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The Microflora of Gills, Gut and Skin of European Eels (*Anguilla anguilla*) in Lakes of Latvia

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Abstract: The microbial contamination of fish is the most important factor in assurance of food safety. The microflora on the surface of skin, gills and gut of fish is constantly under the influence of the water environment and this may cause colonization of microorganisms on fish. The goal of this study was to evaluate the bacterial contamination level on the skin, gills and gut of the European eel (*Anguilla anguilla*), i.e., detecting total bacteria count (TBC) and Enterobacteriaceae counts, as well as *Listeria* spp. and *Salmonella* spp. in freshly caught fish. Among the three lakes, TBC on skin, gills and gut varied from 0.66 CFU/cm^2 to 4.93 CFU/cm^2, from 0.40 CFU/cm^2 to 5.51 CFU/cm^2 and from 0.30 CFU/cm^2 to 6.37 CFU/cm^2, respectively. Enterobacteriaceae count on skin, gills and gut was from 0 CFU/cm^2 to 4.30 CFU/cm^2, from 0 CFU/cm^2 to 2.47 CFU/cm^2 and from 0 CFU/cm^2 to 1.72 CFU/cm^2, respectively. The highest mean count of TBC on gills, skin and gut was found in samples from Sivers lake, while the lowest was in samples from Aluksne lake. Values among the lakes were significanty ($P < 0.05$) different. Also the highest mean counts of Enterobacteriaceae were found on gills and skin of eels from Sivers lake, but the highest count on gut was found in Usma lake. All tested samples were *Listeria monocytogenes* and *Salmonella* spp. negative. TBC and Enterobacteriaceae counts of skin, gills and gut were typical for wild fish in fresh water. Foodborne pathogens, such as *Salmonella* spp. and *L. monocytogenes*, have not been found in the present study, indicating that fish are safe for human consumption.

Key words: Freshwater fish, lake, microbial contamination, European eel, *Salmonella* spp., *L. monocytogenes*.

1. Introduction[1]

Nowadays, fish and fish products comprise a significant part of food for the majority of the world population, and fish meat is considered to be healthier alternative to red meat consumption [1, 2]. According to the Latvian Statistic Bureau, the consumption of fish and fish products was within the range of 6.0-6.6 kg/capita between the years 2008 and 2012 in Latvia [3]. Foodborne disease outbreaks related to the consumption of fish and fish products have been reported from the European countries, indicating that the microbiological quality of fish available on market is an important public safety issue [4].

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Microbiological quality of fish depends on various factors and the quality of water among those factors has a significant impact on fish microflora. Pollution of the water sources frequently is attributed to contamination with wastewaters from agricultural and human sources [5]. Initial contamination of fish with microorganisms alters fish microbiological quality. After catching the fish, the immune system is collapsed, so it does not delay microbial growth, and post-mortem changes enhance their proliferation on gills, skin and gut [6]. Large numbers of microorganisms are present in fish gills and this is related to aeration processes and access to oxygen for microbial growth. Large amount of available organic compounds as well as slight alkaline environment of gills make water microflora to easily colonize on gills

[7]. Gut microflora could penetrate intestinal wall under certain conditions and contribute to the spoilage of fish, raising concerns about fitness of product for human consumption [6, 8-10]. In general, fish is more perishable than red meat [2, 11], therefore it is necessary to evaluate general contamination rates of freshwater fish available for consumption.

Water as same as food can serve as a vehicle for transmission of pathogens, which potentially can cause different outbreaks of infections worldwide [12]. Microbiological pollution of water can directly affect water inhabitants and fish may be contaminated with foodborne pathogens, such as *Salmonella* spp. or *L. monocytogenes*, making them unfit for human consumption. Contamination of *Salmonella* spp. can cause gastrointestinal disorders in human and it may be arisen from agricultural or human sources with subsequent contamination of environment and fish [13, 14]. *L. monocytogenes* can cause foodborne infection characterized with severe disorders, such as meningitis, encephalitis and septicemia in susceptible population. In opposite to *Salmonella*, *L. monocytogenes* is a psychrotrophic pathogen and widely distributed in the environment, including inland waters. The presence of these foodborne pathogens in raw product poses the public health concerns, because of cross-contamnation and possibility of transmission of pathogen during processing if the raw material is contaminated [14]. Studies on microbiological contamination of fish are necessary for identification of existing problems and for implementation of preventive measures among consumers, fishermen and retailers [15, 16]. Usma, Sivers and Aluksne lakes are common destinations for individual fishermen, recreational and economical activities in Latvia. However, environmental pollution problems raise concerns about microbiological quality of inland water and subsequently about fish microbiological quality. Therefore, it is important to estimate the environmental health problems and subsequently the track possible effect to consumers. The aim of the present study was to evaluate the microbiological contamination rates of eels in three lakes in Latvia.

2. Materials and Methods

Samplings of European eels (*Anguilla anguilla*) were done in April and May in 2014, with 31 samples including three lakes of Latvia: Sivers lake (n = 11), Usma lake (n = 11) and Aluksne lake (n = 9), situated in different regions across the country with triplicated water samples from these lakes (Fig. 1).

Alive eels were obtained from fishermen and the water sample from each lake was collected in 0.5 m depth from the surface with 1 L sterile bottle. All samples were delivered into laboratory immediately after sampling and they were stored in ice during transportation. Eels were prepared for testing in the laboratory.

For detection of total bacterial count (TBC) and Enterobacteriaceae, the samples from skin, gills and intestinal tract were investigated separately. Surface

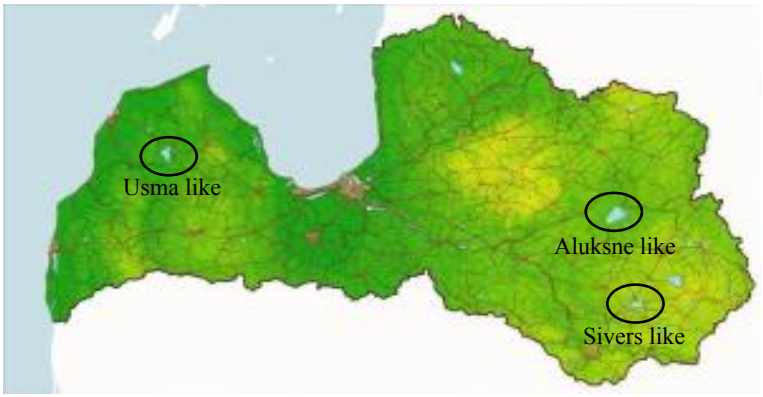

Fig. 1 Location of the lakes on the map of Latvia.

samples of fish skin were collected with Abrasive sponge moisturized with 0.1% peptone water by covering 25 cm^2 or 100 cm^2 area of fish skin depending on the fish size. For collection of intestinal tract samples, the abdomen was incised and the intestinal tract containing the intestines together with their content was separated from surrounding tissues. For gill sampling, the gills were aseptically separated from the surrounding tissues. The skin, gills and intestinal tract of each fish were investigated separately. Not less than 1 g of gills and intestinal tract and a sponge covering 25 cm^2 of skin were used for testing.

For detection of *Salmonella* spp. and *L. monocytogenes*, the pooled sample of skin, muscles and intestinal tract was used. Samples of each fish were investigated separately and 25 g of material were used for each test.

TBC and Enterobacteriaceae were detected by adding 0.1% peptone water to each sample composed of gills, skin and intestinal tract to make a 1:10 dilution. After that, serial dilutions were prepared for the investigation of samples. TBC detection was done, by transferring 1 mL of each serial dilution onto a plate count agar (PCA, Biolife, Milan, Italy) with two plates for each dilution and incubated for 72 h at 30 °C. After incubation, the bacterial colonies were counted.

Enterobacteriaceae was detected by transferring 1 mL of suspension from each serial dilution onto the violet-red bile glucose agar (VRBA, Biolife) with two plates for each dilution and incubated for 24 h at 37 °C. Typical colonies of Enterobacteriaceae were tested from oxidase activity and inoculated into glucose agar (Biolife) with subsequent incubation for 24 h at 37 °C. Only oxidase-negative and glucose-positive colonies were confirmed as Enterobacteriaceae. For Enterobacteriaceae count, typical bacterial colonies were enumerated.

For detection of *Salmonella*, a total amount of 25 g of fish samples were transferred to 225 mL of buffered peptone water and incubated for 18 ± 2 h at 37 °C. After pre-enrichment, 0.1 mL of suspension was transferred to Rappaport-Vassiliadis broth (Biolife) and Mueller-Kauffmann tetrathionate-novobiocin broth (Biolife) for enrichment for 24 h at 41.5 °C and 37 °C, accordingly. The enriched suspension was plated out onto the brilliant green (Biolife) and xylose lysine deoxycholate (XLD, Biolife) agar for subsequent incubation for 24 h at 37 °C and plates were examined for the presence of presumptive colonies.

L. monocytogenes was detected by transferring a sample of 25 g into Half-Fraser broth and incubated for 24 h at 30 °C. After incubation, 0.1 mL of suspension was transferred to Fraser broth and incubated for 48 h at 37 °C. The amount of 0.1 mL of enriched material of Half-Fraser and Fraser broth was plated out on Oxford (Biolife) and ALOA (Biolife) agar plates and incubated for 24-48 h at 37 °C. After incubation, agar plates were screened for the presence of presumptive colonies, which were selected for further confirmation. Presumptive colonies were stained according to Gram and checked for catalase activity, mobility and β-hemolysis. Suspicious *Listeria* spp. isolates were confirmed with API Listeria (BioMérieux, France).

For data analysis, microbial counts data were transformed to decimal logarithms. For evaluation of differences between means of microbial counts for TBC and Enterobacteriaceae in eels from three lakes, the Student's *t* test analysis was applied.

3. Results and Discussion

The microflora of fish and other water inhabitants depends on microbiological quality of water, the particular fish, water temperature and fishing conditions. Results of TBC, Enterobacteriaceae, *Salmonella* spp. and *L. monocytogenes* in water are shown in Table 1.

The highest water TBC count was identified in Aluksne lake 1,603 CFU/mL, while the lowest was in

Table 1 Water testing results (CFU/mL).

Lake	Samples	TBC (CFU/mL)	Enterobacteriaceae (CFU/mL)	*Listeria* spp. (CFU/mL)	*Salmonella* spp. (CFU/mL)
Aluksne	1	1,603	< 1	0	0
Usma	1	16	1	0	0
Sivers	1	86	< 1	0	0

Table 2 The TBC in European eel (*Anguilla anguilla*) samples (CFU/cm^2).

Lake	Gills		Skin		Gut	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Aluksne	1.14 ± 0.45[b]	0.40-1.87	1.31 ± 0.47	0.85-2.00	1.51 ± 2.21	0.44-5.68
Usma	2.41 ± 0.51	1.89-3.25	1.50 ± 0.36	0.66-1.84	1.88 ± 1.59	0.30-5.82
Sivers	4.58 ± 0.77[a]	3.36-5.51	2.94 ± 1.18[c]	1.85-4.93	3.45 ± 1.57	2.56-6.37

SD = standard deviation. [a]Means TBC counts in gills, skin and gut samples from Sivers were significantly higher than in samples from Aluksne and Usma lakes ($P < 0.05$); [b]means no differences in TBC counts among gills, skin and gut in eels from Aluksne lake were observed ($P > 0.05$); [c]means skin of eels from Sivers was significantly less contaminated with TBC than gills and gut ($P < 0.05$).

Usma lake 16 CFU/mL. Enterobacteriaceae count did not exceed 1 CFU/mL in all lakes. All tested lake water samples were *L. monocytogenes* and *Salmonella* spp. negative.

Microbiological eel samples testing results show that TBC counts were different in all sampling sites on skin, gills and gut. TBC counts on ells gills, skin and gut were from 0.40 CFU/cm^2 to 5.51 CFU/cm^2, from 0.66 CFU/cm^2 to 4.93 CFU/cm^2 and from 0.30 CFU/cm^2 to 6.37 CFU/cm^2, respectively, depending on lake of origin (Table 2), which shows that the bacterial load (TBC) is not high according to Nikolajeva [7].

Enterobacteriaceae did not exceed 1 CFU/mL in any of the lakes. All the tested samples were *L. monocytogenes* and *Salmonella* spp. negative.

The mean TBC counts in gills, skin and gut were significantly higher in eels from Sivers than in samples from Aluksne and Usma lakes ($P < 0.05$). This indicates than fish originated from different lakes may show different numbers of TBC counts and fish origin may have impact in microbiological condition of fish. Despite of fish testing results showed the highest TBC counts were found in water from Aluksnes, it is generally accepted that microbiological load of freshwater fish depends on water condition and temperature. However, Gonzalez et al. [17] found that composition of water microflora did not influence fish microflora and had no significance effect on fish microflora.

There were no significant differences between TBC counts for gills, skin and gut samples in eels from Aluksne and Usma lake ($P > 0.05$), however, skin of eels from Sivers was significantly less contaminated with TBC than gills and gut ($P < 0.05$). TBC counts of fish were acceptable and indicated that eels yielded the same counts of organisms as another fishes, such as wild brown trout (*Salmo trutta*), wild pike (*Esox lucius*), Silver bream (*Blicca bjoerkna*), European perch (*Perca fluvialitis*), aquacultured rainbow trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis* spp.) in the previous studies [7, 17-19]. Results of this study indicate that mesophiles counts for wild and farmed fishes from cold and unpolluted waters varies from 10^2 CFU/cm^2 to 10^5 CFU/cm^2 for skin and 10^3 CFU/cm^2 to 10^6 CFU/cm^2 for gut, respectively [17].

Enterobacteriaceae counts on the gills, skin and gut of eel ranged from 0 CFU/cm^2 to 2.47 CFU/cm^2, from 0 CFU/cm^2 to 4.30 CFU/cm^2 and from 0 CFU/cm^2 to 1.72 CFU/cm^2 depending on lake of origin (Table 3). The highest mean counts of Enterobacteriaceae in gills (1.64 CFU/cm^2) and skin (3.20 CFU/cm^2) were found in

Table 3 The Enterobacteriaceae in European eel (*Anguilla anguilla*) samples (CFU/cm^2).

Lake	Gills		Skin		Gut	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Aluksne	0.03 ±0.11[a]	0.00-0.30	0.03 ±0.11	0.00-0.30	0.38 ± 0.71	0.00-2.26
Usma	1.40 ±0.51	0.70-2.11	0.00	0.00	2.43 ± 2.44	0.00-4.15
Sivers	1.64 ± 0.42[a]	1.11-2.47	3.20 ± 1.28	1.34-4.30	0.20 ± 0.50	0.00-1.72

SD = standard deviation. [a]Means there were significant differences in Enterobacteriaceae counts among gills, skin and gut samples from different lakes ($P < 0.05$).

samples from Sivers, but the highest mean counts of Enterobacteriaceae in gut (2.43 CFU/cm^2) was in samples from Usma lake.

The mean counts of Enterobacteriaceae were the highest in skin in comparison with gut and gills and there were significant differences in Enterobacteriaceae counts among gills, skin and gut samples from different lakes ($P < 0.05$). The authors' results are in accordance with Junior et al. [10] who reported the highest numbers of coliforms on skin than on gut in tilapia, and with Gonzalez et al. [17] who evaluated Enterobacteriaceae in gills, gut and skin samples of wild brown trout (*Salmo trutta*), wild pike (*Esox lucius*) and aquacultured rainbow trout (*Oncorchynchus mykiss*) in Spain. Increased counts of Enterobacteriaceae in gills and skin samples of eels corresponded to results of increased TBC counts for eel samples from Sivers lake, however Enterobacteriaceae counts in Aluksne and Sivers water were lower than detection limits.

All tested samples were *Salmonella* spp. and *L. monocytogenes* negative. Results on the prevalence of *Salmonella* spp. are in accordance to previous studies, and the *Salmonella* spp. was not isolated from wild and aquacultured fishes across Europe [15, 19, 20]. In contrast, the presence of *Salmonella* spp. in freshly caught and marketed fish were reported in Africa in Asia and the presence of *Salmonella* spp. in fish was linked to poor hygiene of water source and handling of fish during fishing, processing and marketing [21, 22].

L. monocytogenes was isolated from freshwater and marine fish more frequently than *Salmonella* spp. Results of this study were in agreement with Davies et al. [15], Gonzalez et al. [17], and Pullela et al. [18], who did not find *L. monocytogenes* in wild and aquacultured fish samples. However, Vogel et al. [23] and Miettinen and Wirtanen [24] reported 8.6% and 14.6% prevalence of *L. monocytogenes* in Denmark and Finland, respectively. Absence of *L. monocytogenes* in tested samples was linked to good aquacultural practice [18]. Since tested water samples in this study did not reveal the presence of *L. monocytogenes*, these findings may be attributed to good microbiological quality of lake water and indicate an unpolluted lake environment. In general, absence of *Salmonella* spp. and *L. monocytogenes* in tested samples shows that freshly caught eels do not pose public health concerns and are safe for consumption.

4. Conclusions

Microbiological contamination rates of European eels (*Anguilla anguilla*) in the present study were typical for wild fish in unpolluted environment and were comparable with microbiological contamination tares of another freshwater and marine fishes. Testing of gills, skin and gut of eel did not reveal specific contamination pattern, which could be typical for European eel. Additionally, foodborne pathogens, such as *Salmonella* spp. and *L. monocytogenes*, were not found in eel samples, indicating that fish are safe for consumption.

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