

Bacteria in the digestive system of molluscs from Lithuanian lakes

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The abundance of bacteria (total heterotrophic, proteolytic, total coliform and hydrocarbon-degrading) in the digestive system of bivalve molluscs *Anodonta cygnea*, *Unio tumidus* (Birva and Spėra Lakes) and *Dreissena polymorpha* (Skaistis Lake) has been studied. Bacteria counts in the digestive tract of molluscs were found to differ and vary depending on species and the water basin. In the digestive tract of *A. cygnea* and *U. tumidus* from Lake Spėra the bacterial counts were similar. In Lake Birva, a higher abundance of the heterotrophic and proteolytic bacteria has been found in the digestive tract of *A. cygnea* than in *U. tumidus*. In the digestive tract of molluscs, hydrocarbon-degrading bacteria constituted 0.09% (*D. polymorpha*, Lake Skaistis) to 24.22% (*U. tumidus*, Lake Spėra) of the total heterotrophic bacteria. In the water of the lakes, there were 17.3% (Lake Skaistis) to 33.89% (Lake Spėra) of hydrocarbon-degrading bacteria. A less significant abundance was found in the cenosis of the digestive tract of *D. polymorpha* (Lake Skaistis). According to the average values of hydrocarbon-degrading bacteria and the total number of coliform bacteria, Lake Skaistis ecosystem was least polluted with oil and its products, the Lake Spėra ecosystem was polluted most heavily, and the ecosystem of Lake Birva less than the Lake Spėra ecosystem and more than the Lake Skaistis ecosystem.

Key words: molluscs, digestive tract, bacteria, hydrocarbon-degrading bacteria

INTRODUCTION

The underlying role in the synthesis, transformation and destruction of materials belongs to microorganisms. They actively participate in the biogeochemical conversion of water pools, ensure the processes of their self-purification, and provide the basis for nutritional networks and chains which in turn enable to maintain an integral and balanced functioning of the entire water and digestive tract ecosystem (Шивокене, 1989; Sorokin, 1999).

Molluscs adaptation to the medium, their ability to consume alimentary substances, compete successfully and procreate in particular conditions are among the principal factors determining the formation of a microbial population. Their dietary specialisation, ecosystem pollution and the intensity of their nutritional process may be judged by the abundance and interrelation of the functional groups of bacteria. In relation to abundance of bacteria functional groups in normal microflora of the digestive systems of different aquatic animals is unlike (Шивокене, 1989). The prevalence of certain functional groups in the bacteriocenosis of the digestive tract of bivalve molluscs can provide information on the choice of a nutritional substratum (animal or vegetable) or pollution with xenobiotics.

Bacterioflora of the digestive system of aquatic animals primarily integrates into the protective function of the organism and decomposition of natural and extraneous, useful and harmful substances as well as the synthesis of the missing ones (Шивокене, 1989).

However, xenobiotics may inhibit activity and have a negative impact on assimilation efficiency and adaptation possibilities of the host organism. Under the influence of anthropogenic pollutants, changes not only the composition and enzymatic activity of prevailing water bacteria, but also that of aquatic animals (Mickėnienė et al., 2008; Šyvokienė, Mickėnienė, 2008).

According to the available data (Vestel et al., 1984; Čipinytė, 2000), particular microorganisms can degrade only a certain restricted hydrocarbon substratum, therefore, a wide-ranging enzyme spectrum of bacterial population is required to degrade such a complex hydrocarbon compound as crude oil. Beside the ability of microorganisms to degrade hydrocarbon substrata using enzymes, an important characteristic is chemotaxis – the ability of an organism to identify a substratum using chemo receptors; however, considerable concentrations of oil hydrocarbons in the environment may disorganize chemotaxis.

The spread of organic carbon in nature and the chemical composition of organic substances are under a wide discussion. In oil industry, crude oil or gas are referred to as hydrocarbons, although they consist not only of hydrocarbons but also of nitrogen, sulphur, oxygen, minor amounts of metals like vanadium and nickel (Zdanavičiūtė, 1998).

Since hydrocarbon-degrading bacteria are a significant indicator of pollution in water ecosystems, their development in the digestive system of aquatic animals enables to estimate the peculiarities of pollution with oil and the intensity of this process in numerous water pools: Lake Drūkšiai (Šyvokienė, Mickėnienė, 2000), the Curonian Lagoon (Voverienė et al., 2002; Šyvokienė

et al., 2004), the Baltic Sea (Šyvokienė, Mickėnienė, 2003) and rivers (Šyvokienė, Mickėnienė, 2002 a, b).

New methods of fast identification of hydrocarbon-degrading bacteria are being created accumulating banks of 16 S rDNA clones and applying primers specific to certain species. However, only a few hydrocarbon-degrading bacteria species from sea water have been identified, whereas much more exhaustive identification has been performed in freshwater organisms (Chang et al., 2000). Wilson et al. (1999) generated specific PCR primers for variable rRNA gene sequences of the bacteria *Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, *Serratia marsescens*.

Hydrocarbon-degrading bacteria as a separate fraction in the digestive tract of aquatic animals have been researched in various water ecosystems since 1991 (Шивокене, и др., 1991; Šyvokienė, Mickėnienė, 1994, 1995, 1998, 1999, 2000, 2002 a, b, 2004; Шивокене, Мицкене, 1997; Jankauskienė et al., 1999; Voverienė, 2002).

However, data on the impact of pollution on the bacteriocenosis of the digestive tract of freshwater animals are not abundant. Molluscs in such aspects have been investigated most thoroughly. The aim of the present work was to examine hydrocarbon-degrading bacteria as microbiological markers in the digestive tract of invertebrate aquatic animals.

MATERIALS AND METHODS

Bacteria (total heterotrophic, proteolytic, total coliform and hydrocarbon-degrading) of the digestive tract of the bivalve molluscs *Anodonta cygnea* and *Unio tumidus* collected in Lake Spėra (Širvintos distr.), Lake Birva, and *Dreissena polymorpha* sampled in Lake Skaistis (Trakai distr.) and bacteria of the same groups in the water of the above-mentioned lakes have been studied.

Populations of aerobic and facultative anaerobic heterotrophic bacteria occurring in the digestion system of molluscs were estimated using the dilution plate technique (Кузнецов, Дубинина, 1989; Ringø, Olsen, 1999). The surface of animals was sterilised with 95% ethanol, and then they were dissected to remove the digestion system. The content of the digestive system was removed to sterile Petri dishes. Each set of experiments involved five molluscs, and in total 45 molluscs were examined. 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. Least dilutions (0.1 ml), expected to give 30 to 300 colony-forming units, (CFU) were plated on triplicate plates with solid media. Incubation was carried aerobically at 20 °C for seven days. The number of CFUs in the digestive system of molluscs was established on five media: soya peptone agar was chosen for isolation of total heterotrophic bacteria THB, milk agar for proteolytic bacteria PB as a separate group of heterotrophic bacteria, proteolytic bacteria were identified according to zones of protein (casein) hydrolysis on milk agar; the Mac Conkey agar was used for total coliform bacteria (TCFB), the Voroshilova-Dianova agar with crude oil for hydrocarbon-degrading bacteria (HDB) 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 differences in bacterial counts were assessed using the one-way analysis of variance (ANOVA) technique. In all cases, treatments were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Data on the qualitative composition and abundance of bacteria in the digestive tract of bivalve molluscs *A. cygnea*, *U. tumidus* (from Spėra and Birva lakes) and *D. polymorpha* (from Lake Skaistis) indicate that the qualitative composition of bacteria in the digestive tract of the molluscs was the same, whereas the abundance of bacteria differed significantly and depended on mollusc species and water basin (Figs. 1, 2, 4, 5, 7). However, the abundance of total heterotrophic and proteolytic bacteria in the digestive tracts of simultaneously caught bivalve molluscs *A. cygnea*, *U. tumidus* (from Lake Spėra) was higher in *U. tumidus* than in *A. cygnea* (Figs. 1, 2), probably because of nutrition peculiarities. The numbers of total coliforms indicating pollution of a water ecosystem by wastewater, and hydrocarbon-degrading bacteria indicating pollution with crude oil and its products, in the digestive tract of *A. cygnea*, *U. tumidus* from Lake Spėra were similar. The bacterial counts in the water of Lake Spėra were significantly lower in comparison with their numbers in the digestive tract of molluscs (Fig. 3). The abundance of hydrocarbon-degrading bacteria in the water of Lake Spėra was one order of magnitude lower in comparison with their abundance in the molluscs' digestive tract.

Normal bacteria of the digestive tract of aquatic animals have self-controlling mechanisms and within certain restraints are able to resist the impact of harmful conditions thus retaining the balance of microorganism populations. However, when coliform bacteria and hydrocarbon-degrading bacteria start dominating, the composition and enzymatic activity of normal bacterioflora changes and reduces the assimilation of vitally important substances synthesized by normal bacterioflora (Mickėnienė, Šyvokienė, 1996; Шивокене и др., 2001).

Comparison of bacteria in the digestive tract of bivalve molluscs *A. cygnea* and *U. tumidus* from Lake Birva revealed a much higher abundance of the study bacteria in the digestive tract of *A. cygnea* than in *U. tumidus* (Figs. 4, 5). As regards the abundance of total heterotrophic bacteria, proteolytic and total coliform bacteria were predominant in the digestive tract of *A. cygnea* (Fig. 4). Nevertheless, in *U. tumidus* the abundance of hydrocarbon-degrading bacteria was higher, but did not reach the significance level. The abundance of hydrocarbon-degrading bacteria was significantly (one order of magnitude) higher in the digestive tract of molluscs from Lake Spėra as compared with molluscs from Lake Birva.

Typical features of pollution with oil and its products are long-term presence of oil and its products in the environment, long-distance distribution from the discharge, multiple migrations among water and sediments (Jašinskaitė, 1998). Thus, it could be suggested that toxins disturb the balance of a water ecosystem, affecting not only its organisms, but also their populations and even biocenoses.

Analysis of bacteria of the digestive tract of two bivalve mollusc species enables to reveal regularities and avoid casual generalizations. Comparison of the microbiological data in bivalves

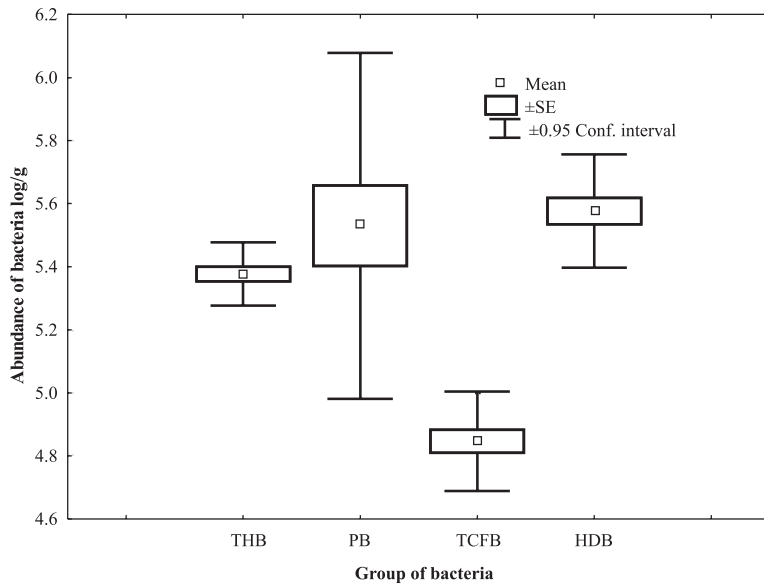


Fig. 1. Abundance of bacteria in the digestive tract of mollusc *A. cygnea* from Lake Spėra

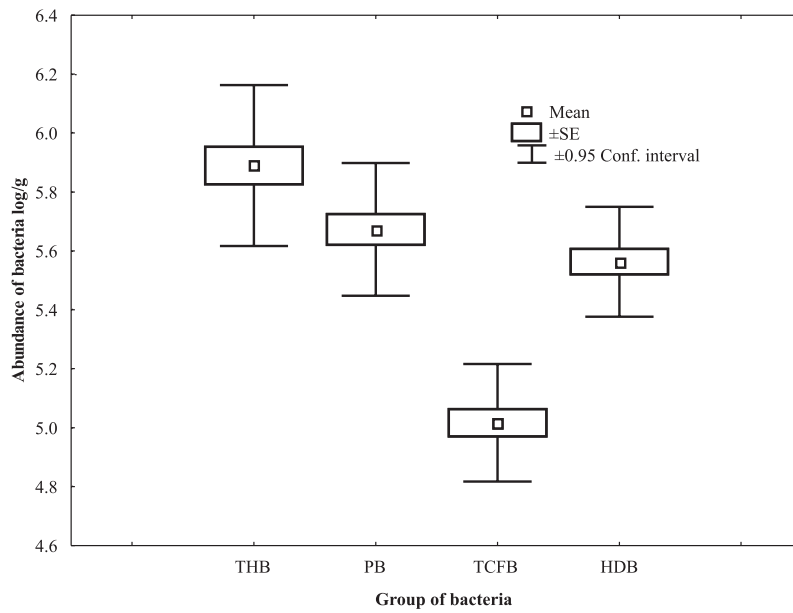


Fig. 2. Abundance of bacteria in the digestive tract of mollusc *U. tumidus* from Lake Spėra

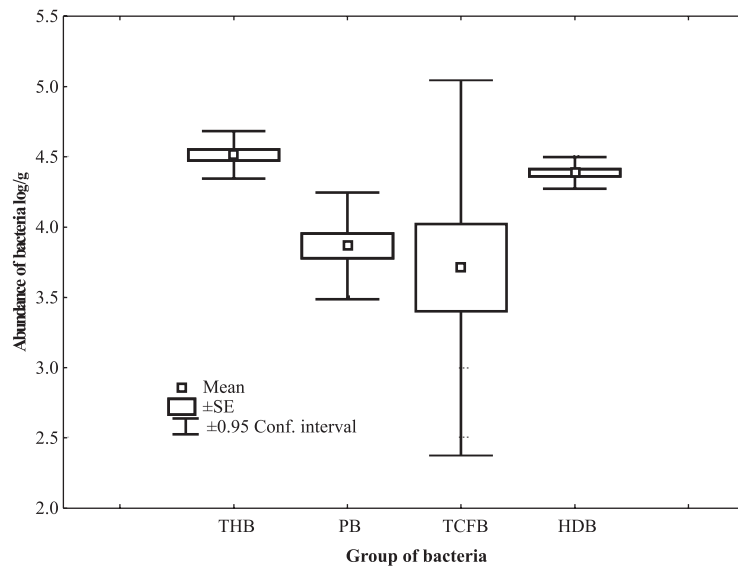


Fig. 3. Abundance of bacteria in Lake Spėra water

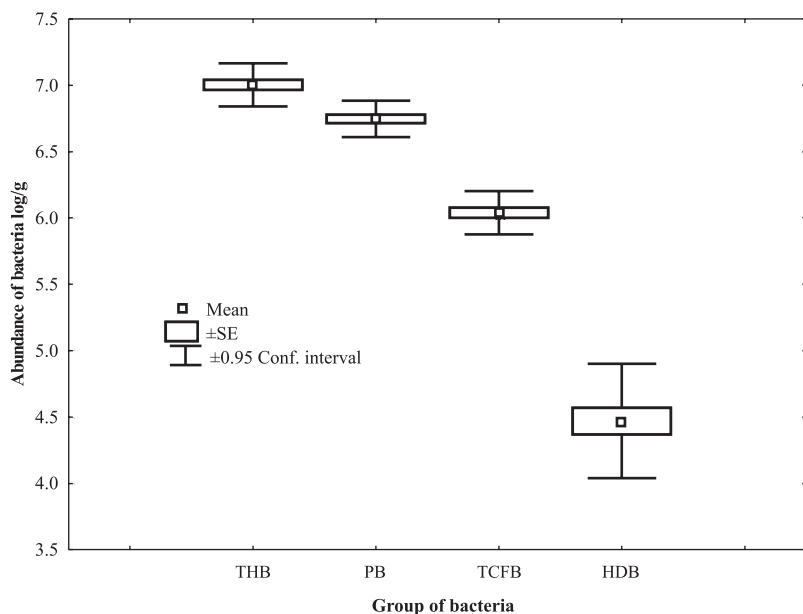


Fig. 4. Abundance of bacteria in the digestive tract of mollusc *A. cygnea* from Lake Birva

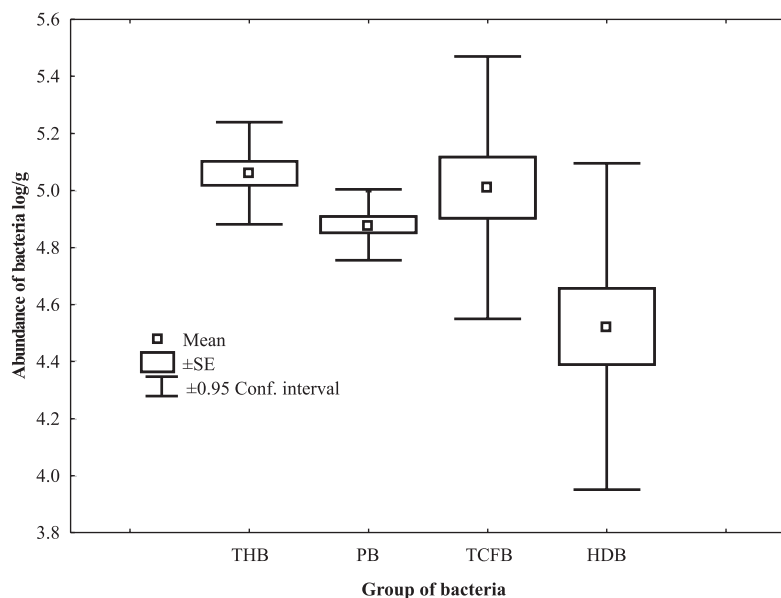


Fig. 5. Abundance of bacteria in the digestive tract of mollusc *U. tumidus* from Lake Birva

from lakes Spėra, Birva and Skaistis regarding the abundance of total coliform bacteria in the bacteriocenosis of their digestive tract indicates that the major pollution of Lake Birva originates from wastewaters, while such kind of pollution is absent in Lake Spėra (Figs. 1, 2, 4, 5, 7).

Analysis of bacteriocenosis of the digestive tract of *D. polymorpha* from Lake Skaistis shows a prevalence of normal bacteria over hydrocarbon-degrading bacteria (Fig. 7). The prevalence of total coliform bacteria could serve as a marker of organic pollution which can appear due to existence of a beach here in summer.

As regards the average values of hydrocarbon-degrading bacteria abundance in water, Lake Skaistis was least polluted with oil and its products (Fig. 8), Lake Spėra being polluted most heavily (Fig. 3) and Lake Birva less than Lake Spėra and more than Lake Skaistis (Fig. 6). Oil-hydrocarbons present in aquatic ecosystems inevitably change the quantitative structure of hydrocarbon-degrading bacteria in them (Ильинский и др., 1998).

In natural habitats polluted with oil and its products, undesirable effects (toxicity, secondary pollution, disbalance of normal bacterioflora, hypoxia, etc.), extensive development of hydrocarbon-degrading bacteria become a real problem not only because of their additional inflow into a water ecosystem. In the digestive tract of aquatic animals, hydrocarbon-degrading bacteria evoke undesirable changes in its bacterioflora.

It should be noted that without a proper assessment of the real abundance of hydrocarbon-degrading bacteria in water and in the digestive tract of animals, their ability to degrade oil and its products, to perform detoxification and transformation is hardly predictable.

It has been scientifically proven that the abundance of hydrocarbon-degrading bacteria and their relation to total heterotrophic bacteria is increasing with the growth of the concentrations of oil and other hydrocarbon pollutants (Delille, Delille 2000; Šyvokienė, Mickėnienė, 1999, 2000). Hydrocarbon-

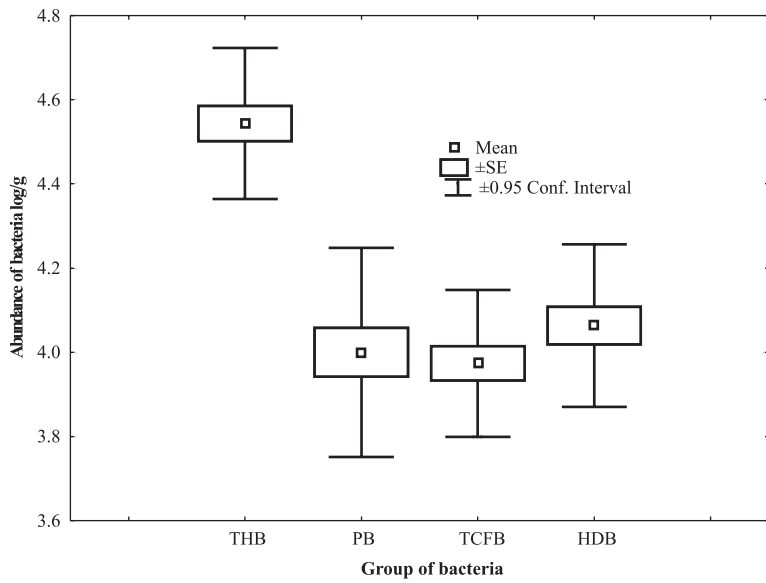


Fig. 6. Abundance of bacteria in Lake Birva water

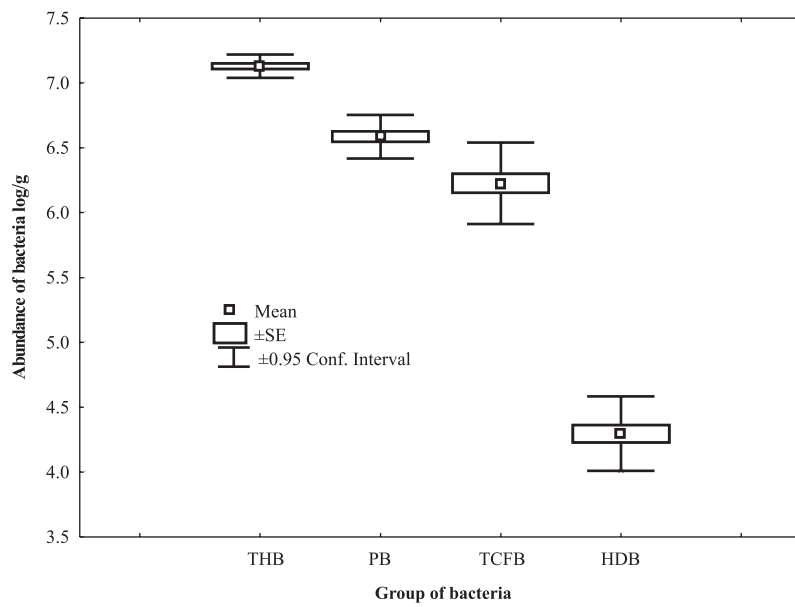


Fig. 7. Abundance of bacteria in the digestive tract of mollusc *D. polymorpha* from Lake Skaistis

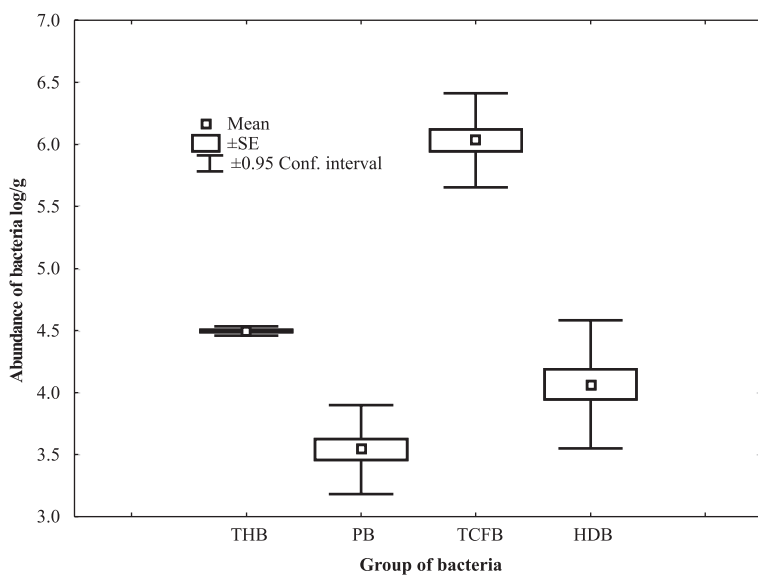


Fig. 8. Abundance of bacteria in Lake Skaistis water

Table. Abundance of hydrocarbon-degrading bacteria (%) in the digestive tract of molluscs and in water of lakes

Lake	Abundance of hydrocarbon-degrading bacteria (%)			
	<i>A. cygnea</i>	<i>U. tumidus</i>	<i>D. polymorpha</i>	Water
Spėra	16.0	24.2		33.9
Birva	0.2	16.0		29.3
Skaistis			0.09	17.3

degrading bacteria usually do not exceed 10% of the total heterotrophic bacteria in freshwater ecosystems (if the method of counting grown bacteria colonies is used). When this quantity exceeds 10%, it is suggested to consider it as an indication of permanent pollution with oil products (Vestal et al., 1984).

In the present study, in mollusc digestive tract the abundance of hydrocarbon-degrading bacteria varied from 0.09% to 24.22% of total heterotrophic bacteria (Table). In the lakes' water, hydrocarbon-degrading bacteria made 17.3% (Lake Skaistis) to 33.89% (Lake Spėra). Hydrocarbon-degrading bacteria may constitute 0.13% to 50% in soil bacteriocenosis, 0.003% to 100% in marine ecosystems (Leahy, Colwell, 1990), 0.05% to 66.67% in the Curonian Lagoon (Voverienė, 2002).

Summarizing data of the literature and of the present study, it can be stated that the abundance of hydrocarbon-degrading bacteria could be used as a biomarker reflecting the level of ecosystem pollution.

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- Janina Šyvokienė, Liongina Mickėnienė, Janina Baršienė**
- BAKTERIJOS LIETUVOS EŽERŲ MOLIUSKŲ VIRŠKINIMO SISTEMOJE**
- S a n t r a u k a*
- Ištirta dvigeldžių moliuskų *A. cygnea*, *U. tumidus* (Birvos, Spėros ežerai) ir *D. polymorpha* (Skaisčio ežeras) virškinimo sistemos bakterijų kiekybinė ir kokybinė sudėtis. Nustatyta, kad virškinimo sistemos bakterijų gausumas tirtų moliuskų virškinimo sistemos cenozėse įvairuoja ir yra skirtingas atskirų rūšių individuose bei priklauso nuo vandens telkinio. Išaiškintos visų tirtų normalios virškinimo sistemos funkcinių grupių bakterijų gausumo vertės dvigeldžių moliuskų iš Spėros, Birvos ir Skaisčio ežerų virškinimo sistemos cenozėje. Moliuskų *A. cygnea* ir *U. tumidus* iš Spėros ežero virškinimo sistemos bakterijų vidurkiai buvo panašūs. Birvos ežero *A. cygnea* virškinimo sistemoje aptikta gausiau bendrų heterotrofinių ir proteolitinių bakterijų negu *U. tumidus*. Nustatyta, kad angliavandenilius skaidančių bakterijų gausumas tirtų moliuskų virškinimo sistemoje sudarė nuo 0,09% (*D. polymorpha*, Skaisčio ežeras) iki 24,2% (*U. tumidus*, Spėros ežeras) bendrų heterotrofinių bakterijų gausumo. Tirtų ežerų vandenyje jų rasta nuo 17,3% (Skaisčio ežeras) iki 33,9% (Spėros ežeras). Gausiausiai angliavandenilius skaidančių bakterijų nustatyta *A. cygnea* ir *U. tumidus* moliuskų iš Spėros ežero virškinimo sistemoje. Mažiau reikšmingas angliavandenilius skaidančių bakterijų gausumas nustatytas tirtų *D. polymorpha* (Skaisčio ežeras) virškinimo sistemos cenozėje. Pagal angliavandenilius skaidančių ir žarnyno grupės bakterijų gausumo vertes galima teigti, kad nafta ir jos produktais Skaisčio ežero vandens ekosistema buvo užteršta mažiausiai, Spėros ežero vandens ekosistema – daugiausiai, o Birvos ežero vandens ekosistema – mažiau negu Spėros ir daugiau negu Skaisčio ežero.
- Raktažodžiai:** moliuskai, virškinimo sistema, cenozė, bakterioflora, angliavandenilius skaidančios bakterijos