

BACTERIOFLORA OF DIGESTIVE TRACT OF FISHES *IN VITRO*

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Summary. Microbiological method was used to assess peculiarities of abundance of autochthonous and petroleum hydrocarbon-degrading bacteria (HDB) in the digestive tract of fish of different trophic groups and the proportion of HDB in the total heterotrophic bacteria (THB). The number and dynamics of petroleum hydrocarbon-degrading bacteria in the digestive tract of fish was registered in different seasons of the year. Regularities of abundance of petroleum hydrocarbon-degrading bacteria in freshwater and marine fish species were pointed out. The bacteriocenoses of the digestive tract of investigated fish were found to be dominated by the total heterotrophic bacteria. The variability of abundance and dynamics of autochthonous and allochthonous bacterioflora of the digestive tract of fish from the Baltic Sea and the Curonian Lagoon was due to fish species, nutrition habits and intensity, and season of the year. The lowest amount of bacteria of investigated functional groups was observed in early spring, and the highest in summer, during intensive fish feeding. The total heterotrophic bacteria in bacteriocenoses of the digestive tract of river perch and gudgeon from the Curonian Lagoon varied from 10^{-7} to 10^{-8} g⁻¹ of intestine content. A similar tendency was observed in fish from the Baltic Sea; however, summer counts of THB in fish from the sea were considerably lower than in fish from the Curonian Lagoon. The greatest part of HDB in THB was observed in summer in the digestive tract of small sandeel (30.95%), European flounder (30.08%) and bullrout (28.33%) from the Baltic Sea. A respective spring proportion was 11.57% in European flounder, 9.88% in sandeel, and 9.24% in bullrout. In autumn this parameter was slightly higher than in spring in the digestive tract of European flounder (18.5%) and the least in bullrout (0.36%). From the proportion of HDB in THB we can assume that the littoral zone of the Baltic Sea near Būtingė is considerably more contaminated with oil than the Curonian Lagoon. The average values of abundance of the total coliform bacteria in the bacteriocenosis of the digestive tract of perch and gudgeon caught in the Curonian Lagoon varied, with the highest values in summer and autumn, whereas in the Baltic Sea the greatest average abundance of total coliforming bacteria (TCFB) was observed in the digestive tract of bullrout in spring and summer.

Keywords: hydrocarbon-degrading bacteria, petroleum, digestive tract, fish.

ŽUVŲ VIRŠKINAMOJO TRAKTO BAKTERIOFLORA *IN VITRO*

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Santrauka. Įvertinti naftos angliavandenilius skaidančių bakterijų gausos dėsningumai gėlavandenių ir jūrinių žuvų rūšyse. Tirtų žuvų virškinamojo trakto bakteriocenozėse daugiausia nustatyta bendrų heterotrofinių bakterijų. Kuršių marių ir Baltijos jūros žuvų virškinamojo trakto autochtoninės ir alochtoninės bakteriofloros gausos dinamikos kaita priklauso nuo žuvų rūšies, mitybos intensyvumo, specifikos, metų sezono. Mažiausiai tirtų funkcinį grupių bakterijų nustatyta ankstyvą pavasarį, daugiausia – vasarą, intensyvios žuvų mitybos metu. Kuršių marių paprastojo ešerio ir gružlio virškinamojo trakto bakteriocenozėse vyraavo bendrosios heterotrofinės bakterijos 10^{-7} – 10^{-8} g⁻¹ žarnyno turinio. Didžiausias bendrų heterotrofinių ir naftos angliavandenilius skaidančių bakterijų santykis nustatytas vasarą Baltijos jūroje sugautuose mažojo tobio (30,95 proc.), upinės plekšnės (30,08 proc.) ir builio (28,33 proc.) virškinamajame trakte. Pavasarį šio santykio išraiška nustatyta upinėse plekšnėse (11,57 proc.), mažajame tobyje (9,88 proc.) bei builyje (9,24 proc.). Rudenį šio parametro išraiška buvo kiek didesnė nei pavasarį plekšnėse (18,5%) ir mažiausia – builyje. Baltijos jūros priekrantė ties Būtinge yra ženkliai užteršta naftos angliavandeniliais palyginti su Kuršių mariomis. Kuršių mariose sugautų ešerio ir gružlio virškinamojo trakto bakteriocenozėje bendrų žarnyno grupės bakterijų vidurkis kito tendencingai, daugiausia vasarą ir rudenį. Baltijos jūros žuvyse šių bakterijų didžiausias vidurkis nustatytas pavasarį ir vasarą builio virškinamajame trakte.

Raktažodžiai: naftos angliavandenilius skaidančios bakterijos, nafta, virškinamasis traktas, žuvis.

Introduction. Extensive reviews have been published on various aspects of microbial flora associated with fish eggs, skin, gills and intestine and on relationships between intestinal microflora and the microflora of aquatic habitats or internal tissues (Ringø et al., 1995; Ringø, Birkbeck, 1999; Ringø et al., 2008; Ghosh et al., 2010). Microbial populations in the digestive tract of fish are rather dense, with numbers of microorganisms much

higher than in the surrounding water, indicating that the digestive tract provides favourable ecological niches for these organisms. It is accepted that fishes possess a specific intestinal microbiota consisting of aerobic, facultative anaerobic and obligate anaerobic bacteria; however, bacterial composition may change with age, nutritional status and environmental conditions.

The autochthonous microflora of the digestive tract of

fish participates in the secretion of enzymes, vitamins, amino acids and other physiologically active materials and, therefore, is necessary for normal metabolism of an organism. However, microflora of the digestive tract of aquatic animals is less abundant and its species diversity is narrower compared to the microflora of the digestive system of homothermal animals. Nevertheless, the bacteria of the digestive tract of aquatic animals participate with their enzymes in the process of degradation of nutrients. Furthermore, the normal microflora of aquatic organisms occupies vital niches on the surface of the body and in the digestive tract, thus preventing from intrusion of pathogenic microorganisms (Шивакене, 1989; Onarheim, Raa, 1990; Мицкене, 1992; Austin, 2002; Bairagi et al., 2002; Mondal et al., 2008; 2010; Roy et al., 2009; Ray, 2010; Ray et al., 2010).

The stability of the ecological balance of microflora of the digestive tract of aquatic organisms depends upon the trophism, productivity, toxicity and other chemical and physical parameters of water bodies. Contamination of water bodies with oil and its products is one of the acutest problems as it is the most widely spread type of anthropogenic pollution. Biodegradation of petroleum hydrocarbons by microorganisms is the principal mechanism to remove petroleum and other hydrocarbons from environment (Leahy, Colwell, 1990; Atlas, 1995; Ringø et al., 2008).

An increasing demand for and utilization of petrochemicals resulted in the rise of the level of petroleum hydrocarbons in marine, coastal and estuarine environments. Biodegradation by natural populations of microorganisms is the basic and most reliable mechanism by which thousands of xenobiotic pollutants, including crude oil, are eliminated from the environment. The effects of environmental conditions on microbial degradation of hydrocarbons and the effects of hydrocarbon contamination on microbial communities are areas of great interest (Delille, 2000; Delille, Delille, 2000; El-Tarabily, 2002).

The sensitivity of some microorganisms to toxic substances is similar to the sensitivity of animals; however, some microorganisms are resistant to pollution. Animals sensitive to toxic substances can feed on microorganisms resistant to toxic substances without any consequences. And on the contrary, toxic substances can be lethal to sensitive microorganisms of the digestive tract of an animal even if concentration of such toxic substances is not dangerous to the animal, and the animal may die from disorder of the digestive system (Pokarzlewskii, 1991).

High adaptability of microorganisms to a new environment and a wide spectrum of their enzymes systems condition their ability to degrade, use and transform different organic and inorganic compounds. The natural microflora of the digestive tract of animals is an accumulator of toxic substances from outside and those generated in the host's organism. Therefore, the normal microflora of the digestive tract might be considered the primary target for substances getting into the organism. The normal microflora is an ecological system, which is the first to participate in the degradation of natural and alien, useful and harmful substances; only after this non-specific barrier is

crossed, protection mechanisms of the organism itself start functioning (Spanggaard et al., 2000; Vine et al., 2004; Izvekova et al., 2008).

On the basis of long-term data on interaction of marine organisms and petroleum hydrocarbons, estimation of petroleum hydrocarbon flows through some organisms was carried out. In the Sevastopol Bay, the bacterial association of seawater and bottom sediments transform 0.2 t and 4.4 t of petroleum hydrocarbons approximately. Mussels settling in the Sevastopol Bay berths accumulate about 0.15 t of petroleum hydrocarbons. Potentially (from calculation data) the zooplankton association of the Sevastopol Bay can accumulate and transfer about 15 t of petroleum per day. A possibility of petroleum hydrocarbon flows through other organisms is discussed (Миронов, 2006).

Fish and mussels intoxication with oil causes certain morphological changes in blood and gills (Baršienė et al., 2006 a, b). Configuration of fish blood cells is changing, and the nucleus is decentralizing. Blood changes cause morphological alterations in surface layers and internal organs. Even low concentrations of oil cause pathogenic changes in fish embryos, which lead to inadequate generations. Oil and its products might adversely affect fish during their early period of development when spawn and larvae of most pelagic fishes are on the surface and occupy the water body's upper layers, which are most often contaminated with oil and its products. It should be noted that fish might avoid contaminated areas; on the other hand, it is practically impossible to follow a fish, which had contacts with petroleum but later left the contaminated zone (Миронов, 1985).

However, reviews on microflora of the digestive tract of fish do not provide information on the presence of hydrocarbon-degrading bacteria in the gastrointestinal tract of fish. Hydrocarbons occur naturally in the environment, and it is surprising that microorganisms have evolved the ability to utilize these compounds. Almost all natural aquatic ecosystems contain populations of bacteria that can metabolize some oil and related compounds even if these systems have not ever been exposed to oil or oil products (Leahy, Colwell, 1990).

Estimation of the abundance and dynamics of petroleum hydrocarbon-degrading bacteria in the digestive tract of fish enables evaluation of participation of such bacteria in biodegradation of pollutants and in the process of self-purification of water. The microflora of the digestive tract of aquatic animals is proved to be the first to be affected by any pollutants appearing in water (Миронов, 2006). Therefore, it is important to know how aquatic animals and their communities are affected by pollutants, evaluate their sensitivity to different substances and investigate transformation possibilities of pollutants. In this respect it is of great importance to identify changes in the microflora of the digestive tract caused by pollution.

Objective. The objective of this research was to investigate abundance dynamics of autochthonous and petroleum hydrocarbon-degrading bacteria of the digestive tract of fish of different trophic groups and regularities of their ratio to the total heterotrophic bacteria.

Material and Methods. For identification of petroleum hydrocarbon degrading bacteria (HDB), fish were caught in different areas of the Curonian Lagoon and in the littoral zone of the Baltic Sea in spring, summer and autumn. Microbiological analysis was carried out with 90 specimens (aged 0+ – 2+). Hydrocarbon-degrading bacteria were examined in the digestive tract of ten different fish species.

Fish fry were caught using a 30 m long net in the littoral of the Baltic Sea, and a 10 m long net in the Curonian Lagoon. Net mesh size was 8-20 mm in the wings, and 3-8 mm in the sack.

The Curonian Lagoon is a shallow freshwater body, which though separated from the Baltic Sea by a narrow spit, still belongs to the sea basin. The Curonian Lagoon is a junction reservoir receiving the flow of the Nemunas River and acting as a sedimentation basin. The following fish species were used for microbiological analysis: European flounder (*Platichthys flesus trachuarus*) (16 specimens), viviparous eelpout (*Zoarces viviparus*) (5), bleak (*Alburnus alburnus*) (7) small sandeel (*Ammodytes tobianus*) (19), bullrout (*Myoxocephalus scorpius*) (10) in the Baltic Sea near the Būtingė, and eelpout (*Lota lota*) (10), dace (*Leuciscus leuciscus*) (8), river perch (*Perca fluviatilis*) (9) and gudgeon (*Gabio gabio*) (6) in the Curonian Lagoon.

Populations of aerobic and facultative anaerobic heterotrophic bacteria occurring in the digestion system of fish were estimated using the dilution plate technique (Кузнецов, Дубинина, 1989; Ringç, Olsen, 1999). The surface of animals was sterilized with 95% ethanol and then animals were dissected to remove their digestive system. The content of the digestive system was removed

onto sterile Petri plates. Each experiment set involved five fish specimens. All digestion system samples were weighed and placed into a test tube and then nine volumes of diluents were added. The tenfold dilution was further done serially. The least dilutions (0.1 ml), expected to give 30 to 300 colony-forming units (CFU), were plated on triplicate with solid media. Incubation was carried out aerobically at 20°C for seven days except for Mac Conkey agar plates, which were incubated at 37°C for 7 days. The number of CFUs in the digestive system of fish was measured on five media: soy peptone agar was chosen for isolation of the total heterotrophic bacteria (THB), milk agar for proteolytic bacteria (PB) as a separate group of heterotrophic bacteria, proteolytic bacteria were identified according to the zones of protein (casein) hydrolysis on milk agar; starch agar for amilolytic bacteria (AB) as a separate group of heterotrophic bacteria were determined according to the zones of starch hydrolysis on starch agar under the action of iodine solution; Mac Conkey agar was used for total coliform bacteria (TCFB), the Voroshilova-Dianova agar with crude oil for hydrocarbon-degrading bacteria as a separate group of heterotrophic bacteria, and the same agar without crude oil was used as a control. Bacterial colonies appearing on each plate were counted, and a CFU per g (wet weight) of intestinal content was obtained. The average values and mean square deviations were calculated (Sakalauskas, 2003).

Results and Discussion. The abundance of bacteria in aquatic organisms was found to depend on fish species, nutrition habits, as well as seasonal and environmental effects. The lowest abundance of bacteria was found in early spring, and the highest in summer, during intensive fish feeding (Fig. 1, 2).

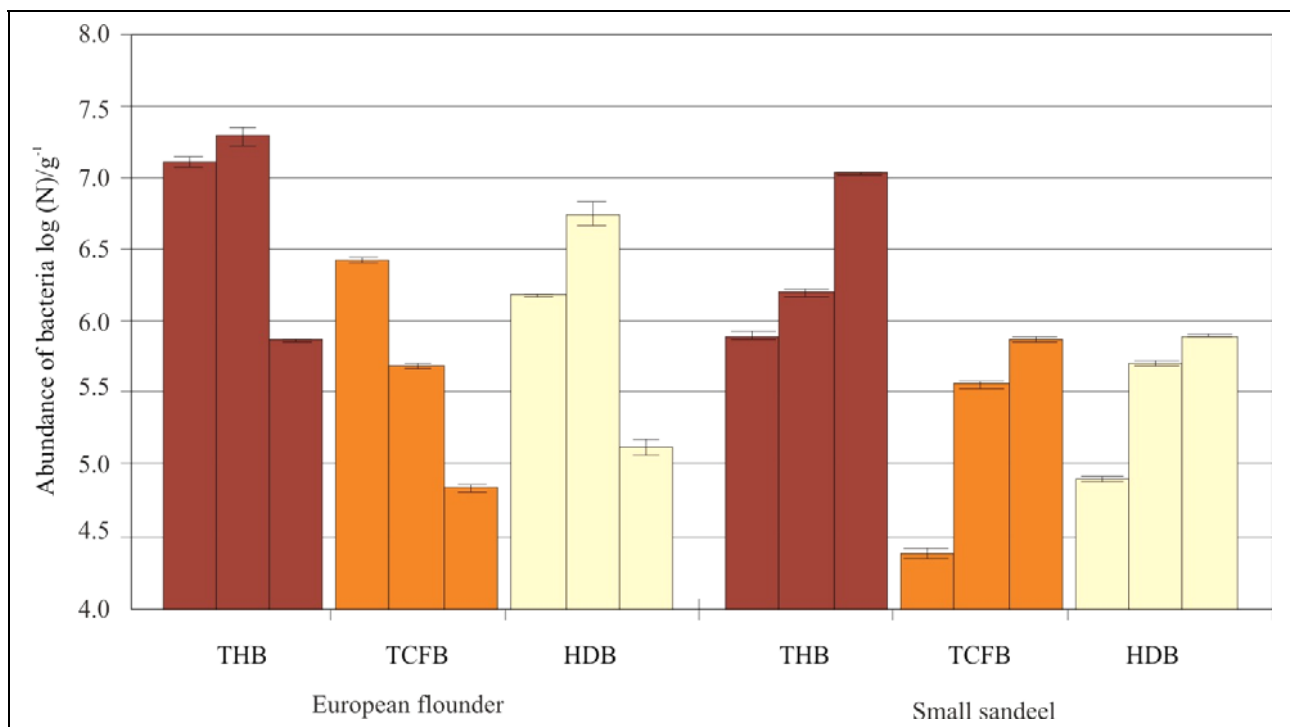


Figure 1. Abundance of bacteria in European flounder and small sandeel digestive tract in the Baltic Sea, depending on the season (1st column – spring, 2nd column – summer, 3rd column – autumn)

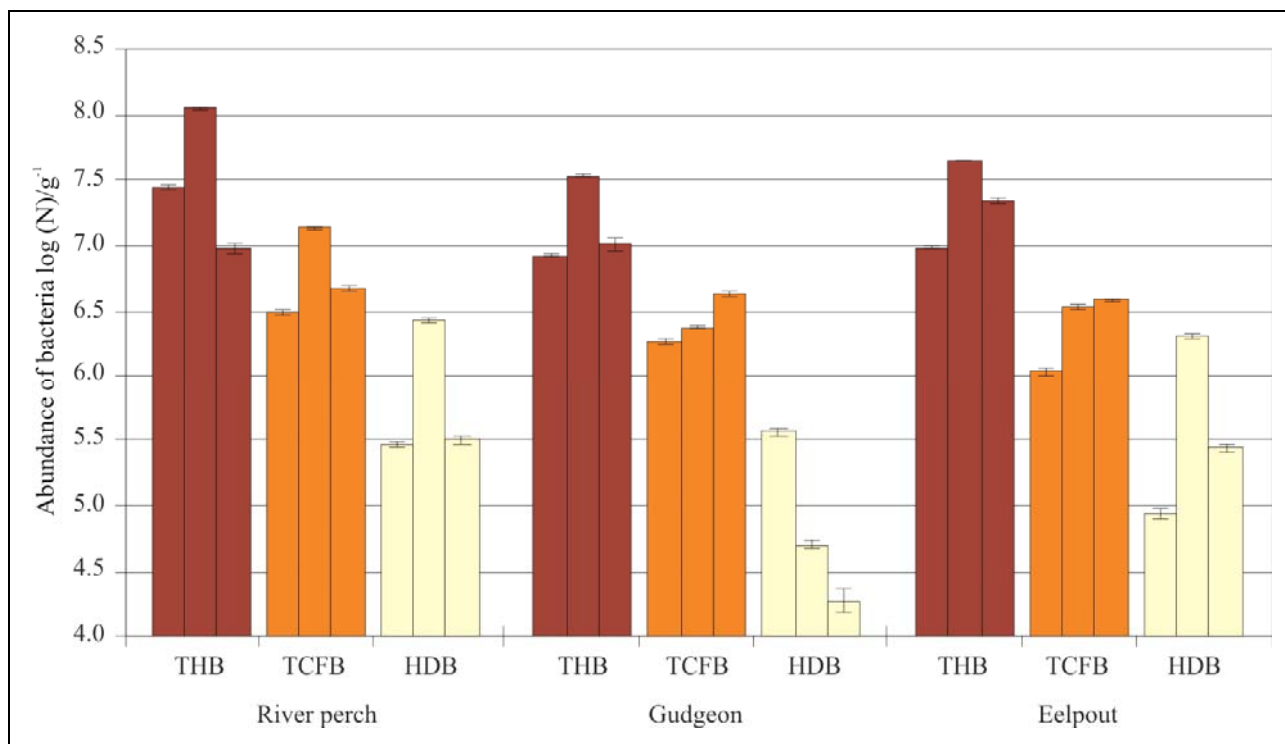


Figure 2. Abundance of bacteria in river perch, gudgeon and eelpout digestive tract in the Curonian Lagoon, depending on the season (1st column – spring, 2nd column – summer, 3rd column – autumn)

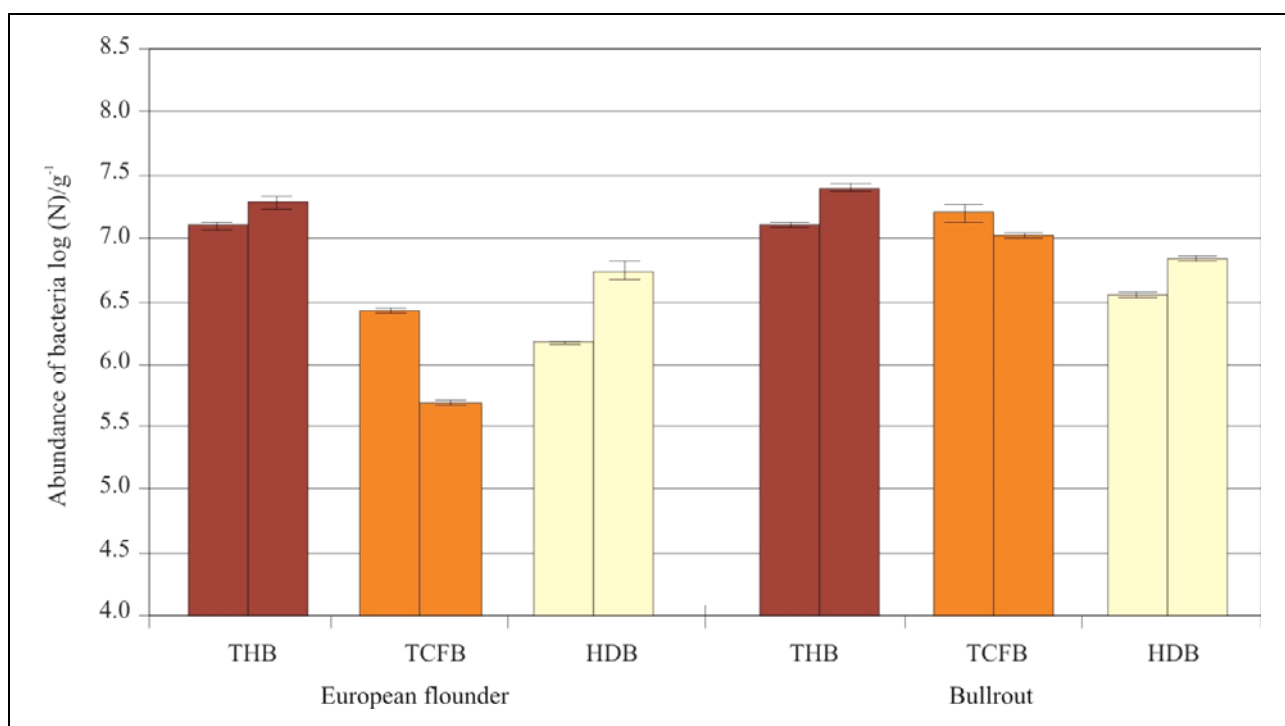


Figure 3. Abundance of bacteria in European flounder and bullrout digestive tract in the Baltic Sea, depending on special distribution of these fish in the ecosystem (1st column – spring, 2nd column – summer)

Similar results have been obtained by other authors who investigated the microfauna of the digestive tract of freshwater and marine fishes (Лубянскене и др., 1989; Izvekova et al., 2008; Ghosh et al., 2010). The bacteriocenoses of the digestive tract of fishes are dominated by

the total heterotrophic bacteria, which constitute one of the main functional groups of fish autochthonous microfauna and determine the formation and functioning regularities of autochthonous bacterioflora in the early ontogenesis of fish (Шивокене, 1989). The bacterioflora of an

autochthonous digestive tract is composed of many populations, including frequently met and accidental species of bacteria, the qualitative and quantitative composition of which depends upon internal and external factors (Ringø et al., 1999, 2008; Spanggaard et al., 2000; Austin, 2002). The greatest abundance of heterotrophic bacteria was

found in carnivorous fish species both from the Baltic Sea (European flounder, bullrout) (Fig. 3) and Curonian Lagoon (river perch, eelpout, gudgeon), compared to omnivorous fish species (dace) (Fig. 4) (Virbickas, 2000). The abundance of bacteria studied was due to the foraging zones and nutrition spectrum of fish (Fig. 5).

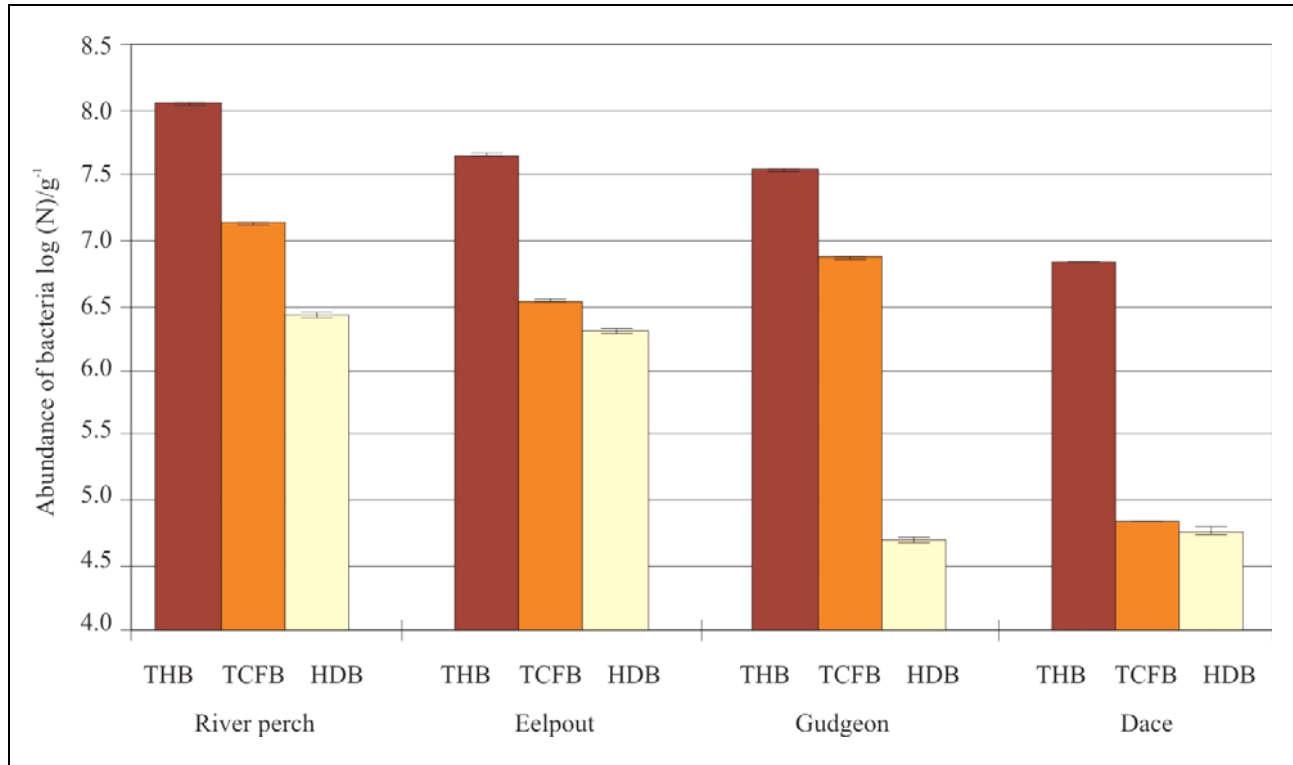


Figure 4. Abundance of bacteria in river perch, eelpout, gudgeon and dace digestive tract in the Curonian Lagoon, depending on fish nutrition habits (summer)

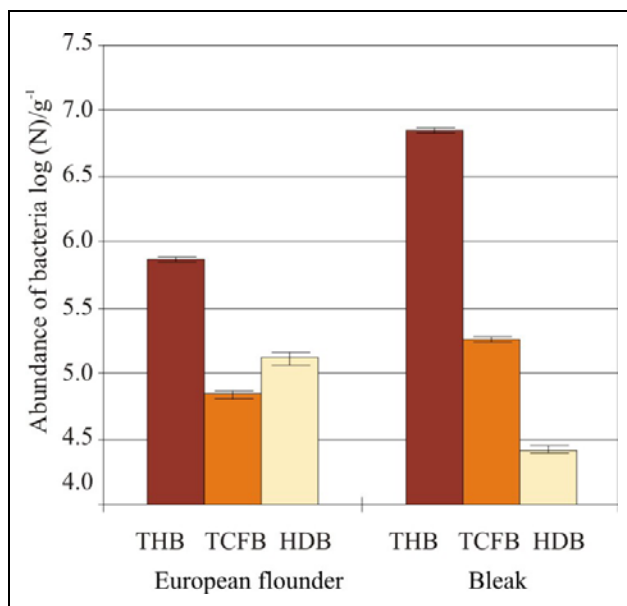


Figure 5. Abundance of bacteria in benthic fish European flounder and pelagic fish Bleak (autumn)

Parallel investigations of bacteriocenoses of the digestive tract of fish in the Baltic Sea and in the Curonian Lagoon enabled disclosing certain regularities and preventing from accidental conclusions. As discussed above, the abundance of bacteria of the digestive tract of fish varies depending on fish species, season of the year, intensity of fish foraging, and temperature regime (Žaromskis, 1998; Šyvokienė et al., 2004).

We examined seasonal regularities of the abundance of bacterioflora of the digestive tract of fish and found that the greatest abundance of the total heterotrophic bacteria was in summer in the Curonian Lagoon, where bacterial abundance in bacteriocenoses of the digestive tract of river perch, gudgeon and eelpout ranged from 10^{-7} to 10^{-8} g⁻¹ of intestine content. Further, abundance of the total heterotrophic bacteria depended on temperature, namely the number of heterotrophic bacteria in fish caught in the Curonian Lagoon in autumn was found to be tens or hundreds of times lower than in summer (Fig. 2). The foraging intensity of poikilothermic animals is affected by decreasing water temperatures when metabolism becomes slower (Voverienė, 2002).

A similar tendency was observed in benthic European flounder caught in the Baltic Sea. As to abundance of

bacteria in the digestive tract of small sandeel, it was increasing in autumn, which was most probably due to species-specific nutrition habits and age of fish caught (Fig. 1).

It should be noted that the functioning of bacterioflora of the digestive tract of fish could result in the production of physiologically active substances (Шивокене, 1989; Ghosh, 2010; Ray, 2010), which can be used by the organism as construction materials, increase intensity and efficiency of the digestion process and stimulate fish growth rates.

The abundance of petroleum hydrocarbon-degrading bacteria of the digestive tract of different fishes caught at the same time was found to be similar, however, abundance of bacteria in the same fish species in different seasons could vary markedly (Fig. 1, 2).

The petroleum hydrocarbon-degrading bacteria most rapidly adapt to environmental changes. By using petroleum hydrocarbons as the only source of carbon, HDB perform biodegradation of pollutants, and when biodegradation products appear other functional groups of bacteria join to utilization of such newly appeared products (Šyvokienė et al., 2005; Youssef et al., 2010).

It is known from literature that if petroleum hydrocarbons get into water, vegetation, phytoplankton, zooplankton, benthos and sediments (through which as food into the digestive tract of animals), they bring about changes in the composition of populations of hydrocarbon-degrading bacteria in the water body or digestive tract of aquatic organisms irrespective of the degree of contamination. The greatest numbers of HDB in water and fish digestive tracts and the biggest part of such bacteria within heterotrophic bacteria are associated with the highest concentrations of petroleum hydrocarbons (Миронов, 2006).

A significantly greater increase (97.67 times) in summer numbers of petroleum hydrocarbon-degrading bacteria was observed in fish caught in the Baltic Sea near Būtingė (5.86 million/g; $\log 6.7 \text{ g}^{-1}$ in European flounder) (Fig. 1) than in the Curonian Lagoon (0.06 million/g; $\log 4.76 \text{ g}^{-1}$ in gudgeon) (Fig. 2).

The greatest part of hydrocarbon-degrading bacteria within the total heterotrophic bacteria was found in the digestive tract of small sandeel (30.95%), European flounder (30.08%) and bullrout (28.32%) caught in the Baltic Sea in summer. In spring, this proportion was 11.57% for European flounder, 9.88% for small sandeel, and 9.24% for bullrout. A respective autumnal parameter was higher compared with spring, namely 18.5% in the digestive tract of European flounder, 7.32% in small sandeel, and 0.36% in bullrout.

The proportion of hydrocarbon-degrading bacteria within heterotrophic community in the digestive tract of eelpout caught in the Curonian Lagoon in summer was 4.9 times greater than in spring and 3.4 times greater than in autumn. A respective proportion in river perch and gudgeon was 1-2 times greater in summer than in spring. As to gudgeon, the summer proportion of hydrocarbon-degrading bacteria was as much as 3.4 times greater than the autumnal proportion. The greatest abundance of total

heterotrophic and petroleum hydrocarbon-degrading bacteria in the digestive tract of fish caught in the Curonian Lagoon in summer was registered in river perch and the lowest in dace.

From microbiological analysis we can assume that the Baltic Sea near Būtingė is considerably more contaminated with petroleum hydrocarbons than the Curonian Lagoon.

The abundance of petroleum hydrocarbon-degrading bacteria in water and their proportion in the total heterotrophic bacteria has already been proved to increase with higher concentration of petroleum and other hydrocarbons (Čipinytė, 2000; Voverienė et al., 2002; Šyvokienė et al., 2004). The abundance of hydrocarbon-degrading bacteria in water and in fish digestive tract shows the degree of contamination of the ecosystem with oil and its products. The ability of bacterial populations of the digestive tract of aquatic organisms to use petroleum hydrocarbons as a source of carbon allows assuming that aquatic organisms participate in biodegradation of oil pollutants and self-purification of water.

As per literature data, the main petroleum hydrocarbon-degrading bacteria occurring in water belong to the following genera: *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Nocardia* and *Pseudomonas*. The *Vibrio* genus is mentioned as one of the main genera in the marine environment (Миронов, 2006; Tekorienė, 2008). The petroleum hydrocarbon-degrading bacteria existing in the digestive tract of fish, otherwise than in water, are dominated by genus *Aeromonas* (60–70%), which is followed by genus *Pseudomonas* accounting for merely 18% and being more frequent in autumn rather than in spring or summer and by bacteria of genera *Micrococcus*, *Flavobacterium* and *Arthrobacter* constituting the remaining 10–15% (Voverienė, 2002; Šyvokienė et al., 2004). According to these authors, the degrading activity of different bacteria varies from 7.49 to 31.25% of degraded substrate. The average degrading activity of genus *Pseudomonas* was found to be 14.68–27.12% of degraded substrate. Genus *Aeromonas* demonstrates the widest range of petroleum hydrocarbon degrading activity (7.49–31.25). *Aeromonas eucrenophila* dominates (30–40%) among hydrocarbon-degrading bacteria from the digestive tract of fish. The speed and degree of biological hydrocarbon degradation depend not only on the activity of microorganisms, but also on the type and concentration of hydrocarbons and on environmental conditions (temperature, aeration, pH) (Chang et al., 2000).

Atlas (Atlas et al., 1998) reported that populations of hydrocarbon-degrading bacteria normally constitute less than 1% of total microbial communities but if other oil pollutants are present, hydrocarbon-degrading populations increase, typically to 10 percent of the community.

The average of hydrocarbon-degrading bacteria within the average of heterotrophic communities fluctuates from 0.2% to 2.1%. Almost all natural aquatic ecosystems contain populations of bacteria that can metabolize some oil components and related compounds even if those systems have not ever been exposed to oil or oil products (Šyvokienė et al., 2002). The numbers of hydrocarbon-

utilizing bacteria and their proportion in the heterotrophic community increase upon exposure to petroleum or other hydrocarbon pollutants, and the levels of hydrocarbon-utilizing microorganisms generally reflect the degree of contamination of the ecosystem.

Along with petroleum hydrocarbon-degrading bacteria, the digestive tract of aquatic animals contains coliform bacteria. The dominance of coliform bacteria in the bacteriocenosis of the digestive system of aquatic animals changes the balance of autochthonous bacterioflora and enzymes activity, whereupon animal healthiness is getting worse (Šyvokienė, 2010).

The average values of abundance of coliform bacteria in bacteriocenosis of the digestive tract of river perch, gudgeon and eelpout caught in the Curonian Lagoon var-

ied, with the greatest values registered in summer and autumn (Fig. 2).

The greatest average values of coliform bacteria in the digestive tract of fish caught in the Baltic Sea were registered in bullrout in spring and summer (Fig. 3), which was due to industrial and household wastewater emissions into the Malkū inlet and pollutants inflowing with the Nemunas River water (Žaromskis, 1998).

The abundance of coliform and hydrocarbon-degrading bacteria in the digestive tract of fish species of similar nutrition habits but living in different habitats differed, namely the number of bacteria in bullrout from the Baltic Sea was greater than in perch from the Curonian Lagoon (Fig. 6).

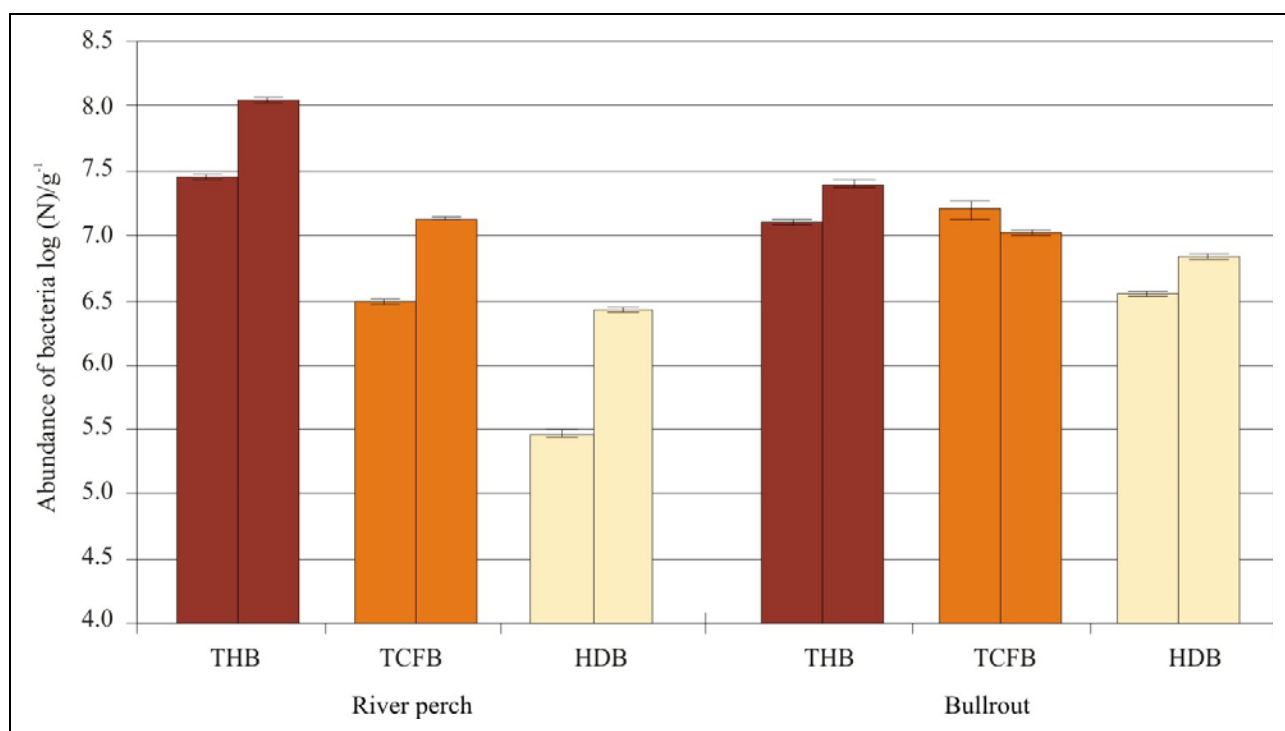


Fig. 6. Abundance of bacteria in river perch from Curonian Lagoon and bullrout from Baltic Sea depending on different habitats (1st column – spring, 2nd column – summer)

A comparison of two fish species of different nutrition habits from the Baltic Sea *Zoarces viviparus* and *Alburnus alburnus* showed that abundance of hydrocarbon-degrading bacteria in the digestive tract of *Z. viviparus* was considerably higher than in *A. alburnus*. The results obtained show the importance of the choice of nutrition objects (Fig. 5).

Bacterial populations of the digestive tract of fish caught in the Curonian Lagoon and in the littoral of the Baltic Sea were analysed seeking to determine ecological trends and indicators of long-term anthropogenic impact. Significant data were obtained on contamination of freshwater and marine ecosystems with oil and its products. The knowledge of abundance and functional activity of hydrocarbon-degrading bacteria, as biomarkers, in the digestive tract of fish opens vast possibilities in estimating participation of aquatic organisms in identification,

biodegradation and transformation of contaminants and self-purification of water. Hydrocarbon-degrading microorganisms in the digestive tract of aquatic animals reflect the degree of pollution of the ecosystem. This is the most time-saving microbiological method to register contamination with oil.

Conclusions

1. The bacteriocenoses of the digestive tract of fish were dominated by the total heterotrophic bacteria as one of the main functional groups of autochthonous microflora of fish. The highest abundance of the total heterotrophic bacteria was detected in carnivorous fish both from the Baltic Sea and the Curonian Lagoon. Changes in the dynamics of autochthonous and allochthonous bacterioflora of the digestive tract of fish from the Curonian Lagoon depend on fish species, nutrition habits and intensity, and season of the year.

2. Seasonal variability of abundance of bacterioflora of the digestive tract of fish was identified. The lowest abundance of bacteria of all functional groups was observed in early spring, and the highest in summer, during intensive fish feeding. The highest abundance of the total heterotrophic bacteria was registered in summer, with their abundance in bacteriocenoses of the digestive tract of river perch, eelpout and gudgeon from the Curonian Lagoon ranging from $34.3 \cdot 10^6$ to $113.72 \cdot 10^6$ g. The autumnal abundance of total heterotrophic bacteria was tens or hundreds of times lower than summer numbers. Similar tendencies were observed in fish caught in the Baltic Sea.

3. The greatest proportion of hydrocarbon-degrading bacteria in the total heterotrophic community was found in the digestive tract of small sandeel (30.95%), European flounder (30.08%) and bullrout (28.33%) caught in the Baltic Sea in summer. In spring, this proportion was 11.57% in European flounder, 9.88% in small sandeel, and 9.24% in bullrout. The autumnal expression of this proportion was 18.5% for European flounder, and 7.32% for small sandeel.

4. A comparison of bacteriological data of the digestive tract of fish from two habitats according to the proportion of total heterotrophic bacteria and hydrocarbon-degrading bacteria shows that the Baltic Sea near the Būtingė zone is considerably more polluted with petroleum hydrocarbons than the Curonian Lagoon.

5. The greatest average values of abundance of coliform bacteria in the digestive tract of fish caught in the Baltic Sea were registered in the bullrout in spring and summer. The average values of abundance of coliform bacteria in bacteriocenosis of the digestive tract of river perch and gudgeon caught in the Curonian Lagoon varied, with the greatest values in summer and autumn, which was due to industrial and household wastewater emissions and pollutants inflowing with the Nemunas River water.

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