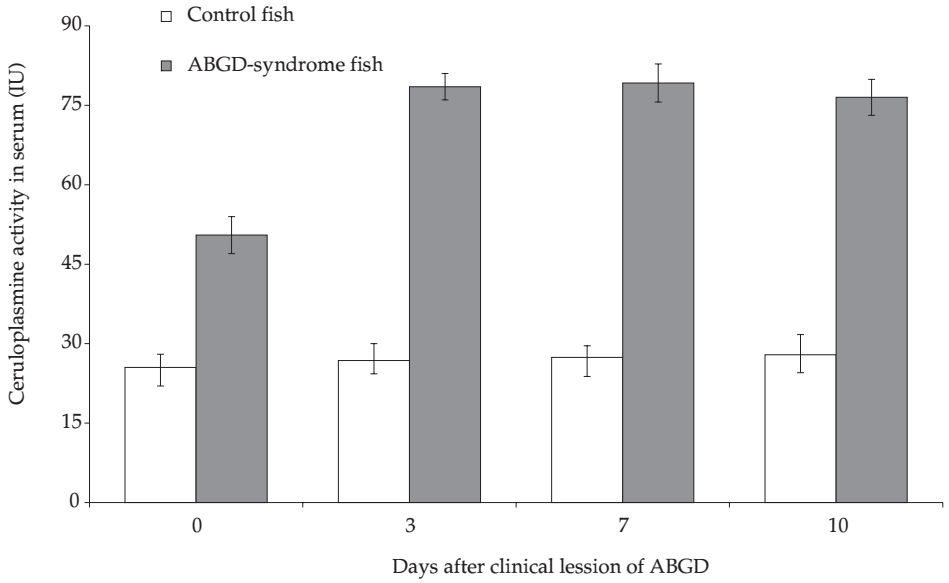

Fig. 2. Kinetics in ceruloplasmine activity during the development of atypical bacterial gill disease in rainbow trout and in disease-free control fish (mean ± SD, n=20).

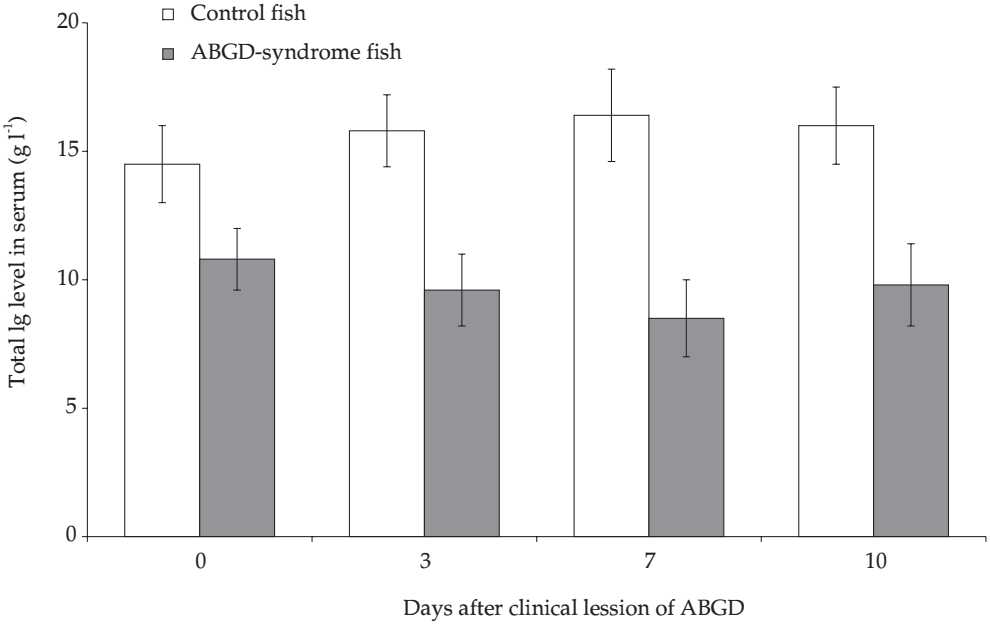

Fig. 3. Kinetics in total Ig levels during the development of atypical bacterial gill disease in rainbow trout and in disease-free control fish (mean ± SD, n=20).

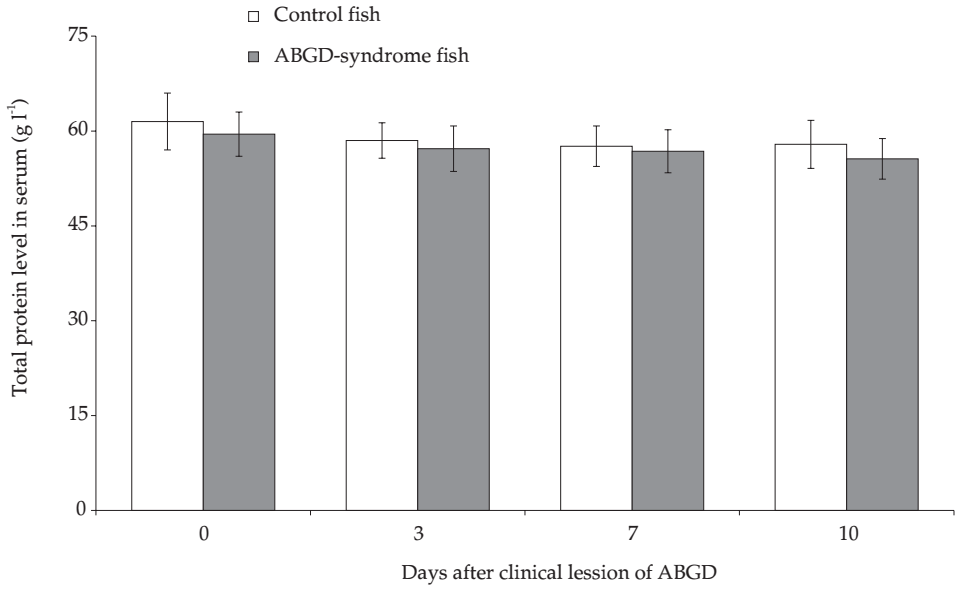

Fig. 4. Kinetics in total protein levels during the development of atypical bacterial gill disease in rainbow trout and in disease-free control fish (mean ± SD, n=20).

with the ABGD infection. All of the immunological parameters were statistically significantly lower after three, seven, and ten days following the onset of disease symptoms in comparison with the disease-free control fish. Mortality increased only until day seven, after which it rapidly decreased.

This preliminary study indicates that there is a strong suppression effect on the nonspecific humoral defense mechanisms in ABGD caused by *F. psychrophilum*. It was also suggested that *F. psychrophilum* has some influence on nonspecific defense mechanisms and protection against disease. Another possible hypothesis is that an alimentary or environmental intoxicant decreased defense mechanisms leading to a predisposition for developing atypical bacterial gill disease. This line of study must be continued in order to isolate, identify and characterize pathogens, diet control and environmental intoxicants, and to permit the early diagnosis of the immunosuppression syndrome and the application of immunomodulators for the restoration of fish immunity.

REFERENCES

Daskalov H., Robertson P.A.W., Austin B. 2000 – Influence of oxidized lipids in diets on the development of rainbow trout fry syndrome – Journal of Fish Diseases 23: 7-14.

Ekman E., Borjeson H., Johansson N. 1999 – *Flavobacterium psychrophilum* in Baltic salmon *Salmo salar* brood fish and their offspring – Diseases of Aquatic Organisms 37: 159-163.

Lorenzen E. 1994 – Studies on *Flexibacter psychrophilus* in relation to rainbow trout fry syndrome (RTFS). PhD thesis, Royal Veterinary and Agricultural University, Copenhagen.

Ostland V.E., Byrne P.J., Lumsden J.S., MacPhee D.D., Derksen J.A., Haulena M., Skar K., Myhr E., Ferguson H.W. 1999 – Atypical bacterial gill disease: a new form of bacterial gill disease affecting intensively reared salmonids – Journal of Fish Diseases 22: 351-358.

Siwicki A.K., Anderson D.P. 1993 – Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs, and total immunoglobulin (Ig) level in serum. Fish Diseases Diagnosis and Prevention Methods, FAO-Project GCP/INT/526/JPN, IFI Olsztyn: 105-112.

Siwicki A.K., Studnicka M. 1986 – Ceruloplasmine activity in carp (*Cyprinus carpio*) - Bamidgeh 38: 126-129.

Speare D.J. 1999 – Nodular gill disease (amoebic gill infestation) in arctic char, *Salvelinus alpinus* – Journal of Comparative Pathology 121: 277-282.

Wakabayashi H., Toyama T., Lida T. 1994 – A study on serotyping of *Cytophaga psychrophila* isolated from fishes in Japan – Fish Pathology 29: 101-104.

STRESZCZENIE

ATYPOWA BAKTERYJNA CHOROBA SKRZELI U PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS*): WPŁYW NA NIESWOISTE HUMORALNE MECHANIZMY OBRONNE

W ostatnich latach obserwuje się znaczny wzrost zachorowań narybku pstraga tęczowego na atypową bakteryjną chorobę skrzeli, która powoduje znaczące straty w hodowli. Zmiany dotyczą jedynie skrzeli z których izoluje się najczęściej *Flavobacterium psychrophilum*, *Flavobacterium branchiophilum*. W prezentowanych badaniach określano wpływ atypowej bakteryjnej choroby skrzeli wywołanej przez *Flavobacterium psychrophilum* na wybrane nieswoiste humoralne mechanizmy obronne u narybku pstrąga tęczowego. Uzyskane wyniki badań jednoznacznie wykazały, że w czasie rozwoju choroby stwierdzono statystycznie istotne ($P < 0,05$) obniżenie aktywności lizozymu oraz poziomu Ig w surowicy badanych ryb, w porównaniu z grupą kontrolną, u której nie stwierdzono zmian chorobowych oraz nie izolowano *Flavobacterium psychrophilum*. W tym okresie stwierdzono statystycznie istotny wzrost aktywności ceruloplazminy, przy braku zmian w poziomie białka całkowitego. Wstępne badania sugerują, że *Flavobacterium psychrophilum* może indukować supresję nieswoistych mechanizmów obronnych, co pozwala na przełamanie bariery odpornościowej i kliniczny rozwój choroby.