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ABSTRACTS
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Downstream transformation of the phytoplankton communities along the Middle Daugava River at different flood-flow discharges

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Key words: chlorophyll *a*, Daugava River, discharge, Lagrangian sampling, phytoplankton, spring floods.

This study is based on the results obtained during annual Lagrangian drift expeditions conducted by Daugavpils University in the upper part of the Naujene-Jēkabpils stretch of the Daugava River in 2007 – 2017.

Within the Piedrujas-Naujenes stretch, the valley of the Middle Daugava River crosses the East Latvian Lowland. The depth of the valley and longitudinal gradient of its riverbed is the smallest one while the width of the main channel and that of the adjacent floodplains the largest one when compared to other stretches of the Daugava River in Latvia (Eberhards 1972). Within this stretch significant changes in main physical characteristics of the flood-flows occur: the current slows down and floodwaters spread across the adjacent floodplain area. Therefore, an active hydrological connection between the main channel of the Daugava River and various floodplain habitats (meadows, wetlands, standing water bodies etc.) is temporarily established. Some of the largest floodplain lakes in Latvia are located here, and their ecosystems clearly benefit from such natural exchange of the fresh water masses, suspended matter, nutrients and living organisms (phytoplankton, zooplankton, fish populations etc.) with the Daugava River flowing nearby (Gruberts et al. 2007).

To understand how (and where) such interaction occurs in real-time regime regarding the water mass dynamics, chemistry and plankton communities, the first Lagrangian-type field study in Latvia (the real-time Lagrangian drift experiment) was conducted on the Middle Daugava on March 28, 2007, at the peak of the spring floods (Gruberts et al. 2012). Since then, there were nine additional drift experiments conducted by Daugavpils University within the upper part of the Naujenes-Jēkabpils stretch (i.e. from Krauja to Dunava, about 60 km long distance), usually in late March, mid-April, at the peak of the spring floods. Each drift experiment was focused on different hydrological, physical, chemical and biological characteristics of the selected flood water masses that were tracked for many hours and sampled repeatedly en route. For example, almost all drift experiments included regular measurements of the average drift speed, the channel's depth and the chlorophyll *a* concentration near the water surface (Gruberts, Paidere 2014). The sub-surface phytoplankton samples were collected during five such drifts, and final results of three of them (conducted in 2007, 2011 and 2015, respectively) were selected for this study.

All three Lagrangian drift experiments mentioned above were conducted by applying a manned drifting research platform "Aventura" (constructed at Daugavpils University in 2007), made of an inflatable boat and a maritime life-raft attached to each other (Gruberts et al. 2012). The research platform was equipped with two floating anchors, a HACH DS-5 multiprobe (with the chlorophyll *a* sensor), a GPS receiver, an echo-sounding device and the phytoplankton sampling equipment. At the beginning of the drift experiment, the platform was deployed and transported to the middle of the main channel (usually at Krauja village 10 km upstream from Daugavpils), then left to drift freely along the current and used by the crew as a constantly moving sampling station.

The Lagrangian sampling strategy was followed by drifting continuously along with the Daugava's current during daytime (for about 10 to 12 h) and by conducting repeated probing and sampling of the selected water masses for their chlorophyll α concentration and phytoplankton composition. The sub-surface phytoplankton sampling was repeated each 60 min during the whole drift resulting in 11 to 13 samples per drift collected en route. The phytoplankton samples were conserved *in situ* and analysed for their composition, abundance and biomass later by an inverted microscope method at the Department of Hydrobiology, University of Latvia (Gruberts et al. 2012; 2018).

To obtain direct insight in the real-time physical processes of downstream transformation of the phytoplankton transported by the Daugava's floodwaters, biological data records (the chlorophyll *a* concentration, number of phytoplankton taxa, its abundance and biomass)

were plotted on the distance axis (i.e. the Lagrangian reference system) and compared to main hydrological characteristics of each sampling site (the average drift speed, the channel's depth and active cross-section area). The average values of main biological parameters for each drift were also calculated and compared to the peak flood discharges of the Daugava River at Daugavpils recorded on the day of particular drift experiment.

According to the results of instrumental measurements, the average chlorophyll *a* concentration in the Middle Daugava River during the spring floods strongly and negatively correlates to the peak flood water discharge at Daugavpils. The largest flood events of the last decade (in 2010, 2011 and 2013, respectively) were characterised by the lowest average concentrations of the chlorophyll *a* in the floodwaters (about 3 μg L^{-1}), and vice versa, therefore pointing to a dilution effect of the melting snowwater run-off formation on the suspended load in the river. In addition, site-by-site variation in the chlorophyll *a* concentration had a random character probably indicating the effect of turbulent mixing of the floodwaters of different origin within the main channel. However, the amplitude of such short-term variation (i.e. its standard deviation) was much lower in the case of the largest spring floods. Therefore, an effect of the flood-flow homogenization within the main channel could be also observed at larger peak flood discharges. Besides, there was an obvious negative relationship between the chlorophyll *a* concentration and the active cross-section area of the flood-flow calculated for the particular sampling site, especially in 2010 and 2013. This phenomenon could be possibly related to the effect of hydraulic washout (spreading out) of the suspended matter across a much larger cross-section area of the flood-water's flow downstream from Daugavpils, especially at the Berezovka (Dviete) River inlet.

According to the results of the sample analysis the phytoplankton communities of the Middle Daugava River during the spring floods are dominated by benthic diatoms (*Melosira varians, Navicula* sp. etc.) and washout periphytic blue-green Cyanobacteria *Oscillatoria* sp. However, the percentage of each systematic group depends on the peak discharge of the Daugava River at Daugavpils. During the very large floods (such as those observed in 2011, when the peak discharge reached 2831 m^3 s^{-1}) the diatoms formed > 86 % of the total biomass, whereas during relatively low floods (such as in 2015 when the peak discharge was only 696 m^3 s^{-1}) it was dominated by Cyanobacteria, especially the filamenous *Oscillatoria* sp. (Gruberts et al. 2018).

Downstream trends in the number of phytoplankton taxa, its abundance and total biomass also depends on the extent (magnitude) of the spring floods. Thus, during the large floods of 2011 the number of phytoplankton taxa and its total abundance gradually decreased downstream. In contrast, during the low floods of 2015 the opposite trends in the same stretch of the river were recorded. These observations are pointing to the conclusion, that during the large floods the phytoplankton of the Daugava River downstream from Daugavpils is lost as a result of the hydraulic washout to the adjacent floodplain, while at low floods it is enriched by a new taxa and organisms from the shoreline habitats, i.e. downstream recruitment of the benthic algae by the floodwater flow. Meanwhile, during the average floods of 2007, when the peak discharge was about 1690 m^3 s^{-1}, no trends were stated at all thus probably indicating that dynamically stable downstream transit of the suspended load (including the phytoplankton) was established in the main channel of the Middle Daugava at that moment.

In conclusion, the presence of several hydrological effects on the phytoplankton communities of the Daugava River could be identified in the upper part of the Naujenes-Jēkabpils stretch during the annual spring floods, namely, the dilution effect, turbulent mixing, flood-flow homogenization, hydraulic washout, downstream recruitment, and downstream transit, therefore highlighting the complex and still poorly understood functioning of the Middle Daugava river-floodplain system at different locations and under different hydrological conditions.

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Success of long-term salmonid restocking in Latvia

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Key words: Daugava River, fry, release, *Salmo salar, Salmo trutta*, smolt.

Artificial propagation of salmon and sea trout in Latvia began more than 130 years ago. Aquaculture specialist Alvins Kirsh (Alvins Kiršs) in 1885 started salmon egg incubation and fry releasing. He founded the first private salmon and sea trout hatchery in Carnikava. First artificially reared salmon larvae were released in the Gauja River, and few years later in the Daugava River. In 1892, hatchery was transferred to Vecsalaca village, located by the Salaca River and in 1893 salmon larvae were released in the Salaca River for the first time. Altogether, 35 000 artificially reared salmon larvae were released in 1894, 1 220 000 in 1894, 1 million in 1896, and 1 673 000 in 1897. In 1898, the Riga City Council bought 125 000 salmon fry for 600 gold rubles and released them in the Daugava River, and its tributaries, Ogre and Perse. To make fish larvae transportation from the Salaca River to the Daugava River easier, in the spring of 1898, a small salmon hatchery of Manor Riteru was equipped especially for the Daugava River needs. The difficulties of fish fry transfer were overcome. In 1909, A. Kirsh continued to rear salmon larvae in Vecsalaca hatchery and in its branch Berkavina, located by the Daugava River in front of Ikskile City. Altogether, from 1893 till 1916, 368 000 salmon fry were released in the Lielupe River, 2 617 000 salmon fry in the Daugava River, and 6 308 000 in the Salaca River (Eglitis 1939).

World War I interrupted salmon artificial rearing and release, nevertheless, after the War rearing was reestablished. In the 1930-ies salmonid rearing concentrated in three hatcheries: Tome, Karli (Kārļi) and Pelci (Pelči), situated in the Daugava, Gauja and Venta River basins, releasing altogether about 3 millions of fry yearly (Mitans 1975).

In the post-World War II period fish farms continued their work, and gradually increased their efficiency till 10 – 12 millions of released salmon larvae and fry per year. To increase salmon release productivity the scientific team of the Baltic Fisheries Research Institute intensively developed rearing methodology. The necessity to increase salmon rearing productivity was closely related to perspective government plans to dam the largest rivers by hydroelectric power plants (HPP). It was important to maintain natural salmon stock, especially by developing artificial rearing of Daugava salmon, because natural salmon from Daugava constituted 70 – 80% in fishermen catches at that time.

After the Daugava River damming by Riga HPP in 1974, the main salmon river of Latvia lost its natural salmon migration routes and breeding grounds. The question of artificial rearing and salmon population maintenance was solved by reconstruction of state hatcheries to maintain salmon fisheries sector and natural populations. To maintain natural salmon spawning, as well as other natural values, the biosphere reservation was gradually intended to establish in the Salaca River basin (Malikova 1966). From 1970 till 1975 state hatcheries of Latvia released 423 000 to 735 400 salmon smolts yearly (Hasina, Orlova 1976).

Since 1974 salmon population in the Daugava River is maintained artificially. Nevertheless, salmons continue to return for spawning to their native river proving the effectiveness of National Restocking Programme fulfilled through decades.

Today research institute "BIOR" is responsible for salmon and sea-trout artificial reproduction in the Daugava, Gauja and Venta rivers, releasing altogether 1.25 millions of salmon and sea trout smolts and parr yearly. Artificial reproduction of salmon and sea-trout resources is implemented in accordance with the National Artificial Fish Resources Reproduction Programme Guidelines. The main principle of the Guidelines is to maintain the stock in long-term sustainable condition, keeping salmonid homing reflex, adaptiveness to natural water, etc. The fishery of the salmon spawners for the rearing purposes takes place in the same rivers where they were born and bred. Rearing occurs in natal river water in flow-through systems. Such kind of rearing determines successful long-term maintaining of homing reflex in population, recognition of natal river water chemical content, which finalizes in successful adult fish return for spawning. For monitoring purposes all smolts and one year old parr are marked by adipose fin-clipping. According to salmon migration data gained from contract fishermen, artificially reared salmon constitutes around 99% in Daugava, 86% in Gauja and 58% in the Venta River. Restocked Sea trout constitutes 100% in Daugava, 67% in Gauja and 37% in the Venta River.

At present, in the whole Baltic Sea the amount of stocked salmon exceeds natural salmon production in rivers (ICES 2018). It has been proven that artificial salmonid propagation without natural spawning is not a long-term

perspective. There is a threat of genetic depletion of the natural populations (Samuilovienė 2012). The balance between artificial salmon restocking and maintenance of healthy natural population is of critical importance in a long-term conservation of the salmon and sea-trout stocks in Europe and particularly, in Latvia.

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Limnological characteristics of small shallow lakes situated in Garkalne County, Latvia

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Key words: brown water, forest lakes, macrophytes, macrozoobenthos, sphytoplankton.

The aim of this study was to detect limnological status and water quality of shallow brown water lakes situated in Garkalne county. Phytoplankton, macrophyte and macrozoobenthoss communities were observed in Lake Līņezers, Lake Maku ezers, Lake Piekūnītis, Lake Lielais Jūgezers and Lake Mazais Jūgezers in July 2017 and May 2018.

One of investigated lakes – Lake Līņezers – in summer period of 2018 was under anthropogenic impact due to digging works performed in coastal zone of the lake. Smallest of lakes – Lake Piekūnītis – is situated in sphagnum bog and practically is not affected by any human activities. Lakes are characterized by low water conductivity and very low Secchi water visibility characteristic for waters rich in humic substances. Observed hydrophysical indices at the sampling time are shown in Table 1.

In May 2018 oxygen concentration in observed lakes decreased in comparison to that in July 2017. It could be explained by different sampling time, as in July 2017 it was summer maxima when macrophytes and phytoplankton algae dominated. Lowest concentration of oxygen was observed in May 2018 in lake Līņezers where digging works in coastal zone were performed (Fig. 1)

Lakes are shallow, embosomed with typical bog and forest vegetation. It is possible to find macrophytes in all the aquatorium of lakes. *Nuphar lutea, Potamogeton* spp., *Stratiotes aloides, Elodea canadensis, Nymphaea alba, Hydrocharis morsus-ranae* and *Lemna trisulca* were dominating macrophyte species. Overhangs were typical for coastline where communities of marsh vegetation is formed by *Sphagnum* spp., *Calla palustris, Vaccinium uliginosum, Comarum palustre, Rubbus chamaemorus, Vaccinium vitis-idaea, Menyanthes trifoliata* and *Carex* spp. (Poppela, Poppels 2018). Coastal zone are covered by *Typha angustifolia, Equisetum fluviatile* and *Phragmites australis*.

Phytoplankton communities in investigated lakes was characterised as poor and typical for small forest and bog waters. In May 2018 highest phytoplankton biomass was observed in Lake Līņezers dominated by nuisance Raphidophyte algae *Gonyostomum semen* (1.596 mg L^{-1}) and green algae Ankistrodesmus sp. (1.49 mg L^{-1}). Euglenophytes *Euglena acus* and *Phacus* sp. formed comparatively high biomass, 0.918 mg L^{-1} (Fig. 2).

Dominating algae species in Lake Līņezers were *Gonyostomum semen, Ankistrodesmus* spp., *Euglena acus, Phacus* sp., *Phacus pleuronectes*. Phytoplankton of Lake

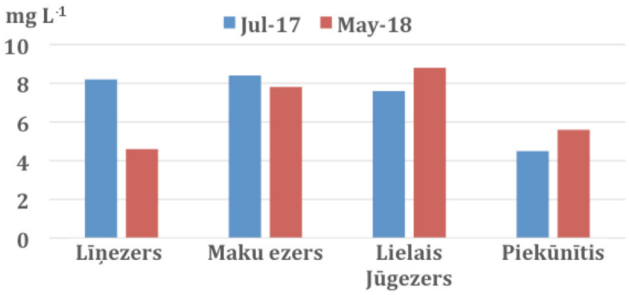

Fig. 1. Oxygen concentration (mg L^{-1}) in the water column (depth 0.5 m) in the observed lakes (Lake Līņezers, Lake Maku, Lake Lielais Jūgezers, Lake Mazais Jūgezers, Lake Piekūnītis) in July 2017 and in May 2018.

Table 1. Hydrophysical indices in small lakes situated in Garkalne County

Lake	Temperature (°C)		pH		Secchi visibility (m)		Conductivity (µs cm^{-1})	
	May 2017	July 2018	May 2017	July 2018	May 2017	July 2018	May 2017	July 2018
Līņezers	24.4	24.4	8.3	8.4	0.18	0.18	28.0	50.6
Maku ezers	22.1	26.4	5.4	5.4	0.1	0.2	34.0	50.6
Lielais Jūgezers	26.2	18.5	7.6	7.12	0.7	0.8	47.7	43.3
Mazais Jūgezers	26.4	–	7.5	–	0.25	–	47.0	–
Piekūnītis	23.2	24.1	4.3	5.26	0.25	0.27	66.4	68.0

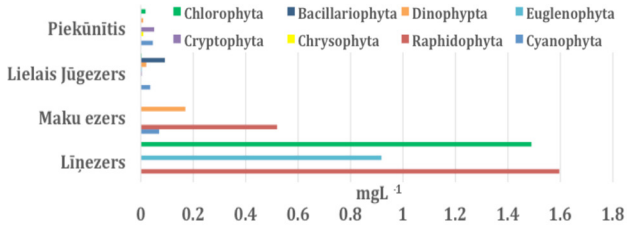

Fig. 2. Structure of phytoplankton biomass forming algal taxa (mg L^{-1}) in Lake Līņezers, Lake Maku ezers, Lake Lielais Jūgezers, Lake Piekūnītis) in May 2017.

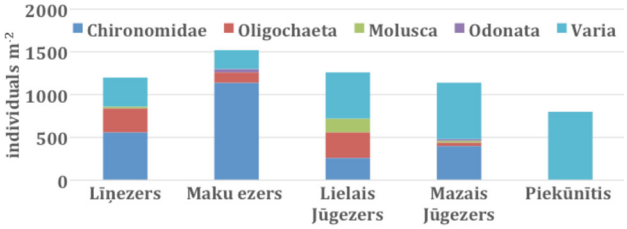

Fig. 3. Number of macrozoobenthoss animals in medium part of observed lakes (Lake Līņezers, Lake Maku, Lake Lielais Jūgezers, Lake Mazais Jūgezers, Lake Piekūnītis) in July 2017.

Maku was dominated by *Gymnodinium mirabile* and *Gonyostomum semen*. Phytoplankton of Lake Lielais Jūgezers was dominated by *Cryptomonas* sp., *Gymnodinium* sp., *Glenodinium* sp., *Aulacoseira italica, A. italica* var. *tenuissima, Gomphonema* sp., *Asterionella* sp., *Botryococcus braunii, Tetrastrum* spp., *Desmidium* sp. *Phacus* sp. Phytoplankton of Lake Piekūnītis was dominated by algae typical for bog lakes: *Dinobryon* spp. and *Cryptomonas* sp.

Potentially toxic Cyanobacteria (*Microcystis* spp., *Anabaena* spp.) were found in all investigated lakes but in very small amounts.

Highest biomass of *G. semen* in lakes of Garkalne County was observed in July 2017 in Lake Maku (15.7 mg L^{-1}) (Druvietis 2018). Former studies of *G. semen* in Latvia and in neighbor countries shows that *G. semen* now is a widespread species at Baltic region humic and mesohumic inland waters (Laugaste, Noges 2005; Karosiene et al. 2014; Druvietis et al. 2010; Klavins et al, 2010; Druvietis, 2018).

Bottom of the lakes was formed by mud, detritus and peat. Submerged snags and trees were very common, forming suitable habitats for invertebrate animals, especially for larva of *Odonata* spp,. which is favorite food for *Cybister lateralimarginalis*. Amount of Odonata larva in overhangs consisted of 1300 to 11600 individuals per m^2. Another groups of macrozoobenthoss animals (Mollusca, Chironomidae and Varia dominated by *Chaoborus* sp.) in overhangs were found in high amounts. Macrozoobenthoss communities from profundal part of observed lakes, except Lake Maku ezers due to high amount of Chironomidae, were scanty (Fig. 3).

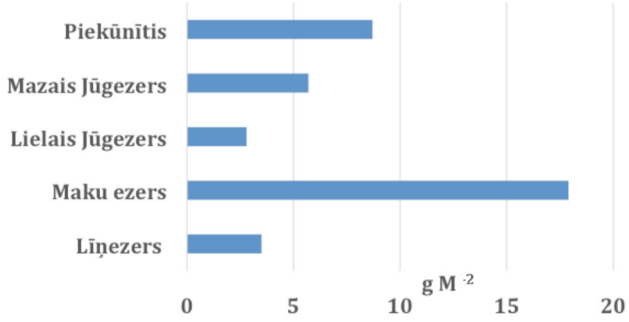

Fig. 4. Average biomass (g m^{-2}) of macrozoobenthoss animals observed in Lake Līņezers, Lake Maku, Lake Lielais Jūgezers, Lake Mazais Jūgezers, Lake Piekūnītis in July 2017.

Average biomass (g m^{-2}) of macrozoobenthoss animals shows that macrozoobenthoss of investigated lakes except Lake Maku ezers would be scanty food for fish (Fig. 4).

In general lakes are characterized by low water conductivity and very low water visibility characterized for waters rich in humic substances. Lakes are embosomed with typical bog and forest vegetation. Phytoplankton communities characterized as poor and typical for clean forest and bog waters where nuisance Raphidophyte *G. semen* is common. Potentially toxic Cyanobacteria is detected in all lakes but in very small amounts – few colonies or filaments in the sample. Macrozoobenthoss communities are scanty, typical for bog lakes. Investigated lakes Līņezers, Lake Maku ezers, Lake Lielais Jūgezers, Lake Mazais Jūgezers are characterized as dyseutrophic. Lake Piekūnītis could be characterized as typically dystrophic lake.

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Biomass objective function and its role in metabolism modelling

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Key words: biomass objective function, metabolic modeling, stoichiometric modelling.

Stoichiometric metabolic modelling is used to reveal topological properties, biophysical and biotechnological capabilities of microorganism. These network models contain metabolic reactions of organisms and genes that encode them. Using stoichiometric modelling approaches like FBA it is possible to calculate reaction rates in steady state or even substrate product maximum theoretical yields. However, to take into account biologically relevant organism metabolic processes, there is need to integrate biomass objective function into stoichiometric model.

To describe mathematically biomass objective function, it is crucial to know the composition of the cell and energy requirements which are necessary to develop biomass content from metabolic precursors. Biomass objective function can be described at several detailed levels: (1) basic level describes cell macromolecular content (like protein, RNA, DNA, lipids, cell wall and other small molecules) and its building metabolites which make up each macromolecular group (amino acids, nucleotides, fatty acids, phospholipids, fatty acids and others if necessary). That way biomass objective function describes energetic conversion to drive bioprocesses, and polymerization process. (2) Advanced level of biomass objective function should include additional description of vitamins, elements and cofactors, which are required for cell growth process. (3) Additional biomass objective function includes detailed information about advanced biomass composition (example for wild type) but has alternative biomass objective function with core macromolecular content taking into account mostly central metabolism metabolic precursor amounts, which allows to use it for different kind of mutation, deletion and other cell manipulations, where detailed wild type biomass is specified for exact species analysis (Feist, Palsson 2010).

This information was summarized and was applied for gram negative bacteria *Zymomonas mobilis* previously published metabolic model (Pentjuss et al. 2013). *Z. mobilis* core biomass objective function was created using previously published ^{13}C experimental data (De Graaf et al. 1999). Core biomass objective function macromolecular metabolite precursors were recalculated in units of measurements:

$$\frac{\mu mol}{gdw * h}$$

This allowed to revalidate previously published modelling results and get rid of biologically irrelevant steady states, where glycerol transporter was introduced into metabolic model and core biomass objective function showed that in this condition *Z. mobilis* can not maintain steady state on pure glucose or fructose medium.

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Trichoderma asperellum, Bacillus subtilis, Botrytis cinerea and their consortium effects on soil microbiota

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Key words: microbial consortia, microorganisms, soil.

Soil, the naturally occurring unconsolidated mineral and organic material at the earth's surface, provides an essential natural resource for living organisms (Paul 2015). Interest in the unique nature of the microbiota of specific soils has been growing recently, keeping in mind the properties of the microorganisms that constitute them, and the importance of these microorganisms for the healthy growth of certain crop plants. A positive effect can be achieved by promoting the development of beneficial microorganisms (such as *Trichoderma asperellum* and *Bacillus subtilis*) that are used as growth stimulators in the soil, using both natural microbial associations and laboratory-constructed consortia (Bioefekts 2012).

The goal of the study was to determine if *T. asperellum*, *B. subtilis* and their consortium affect the microbiota of the soil, with a hypothesis that treating the soil with microbial consortia increases the CFU of microorganisms after a certain time and reinforces the utilisation of certain substrates and inhibits the development of Botrytis cinerea pathogen.

During the study, six experimental soil samples were prepared and treated with *T. asperellum, B. subtilis*, and their consortium. The CFU value was determined for bacteria and fungi immediately and after a certain number of days (0, 30, and 60 days) making inoculations in R2A (for bacteria) and malt extract (for fungi) media, identifying the dominant groups of microorganisms. It was tested, if there were any antagonistic effects of the microorganisms against the *Botrytis cinerea* pathogen. Using the Biolog EcoPlates method, the ability of the microorganisms to metabolise carbon compounds was determined (Chazarenc et al. 2010).

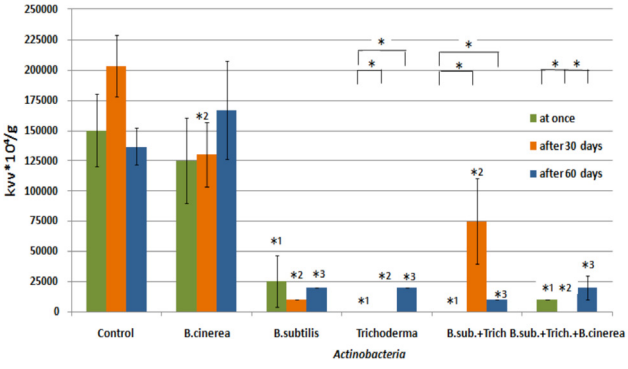

Fig. 1. Number of actinobacteria CFU in soil samples treated with different microorganisms and their consortia over time. * significant ($p < 0.05$) difference between samples; * 123 significant difference from control.

The overall conclusions based on the results are that the CFU value for fungi significantly dropped over the 60-day period in all the treated soil samples, except for the control. The total CFU value for bacteria did not significantly grow in any of the samples compared to the control, while the total bacterial CFU value for samples with *B. cinerea, T. asperellum,* and three-species microbial consortium reduced over the 60-day period. The CFU value of *B. subtilis* significantly increased in the soil samples that were treated with *B. subtilis* or its consortium with the fungi. The CFU value of actinobacteria fell significantly in all the samples, except for those that contained *B. cinerea* (Fig. 1). Based on the Biolog EcoPlates method, it can be stated that, within 60 days, the utilisation of carbohydrates and complex carbon compounds in the soil increases, while the utilisation of organic phosphorous compounds drops.

Acknowledgements

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Microbial population dynamics of freshwater sludge during fermentation of crude glycerol

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Key words: biogas, biohydrogen, crude glycerol, freshwater sludge.

As the global production of biodiesel increases, so does the production of its main by-product, crude glycerol, which is often considered as a waste product with a disposal cost attributed to it (Yazdani, Gonzalez 2007). Therefore, to increase the economic viability of biodiesel, effective utilization of crude glycerol is necessary. One potential application of crude glycerol is as a co-substrate to enhance hydrogen and methane production during the anaerobic treatment of different waste materials (Yang et al. 2012). Traditionally, microbial fermentation is studied using pure cultures. However, since cheap biomass sources contain many complex compounds and impurities, it is very difficult for a single microorganism to utilize them effectively so a promising alternative could be the use of mixed microbial consortia (Jiang et al. 2017). The aim of this study was to determine the changes in the composition of freshwater sludge microbial communities during fermentation of crude glycerol.

Freshwater sludge sample was obtained from a quarry in Dalbe (Ozolnieki municipality, Latvia). It was used for inoculation of 70 mL anaerobic liquid media consisting of tryptone (1.0 g L^{-1}), yeast extract (2.5 g L^{-1}) and cysteine hydrochloride monohydrate (0.5 g L^{-1}). Selected samples were supplemented with 6 g L^{-1} crude glycerol (Bio-Venta, Latvia). Incubation was carried out at 20 and 37 °C temperature for 4 and 8 days. Volume of gas produced during fermentation was measured using a syringe. Total DNA of samples was extracted using PowerSoil DNA Isolation Kit. DNA concentrations of specific microbial groups were determined by performing qPCR using taxon specific 16S rDNA primer pairs: Gammaproteobacteria (Gam877F, Gam1066R), Firmicutes (Lgc353, Eub518R), Actinobacteria (Actino235, Eub518R) and Archaea (Arch 967F, Arch 1060R). Quantifying of culturable microorganisms was done using serial dilution method on aerobically and anaerobically incubated R2A agar plates. Based on colony and cell morphology, predominant cultures were isolated and identified biochemically with BD BBL Crystal identification system.

The results showed higher volume of evolved gases in samples incubated at 37 °C, compared to samples incubated at 20 °C. The highest amount of colony forming units (CFUs) of anaerobically growing microorganisms was calculated in a crude glycerol containing sample incubated for 4 days at 20 °C, while the lowest amount of CFUs was in an identical sample incubated for 8 days (Fig. 1). Higher incubation time reduced the amount of CFUs in samples containing crude glycerol, whereas it increased the amount of CFUs in samples, which did not contain crude glycerol.

The majority of isolated anaerobically growing microorganisms belonged to the phylum Firmicutes (mainly the genus *Clostridium*, such as *Clostridium butyricum*, which has high hydrogen production capabilities). Isolated aerobically growing microorganisms mostly consisted of Firmicutes (*Brevibacillus brevis, Bacillus licheniformis* etc.) and in smaller quantities Actinobacteria and Gammaproteobacteria.

Of the four microbial groups tested using qPCR Firmicutes showed the highest DNA concentrations with maximum concentration (107.08 ± 11.05 ng mL^{-1}) in

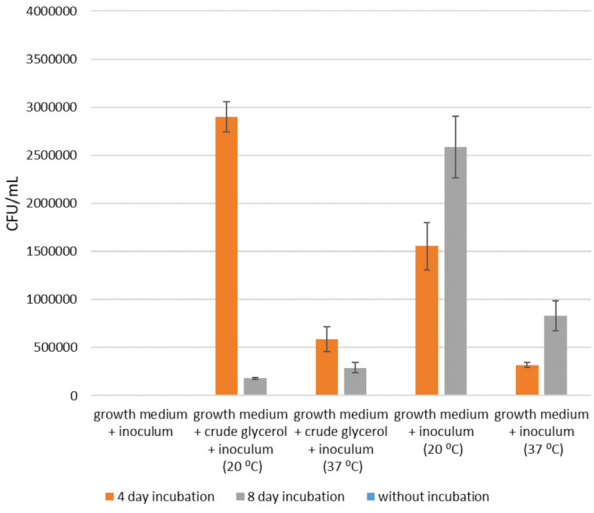

Fig. 1. Colony forming units of anaerobically growing microorganisms (CFU mL^{-1}).

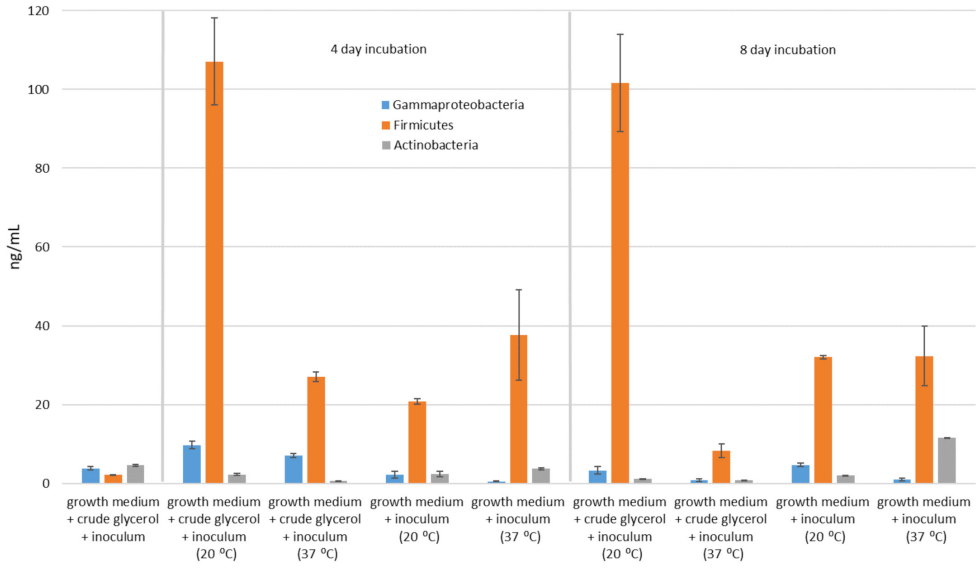

Fig. 2. Gammaproteobacteria, Firmicutes and Actinobacteria DNA concentrations (ng mL^{-1}).

sample containing crude glycerol incubated for 4 days at 20 °C (Fig. 2). Archaea showed very low DNA concentrations (under 1 ng mL^{-1}) and they were lower in samples, which were incubated, compared to samples with added freshwater sludge inoculum without incubation. Incubation also reduced the DNA concentration of Actinobacteria with an exception of the sample, which did not contain crude glycerol and was incubated for 8 days at 37 °C.

Of the four studied microbial groups found in freshwater sludge the highest capacity to grow in crude glycerol containing medium was determined for Firmicutes. From this phylum *Clostridium* bacteria with high hydrogen production capabilities were isolated. As the number of CFUs in crude glycerol containing medium in both aerobic and anaerobic conditions decreased, when incubation time extended from 4 to 8 days, it can be concluded that at 8 days of incubation bacterial death phase had already begun. Although the optimal growth temperature of *Clostridium* is closer to 37 °C, in this study a higher bacterial growth for Firmicutes was observed at 20 °C, which is closer to the natural temperature of freshwater sludge.

Acknowledgements

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Effect of foliar fertilization of microelements on yield and quality of highbush blueberries (*Vaccinium corumbosum*) in cutover peatlands

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Key words: blueberry, field experiment, leaf nutrient status.

Commercial cultivation of highbush blueberry in Latvia was successfully started during the last 20 years. In 2018, 280 ha of highbush blueberries was estimated in Latvia with increasing annual hectarage. In general, blueberry has low nutrient needs and is sensitive to excessive nutrient levels in the soil. However, balanced and precise mineral nutrition is essential for producing high and quality yield. Considering that blueberry plants are shallow-rooted, fruit production can be significantly reduced even with a moderate nutrient deficiency. Excessive or inadequate fertilization is potentially damaging to blueberry cultivation especially in plantations established in environmentally sensitive areas such as excavated peat bogs.

Results from the previous studies in 2006 – 2017 highlighted problems in nutrient supply for highbush blueberry that could be a significant restrictive factor for obtaining high, qualitative and sustainable berry yields in Latvia (Osvalde et al. 2018). Considering that average yield in Latvia (1.49 t ha^{-1}) is significantly lower in comparisonn to that in the United States of America (6.91 t ha^{-1}), Canada (3.28 t ha^{-1}) and Poland (3.07 t ha^{-1}) (FAO 2019), research on mineral nutrition as one of the potential limiting factors of reduced yield of blueberry in Latvia are critically important. Availability of nutrients in soil may be limited in conditions of improper soil pH, ionic antagonism, or unfavorable weather conditions. Under such circumstances, foliar sprays are a simple, fast and effective method for supplying nutrients to plants (Wach, Błażewicz-Woźniak 2012).

The study was carried out to determine the effect of foliar fertilization with micronutrients on the productivity, content of mineral elements in leaves and photosynthesis of blueberries. Field experiment was performed in 2018 on a production farm (56°70'N, 23°60'E) established on a excavated peat bog abandoned after industrial peat production (region of Jelgava). Experiment included foliar fertilizer treatments with microelements (Fe, Zn, Cu, Mo, B) 0 to 3 times per season and followed a randomized sub-block design. Together 128 (10 years old) blueberry plants (32 for each treatment) of 'Patriot' cultivar was used in the spacing of 1.2 × 2.0 m (density 3888 plants ha^{-1}).

Overall, the results from the study demonstrated a significant influence of the applied foliar fertilizer on the content of Fe, Zn and B in the highbush blueberry leaves. Only small impact or trend with no significant differences among treatments was established in the case of Cu and Mo. In general, severe Fe, Zn and B deficiency in blueberry leaves, collected prior to the use of foliar micronutrients, were detected at all experimental plots. After the first spray, the Fe and Zn and after the second spray B concentration in the leaves exceeded the minimum sufficiency level and reached the optimum range. Highest Fe, Zn and B concentration in leaves were recorded when plants were treated 3 times per season with foliar fertilizer.

Chlorophyll *a* fluorescence parameter Performance Index (PI) and partly F_V/F_M value reflected the direct effect of increased microelement supply on blueberries. The lowest PI values were established in spring when Fe deficiency and suboptimal concentrations of Zn and B was evident for all treatments. The highest PI and F_V/F_M, above recommended 0.8, were detected after the third spraying of fertilizers.

Application of the foliar fertilizer three times per season provided the highest berry yield (134% compared to control), largest berries and the highest mass of one berry (23.0 mm and 3.36 g accordingly). Correct foliar fertilization can optimize the content of Fe, Zn and B in blueberry leaves. Our study reveals that foliar fertilization can become a very practical and effective method for increasing blueberry yields and berry quality.

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Investigation of bilberry (*Vaccinium myrtillus*) genetic diversity and population structure

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Key words: genetic diversity, Vaccinium myrtillus.

Systematic research and conservation activities for crop wild relatives (CWR) and wild harvested plants (WHP) have been initiated in many European countries. Population and genetic diversity studies are important sources of information for the development and implementation of conservation strategies. The main CWR and WHP plant groups in Latvia are forage grasses, aromatic and medicinal plants, and forest fruits and berries.

Vaccinium myrtillus L. belongs to the genus *Vaccinium*. It is a dwarf shrub (5 to 90 cm) typical in the northern hemisphere (Nestby et al. 2010). This species can propagate both vegetatively and generatively, and can form colonies with a diameter of up to 15 m. An investigation of the population structure and genetic diversity of *V. myrtillus* has not been previously undertaken in Latvia.

The majority of molecular studies, including the use of EST-SSR markers, have been done on species of the section Cyanococcus (Rowland et al. 2003; Boches et al. 2005). The species endemic to Latvia belong to other sections (Nestby et al. 2010), therefore the available DNA markers need to be tested and adapted for use in *V. myrtillus*. From 18 tested SSR markers (Boches et al. 2005), analyses were performed with eight markers on bilberry samples collected within Latvia (19 locations), Estonia (seven locations) and Lithuania (nine locations). Genotyping was done with Applied Biosystems ABI Prism 3100xl Genetic Analyzer. SSR genotype data were analysed with GenAlEx 6.501 (Peakall, Smouse 2012) and STRUCTURE 2.3.4 (Pritchard et al. 2000).

Initial results indicate that 5% of the genetic diversity was found among populations, while most of the genetic diversity was found within individuals (64%), and between individuals (30%). The number of alleles unique to one population was low, and these unique alleles were found in only three populations in Latvia, three populations in Estonia and three populations in Lithuania. The expected heterozygosity (He) varied from 0.44 to 0.58. Populations are not structured e.g. there are no isolated, genetically differentiated populations. The correlation between genetic and geographic distances between populations is positive (Fig. 1), indicating genetic differentiation of Latvian bilberry population due to isolation by distance, but no other potential gene flow barriers were detected.

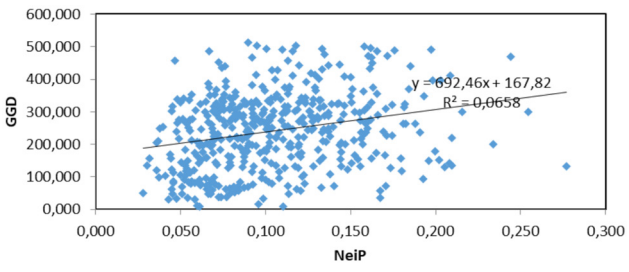

Fig. 1. Correlation between geographic (GGD) and genetic (NeiP) distances.

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Traditional beekeeping and its sustainable development in eastern Latvia

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Key words: consumption of apiculture products, honey bee, pollination.

Nowadays, the problem of declining pollinators, including honey bees, has become topical in the world (Neuman, Carreck 2010). In many countries, development of agricultural branches dependent from success of pollination is threatened by increasing bee mortality (Gallai et al, 2009; Pettis, Delaplane 2010). So far, there is no data whether there is an increase in honey bee mortality in Latvia. Reduction in the number of honey bees, can be caused either by adverse environmental factors, such as environmental pollution, diseases and parasites, or by the decline in demand for apiculture products in the market. Therefore, we launched a study on the state of beekeeping in Latvia. This article summarizes the first results of research in Latgale (Eastern Latvia).

Latvia has not traditionally developed beekeeping regions. The sector is equally well developed throughout the country (Ministry of Agriculture 2018, Europa.eu). Bee colonies in the country exceeds 80 thousand (Table 1). Latgale region of Latvia also have professional apiaries with modern, well-maintained apiary premises and a relatively large volume of production. In 2015, there were 23% of all Latvian bee farms in Latgale. There is an average 150 bee apiaries within one parish producing 62 t (13%) of honey. There is an average of 25 bee haves in one apiary producing 700 kg of honey per season. However, at present the amount of honey produced in the region is decreasing.

In 2015 – 2017 an assessment of the situation of Latgale beekeeping was performed. by questioning beekeepers of the region and also the Latvian Beekeepers' Association leaders. As the local beekeeping market plays an important role in the successful development of beekeeping, a population survey on the use of apiculture products was carried out in parallel. Answers to the questionnaire were collected on the purchase and consumption of apiculture products in the city of Riga as a main consumer of apiary products. The main findings of the study: the majority of repositories use apiculture products, especially honey and wax.

Interviews with 23 beekeepers of Latgale showed that most of them do beekeeping as a hobby. Most beekeepers plan to expand production, so development can be viewed as positive. Mass death of bee mortality was not detected. Beekeeping and the problem of pollination of plants in Latgale are currently not under threat.

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Table 1. Summary of the structure of the Latvian beekeeping industry (prepared by the author according to the Latvian Beekeepers' Association data)

Parameter	2012	2015
Professional beekeeper bee colonies (with 150 flocks and more)	15 348	22 498
Total number of bee colonies	83 801	95335
Professional Beekeepers	70	90
Total number of beekeepers	3346	3393
Direct sales to consumers (%)	71	58
Direct sales to retail businesses (%)	9	12
Sales to market / wholesalers (%)	16	15
Manufacturers (%)	4	6
Exported honey to other countries (%)	(was not asked)	9

Algae as indicators of the ecological quality of Latvian rivers

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Key words: benthic diatoms, ecological quality, macrophytes, macroscopic algae.

Development of phytobenthos depends on the availability of inorganic nitrogen and phosphorus compounds. Increase in concentrations of these nutrients can lead to increased biomass of phytobenthos as well as to shift in species composition. "Macrophytes and phytobenthos" is among the parameters required by the Water Framework Directive (Directive 2000/60/EC) to assess the ecological quality in rivers. Currently, there is developed and intercalibrated the assessment method based on macrophytes in Latvia. This method includes only few taxa of macroscopic algae which is not sufficient to represent "phytobenthos" part. Due to lack of experienced specialists, there is still not enough data to develop and intercalibrate the assessment method according to microscopic benthic algae. Most of the EU member states have developed the assessment method based on diatoms.

In summer 2017, survey of macroscopic algae in 40 small and medium size rivers were carried as described in Kelly and Krokowski (2015). Benthic diatoms were also sampled in the same rivers. Hydromorphological modifications were assessed by the Lithuanian Hydromorphological River Index (HMI). Data on water quality parameters were obtained from the Latvian Environment, Geology and Meteorology Center.

In total, 21 taxa of macroscopic algae were found during the survey of 40 rivers. The most abundant genus was *Cladophora* (found in 34 rivers), *Melosira* (27 rivers), *Oscillatoria* (22 rivers) and *Rhizoclonium* (20 rivers). The highest number of genus was found in the River Svētupe (10), nine genera of macroscopic algae was found in the Rivers Bērze, Svitene and Ķekaviņa. In heavily modified rivers Krišupe only *Cladophora* was found and in Bubieris only *Melosira* was found. Massive density of macroscopic algae was found in the rivers Taļķe, Tērvete and Bērze. These rivers are in the nitrate vulnerable zone, where high concentrations of nitrates are observed.

In total, 188 diatom taxa from 53 genera were found in this study. The most abundant genera were *Nitzschia* by 25 diatom species, *Navicula* by 24 species, and *Gomphonema* by eight species. The highest number of taxa (65) was found in the river Tirza, and the lowest number (24) in the river Aģe. Calculated IPS index was 11.9 to 16.7 and WAT index 11.7 to 18.2. According to these indices, provisional ecological quality of rivers is good and high. Values of TDI index was in range 58.6 to 86.7 that mostly corresponds to average and bad ecological quality.

This study shows that diatom communities are not sensitive to hydromorphological modifications. Correlation between yearly average phosphorus concentrations and diatom indices as well as the cover of macroscopic algae is weak. Macroscopic algae and diatoms could be potentially used as indicators of increasing concentrations of nitrates. However, to prove this relationship, more data from the rivers with high concentrations of nitrogen are necessary. Springe et al (2006) have found correlation between diatom indices and ammonium concentrations in rivers. The results of this study did not confirm such relationship. It should also be mentioned that other factors such as alkalinity, concentrations of silica and organic matter can have an impact on communities of diatoms and macroscopic algae.

Acknowledgements
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Efficiency of stocking of river lamprey ammocoetes – first results

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Key words: ammocoetes, European river lamprey, *Lampetra fluviatilis*, stocking.

River lamprey *Lampetra fluviatilis* L. is a protected species listed in Council Directive 92/43/EEK on the conservation of natural habitats and of wild flora and fauna as wells as other legislative acts of nature protection. At the same time this is one of the most important target species in Latvian inland water fishery. In recent years share of river lamprey often reaches one third of the total landings in Latvian inland waters. Stocking of lamprey ammocoetes in Latvia begun in the last decades of the 20th century. Recently on average more than 10 million ammocoetes are stocked annually and intensity of stocking has an increasing trend. Despite the long stocking history and remarkable number of ammocoetes stocked its effectiveness has never been tested. The aim of this research was to survey the changes of abundance of ammocoetes after stocking and to start a discussion on the efficiency of lamprey stocking and possibilities of its increase.

Research area for this survey covers ~5 km long lower reaches of two adjacent right coast tributaries of Gauja River. Before-after-control-impact approach was used. Strīķupe River (impact) is ~12 km long with ~90 km^2 catchment area and has a small hydro power plant (HPP) ~10 km from its rivermouth. Lenčupe River (control) is ~20 km long with 120 km^2 catchment area and has an HPP ~11 km from its rivermouth. Both rivers host a natural river lamprey population. Survey lasted for two years. In September 2017 sampling of ammocoetes was performed in both rivers, in May 2018 ammocoetes were stocked in Strīķupe River and in September 2018 sampling in both rivers was repeated. In total ~250 000 ammocoetes were stocked. Stocking was performed by wading down the riverbed and releasing small portions of ammocoetes every 5 to 15 meters. Ammocoetes used for stocking were one week old, and their hatching was performed in hatchery "Brasla" by using spawners from Gauja River. Sampling of ammocoetes was performed by taking ground samples with a special box-shaped shovel (length 25 cm, width 20 cm and height 8 cm) and collecting ammocoetes found in the sample. Area sampled and abundance of ammocoetes (individuals per m2) for each sampling spot as well as mean abundance for all sampling spots in one river were calculated. Only subyearling ammocoetes (0+ age group) were included in the analysis. On the basis of the length data, the upper border for this age group was set to 20 mm in Strīķupe River and to 22 mm in Lenčupe River.

Changes of the abundance of ammocoetes of 0+ age group were different in each river. In Strīķupe River mean abundance of ammocoetes increased from 1.8 in 2017 to 2.1 individuals per m^2 in 2018. At the same time in Lenčupe River mean abundance of ammocoetes decreased from 14.7 to 6.6 individuals per m^2. However, in both rivers this change was not statistically significant (Mann–Whitney U test, p ~0.75 in Strīķupe and p ~0.25 in Lenčupe). It should be noted that in 2017 the difference between both rivers

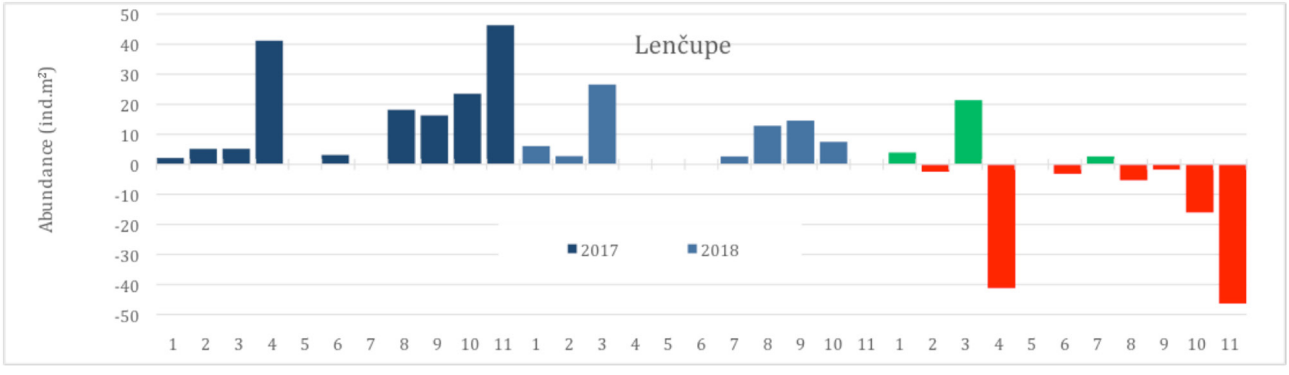

Fig. 1. Abundance of ammocoetes in each year and difference between years in different sampling sites in Lenčupe River.

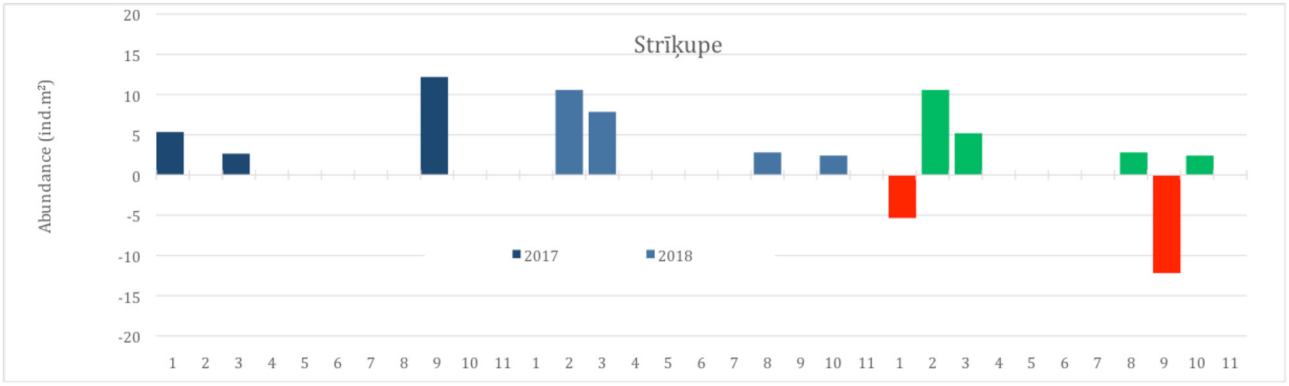

Fig. 2. Abundance of ammocoetes in each year and difference between years in different sampling sites in Strīķupe River.

was statistically significant (p ~0.01) but after stocking in 2018, statistically significant difference was not observed. Possible positive effect of stocking is also pointed out by other indirect indicators. In Strīķupe River the number of sampling sites where 0+ ammocoetes were caught increased from three in 2017 to four in 2018 while in Lenčupe River number of such sites decreased from nine to seven (Fig. 1 and 2). Changes of abundance of ammocoetes in the sites where they were caught in subsequent years were also different. In Strīķupe River ammocoetes of 0+ age group in 2017 and 2018 were repeatedly caught in only one sampling site and abundance of ammocoetes in this site increased from 2.6 to 7.8 individuals per m^2. In Lenčupe River ammocoetes of 0+ age group in subsequent years were repeatedly caught in six sampling sites and increase of abundance was observed only in two of them.

Results of this research may be influenced by several factors. First, it is possible that survival rate of stocked ammocoetes was reduced by unusually dry summer of 2018. It is also possible that the impact of stocking was not detected because of the small sample size or it was masked by the great difference of abundance of subyearling ammocoetes often found in one sampling site (unpublished data) which greatly depends on the bottom substrate (Aronsuu, Virkkala 2013). In order to get more reliable information on efficiency of performed stocking measures, stocking of ammocoetes and monitoring of their density should be continued for several years. However, it is also possible that efficiency of stocking of ammocoetes is lower than expected and measures for its increase might be needed. One of such measures is stocking of older ammocoetes with a greater survival potential (Ryapolova, Mitans 1991; Kujawa et al. 2018). It is also possible that in the future it will be necessary to do a more careful selection of stocking sites and gather additional information on area of habitats suitable for subyearling and older lamprey larvae as well as analyse water quality and other factors before stocking.

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I would like to thank all my recent and past colleagues Jānis Bajinskis, Jānis Aizups, Atis Minde, Sarmīte Inberga and Toms Zalāns for help in the field works, colleagues Santa Purviņa and Rūdolfs Tutiņš for stocking the ammocoetes and staff of hatchery "Brasla" and Jānis Balodis personally for providing ammocoetes for stocking.

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Detection of *Salmonella* spp. presence in food and samples from primary steps of food production chain using microbiological and molecular biology methods

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Key words: food, food production chain, real time PCR, *Salmonella* spp., standard method ISO 6579-1: 2017.

There are 100 000 registered cases of salmonellosis in European Union (EU) every year, 19.7 cases per 100 000 of inhabitants. The most common serotypes are *Salmonella enteritidis, Salmonella typhimurium*, monophasic *S. typhimurium, Salmonella infantis* and *Salmonella newport* (EFSA, ECDC 2018). Since 2003 EU is implementing National control programs for the reduction of *Salmonella* presence in primary production of poultry (laying hens, broilers, turkeys) and pigs (EFSA 2018). Registered human cases in Latvia have decreased from 1088 cases in 2011 to 400 in 2018 (CDPC 2018).

Presence of *Salmonella* spp. in food can be detected by classical microbiology methods that are time consuming. Faster commercial real time PCR based test systems have been evaluated in previous studies (Margot et al. 2013).

The aim of the work was to compare presence of *Salmonella* spp. in foodborne products and primary production stage products with commercial real time PCR kit and standard method in order to assess whether the molecular biology method is equally sensitive as the standard method. This investigation was carried out during the period from September 2017 to May 2018. Real time PCR based molecular biology method (mericon *Salmonella* spp. kit, Qiagen) and the ISO standard method ISO 6579-1: 2017 "Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp." were used. Mericon *Salmonella* spp. kit has been validated by the producer for the application for food and feed samples after pre-enrichment. The detection limit is 10 *Salmonella* DNA copies per PCR reaction. The producer has carried out the specificity tests and it has been proved that no amplification was obtained with 2500 copies of any of the following bacteria: *Staphylococcus aureus, Clostridium perfringens, Listeria monocytogenes, Yersinia enterocolitica, Shigella flexneri, Escherichia coli, Cronobacter sakazakii, Bacillus*

Table 1. Comparison of both methods: molecular biology method (mericon *Salmonella* spp. kit, Qiagen) and the ISO standard method ISO 6579-1: 2017 "Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp."

Sample type	Sample number	Results obtained with LVS ISO 6579-1:2017	DNA extraction method	Results obtained with mericon *Salmonella* spp. kit
Food	109	Positive 20	InstaGene Matrix	Positive 0
		Negative 88		Negative 55
		Suspicious 1	*mericon* DNA	Positive 21
			Bacteria Kit	Negative 33
Washings of food surfaces and contact surfaces	18	Positive 0	*mericon* DNA	Positive 0
		Negative 18	Bacteria Kit	Negative 18
Faeces	54	Positive 4	*mericon* DNA	Positive 2
		Negative 50	Bacteria Kit	Negative 52
Environmental samples from poultry farms	15	Positive 3	*mericon* DNA	Positive 3
		Negative 12	Bacteria Kit	Negative 12
Poultry feed	3	Positive 0	*mericon* DNA	Positive 0
		Negative 3	Bacteria Kit	Negative 3

cereus, Legionella erythra, Campylobacter jejuni, Legionella pneumophila, VTEC stx1/stx2.

In total 199 samples were tested, from those 127 were food samples and 72 were samples from primary steps of food production chain. Food samples were mainly minced meat (beef, pork, mixed), frozen and fresh meat, smoked meat products, washings of food surfaces and contact surfaces, fresh, frozen and smoked fish, milk products (ice cream, mayonnaise, yogurt, kefir), eggs and egg shells, spices and food additives. Samples from the primary food production were faeces of laying hens, broilers, ducks, goose and turkey, dust samples from chicken farms, waste from broiler incubators, and poultry feed.

All samples (25 g in five replicates) were subjected to pre-enrichment in buffered peptone water. After 18 h of incubation at 34 to 38 °C, further microbiological tests were performed according to ISO 6579-1: 2017, and the pre-enrichment suspension was used also for the DNA extraction. At the beginning of the investigation DNA was extracted from 55 food samples using InstaGene Matrix (Bio-RAD), but due to PCR inhibition problems it was changed to mericon DNA Bacteria Kit (Qiagen).

Obtained results of the method comparison are summarized in Table 1. *Salmonella* presence was detected in both food samples and samples from primary step of food production. Results were consistent in 96.3% of positive samples and 99.42% of negative and suspicious samples. Mericon *Salmonella* spp. kit has not been validated by producer for the samples from primary step of food production and during the investigation it was observed that in two cases of positive poultry faeces it was not possible to obtain positive result with real time PCR kit.

The main conclusion is that since it was not possible to obtain 100% consistent results with both methods the real time PCR method can be used as additional test or fast screening test for large number of samples in problem situations at food production chain.

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Presence of shiga toxin producing *Escherichia coli* in Latvia in food, veterinary and clinical samples

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Key words: EPEC, *Escherichia coli*, STEC, VTEC.

Humans and animals harbour *Escherichia coli* bacteria in the intestinal tract as part of normal microflora, and usually they are harmless. However, certain *E. coli* strains may be dangerous to human health, including those that are capable of producing toxins. These strains are referred to as STEC or VTEC (Shiga toxin or verotoxin-producing *E. coli*) or EHEC (enterohemorrhagic *E. coli*). Their toxins have the properties to cause bloody diarrhea and hemolytic uremic syndrome (HUS) and serious complications that can be fatal. People get infected by dealing with or eating contaminated foods, or drinking contaminated water, as well as contacting infected animals. The most common source of infection is beef meat (EFSA 2018). The highest number of cases in the European Union (EU) have been registered in 2011 – 9487, of which 5558 in Germany, caused by the enteroaggregative and concurrently shiga toxin producing *E. coli* O104:H4. The source was contaminated biological sprouts from Germany (EFSA 2011). In 2017 in EU 6073 STEC cases were registered, mortality rate was 0.5% (EFSA, ECDC 2018). In Latvia in 2018 there were six registered human cases, in 2017 five, corresponding to 0.3 and 0.1 cases per 100 000 of population, respectively (CDPC 2018). The goal of the present investigation was to evaluate the current situation of the presence of STEC in Latvia in food, veterinary and clinical samples within the period from 2015 till 2018.

Food samples (n = 122) were obtained within the Food and Veterinary Service (FVS) Food Monitoring Programs. They were cold smoked, dried or boiled sausages, dried meat, fresh meat, sprouted seeds, sprouts, fish products, minced turkey meet, game sausages, vegetables and juices. Countries of origin were Latvia (52.46%), Bulgaria, Estonia, Italy, Canada, Lithuania, Norway, Poland, Spain. Veterinary *E. coli* isolates (n = 54) were obtained within the FVS annual plans for the surveillance of animal infectious diseases and individual disease cases. They were from sheeps, pigs, cattle, dogs, cats, domestic and wild birds. Clinical isolates from faeces (n = 42) were obtained within contracts with the Centre for Disease Prevention and Control of Latvia and regional hospitals. Food samples have been tested according to ISO/TS 13136:2012 "Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing *E. coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups". From the veterinary samples isolates of *E. coli* were obtained from pathological material and faeces (according to World Organisation for Animal Health (OIE) Manual chapter 2.9.10.; Clinical Veterinary Microbiology, 2013, chapter 17) and screened for the presence of verotoxin encoding genes (Perelle et al. 2014). The same method was used for *E. coli* isolates from human faecal samples. *E. coli* serotypes were determined by agglutination reaction with commercially produced *E. coli* antisera (Statens Serum Institut, Denmark) as well according to the OIE Manual Terr., ch.2.9.10.

Data about the percentage of food samples and veterinary isolates containing STEC or EPEC (enteropathogenic *E. coli*) genes are given in Table 1. The retrospective data evaluation indicates that *E. coli* serotypes O26, O103, O145

Table 1. Data about the percentage of food samples and veterinary isolates containing STEC or EPEC isolates

Year	Percentage of food samples containing STEC/EPEC (%)	Percentage of veterinary isolates containing STEC/EPEC (%)
2015	8.82	25.00
2016	26.53	0
2017	5.00	0
2018	0	25.00

and O157 have been detected in both food and veterinary samples within years 2015 – 2018, while serotypes O121 and O91 were present only in food samples. Serotype O91 was found once in a sample of dried sausage originating from Lithuania. Serotype O121 has been detected twice, in cold smoked meat products originating from Lithuania. VTEC (belonging to serotypes O26, O103, O121, O145) and EPEC (enteropathogenic *E. coli*, serotypes O91, O103, O121, O145, O157) have been detected in food samples originating from Lithuania or Latvia. In human faeces presence of verotoxin encoding genes have not been recorded. The occurrence of pathogenic *E. coli* and the most dangerous serotypes in veterinary samples indicates that farm animals in Latvia is a serious reservoir of these bacteria from which they can enter the food chain.

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Application of enzyme activity assay for an improved enumeration of culturable nitrifiers

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Key words: colony forming units, dehydrogenase activity, nitrification.

Nitrification is an important step of wastewater treatment as it biodegrades ammonia, one of the more common compounds in wastewaters and which has toxic properties to aquatic life (Randall, Tsui 2002). Therefore, detection of nitrification bacteria in activated sludge is a major indicator of wastewater treatment plant effectiveness. In the process of nitrification, ammonia is oxidized to nitrite later nitrate by archaea and autotrophic bacteria like *Nitrosococcus mobilis* (Wagner et al. 2002).

In our study, enumeration of culturable nitrifiers in the activated sludge was determined by a droplet microdilution method on the agarized Winogradsky medium for nitrification phase I with the following composition (g L^{-1}): $(NH_4)_2SO_4$ 2.0, K_2HPO_4 1.0, $MgSO_4$ $7H_2O$ 0.5, NaCl 2.0, $FeSO_4$ $7H_2O$ 0.4, $CaCO_3$ 0.01, agar 15.0. The colonies were counted after 4 days of incubation at 30 °C. As shown in Fig. 1A, white colonies on the white background were not clearly distinguishable. It was hypothesized that a dehydrogenase (DHA) assay, which results in a formation of red formazan in physiologically active cells, can make the colonies more contrast.

DHA activity was determined by reduction of 2-p-iodo-3-nitrophenyl-5-phenyltetrazolium chloride (INT) to iodonitrophenylformazan (INTF) (Camiña et al. 1998). Five mL reaction mixture (10 mg INT in 0.25 M TRIS) was applied on the Petri dish with colonies. The red colour in the colonies appeared already after 2 min, while after 30 min the enumeration of CFU has been performed (Fig. 1B).

Visualization of the colonies under light microscopy provides with additional information regarding colonies morphology and diversity. Thus, DHA assay could serve for a more precise enumeration and characterization of culturable nitrifiers.

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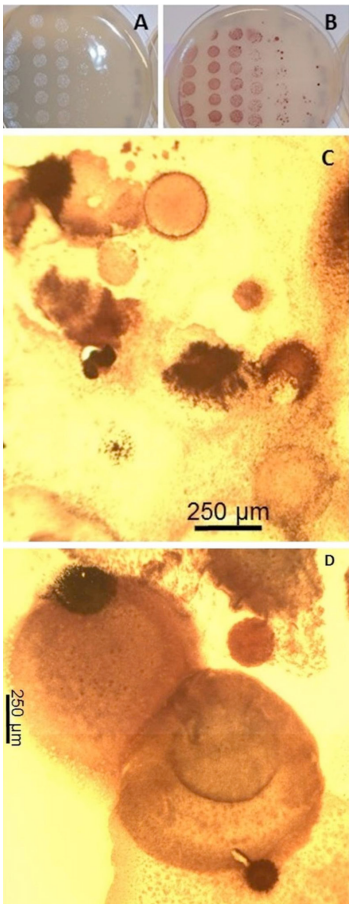

Fig. 1. Colonies of nitrifiers formed on the agarized Winogradsky medium for nitrification phase I. A, colonies after 4 days of incubation; B, colonies after 30 min reaction with the reaction mixture for DHA assay; C, D, micrographs of colonies subjected to DHA assay.

Monitoring of potential insect pests in commercial plantations of Japanese quince *Chaenomeles japonica* in Latvia

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Key words: *Chaenomeles japonica*, insect pests.

Area of commercial plantations of Japanese quince *Chaenomeles japonica* has risen substantially in Latvia during the last decade. There is an insufficient information on distribution of potential invertebrate pests in Japanese quince plantations. Latest available data about pests of *C. japonica* in Northern Europe is more than 15 years old, and only few samples from Latvia were included there.

ERAF project "Environment-friendly cultivation of emerging commercial fruit crop Japanese quince – *Chaenomeles japonica* and waste-free methods of its processing" (No. 1.1.1.1/16/A/094) was started in 2017.

One of the aims of the respective project was monitoring of potential *C. japonica* invertebrate pests in eight Japanese quince plantations in Latvia. This investigation is important for evaluation of phytosanitary status of Japanese quince plantations and identification of potential harmful organisms for which control plant protection methods would be necessary. The monitoring of invertebrates was performed in one plantation with integrated agricultural management system on three Japanese quince varieties (Institute of Horticulture, Dobele). In the rest seven plantations organic agricultural management system was used.

To perform an objectives at each quince plantation, one transect with 20 observation points was developed. For assessment of potential invertebrate pests and their damage on *C. japonica* five methods were used in vegetation seasons 2017 – 2018: (1) visual assessment of European red spider mite *Panonychus ulmi* eggs on *C. japonica* twigs, (2) yellow sticky traps, (3) Delta traps with sex pheromones of tortrix moth *Archips rosana, Archips podana, Rhopobota naevana* and *Cydia pomonella*, (4) visual observations of quince buds, flowers and leaves, (5) visual assessment of quince fruit.

As a potential invertebrate pests in *C. japonica* plantations can be assumed several polyphagous species: European red spider mite *P. ulmi*, Tortricidae moth species *A. podana, A. rosana, C. pomonella*, black-veined white *Aporia crataegi*, and garden chafer *Phyllopertha horticola*. These invertebrates in case of favourable for their development and reproduction conditions can cause a damage to Japanese quince.

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Evaluation of antimicrobial properties of hematite sphere- and wire-like nanoparticles in the visible-light process

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Key words: antimicrobials, iron-oxide nanoparticles, photocatalytic process, *Saccharomyces cerevisiae*.

Microorganisms and effects caused by their vital activity are undesirable and unacceptable for sterility requirements of different industries. These circumstances are challenging science in order to constantly keep this problem in field of vision and to test new methods of microbial activity prevention. Antimicrobial effects of nanoparticles (NP) could be the one of alternative ways of using antimicrobial compounds. Various ways of NP effects on microorganisms were detected during the numerous studies on toxicity of different size NPs consisting of different materials and different shape. Chemical properties of particles and physicochemiclal reactions during cell/NP interaction are the main way of the NP antimicrobial effect (Adams et al. 2006; Thill et al. 2006). A high surface-to-volume ratio increases reactivity and biochemical activity of NPs. Nevertheless, up to date, the interaction mechanism between NPs and microbial cells is largely unknown (Auffan et al. 2009; Ali et al. 2016).

In particular, Fe_2O_3 NPs, with a band gap of 2.2 eV, have received increasing attention as antimicrobial agents due to its visible light absorption properties (564 nm), unique magnetic properties and biocompatibility (Long et al. 2017).

This study was aimed at evaluating the antimicrobial effect of newly developed sphere-like and wire-like hematite (Fe_2O_3) NPs on the growing culture of *Saccharomyces cerevisiae* 14 in suspension. The Fe_2O_3 sphere-like NPs were synthesized by hydrothermal method. To obtain Fe_2O_3 nanowires, goethite nanowires were synthesized by precipitation method. Both types of NPs were tested without dialysis and after dialysis in a cellulose tube in distilled water for 72 h.

Effect of the tested NPs (concentrations range of 1 to 100 μg mL^{-1}) on the growth of *S. cerevisiae* was evaluated in YPG (Yeast Extract Peptone Glucose) broth in microplates, which were incubated at 23 °C, in dark and under LED light.

Cultivation of *S. cerevisiae* 14 under tested conditions showed the inhibitory effect of both types of NPs on the culture growth, exposed by LED light. In particular, the HemaS-D with concentration in the range from 1 to 100 μg m^{-1} reduced the culture OD_{620} after 48 h cultivation by ≈45% compared to control (Fig. 1).

Wire-like and sphere-like hematite NPs were shown to inhibit the growth of *S. cerevisiae* 14 under tested conditions with a more pronounced inhibitory effect being under LED light. This effect is expected to study in further experiments with yeast and bacterial strains, in order to develop antimicrobial coatings for different types of surfaces.

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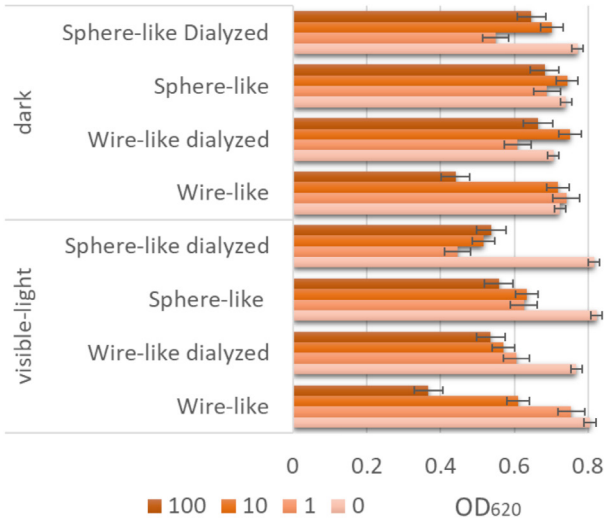

Fig. 1. Nanoparticle impact on *Saccharomyces cerevisiae* 14 growth (OD_{620}) under LED light and in the dark. Cultivation was performed in YPG medium (with 100 mg L^{-1} tetracycline) in microplate, for 48 h at 23 °C. Concentration of NPs 0; 1; 10; 100 μg mL^{-1}. Culture volume 300 μL. HemaW, wire-like hematite NPs; HemaS, sphere-like hematite NPs; D, dialysis.

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The actualisation of scanning electron microscopy-based morphological research on invertebrate microstructures at the University of Latvia: an example of Cyclophoroidea Grey, 1847 snails from the Papuan region

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Key words: Cyclophoroidea, microstructures, Papuan Region, scanning electron microscopy, shell morphology.

Scanning electron microscopy (SEM) is an indispensable tool for a wide range of morphological and anatomical studies in many sub-disciplines of life sciences including zoology. This tool is used intensively not only by scientific institutions but also by academic facilities worldwide. External microstructures of small-size gastropod shells (less than 7 mm) are used as one of the most effective characters for species determination. However, SEM has been used hardly ever for invertebrate research at scientific institutions in Latvia.

In order to actualise SEM in invertebrate zoology at the University of Latvia, a pilot study of gastropod microscopy was performed. By using tabletop Hitachi-3000 microscope microstructures of cyclophoroid (Caenogastropoda) snail shells from the Papuan Region (zone of Indo-Australian transition) were examined and photographed, as well as various preparation techniques tested within the last three years. In parallel, identified museum material was

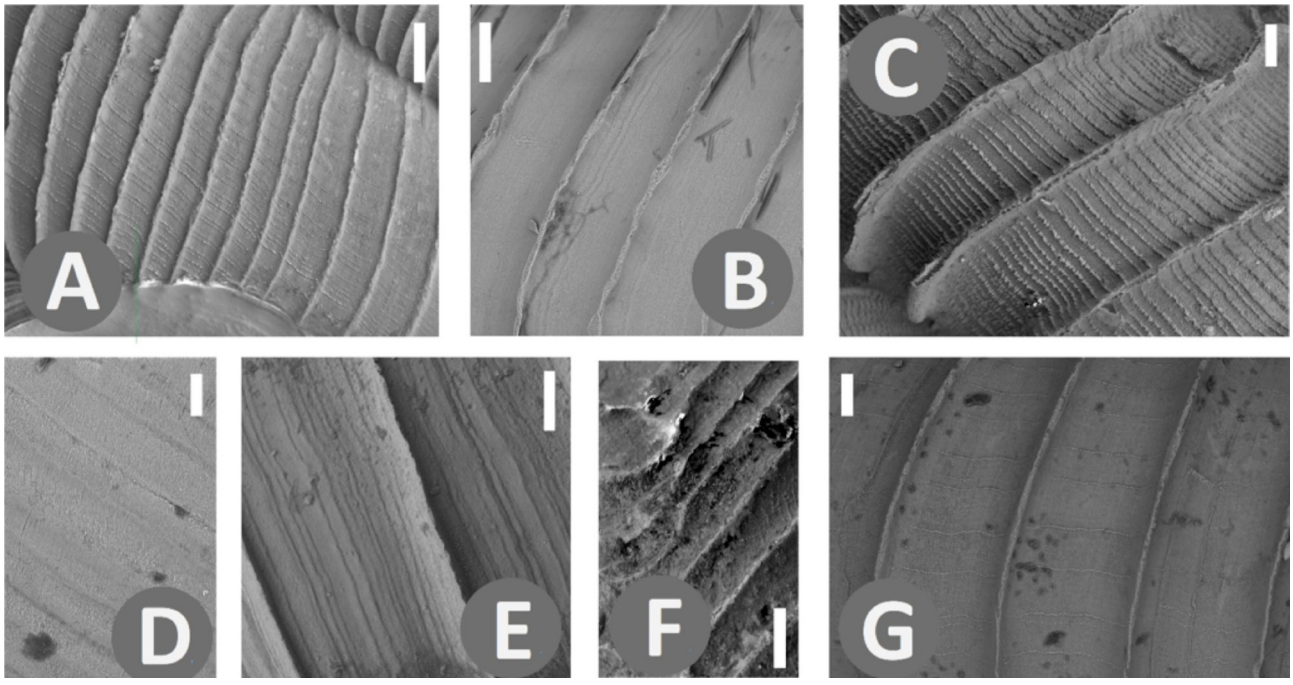

Fig. 1. SEM micrographs of the ultimate whorl in apertural view for various Cyclophoroidea snails. A, *Palaina* sp. nov. 1*; B, *Palaina* sp. nov. 2*; C, *Palaina attenboroughi* Greķe, 2017; D, *Palaina* sp. nov. 3*; E, *Diplommatina* sp. nov. 1*; F, *Diplommatina telnovi* Greķe, 2017; G, *Palaina* sp. nov. 4*. Scale bars 50 μm; species that have not yet been described are marked with an asterisk (*).

also investigated using SEM. In a process of preparation, all shells were mounted air-dry on specimen stub using double-sided carbon electron-conductive tape and were not sputtered with a coat of conductor (Au, Pb etc.) afterwards. More than 250 scanning electron micrographs were taken representing over 20 different Cyclophoroidea taxa that were afterwards analysed methodologically, morphologically and classified taxonomically.

Microstructures were best visible at low magnification of 300 to 1500 times. In accordance to the features visible on micrographs, eight species have been confirmed as new to science as a result (Greķe 2017; Greķe 2019). Certain species-specific morphological variation has been observed on micrographs (Fig. 1 A – G). Sample preparation affected visibility of the detail of microstructures (Fig. 1 C, G) and behaviour of the charge-up (Fig. 1 A, F). Most likely, sputtering of shell surface with conductive coating would improve visual results. Both mechanical cleaning with ethanol using soft thin brush and ultrasonic cleaning were necessary procedures to increase the quality of SEM imaging but were not resulted into fully cleaned shells whatsoever (Fig. 1 B – E, G). The cleaning effect strongly varied according to different places against the ultra-sonic beam towards which specimen was positioned in a tank of a cleaner. Therefore, all specimens were cleaned similarly i.e. positioned in the centre of the tank (centre of the ultrasonic beam). Too intensive ultrasonic cleaning (longer than 10 to 15 s) caused significant damage to the thinnest shells. Washing with 5% KOH solution for 60 min eliminated soft tissues from the surface of shells with different success depending on the specimen (Fig. 1 F).

According to our results, no single preparation technique can be suggested as the most effective for gastropod microstructure imaging, and preliminary testing is always required before SEM. However, high importance of using SEM for taxonomic studies in malacology is highlighted in a wide range of previous publications and is suggested to be used more frequently during various morphological and taxonomic studies of gastropods and other invertebrates (e.g., Araujo, Korniushin 1998; Geiger et al. 2007; Güller, Zelaya 2017).

Authors do believe that activities related to invertebrate SEM among researchers and students of Department of Zoology and Animal Ecology (under the branch of animal biodiversity, morphology, and functions) at the University of Latvia, will increase in nearest future.

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Shape variation in taxonomically problematic genus *Phthiracarus* Perty, 1841 of armoured mites (Acari: Oribatida) investigated by using methods of geometric morphometrics: preliminary results

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Key words: geometric morphometrics, landmarks, quantitative morphological traits, *Phthiracarus*, shape variation.

Phthiracarus Perty, 1841 – a ptyctimous armoured mite genus – has been characterised as a taxonomically challenging group (Niedbala 2011). Till now, only four species have been registered in the territory of Latvia (Kagainis 2011) of this worldwide and species-rich genus. According to the Europe-associated taxonomic literature, *Phthiracarus* species can be identified by traits that usually are difficult to interpret. Often, relatively variable, hardly visible by light microscopy and non-quantitatively characterizable traits are put on focus (Weigmann 2006; Niedbala 2011). More quantitative traits are necessary to incorporate in species identification. Shape variability may be taken into consideration using a geometric morphometric analysis to improve species determination of the *Phthiracarus* fauna of the Baltic region and abroad.

Phthiracarus mites with non-damaged exoskeleton were selected randomly from various studies in the past and represented three different habitats that mites were previously sampled from. Specimens were positioned laterally, facing left and prepared either in Berlese media or lactic acid. Thirty landmarks on the lateral side were selected (Fig. 1 A) for two-dimensional geometric morphometric analysis (MorphoJ software package, Klingenberg 2011). Canonical Variate Analysis (CVA, Baran et al. 2011) was executed in order to investigate a geometric deformation. For morphometric observations, transmitted light microscopy was used. Species were confirmed after micrographs taken generated by the scanning electron microscope Hitachi-3000.

Three different *Phthiracarus* species were confirmed:

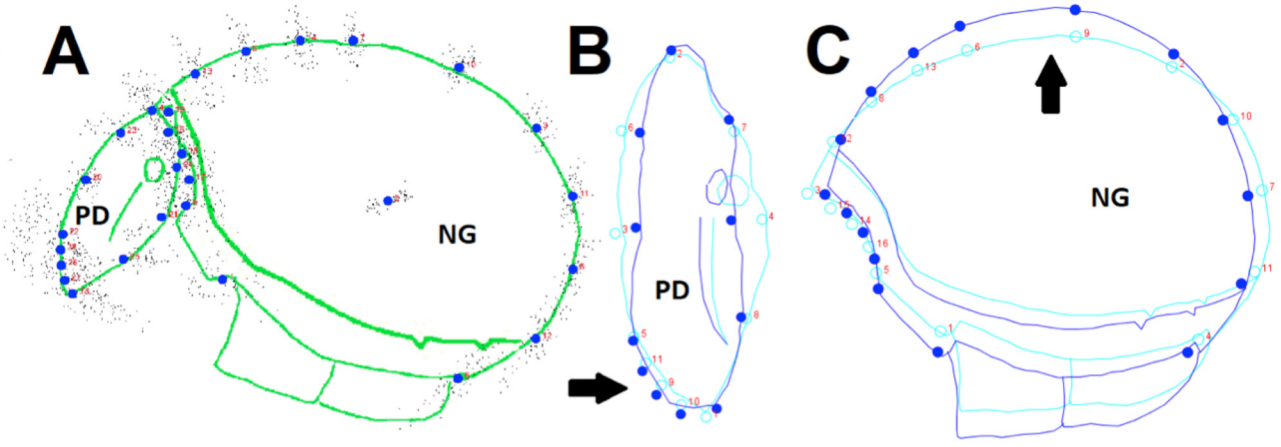

Fig. 1. Distribution of landmarks and shape deformation (outlines of consensus in light blue and shape deformation in dark blue) observed on *Phthiracarus* genus mites for geometric morphometric analysis. A, consensus landmarks (large blue dots, n = 30) and distribution of all landmark data (small black dots, n = 2490), green curves represent the contour outlines of the prodorsum (PD) and the notogaster (NG) after Weigmann 2006, modified. B, shape deformation by the landmarks (CVA) on the prodorsum (PD), location of the median crista pointed by arrow. C, shape deformation by landmarks (CVA) on the notogaster (NG), location of globular shape deformation pointed by arrow.

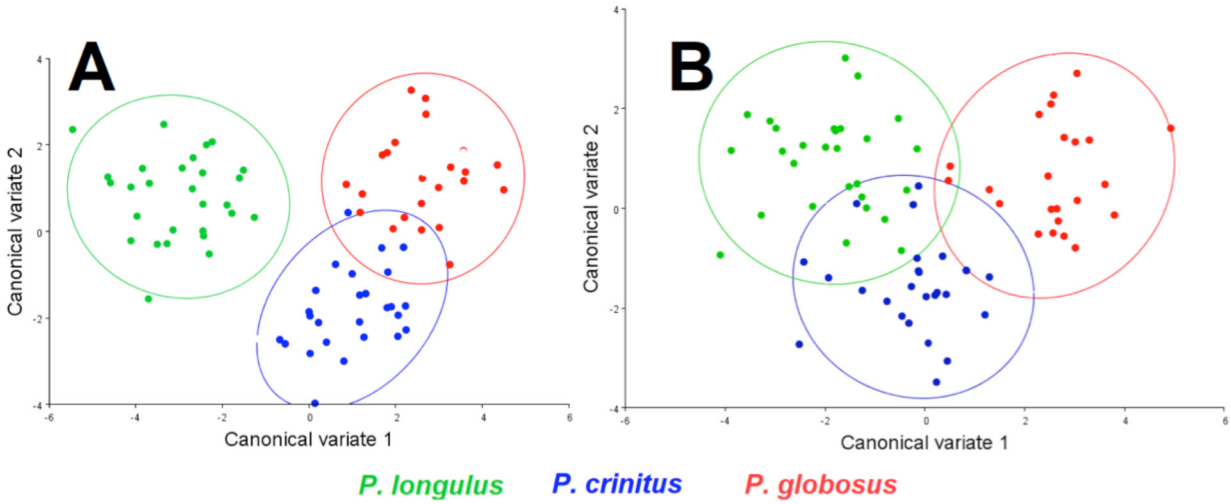

Fig. 2. CVA ordinations of shape variation for three *Phthiracarus* species (ellipses confidence for both analyses 0.95). A, an ordination for the landmark dataset used on the prodorsum (PD). B an ordination for the landmark dataset used on the notogaster (PD).

Phthiracarus crinitus ($n = 28$), *Phthiracarus globosus* ($n = 27$) and *Phthiracarus longulus* ($n = 28$). Species confirmation results showed uneven distribution by means of a number of mites for each species among the habitats. Description of the shape deformation compared to the consensus shape was given for each species. An overlapping for shape variability data [two data sets: prodorsumal and notogastral landmark groups (Fig. 1 A)] was registered in CVA between two or even all three species depending on the trait (3.2 to 28.0% of the shape variability).

Some traits did separate two species significantly with no overlapping in the shape variability. General shape deformation (broader or narrower) of the prodorsum (PD) was significantly species-specific only between *P. globosus* and *P. longulus* and characterised the trait mostly by the presence of median crista and its curves (Fig. 1 B, arrow; Fig. 2 A). Curves of the shape of anterior and posterior part of notogaster (NG) were significantly more globular for *P. globulus* and *P. crinitus* compared to *P. longulus* (Fig. 1 C, arrow; Fig. 2 B). A shape of the anterior edge of the notogaster was detected significantly specific only between *Phthiracarus* mites sampled from different habitat and showed no species-specific variability. It is expected that future studies with larger and standardised samples from various habitats will improve our explanations for habitat-specific shape variation among the three *Phthiracarus* mite species.

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Activated sludge stimulation by adding yeast biomass

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Key words: activated sludge, enzyme activity, respiration, *Saccharomyces cerevisiae*, wastewaters.

Yeasts have been used for making beer, wine and bread since ancient ages (Barnett, Barnett 2011). Nowadays yeasts have different applications in industry: in production of alcoholic beverages, manufacturing of functional food, enzymes and other valuable nutraceutical production (Rai et al. 2019). Particularly important in biotechnological production is the yeast, which is used also for the biodegradation of xenobiotics (Ubiyvovk et al. 2006).

Inasmuch as yeasts have been widespread used in the industry, manufacturing often has an issue: spent yeast biomass that remains after production processes. Spent yeast biomass cannot be re-used, but it is a valuable by-product that can be used to produce products with a high added value (Coldea et al. 2017). One of the biggest industries using yeasts is the beer industry. Beer fermentation involves multiple yeast cell division leading to high biomass accumulation. The environmental legislation is becoming stricter and the associated costs higher; as a consequence, the global brewing industry must reduce production losses and keep waste to a minimum (Jurado, Sorensen 2012). Therefore, it is necessary to look for new approaches to the applications of spent yeast biomass.

The spent yeast biomass of *Saccharomyces cerevisiae* is a valuable by-product with a high nutritional value. It contains large amounts of proteins, amino acids and B group vitamins (Podpora et al. 2015).

The aim of our study was to determine an impact of adding yeast biomass on the activated sludge (AS) activity. These experiments were performed with „fresh" (not „spent") biomass of *S. cerevisiae* 14, which was collected from the agarized medium after 48 h incubation. These data would serve as a control for our further experiments with „spent" yeast biomass. Synthetic wastewaters and activated sludge from the chemical industry were used. Microbial activity was evaluated by respiration intensity (OxiTop™) and FDA (fluoresceine diacetate hydrolysis) enzyme activity.

The impact of yeast biomass to the respiration intensity of the AS is shown in Fig. 1A. After 24 h incubation in the OxiTop™ device, respiration of the yeast-amended AS was higher, than in control, however this difference was not statistically significant ($P > 0.05$) (Fig. 1A). Further incubation did not reveal any considerable changes in a respiration intensity of the tested AS.

Another important criterion for characterizing activated sludge is the enzymatic activity of microorganisms. Since several enzyme groups – lipases, esterases, proteases and hydrolases – are involved in the FDA hydrolysis reaction,

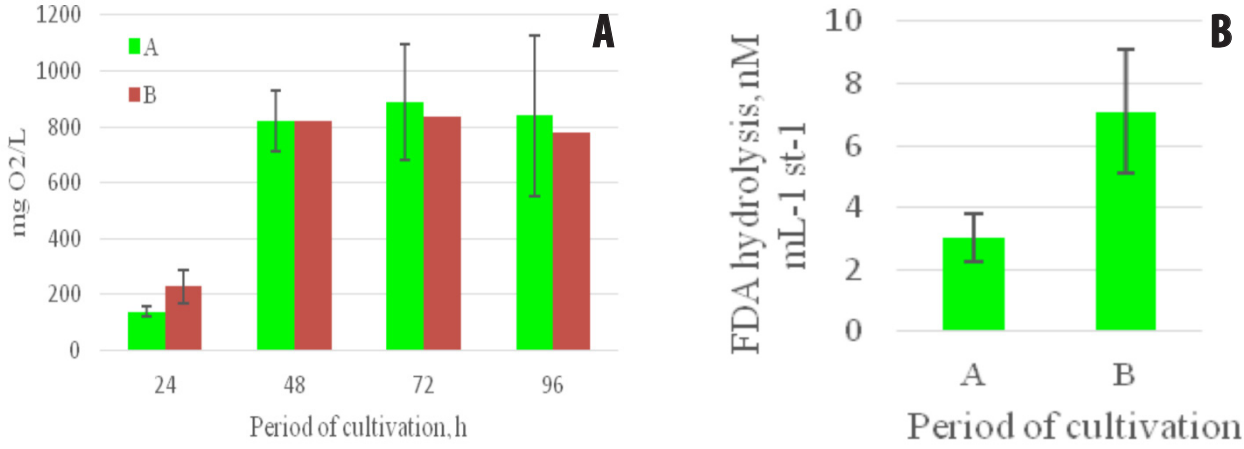

Fig. 1. Respiration intensity in dynamics (A) and FDA hydrolysis after the 4-day-experiment (B) of the activated sludge incubated in the OxiTop™ device. Incubation was performed at 23 °C. Initial concentration of yeast CFUs added to the AS, was equal to bacterial CFU number in AS (10^7 CFU mL^{-1}). CFU, Colony Forming Units; A, activated sludge; B, activated sludge amended with *S. cerevisiae* 14 biomass.

this method is widely used to evaluate the total activity of microorganisms in different environmental samples: soil, sludge and water. Activity of FDA hydrolysis in the AS after 4-day-incubation in the OxiTop™ device is shown in Fig. 1B. Addition of yeast biomass to AS yielded the significantly ($P < 0.05$) higher FDA hydrolysis activity, as compared to the control (Fig. 1B).

Summarizing the obtained results, it can be concluded about the stimulating role of *S. cerevisiae* biomass for the total respiration and enzymatic activity of AS. Further experiments will be focused on the evaluation of the different physiological state of yeast biomass on the efficiency of AS in the wastewater treatment process.

Acknowledgements

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Effects of *Lentinula edodes* extract on melanization reaction un hydrogen peroxide resistance in *Drosophila melanogaster*

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Key words: antioxidative response, *Drosophila melanogaster*, hot water extract, *Lentinula edodes*, melanization reaction.

Shiitake *Lentinula edodes* (Berk.) Pegler is edible and cultivable basidiomycete with potential applications in medicine and biotechnological processes. Hot water extraction is the basic method to isolate polysaccharide rich fraction (Xu et al. 2014), as well presence of polyphenols in hot water extracts (HWE) are reported (Kozarski et al. 2012). Shiitake derivate polysaccharides are revealed as immunomodulators primarily influencing innate immunity (Xu et al. 2014). Additionally, polysaccharides (Kozarski et al. 2012) and polyphenols are studied regarding to its antioxidative properties. In this study we continue to evaluate *L. edodes* HWE effects on induced immune response and oxidative stress in *Drosophila melanogaster*.

HWE was obtained from *L. edodes* (DSM 3565) fruiting bodies as described previously (Xu et al. 2014). Extract was standardized by dry weight (105 °C) of ethanol precipitate, pasteurized, lyophilized and stored at 4 °C. Prior to use in experiments, HWE was dissolved in distilled water and mixed in drosophila media, the final concentrations 0.001, 0.003, 0.015, 0.060, 0.100% of ethanol precipitate were achieved; in the control medium the appropriate amount of water was added.

Normal (wild-type) *D. melanogaster* eggs laid in 12-h period were placed in experimental media. Eggs were allowed to hatch, feed and proceed L1 and L2 stages. At the end of L3 stage larva were collected by 20% sucrose, washed in 0.9% NaCl and selected for the melanization test, catalase activity and hydrogen peroxide resistance test.

Melanization is a part of drosophila innate immune response and it could be triggered by both septic and sterile injuries (Dudzic et al. 2015). To induce melanization drosophila larvae were pricked by sterile needle (0.26 mm) in dorsal side near the posterior spiracles. After a 2-h period melanization intensity was estimated visually and valued by relative units 0 to 5. Relative melanization intensity was significantly increased in HWE-fed larvae (Fig. 1).

Hydrogen peroxide oxidize macromolecules and triggers organism antioxidative defence system. Many organisms can decompose H_2O_2 by enzyme catalase (Subedi et al. 2012). In the current experiment catalase activity in

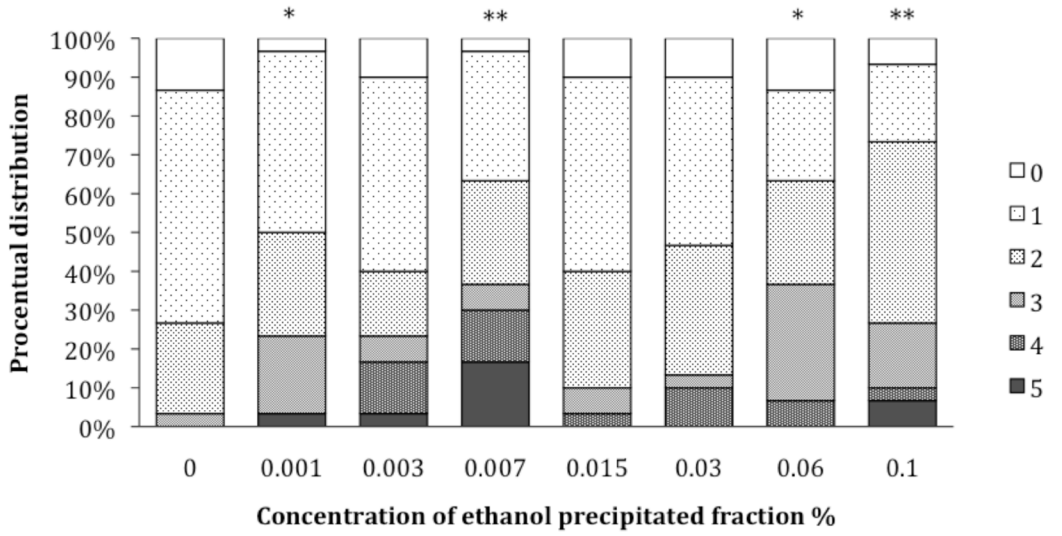

Fig. 1. Intensity of melanization reaction in HWE pre-fed *D. melanogaster* larvae. **P* < 0.0.5, ***P* < 0.001 values determined between control and HWE, fed groups by Mann-Whitney U-test (SPSS 18 for Windows).

Table 1. Hydrogen peroxide resistance in HWE pre-fed *D. melanogaster* larvae. *$P < 0.0.5$, **$P < 0.001$ values determined between H_2O_2 exposed control and HWE-fed groups by one-way ANOVA (LSD test)

Groups	Larvae mouth hook contractions in 30 s (mean ± SE)	Larva-imago viability (%)	Imago thorax size measurements in mm (mean ± SE)	
			Male	Female
Control	69.0 ± 2.3	83.3	0.82 ± 0.03	0.95 ± 0.02
Control 50 mM H_2O_2	40.3 ± 4.8	36.8	0.77 ± 0.02	0.86 ± 0.02
HWE 0.001%	53.0 ± 2.9*	–	0.74 ± 0.02	0.90 ± 0.00
HWE 0.003%	59.1 ± 5.1**	68.8	0.77 ± 0.02	0.87 ± 0.02
HWE 0.007%	68.1 ± 2.4**	75.0	0.78 ± 0.01	0.92 ± 0.02
HWE 0.015%	63.7 ± 3.0**	61.9	0.74 ± 0.02	0.91 ± 0.02
HWE 0.030%	58.9 ± 1.4**	83.3	0.78 ± 0.02	0.92 ± 0.02
HWE 0.060%	55.1 ± 4.1*	50.0	0.79 ± 0.01	0.86 ± 0.02
HWE 0.100%	53.0 ± 1.5*	75.0	0.79 ± 0.02	0.90 ± 0.01

HWE-fed larvae did not differ significantly compared to control group.

To assess H_2O_2 resistance, drosophila larvae were maintained in 50 mM H_2O_2 and 5% sucrose solution. At the end of 24-h period drosophila larvae was rated by mouth hook contractions and placed in standard media to observe larva-adult viability and thorax size of hatched adults.

Twenty-four-hour exposure to 50 mM H_2O_2 significantly decreased L3 larva mouth hook contraction frequency and delayed further development accompanied by minor decrease of thorax size. HWE pre-treatment raised H_2O_2 resistance in *D. melanogaster*. Pre-fed L3 larvae exerted more pronounced mouth hook contractions and viability rate; while imago thorax size did not significantly differ from H_2O_2 exposed control group (Table 1).

Current results correspond to previous observations that *L. edodes* HWE ameliorates induced oxidative stress and modulates immune response (Azena et al. 2018) in *D. melanogaster*. Regarding to obtained results it could be proposed that drosophila is appropriate model system to study immunomodulator and antioxidative effect of medical mushroom extracts.

Acknowledgements

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Effect of nutrient amendments on the growth and dehydrogenase activity of lactic acid bacteria under oxidative stress conditions

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Key words: dehydrogenase activity, lactic acid bacteria, oxidative stress, Tween.

The stress physiology of lactic acid bacteria (LAB) is intensively studied. These investigations are related to the technological implications of LAB robustness in the food industry, including the preparations of probiotics (Papadimitriou et al. 2016). Particularly, the efficacy of high-potency probiotic preparations on prevention of a radiation-induced disbacteriosis in cancer patients represents an important topic in this field.

Zotta et al. (2014) have screened 184 strains of *Lactobacillus* sp. for the oxygen and oxidative stress tolerance. Cultivation of lactobacilli under aerobic conditions resulted in an increased biomass production. Strains from the *Lactobacillus casei* group showed the typical traits of aerobic and respiratory metabolism (increased pH and biomass under aerobic or respiratory conditions) and unique oxidative stress response properties (Zotta et al. 2014; Maresca et al. 2018). Oxidative damages of cells are attributable also to ionizing radiation exposure (Azzam et al. 2012).

The objective of this study was to evaluate the impact of Tween 20, Tween 60 and Tween 80 added to Trypticase Soy Broth (TSB) broth, on the physiological response of the consortium of LAB to the oxidative stress. TSB is considered (among the MRS) to be appropriate for cultivation of lactic acid bacteria (Cálix-Lara et al. 2012). It was reported earlier that unsaturated fatty acids are essential growth factors

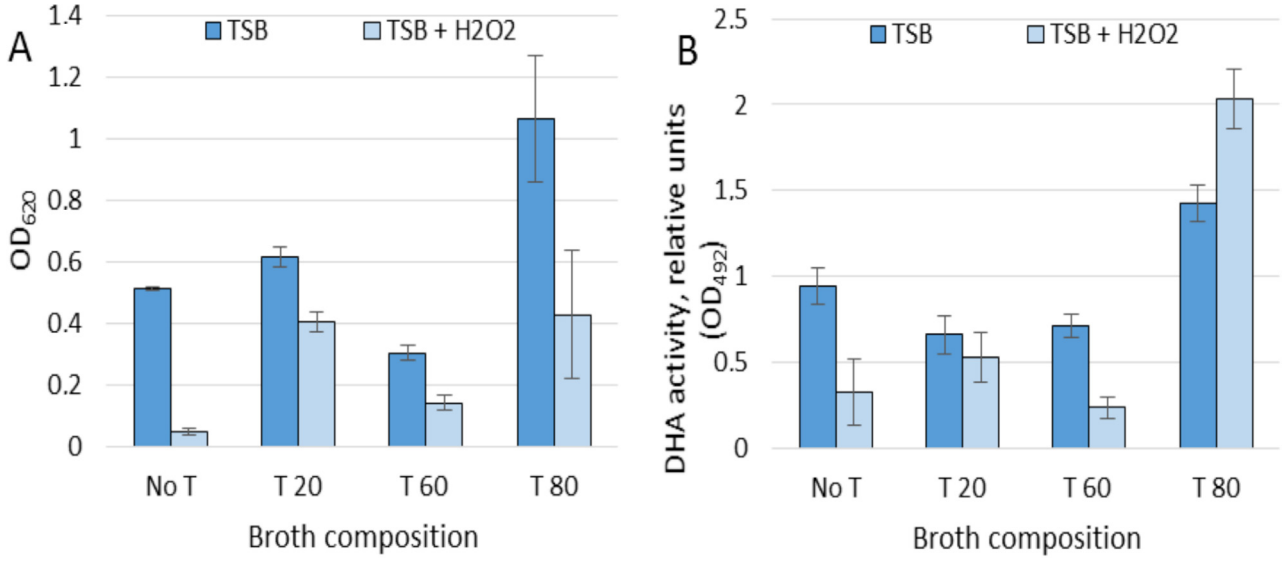

Fig. 1. Effect of H_2O_2 on the growth (A) and dehydrogenase activity (B) of the LAB consortium in TSB broth amended with 0.1% Tween 20, Tween 60 and Tween 80. Culture was incubated in microplates (300 µL) aerobically at 30 °C for 72 h with periodic agitation. Dehydrogenase activity was measured after 72 h incubation (30 min assay with INT). Data are means ± SD from 3 replicates.

for most lactobacilli (Partanen et al., 2001; Malheiros et al., 2015). Oxidative stress was initiated by 1 mM H_2O_2. Dehydrogenase (DHA) activity was determined by reduction of 2-p-iodo-3-nitrophenyl-5-phenyltetrazolium chloride (INT) to iodonitrophenylformazan (INTF) in 0.25 M TRIS (Camiña et al. 1998).

As shown in Fig. 1A, the highest culture OD_{620} after 72 h cultivation was detected in the set with TSB broth supplemented with 0.1% Tween 80.

In turn, the both supplements, Tween 20 and Tween 80 were capable of protecting bacterial culture from H_2O_2. The tested lactic acid bacterial consortium in the non-supplemented TSB showed the most dramatic inhibition in the presence of H_2O_2 (Fig. 1A).

DHA activity of bacterial culture was expected to reveal some specific changes in the cell metabolism under oxidative stress. The highest DHA activity among the tested variants has been revealed in the culture grown in TSB supplemented with Tween 80 and 1 mM H_2O_2 (Fig. 1B).

Further optimization of cultivation conditions of LAB will be focused on the enhancement of probiotic resistance to oxidative stress with emphasis on industrial applications.

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Functional chemistry of coastal plant species: towards a definition of Na and K metallophytes (electrolytophytes)

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Key words: Baltic Sea, coastal habitats, electrical conductivity, K^+ concentration, metallophytes, Na^+ concentration.

Halophytes are most commonly defined as plants native to salt-affected habitats with soil NaCl concentration above 0.5%. Surprisingly, no threshold concentration for Na^+ accumulation in halophyte tissues has been defined so far, in a manner similar to that for heavy metal hyperaccumulators in native habitats. Besides heavy metals, a concept of "plants-metallophytes" has been used to describe plants growing natively on soils with high concentration of certain metals and being able to accumulate these ions in concentration significantly exceeding tissue toxicity level of non-metalophhytes. No studies have been described so far on comparative analysis of Na^+ concentration in different tissues of plants native to salt-affected soils, i.e., these growing on coastal habitats. In addition to Na^+, K^+ has a special importance for salinity tolerance. While Na^+ has no apparent functional role for majority of plant species, due to chemical similiarity between the two elements, it is argued that Na^+ can replace K^+ for halophytes, at least, partially (Subbarao et al. 2003). Therefore, the aim of the present study was to establish criteria for Na^+ and K^+ accumulation and hyperaccumulation for plants from coastal habitats of the Baltic Sea.

Determination of ionic strength in living cells is of critical importance for understanding cellular processes affected by electrostatic interactions, i.e. enzyme activation etc. (Liu et al. 2017). In several relatively simpler systems, ionic strength can be predicted by electrical conductivity, as in the case of soil solution (e.g., Dolling, Ritchie 1985). No such relationship has been addressed for plant tissues. By definition, halophytic and metallophytic plant species need to possess an ability to withstand high internal concentration of metal ions. In the present study, electrical conductivity (EC) of tissue water extracts was used to characterize total concentration of solute ions in plant tissues as an indirect indication of ionic strength in their cells.

Plant sampling was performed in July – August during 2017 and 2018 in different coastal habitats of the Baltic Sea (including both dry and wet sandy beach, driftlines, shingle beach, rocky seashore, coastal lagunes, embryonic dunes, sand dunes, grey dunes, dry dune meadow, wet meadow, wetland) in Latvia, Estonia, Sweden and Denmark. In total, 379 samples of different plant parts (leaves, stems, flowers, roots) from 37 species were collected. Plant material were dried in a thermostat at 60 °C until a constant mass. Tissues were homogenized by crushing to small pieces and a sample (0.2 g) was taken for analysis of EC, Na^+ concentration and K^+ concentration in water extract by LAQUAtwin compact meters (Samsone, Ievinsh 2018).

All measured values were species-specifically variable and tissue-dependent. The lowest Na^+ concentration was found in flowers of *Polygonum lapathifolium* (0.5 g kg^{-1}) and *Atriplex calotheca* (0.7 g kg^{-1}), as well as in leaves of *Artemisia absynthum* and *Bunias orientalis* (both 1.0 g kg^{-1}). The highest Na^+ concentration was found in lateral stems of *Atriplex calotheca* (222 g kg^{-1}). *Rumex crispus* had the lowest EC (9.5 – 13.0 mS cm^{-1}), but the highest EC was found in leaves of *Atriplex calotheca* (410 mS cm^{-1}). Based on the relative distribution of the results of chemical analysis in coastal plants, criteria were set up for definition of degree of internal Na^+ and K^+ concentration, K^+/Na^+ concentration ratio, and EC according to the five-point scale (Table 1).

When average values of measurements in plant leaves were attributed to the defined degrees, all tested species

Table 1. Criteria for definition of degrees of internal Na^+ and K^+ concentration, K^+/Na^+ concentration ratio and electrical conductivity (EC) in tissues of coastal plant species expressed on dry mass basis

Parameter	Very low (1)	Low (2)	Moderate (3)	High (4)	Very high (5)
Na^+ (g kg^{-1})	≤ 3.7	3.8 ÷ 7	8 ÷ 15	16 ÷ 38	≥ 39
K^+ (g kg^{-1})	≤ 13	14 ÷ 23	24 ÷ 35	36 ÷ 45	≥ 46
EC (mS cm^{-1})	≤ 49	50 ÷ 100	101 ÷ 150	151 ÷ 200	≥ 201
K^+/Na^+ concentration ratio	≤ 0.1	0.2 ÷ 0.8	0.9 ÷ 1.7	1.8 ÷ 3.2	≥ 3.3

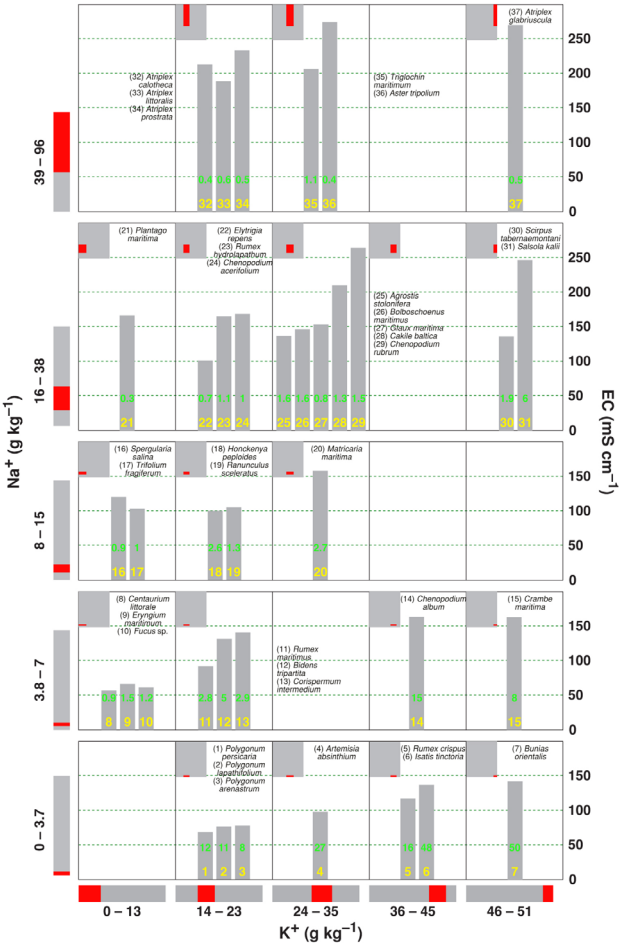

Fig. 1. Distribution of coastal species according to Na^+ and K^+ concentration in their leaves. Bars show tissue electrical conductivity (EC), green numbers on bars represent K^+/Na^+ concentration ratio.

were arranged in several groups (Fig. 1). No species were attributed to "very low Na^+/very low K^+" group, but several *Polygonum* species corresponded to "very low Na^+/low K^+" group. Interestingly, several sea coast-specific species were typical Na^+ excluders, occupying "low Na^+" range, where *Eryngium maritimum* had "very low K^+" and *Crambe maritima* had "very high K^+". A group of typical coastal species (*Spergularia salina, Trifolium fragiferum, Honckenya peploides* and *Matricaria maritima*) had "moderate Na^+" together with very low to moderate K^+ concentration. "High Na^+" range was characteristic for typical halophytes *Plantago maritima* and *Salsola kali*, with "very low K^+" and "very high K^+", respectively. Two grass species (*Elytrigia repens* and *Agrostis stolonifera*), as well as two typical wetland monocotyledonous species (*Bolboschoenus maritimus* and *Scirpus maritimus*) also occupied "high Na^+ range", with significant differences in leaf K^+ concentration. "Very high Na^+" range was occupied by typical sea coast species, where several *Atriplex* species were characterized as "low K^+", but *Atriplex grabriuscula* showed exceptionally high both Na^+ and K^+ concentrations. Two sea coast-specific species (monocotyledonous *Triglochin maritimum* and dicotyledonous *Aster tripolium*) had "very high Na^+" together with "moderate K^+".

No species in "very low Na^+" range had "high EC", but *Chenopdium album* and *Crambe maritima* from "low Na^+" range with high and very high K^+ had "high EC". In a "moderate Na^+" range, *Matricaria maritima* had "high EC". In a "high" and "very high Na^+" range, all species corresponded to "high EC" or "very high EC", depending on their K^+ concentration range.

In a light of two common strategies of salinity tolerance, salt exclusion or salt accumulation, it is evident that plants with both strategies are coexisting in coastal habitats. However, only coastal species, accumulating high concentration of Na^+ and/or K^+, can be designated as metal hyperaccumulators and electrolytophytes. From a practical point of view, these species are potential models for phytoremediation studies.

Acknowledgements

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Does *Ranunculus sceleratus* from coastal wetland is a potential electrolyte-accumulating species?

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Key words: coastal wetland, electrical conductivity, K^+ concentration, Na^+ concentration, *Ranunculus sceleratus*.

Ranunculus sceleratus is a semiaquatic plant species, typical for shallow waters and wet coasts. *R. sceleratus* can be found in a sea coast habitats, evidently affected by high soil salinity and flooding with sea water. During survey of Na^+ and K^+ accumulation capacity of coastal plants, it was found that native *R. sceleratus* plants had moderate Na^+ (12.5 g kg^{-1}) and low K^+ (16.0 g kg^{-1}) concentration in shoot tissues (Ievinsh et al. 2019). The aim of the present study was to investigate Na^+ tolerance of *R. sceleratus* plants in controlled conditions with a special emphasis on tissue Na^+ and K^+ concentration, as well as electrolyte accumulation potential.

Plants were brought in culture by a seed collected at natural sea-affected coastal site near Salacrīva, Latvia and grown in 0.5 L plastic containers in a substrate consisting of commercial garden soil (Biolan, Finland) and quartz sand (1:1, v/v). Plants were cultivated in an experimental automated greenhouse (HortiMax, Netherlands) with supplemented light from Master SON-TPIA Green Power CG T 400 W (Philips, Netherlands) and Powerstar HQI-BT 400 W/D PRO (Osram, Germany) lamps (380 μmol m^{-2} s^{-1} at the plant level), 16 h photoperiod, day/night temperature 23/15 °C, relative air humidity 60 to 70%. After establishment, plants were irrigated with gradually increasing concentration of NaCl in deionized water, reaching final concentration of Na^+ after three weeks. The final concentration of Na^+ in substrate was 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 g L^{-1}. Five plants per treatment were used. Eight weeks after the start of the treatment, the experiment was terminated and plants were separated in roots, rosette leaf petioles, rosette leaf blades, stems, stem leaves and flowers (including fruit). All parts were weighed and dried in a thermostat at 60 °C until a constant mass, then dry mass was measured. Tissues were homogenized by crushing to small pieces and a sample (0.2 g) was taken for analysis of electrical conductivity (EC), Na^+ concentration and K^+ concentration in water extract by LAQUAtwin compact meters (Samsone, Ievinsh 2018).

Morphology of *R. sceleratus* plants was not dramatically affected by increasing Na^+ concentration, but stem height significantly decreased starting from 1 g L^{-1} Na^+ treatment and number of rosette leaves significantly decreased from 5 g L^{-1} (Fig. 1). Total dry mass of shoot decreased with increasing Na^+ concentration, and changes of distribution of relative dry mass among different plant parts were evident (Fig. 2). There was a decreasing trend of mass for all plant parts with increasing Na^+, except for rosette leaf petioles and blades at high Na^+ concentration, where even significant growth stimulation was evident for plants treated with 10 g L^{-1} Na^+.

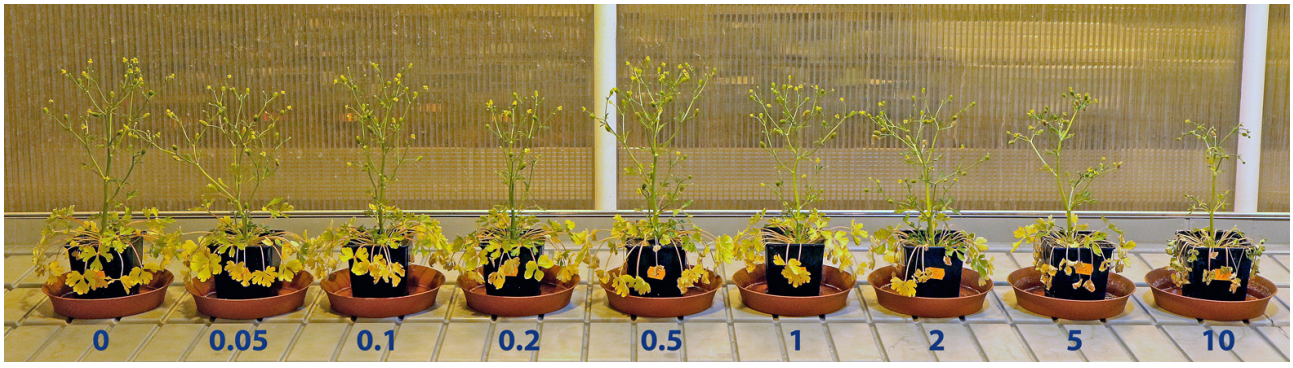

Fig. 1. Morphology of typical *Ranunculus sceleratus* plants grown at different substrate Na^+ concentrations (indicated by numbers in g L^{-1}) for eight weeks.

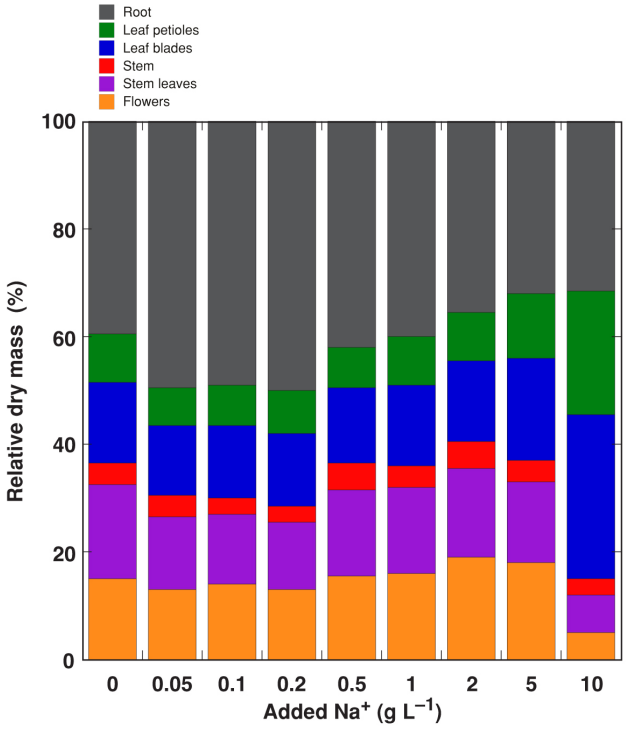

Fig. 2. Relative distribution of dry mass in different *Ranunculus sceleratus* parts for plants grown at different substrate Na^+ concentrations for eight weeks.

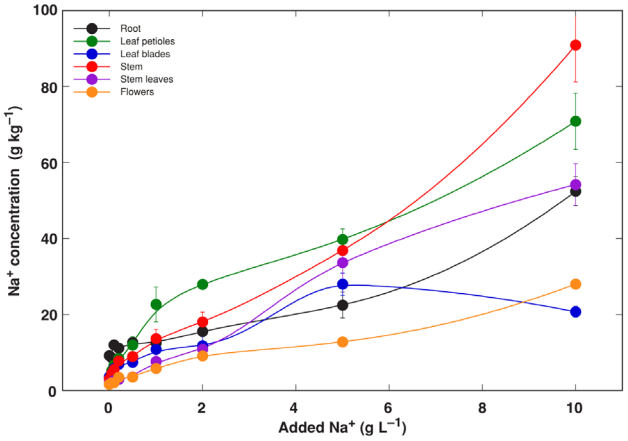

Fig. 3. Concentration of Na^+ in different parts of *Ranunculus sceleratus* plants grown at different substrate Na^+ concentrations for eight weeks.

In control plants, the highest Na^+ concentration was in roots, reaching 9.3 g kg^{-1}. In blades of rosette leaves it was 3.7 g kg^{-1}, followed by petioles of rosette leaves (3.3 g kg^{-1}), stems (3.1 g kg^{-1}), stem leaves (1.8 g kg^{-1}) and flowers (1.7 g kg^{-1}). Tissue Na^+ concentration increased in all plant parts with increasing substrate Na^+ concentration, but the character of the response was different for various parts (Fig. 3). Fastest increase of Na^+ concentration was seen for leaf petioles, followed by that for stem, stem leaves and roots. Na^+ concentration in leaf blades increased with increasing substrate Na^+ similar to that in roots up to 5 g L^{-1}, but decreased in plants treated with 10 g L^{-1} Na^+ to the level even lower than that in flowers, where there was the lowest Na^+ concentration in all other treatments. Maximum Na^+ concentration was found in stems of plants treated with 10 g kg^{-1}, reaching 90.8 g kg^{-1}.

K^+ concentration significantly increased in rosette leaf blades, stems, stem leaves and flowers in all Na^+ treatments up to 5 g L^{-1}, but remained relatively low in roots and rosette leaf petioles. Tissue EC increased gradually and relatively similarly in all plant parts with increasing substrate Na^+ concentration except in flowers and rosette leaf blades at the highest treatment rate. Similar to Na^+ concentration, EC was the highest in stems of plants treated with 10 g L^{-1} Na (378 mS cm^{-1}).

According to the previously defined criteria, *R. sceleratus* are characterized as highly electrolytophytic, Na^+ tolerant species, accumulating elevated concentration of both Na^+ (at very high level) and K^+ (at low to moderate level) in shoots and roots at susbtrate salinity characteristic for sea-affected wetland habitats. However, Na^+ was partially excluded from leaf blades at higher salinity, indicating presence of some control mechanism.

Acknowledgements

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Potential for electrolyte accumulation in tissues of a Na/K hyperaccumulator species from coastal drift lines, *Atriplex glabriuscula*, in controlled conditions

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Key words: *Atriplex glabriuscula*, electrical conductivity, K^+ concentration, Na^+ concentration.

Annual vegetation of drift lines (EUH code 1210) is of major interest for functional studies because of relative rarity and both floristic and environmental significance of this protected habitat. Spatial segregation and temporal instability of drift line communities in nature make them especially vulnerable and difficult to assess functionaly. The total area of this habitat in Latvia is as low as 26 ha, and it is the only place of existence for several rare plant species of special protection status (i.e., *Atriplex calotheca, Atriplex glabriuscula, Atriplex longipes*). During comparative analysis of Na^+ and K^+ concentration in species native to coastal habitats, it was found that *A. glabriuscula* is exceptional species in respect to average metal accumulation ability in shoots, with Na^+ concentration reaching 96 g kg^{-1} and that for K^+ 50 g kg^{-1} in plant leaves (Ievinsh et al. 2019). The aim of the present study was to assess Na^+ and K^+ accumulation ability in *A. glabriuscula* plants in controlled conditions, with emphasis on light intensity effects.

Several flowering *A. glabriuscula* plants were taken from a native coastal drift line habitat in the territory of Jūrmala city near estuary of river Lielupe, Latvia. Plants were transplanted in plastic containers and kept in greenhouse until seedlings were established sporadically from fallen seeds. Seedlings were transplanted to 200 mL plastic containers filled with commercial garden soil (Biolan, Finland) and quartz sand (1:1, v/v). Plants were cultivated in an experimental automated greenhouse (HortiMax, Netherlands) with supplemented light from Master SON-TPIA Green Power CG T 400 W (Philips, Netherlands) and Powerstar HQI-BT 400 W/D PRO (Osram, Germany) lamps (380 μmol m^{-2} s^{-1} at the plant level), 16 h photoperiod, day/night temperature 23/15 °C, relative air humidity 60 to 70%.

Two separate experiments were performed. In the first experiment, different regimes and concentration of NaCl were used for treatment of *A. glabriuscula* plants, resulting in increasing concentration of Na^+ in a substrate, reaching from 0.88 g kg^{-1} Na^+ in control up to 11.9 g kg^{-1} Na^+ in the highest treatment (Fig. 1A). In the second experiment, plants were subjected to different light intensity by placing containers with plants in larger containers of various height, resulting in 25, 50, 75 and 100% light intensity. Half of the plants in each light treatment were irrigated with 200 mM NaCl. Five plants per treatment were used.

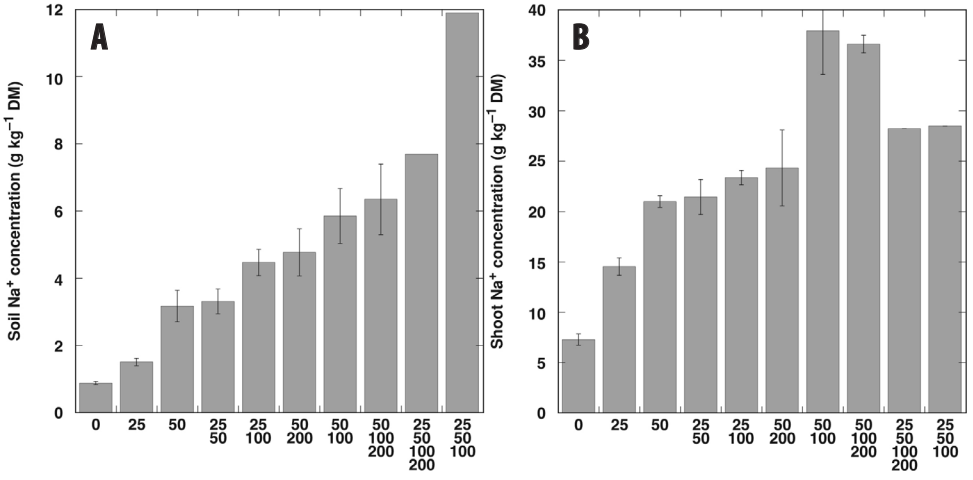

Fig. 1. Concentration of Na^+ in substrate (A) and shoot of *Atriplex glabriuscula* plants (B) under different treatment regimes with NaCl.

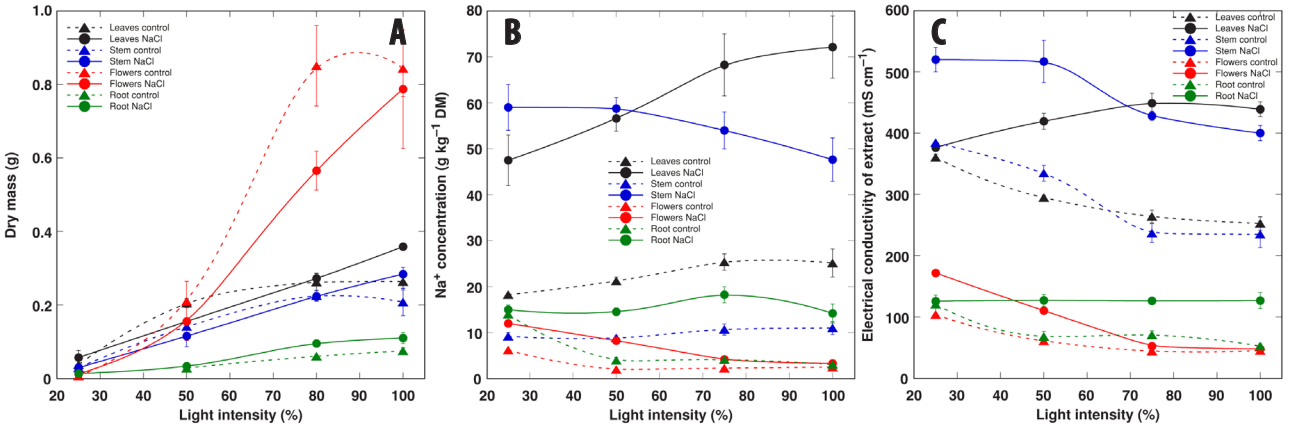

Fig. 2. Effect of light intensity and NaCl treatment on dry mass of different parts (A), concentration of Na^+ in different parts (B) and electrical conductivity in water extracts from different parts (C) of *Atriplex glabriuscula*.

Six weeks after the start of the treatment, the experiment was terminated and plants were separated in roots, leaves, stems, and flowers (including fruit). All parts were weighed and dried in a thermostat at 60 °C until a constant mass, then dry mass was measured. Tissues were homogenized by crushing to small pieces and a sample (0.2 g) was taken for analysis of electrical conductivity (EC), Na^+ concentration and K^+ concentration in water extract by LAQUAtwin compact meters (Samsone, Ievinsh 2018).

In the first experiment, Na^+-treated *A. glabriuscula* plants showed concentration-independent significant increase in shoot fresh and dry mass, but root mass was not significantly affected. Maximum shoot dry mass increase was 59% over control. Character of increase of Na^+ concentration in shoots due to increasing substrate Na^+ corresponded to a relationship typical for accumulating species (Fig. 1B). Similarly, root Na^+ concentration increased from 1.78 g kg^{-1} in control to 3.43 g kg^{-1} in the lowest Na^+ treatment, and further gradually increased up to 5.25 g kg^{-1} in the highest treatment.

In the second experiment, dry mass of leaves and stems of control plants linearly increased with increasing light intensity (Fig. 2A). Root mass increased relatively less, but the more pronounced increase was evident for mass of flowers. NaCl treatment had relatively low effect on plant growth, a significant increase was seen for flowers at 75% light intensity and decrease for leaves and stems at 100% light intensity.

A. glabriuscula plants grown at 100% light intensity in substrate with no additional Na^+ accumulated up to 20 g kg^{-1} Na^+ in leaves, which corresponds to their high Na^+ accumulation ability (Ievinsh et al. 2019). In stems, Na^+ concentration was 11 g kg^{-1}, but in flowers and roots only 2.5 and 4.1 g kg^{-1}, respectively (Fig. 2B). NaCl treatment resulted in a large increase of Na^+ accumulation in both leaves and stems, but less pronounced increase was evident for roots and flowers. Increasing light intensity stimulated NaCl-dependent Na^+ accumulation in leaves, but decreased in stems and flowers. K^+ concentration was negatively affected by increasing light intensity in control plants and was decreased by NaCl treatment in leaves and stems with no significant effect in flowers and roots. EC in tissue extracts of control plants was identical in leaves and stems as well as in roots and flowers, and was similarly affected by changes of light intensity (Fig. 2C). However, in NaCl-treated plants, EC was not affected by light intensity in flowers, increased by increasing intensity in leaves and decreased in stems and flowers. Absolute values of EC in shoots were extremely high, with the maximum level of 520 mS cm^{-1} in stems of plants at 25% light intensity. It seems that in *A. glabriuscula* plants tissue EC is tightly regulated by accumulation/exclusion of both Na^+ and K^+, and is under light control, confirming a special status of the species as an extreme coastal electrolytophyte.

Acknowledgements

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Alien species of legume family in the flora of Latvia

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Key words: alien species, flora, Leguminosae.

Legume family (Leguminosae Juss.) is one of the largest families in the vascular flora of Latvia with 24 genera and 110 species, 52 of them being alien species (Roze 2015).

During the study, Latvian Herbaria collections (LATV, RAS, SVR, LLU, LAAC, AB, VEINB, RIG, LDM, DAU), species lists of flora research routes of Laboratory of Botany (Institute of Biology, University of Latvia) were studied, as well as personal observations of field studies were used.

Alien species of legume family can be divided into four groups: trees and shrubs (nine species), ephemerophytes (22 species), rare species (19 species) and naturalized cultivated species (two species).

Trees and shrubs are only escaped cultivated plants, six of them are native in Europe and Asia (*Sarothamnus scoparius* (L.) Wimm. ex W.D.J. Koch, *Chamaecytisus ratisbonensis* (Schaeff.) Rothm., *Genista tinctoria* L., *Genista pilosa* L., *Caragana arborescens* Lam., *Caragana frutex* (L.) K. Koch) and three are native in North America (*Amorpha fruticosa* L., *Robinia pseudoacacia* L., *Robinia luxurians* (Dieck) C.K. Schneid.).

Ephemerophytes (*Astragalus filicaulis* Fisch. et C.A. Mey ex Kar. et Kir., *Trifolium angustifolium* L., *Trifolium retusum* L., *Trifolium resupinatum* L., *Trifolium lupinaster* L., *Melilotus dentatus* (Waldst. et Kit.) Pers., *Trigonella noëana* Boiss., *Medicago minima* (L.) Bartal., *Medicago arabica* (L.) Huds., *Medicago denticulata* Willd., *Vicia pisiformis* L., *Vicia dumetorum* L., *Vicia varia* Host., *Vicia grandiflora* Scop., *Vicia lutea* L., *Vicia pannonica* Crantz, *Lathyrus tingitanus* L., *Lathyrus hirsutus* L., *Lathyrus sativus* L., *Lathyrus pallescens* (M. Bieb.) K. Koch, *Lathyrus aphaca* L., *Lathyrus latifolius* L.) are a group of species each found only once, twice or three times in Latvia; mostly at landfills or at the edges of railways.

The group of rare species is the one who needs to pay attention to detect the prevalence trends in Latvia. These are adventive species or escaped cultivated species that are natural in Europe and Asia (*Lupinus luteus* L., *Lupinus angustifolius* L., *Glycine max* (L.) Merr., *Coronilla varia* L., *Ornithopus sativus* Brot., *Oxytropis pilosa* (L.) DC., *Astragalus cicer* L., *Galega orientalis* Lam., *Onobrychis viciifolia* Scop., *Trifolium pannonicum* Jacq., *Trifolium incarnatum* L., *Melilotus wolgicus* Poir., *Melilotus altissimus* Thuill., *Trigonella caerulea* (L.) Ser., *Medicago romanica* Prodán, *Medicago prostrata* Jacq., *Vicia faba* L., *Pisum arvense* L., *Pisum sativum* L.). At present, these species have no significant impact on natural habitats.

The most important group is naturalized cultivated species, which includes *Lupinus polyphyllus* Lindl. and *Medicago* × *varia* Martyn. Both are found throughout the territory of Latvia. *L. polyphyllus* expands intensively in unmanaged meadows and other open areas such as roadsides. After herbarium analysis and field observations it can be concluded that *M.* × *varia* displaces *Medicago falcata* L. from flora of Latvia. A similar situation was found in Lithuania (Gudžinskas 2018).

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