

Recent doctoral theses (biochemistry, biology, biophysics, botany, ecology and environmental science) in Lithuania

Prepared by Indrė LIPATOVA

EXTENDED INVESTIGATION OF LIPASES FOR BIOTECHNOLOGICAL APPLICATION (Biochemistry)

Vita Kiriliauskaitė

Scientific supervisor:

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Vilnius University

The dissertation defended:

17 March 2015

Due to their versatility, lipases are among the most investigated hydrolytic enzymes widely used for the synthesis of various compounds. Enzymatic synthesis has some great advantages, including mild reaction conditions, a high substrate specificity and eco-friendly processes. However, there are a variety of problems including a necessity of large quantities of enzymes and inhibition by the components of reaction mixture. In order to optimize enzymatic production, it is necessary to select an enzyme with suitable properties under various conditions. This study was carried out to investigate the possibilities of commercial lipases application for the synthesis of various esters. It was found that t-butanol is more suitable for the hydrolysis reactions as compared to transesterification. In order to increase the productivity of oil transesterification (biodiesel synthesis), various additives, new solvents, and different acyl acceptors are used. However, it was not an effective strategy for enhancing the productivity of the processes under studied conditions. Oleic acid esterification with trimethylolpropane was determined to be an effective way for the production of trimethylolpropane triesters. The yields of the products obtained under mild conditions show the potential to be applied for the industrial production of biolubricants. Coconut oil transesterification with terpenol – β -citronellol – is an effective strategy for the production of valuable esters. Depending on the structure of the organic acid, lipase can catalyze the esterification of dicarboxylic acids with various alcohols. The transesterification and esterification of soap manufacturing wastes – suds – is an effective way of utilization of these wastes.

IN VITRO RECONSTITUTION OF DNA INTERFERENCE IN A TYPE I CRISPR-CAS SYSTEM (Biochemistry)

Tomas Šinkūnas

Scientific supervisor:

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The dissertation defended:

19 March 2015

The Cascade complex together with the signature protein, Cas3, of the type I CRISPR-Cas system confers resistance against the target DNA; however, the molecular mechanism of DNA interference stage remained to be elucidated. Therefore, the main objective of this study was to establish the mechanistic details of the DNA interference stage in type I CRISPR-Cas systems. Type I-E CRISPR4-Cas from *Streptococcus thermophilus* DGCC7710 strain was used as a model system. In this work, we for the first time provide the biochemical characterization of the Cas3 protein that is a hallmark protein for type I systems. We show here that Cas3 combines both an ssDNA nuclease and ATPase/helicase catalytic activities. We isolated and established the molecular composition of the *S. thermophilus* Cascade complex and demonstrated that it binds DNA targets containing promiscuous PAM sequence. Furthermore, we provide the first experimental evidence for the R-loop formation by a single Cascade molecule that revealed the locking step of the stable R-loops. Last but not least, we show the Cascade-triggered directional degradation of the DNA target by the Cas3 protein. Summarising our results, we propose a detailed mechanism of DNA interference stage for the type I CRISPR-Cas systems.

TOPOGRAPHY AND PECULIARITIES OF STRUCTURAL ORGANIZATION OF RABBIT INTRINSIC CARDIAC NERVE SYSTEM (Biology)

Ligita Gukauskienė

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The dissertation defended:

9 February 2015

The rabbit is very popular experimental animal model used for treatment of the heart disease and developing of the new pharmaceutical and invasive cardiac disease methods. The aim of the present study was to investigate the topography and structural organization of the rabbit intrinsic cardiac nerve plexus and ganglia. These dates were received using AChE histochemical and immunofluorescence methods. The goals of this study were: (1) to determine the locations of the accessing nerves into the heart hilum; (2) to examine the distribution of the nerve ganglia; (3) to investigate the distribution of the epicardial nerve plexus; (4) to evaluate the variability of the epicardial nerve plexus; (5) to examine the structural organization of the cardiac ganglia, i. e. the number of the neurons and age-related changes of

the neuronal number; (6) to estimate qualitatively and quantitatively the neurochemical phenotype of the somata of the intrinsic cardiac neurons using the choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), neuronal nitric oxide synthase (nNOS) markers; and (7) to evaluate qualitatively the neurochemical phenotype of the nerve fibers of the cardiac nerve plexus using substance P (SP) and TH, ChAT, TH, nNOS markers. This dissertation gives information about the morphology, topography, and neurochemical phenotype of the rabbit cardiac neuronal structures. These data will be used for treatment of the heart diseases and developing of the new pharmaceutical and invasive cardiac disease methods as well as the autonomic nervous system that influences the formation of the heart diseases.

PHYSIOLOGICAL-BIOCHEMICAL PECULIARITIES OF OILSEED RAPE (*Brassica napus* L.) COLD ACCLIMATION (Biology)

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The dissertation defended:

27 March 2015

The dissertation is devoted to the investigations of the physiological-biochemical mechanisms of plant cold acclimation and preparation for the wintering period. Using winter oilseed rape cultivars different for their wintering resistance, 'Valesca' (more resistant) and 'Casino' (less resistant), changes in the composition of easily (ESP) and heavily (HSP) soluble proteins, indole-3-acetic acid (IAA) amount and its status in organs important for wintering – terminal bud and root collum – were analyzed during the autumn growth period and in various periods of cold acclimation. Peculiarities of reorganization of proteins ESP and HSP fractions, polypeptides and dehydrins composition in the investigated organs during the cold acclimation process and transition to dormancy were revealed. In order to elucidate the role of IAA for the cold acclimation and wintering resistance process, auxin physiological analogues TA-12 and TA-14 were applied. During the cold acclimation process, the amount of free – physiologically active form of IAA is decreasing, while the amount of IAA conjugates (IAA-amides and IAA-esters) is increasing. After TA-12 and TA-14, the significance of protein composition and IAA amount and status in oilseed rapeseed organs transiting to dormancy was confirmed. A conclusion is made that composition of proteins, the IAA amount and status are important biochemical factors determining plant (oilseed rapeseed) cold acclimation and preparation for the wintering period.

COLD STRESS INDUCED SEX-SPECIFIC PHYSIOLOGICAL RESPONSES (Biology)

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The dissertation defended:

28 April 2015

It has been suggested that one of the factors that affects responses to thermal stress is sex. Women experience a faster core cooling rate than men during cold water immersion; however, it is not clear if faster core cooling would induce differences at neuroendocrine responses, motor and cognitive performance between men and women. Furthermore, it was established that when core temperature was above 36 °C there were no differences in physiological adjustment strategies to cold stress in men and women; however, it is not clear if more extreme core cooling induces differences in responses between men and women. Thus, the aim of the research was to identify if there were any sex-specific differences in neuroendocrine responses, physiological adjustments to cold stress strategies, cognitive performance, and motor performance due to the whole body immersion at cold water-induced stress. The present study indicated that despite a similar level of cold stress in men and women, men exhibited a greater neuroendocrine response of the hypothalamic-pituitary-adrenal (cortisol) axis and sympathetic-adrenomedullary (epinephrine) axis, greater perturbations in the cognitive function, decrease in brief maximal voluntary contraction (MVC), and sustained 2-min MVC fatigability. Besides, physiological adjustments to cold stress also differed: women were more reliant on the insulative response, and men were more reliant on the metabolic response, particularly shivering. Thus, whole body cooling induced sex-specific differences in physiological responses.

THE EFFECT OF SPECIFICITY OF PHYSICAL TRAINING ON PULMONARY GAS EXCHANGE AND EMG DURING VARIOUS INTENSITY RUNNING (Biology)

Sandra Kilikevičienė

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Lithuanian Sports University

The dissertation defended:

19 June 2015

The indexes of aerobic capacity (maximal oxygen uptake, VO_{2max}), ventilatory thresholds (VT), and oxygen uptake (VO_2) kinetics are major determinants of physical performance and tightly related to the health state. The most efficient way to improve these indexes is exercise training; however, optimal capacity, intensity and type of training are still not unraveled. Therefore, additional studies are required. The aim of the research was to determine the effect

of various exercise trainings on indexes of pulmonary gas exchange and electromyography during the distinct intensity treadmill loads. The results of this study showed that the aerobic capacity and VO_2 kinetics of sprint-power athletes were affected by power endurance and high intensity power training. Two months of moderate intensity interval running training improved ventilatory thresholds, however had no effect on the speed of VO_2 kinetics. The endurance athletes possessed higher indexes of aerobic capacity and faster VO_2 kinetics than sprinters and untrained individuals. The most of these indexes are comparable between sprinters and untrained individuals.

AN APPLICATION OF THE CONCEPTION OF CENTROID BIASES IN EXPLANATION OF GEOMETRICAL ILLUSIONS (Biology)

Gitana Kadžienė

Scientific supervisor:

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The dissertation defended:

22 June 2015

Experimental investigations of geometrical optical illusions appear to be useful for better understanding the neuro-physiological algorithm of visual information processing because the fact of illusions emergence suggests the imperfectness of the corresponding neural mechanisms and their impossibility to perform correctly the visual tasks in some specific observation conditions. Therefore, a comprehensive illusions study can narrow considerably the range of possible theoretical interpretations and may be helpful for further investigations of the most general principles of the visual perception. In the present study, new illusory stimuli, which were never used before in psychophysical experiments, have been suggested, and new experimental data have been collected. A new experimental method of rotation of distracters around the terminators of stimuli has been proposed for verification of the conception of centroid biases. The mathematical model has been improved in order to provide quantitative estimation of the influence of observer's gaze fixation strategies on the illusion manifestation. A quantitative approximation of experimental curves by the model functions has been performed.

INVESTIGATION OF THE MECHANISMS OF ISCHEMIA-INDUCED ELECTRICAL SIGNAL ALTERNANS (Biology)

Rūta Vosyliūtė

Scientific supervisor:

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The dissertation defended:

30 June 2015

The aim of the study was to investigate the mechanisms of ischemia-induced electrical signal alternans using optical and electrical signal registration simultaneously and to evaluate information of the registered optical signal using voltage sensitive dyes. During this work, we studied high-intensity illumination effects on electrical signals and the effect on myocardium of newly synthesized voltage sensitive fluorescent dye (di-4-ANBDQBS). The application of the dyes in the clinical level is a very important step; therefore, experiments have provided new information about dyes and their use in cardiac electrophysiological studies. In other experiments, the complex of two methods was used at the same time: optical mapping and transmurally microelectrode recordings. The simultaneous recordings of a transmural action potential and optical action potential allowed us to determine the probing-depth constant of the fluorescence measurement, which evaluates the distribution of both excitation and emission lights in the myocardium, during each experiment. When using the calculated transmural AP upstroke, we were able to split the optical action potential (OAP) upstroke into two components: depth weighted transmural action potential (DWTAP) and lateral scattering (LSCATT). Experiments with rabbit hearts allowed us to investigate the development of the spatiotemporal pattern of action potential alternans during regional ischemia and prove that alternation is a consequence of 2:1 conduction blocks.

EFFECTS OF SEVOFLURANE VERSUS PROPOFOL ON CARDIAC MITOCHONDRIAL FUNCTIONAL ACTIVITY AND CLINICAL PARAMETERS AFTER HEART SURGERY WITH CARDIOPULMONARY BYPASS (Biology)

Aida Kinderytė

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The dissertation defended:

31 August 2015

The coronary artery bypass grafting surgery is now commonly used as a method for coronary insufficiency treatment. Myocardial dysfunction after coronary bypass graft surgery (CABG) is a well-known phenomenon called “ischemia/reperfusion injury”, which may significantly affect postoperative prognosis. There is increasing evidence that mitochondrial dysfunction plays a central role. We have investigated the effect of sevoflurane vs. propofol on cardiac

mitochondrial functional activity and clinical parameters after heart surgery with cardiopulmonary bypass (CPB). This perspective study enrolled 72 patients (36 to sevoflurane and 36 to propofol group) scheduled for the first-time elective CABG surgery with CPB. After ischemia/reperfusion, mitochondrial respiratory rate decreased in both groups. Ischemia/reperfusion caused the increasing damage of inner and outer mitochondrial membrane in both anesthetics groups. The damage of outer mitochondrial membrane caused the loss of cytochrome c, and the damage of inner mitochondrial membrane caused an increasing permeability to ions, without statistical differences between anesthetics groups. Ischemia/reperfusion induced a significantly lower heart muscle damage in sevoflurane group than in propofol group, as TnI level was significantly lower in sevoflurane group compared with propofol group. Hemodynamic stability, mechanical ventilation, and stay of length in the intensive care unit during the early post-operative period in two anesthetic groups did not differ.

EFFECTS OF AGE, SEX, MYOSTATIN AND MUSCLE REGENERATION ON CONTRACTION-INDUCED PRIMARY DAMAGE OF SKELETAL MUSCLE (Biology)

Juozas Baltušnikas

Scientific supervisor:

Doc. Dr. Tomas Venckūnas,
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The dissertation defended:

8 September 2015

The aim of the study was to investigate how muscle creatine kinase (CK) efflux in response to contraction-induced primary damage is dependent on age, sex, myostatin dysfunction, and muscle regeneration. A better understanding of the mechanisms of muscle damage is important in sports practice and could also be useful in treating muscular dystrophies. By using activity of creatine kinase released from isolated mouse muscle as an indicator of primary muscle damage in all our experiments, we made the following conclusions: (1) Eccentric contractions induce the largest mice primary damage of soleus muscle when compared with passive stretches or isometric contractions. (2) Primary damage of soleus muscle after eccentric contractions is smaller in females compared with males and smaller in female adult mice compared with female young mice. (3) Myostatin dysfunction increases susceptibility of eccentric contraction-induced primary damage of soleus, but not EDL muscle. (4) Regenerated muscle is much more resistant to eccentric contraction-induced primary damage.

INHERITED PHENOTYPIC INSTABILITY OF BARLEY HOMEOTIC SINGLE AND DOUBLE MUTANTS AND ITS POSSIBLE CAUSES (Biology)

Raimondas Šiukšta

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The dissertation defended:

2 December 2015

This investigation is dedicated to evaluate the effects of ethylene, synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), and auxin inhibitors HFCA and PCIB on the flower/inflorescence development of barley double mutants Hv-tweaky spike 2; Hooded/Kap1 (Hv-tw2;Hd) that are characterized by inherited phenotypic instability and by several new features, such as leaf-like structures, long naked gaps in the spike, and variations in the basic and ectopic flowers, which are absent in parental mutants. Experimental data showed that exogenous ethylene induces the partial normalization of the awn development and its effect is genetic background-dependent. Furthermore, plant treatment with 2,4-D causes the partial normalization of the spike and ectopic flower structures, while the opposite effect was noted for both auxin inhibitors. On the ground of the modifications of phenotypic instability features caused by the auxin inhibitors and 2,4-D, auxin imbalance has been described as a cause of phenotypic instability for the first time; moreover, barley Hv-tweaky-type mutations were demonstrated to be associated with defects in the auxin function. Preliminary results of the differential display analysis showed that the mutant Hv-tw2 differs from WT in the expression of several cDNA fragments that are homologous to the genes participating in epigenetic regulation, stress responses, and flower development and that may be related to phenotypic instability induction in barley double mutants.

PRODUCTION OF DIFFERENT B-GLUCAN PREPARATIONS AND INVESTIGATION OF THEIR IMPACT ON THE IMMUNE SYSTEM COMPONENTS *IN VITRO* AND *IN VIVO* (Biology)

Artur Javmen

Scientific supervisor:

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State Research Institute
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The dissertation defended:

14 December 2015

β -Glucan is one of the most abundant polymers in nature. It is produced by a variety of different organisms – bacteria, fungi, and plants. It is determined that β -glucan has a notable physiological effect on mammalian immune systems, can activate the immune response via immune cells, and trigger the immune response. Thus, β -glucan can be potentially used in the treatment of the different infections and can possibly increase immune cell cytotoxicity for the tumour cells. The immune-stimulating activities of β -glucans

can depend on their physicochemical parameters, such as their solubility in water and molecular weight. It is believed that β -glucan in future can be used as a safe, effective, therapeutic or prophylactic agent, either alone or as adjuvant, to enhance or prime the immune response in mammals with a normal or decreased immunological function. *Saccharomyces cerevisiae* baker's yeast is a frequently used source of β -glucan. Recent studies have shown that yeast cell wall β -glucan can exhibit antimicrobial and antitumor effects. The aim of the dissertation work was to investigate the effect of different β -glucan preparations on the components of the mammal's immune system. During the recent research project, it was investigated how different β -glucan preparations affect proliferation and phagocytosis of the BALB/c macrophages and dendritic cells, cytokine synthesis in these leukocytes and maturation of the dendritic cells *in vitro*. Also, it was determined how different β -glucan preparations affect IFN γ synthesis *in vivo* in BALB/c mice. In addition, it was examined how β -glucan and blood leukocytes affect viability of the MH22a hepatoma cells. Finally, the author tested if β -glucan can induce acute toxicity in mammals.

THE DIVERSITY OF FLEA (SIPHONAPTERA) SPECIES AND FLEAR-BORNE PATHOGENS (Biology)

Indrė Lipatova

Scientific supervisor:

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The dissertation defended:

21 December 2015

Fleas (Insecta: Siphonaptera) are common ectoparasites of mammals and birds in different habitats throughout the world. The Lithuanian flea fauna has not been exhaustively researched, and the real situation is not known as compared with other world regions. The flea species described so far in Lithuania do not include all Siphonaptera fauna that is possible in Lithuania. The aim of the dissertation was to investigate the diversity of flea species on different hosts and habitats and flea-borne pathogens. The obtained results of the conducted research allowed to assess the diversity of flea species in Lithuania and to supplement the list of Lithuanian Siphonaptera. The results of the dissertation are as follows: seven flea species new for the Lithuanian flea fauna were determined: *Hystrichopsylla orientalis*, *Ceratophyllus gallinae*, *C. affinis*, *C. rusticus*, *C. hirundinis*, *C. farreni*, *C. styx*. For the first time in Lithuania, *Bartonella grahamii*, *B. taylorii*, *B. rochalimae*, and *Rickettsia helvetica* pathogens were identified in fleas of small mammals.

GENETIC DIVERSITY OF INVASIVE POPULATIONS OF HIMALAYAN BALSAM (*IMPATIENS GLANDULIFERA ROYLE*) (Biology)

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Scientific supervisor:

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The dissertation defended:

21 December 2015

Impatiens glandulifera Royle (Himalayan balsam, *Balsaminaceae* family) is native in a small district of the Himalayan mountains and invasive in almost all Northern hemisphere. The present study aimed at investigating genetic diversity of *I. glandulifera* populations of Lithuania and other countries within the invasive range. Twenty populations from Lithuania (400 individuals) and 47 from Europe and other continent countries (700 individuals) were analyzed according to the complex of dominant and codominant markers. A comparison of genetic diversity of two *Impatiens* species showed that according to the country a bigger differentiation was documented between populations of invasive species (*I. glandulifera*) when compared to the populations of native species (*I. noli-tangere*). For both sets of *I. glandulifera* populations – from Lithuania and from Europe and other countries – genetic differentiation was high, while diversity was low. Bayesian computation subdivided individuals of populations into 3 genetic clusters. No correlations were found between genetic and geographical distances or habitat features of populations. It is difficult to relate geographic distances with genetic evolution of *I. glandulifera* populations within the invasive range due to multidirectional, unequal frequency erratic spread by natural ways (autochory or hydrochory) and human-mediated intentional and unintentional dispersal. Our results might indicate multiple introduction of this species within the invasive range.

EFFECTS OF STIMULUS CONTEXT AND BEHAVIORAL CONTEXT ON INFORMATION PROCESSING IN THE EARLY VISUAL SYSTEM OF THE MOUSE (Biophysics)

Agnė Vaičeliūnaite

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The dissertation defended:

31 March 2015

A lot of contemporary neuroscience research focuses on the mechanisms by which cortical neurons obtain their well-known response properties. There is a common agreement that multiple mechanisms can play a role, but we do not yet understand their relative contributions. The aim of this thesis is to investigate effects of stimulus context and behavioral context on information processing in the early visual system of the mouse. In this work, we show how visual responses are changed by internal context as a surround suppression

and external context as a brain state. As a brain state here we focus not only on how anesthesia and awake states influence visual processing, but also divide the awake state into stationary and locomotion. To investigate these issues, we used *in-vivo* extracellular recordings and the circuit disruption method (optogenetics) in mice. We noticed that there is a laminar profile of spatial integration (SI) in mouse primary visual cortex: the receptive field (RF) center size is the smallest and the surround suppression (SS) is the strongest in superficial layers. Also, the strength of SS increases over time. Under anesthesia, the laminar specificity of SI is decreased, the strength of SS is reduced, and RF size is increased. Activation of parvalbumin cells also increases the size of pyramidal cells' RF and decreases SS strength. We are the first to show that locomotion-based response enhancements occur already at the level of the thalamus. Finally, we showed that pupil size increases with locomotion speed.

VOLTAGE SENSITIVE GATING OF CONNEXIN BASED GAP JUNCTION CHANNELS: MODELING AND EXPERIMENTAL STUDIES (Biophysics)

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The dissertation defended:

25 August 2015

Connexins (Cx) are proteins forming gap junctions between adjacent cells allowing contacting cells transfer electric and metabolic signals. Gap junctions (GJ) play an important role in different biological processes like development, differentiation, cell synchronization, neuron activity, and immune response. Mutations in GJ are related with different skin diseases, deafness diseases, and development anomalies. Each GJ channel is composed of two hemichannels each of which contains fast and slow gates sensitive to transjunctional voltage. The aim of this work was to create GJ channel models for biophysical properties research and verify their adequacy using physical experimental data. The model described in this work evaluated transjunctional voltage distribution, fast and slow gate mechanisms and its rectifications. Developed model applications allow to explore GJ channels in a single channel level (stochastic model), in dynamics (stochastic and dynamic models), and in steady state (discrete and continuous time Markov chain models). Each model was verified in real experimental conditions by registering intercellular interaction in HeLa cells, expressing different Cx isoforms, under normal and pathological conditions and using global optimization algorithms for model parameter estimation.

APPLIANCE OF MULTIVARIATE ANALYSIS METHODS FOR BIOMEDICAL IMAGE AND SIGNAL ANALYSIS AND EVALUATION (Biophysics)

Robertas Petrolis

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The dissertation defended:

9 December 2015

The aim of the dissertation was the development of methods based on biophysical models for diagnostically important feature extraction from biomedical images and signals and estimation of the possibilities and revealing the peculiarities of usage of multivariate analysis methods for quantitative evaluation of extracted diagnostically important features. Objectives: (1) To investigate the possibilities and peculiarities of multivariate analysis methods usage for quantitative evaluation of diagnostically important features. (2) To develop a method based on biophysics models and multivariate analysis for quasiperiodic biomedical signal quantitative evaluation. (3) To develop a method based on multivariate analysis for extraction of diagnostically important features from biomedical images and their evaluation. The scientific novelty and practical usefulness lies in developing methods based on biophysics models and multivariate analysis principles enable exposure of analyzed biological system operating principles, disorder pathogenesis and its feature, extracted from biomedical images and signals, variation origin.

WOMEN'S OF HEALTHY AND REPRODUCTIVE AGE REACTION TO EFFECTIVE IMAGES: RESEARCHES ON SUBJECTIVE EVALUATIONS, CENTRAL AND AUTONOMIC NERVOUS SYSTEM (Biophysics)

Laura Mačiukaitė

Scientific supervisor:

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The dissertation defended:

28 December 2015

The aim of the dissertation study was to investigate the influence on the ratings of affective images based on the levels of healthy women's sex steroid hormone and to compare the responses of central and autonomic nervous systems. The present dissertation is based on three studies. Based on the results of the first study, it was concluded that the International Affective Picture System (IAPS) can be used for emotion studies in Lithuanian female. The second study demonstrated a significant positive relationship between progesterone level and valence ratings of unpleasant images. The third study showed that the menstrual cycle phase is a significant factor for the subjective rating of affective images. Unpleasant images are more arousing than pleasant ones for women in the luteal phase. The correlation analysis confirms that women sensitivity to pleasant and unpleasant

stimuli in the luteal phase could be influenced by progesterone levels. The mean late positive potential amplitude (LPP) depends on the content of affective stimulus, but does not depend on the levels of sex steroid hormone. Phasic heart rate response depends on the content of affective stimulus, but does not depend on the levels of sex steroid hormone. In conclusion, the findings indicate the effect of the menstrual cycle phase on subjective affective image ratings in healthy women reporting a regular menstrual cycle.

CYANOTOXINS AND THEIR ACCUMULATION IN THE CURONIAN LAGOON (Ecology and environmental science)

Aistė Paldavičienė

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The dissertation defended:

29 May 2015

Certain cyanobacteria form nuisance blooms due to their dense occurrence in open water or in coastal areas close to beaches. The first reports on algal blooms in the basin of the Baltic Sea have been known since the nineteenth century. The Curonian Lagoon, the largest coastal lagoon in Europe, is mostly a freshwater body where autotrophic plankton communities are mainly dominated by cyanobacteria, plagued by such eutrophication-related issues as heavy planktonic blooms and hypoxia. The study aims to assess the hepatotoxins produced by cyanobacteria and their accumulation in mussels, fish and bottom sediments in the Curonian Lagoon. For the first time cyanobacterial secondary metabolites in the Curonian Lagoon were detected. The concentrations of hepatotoxins determined in seston exceeded the World Health Organization's guideline value of $1 \mu\text{g l}^{-1}$ for drinking. MC-LR is the most abundant toxin, the concentrations of which exceeded the normative value for recreational waters ($20 \mu\text{g l}^{-1}$). MC-LY, one of the rare but highly toxic variants, was detected, which could make harm to hydrobionts and humans. The presence of nodularin was recorded in the Curonian Lagoon for the first time. This is the first study that reports hepatotoxin accumulation in mussels and fish. The study results point towards cyanotoxins resuspension as a possible mechanism of secondary contamination in the lagoon.

THE IMPACT OF PROJECTED CLIMATE CHANGE AND DIFFERENT NUTRIENT SUPPLY TO AGRICULTURAL PLANTS AND WEEDS (Ecology and environmental science)

Austra Dikšaitytė

Scientific supervisor:

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The dissertation defended:

26 June 2015

Investigations in the controlled environment plant growth chambers showed that agricultural plants and weeds respond differently to elevated atmospheric. Under elevated atmospheric CO₂, the stomatal conductance and the transpiration rate of agricultural plants decreased more, water use efficiency increased more, and the photosynthesis and growth of agricultural plants were stimulated more than for weeds. When both groups of plants photosynthetically acclimated to elevated CO₂, the initial stimulation of photosynthetic rate for weeds decreased significantly more than for agricultural plants. Under elevated atmospheric CO₂, concentration of non-structural carbohydrates in the leaves of weeds considerably increased, and this increase was very closely related to the down-regulation of photosynthesis. It confirms that the capacity of weeds to re-locate synthesized assimilates to the roots is lower, and weeds respond to rising CO₂ less than agricultural plants do. Increase of air temperature by projected 4 °C had no significant effect on the growth of investigated plants. However, positive interaction between elevated CO₂ and air temperature led to the improved growth of all plants, especially weeds, as compared with the single impact of analogous CO₂ concentration. In simulated warmer climate conditions, the efficiency of fertilizers for both groups of plants decreased. Under additional nutrients supply, the capacity of weed roots to store assimilates increased more than that of agricultural plants therefore, their photosynthetic acclimation losses decreased and the difference in the response of agricultural plants and weeds to the warming climate decreased.

DESIGN AND APPLICATIONS OF CONTACTLESS CAPILLARY FORMAT DETECTORS FOR CHEMICAL AND ENVIRONMENTAL ANALYSIS (Ecology and environmental science)

Tomas Drevinskas

Scientific supervisor:

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The dissertation defended:

25 August 2015

The obvious trends in the development of current analytical instruments for environmental and chemical analyses and bioanalysis are miniaturization and integration. Miniaturized techniques improve performance, throughput and reliability. Many of real samples, particularly in environmental analysis or bioanalysis are very complex and for qualitative and quantitative assessment have to be separated prior to measurement. Capillary electrophoresis and related micro-separation techniques are frequently used in environmental, biological and many other fields of analysis. It can be miniaturized and automated. Coupling microseparation technique to the miniaturized detection technique leads to the design of perspective instrumentation, which can be applied for environmental analysis. One of the perspective and universal detection techniques capable of detecting various compounds in environmental analysis is contactless conductivity (impedance) detection. The aim of this work was to design a miniature, potentially field-portable detector, compatible with capillary format microseparations, suitable for environmental and other types of analyses, and based on the measurement of complex impedance. The developed system is portable, of miniature size, uses wireless data transmission and provides high performance detection characteristics. The detector can be used in the field or on a stand-alone rover. Distant experiments can be performed without leaving the laboratory. In addition, experiments can be performed in different environmental sites, including environmentally savage sites. Obtained evaluation and application data can be used as a guideline for designing similar and more advanced miniaturized detection systems.

INTEGRATED ASSESSMENT OF ECOLOGICAL, ECONOMIC AND SOCIO-CULTURAL RESOURCES FOR THE SUSTAINABLE DEVELOPMENT OF THE LITHUANIAN BALTIC SEA COAST (Ecology and environmental science)

Daniel Depellegrin

Scientific supervisor:

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The dissertation defended:

17 September 2015

The objective of this study is to identify marine ES of the Lithuanian coastal zone, assess its environmental and socioeconomic importance through application of spatial integrated assessment models, test impacts from present and future anthropogenic activities related to offshore wind energy, and deliver recommendations for planning and decision making. The study provides the first comprehensive methodological framework to assess the biological and socioeconomic baseline for MES in the Lithuanian coastal zone. The study area was the Lithuanian coastal stripe (532 km²) bordering the seawater up to the 20-m isobaths in the west and 300 m inland, including the entire terrestrial part of the coastal stripe in the east. The extension of coastline is 98 km. Damage costs from oil spill to vulnerable resources were simulated using oil spill scenarios developed with the Baltic Sea's Particle Tracking Module Sea Track Web with the following parameters: oil spill of 17,000 tons for light, medium and heavy oil on the Lithuanian mainland coast, wind direction NW to SW, wind speed 6 m/s, and current speed of 1 knot. The overall oil spill sensitivity shows that coastal areas of 25 km from Nida to Juodkrantė and of 12 km between Palanga and Šventoji are of high sensitivity. Results from visual impact assessment evidence that the geomorphological features, such as beaches, dune ridges, cliffs, and pine tree forests, function as a natural visibility barrier contributing to the landscape carrying capacity.

HERBIVOROUS INSECTS INTERACTION WITH ENTOMOPATHOGENIC FUNGI (BASED ON SPECIES OF HYMENOPTERA AND LEPIDOPTERA ORDERS) (Ecology and environmental science)

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The dissertation defended:

9 October 2015

Lepidoptera and Hymenoptera are the major orders of pests. Chemical pest control is still the predominant type of pest control today. One of alternatives to chemical insecticides is the use of biological preparations based on entomopathogenic fungi. The aim of the research was to determine the diversity of entomopathogenic fungi in the natural population of conifer defoliators, to establish sensitivity of these insects to entomopathogenic fungi and to test insect ability

to recognise fungus-infected substrates. The results based on the model species from Hymenoptera and Lepidoptera orders expanded the knowledge of economically important conifer pests (*Bupalus piniaria*, *Dendrolimus pini*, *Diprion pini*), their ecology and interfaces with the pathogenic fungi complex. Also, insect (*Plodia interpunctella*) chemoreception on fungus-infected and fungus-uninfected substrates was estimated. For the first time it was shown that an insect not only distinguishes the substrate for egg laying, but also evaluates its quality. It was found for the first time that 4-oxoisophorone can affect mated *Plodia interpunctella* females as a repellent and 3-methyl-1-butanol acts both as a repellent (in high doses) and as an attractant (in lower doses). It was proved for the first time that 3-methyl-1-butanol can be a marker to recognize fungus-infected substrates.

BIOLOGY AND PECULIARITIES OF THE POPULATION-GENETIC STRUCTURE OF LITHUANIAN CEPHALASPIDOMORPHS (CEPHALASPIDOMORPHI) (Ecology and environmental science)

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The dissertation defended:

23 October 2015

Typically, studies of ichthyocenoses in Lithuania are being carried out to investigate the distribution, abundance and conservation status of bony fish communities. Cephalaspidomorphs as a group possess a distinctive life cycle. Therefore, researches into lampreys in Lithuania are fragmented, and knowledge of species diversity, distribution, abundance and status of lamprey populations in Lithuania is poor. The present thesis reviews the distribution and abundance of *Lampetra fluviatilis* (Linnaeus, 1758) and *Lampetra planeri* (Bloch, 1784) in Lithuanian rivers, as well as the factors that affect dispersion and abundance of the larval stage of lampreys. By applying a molecular approach to identification of lamprey species, representatives of the Ukrainian lamprey *Eudontomyzon mariae* (Berg, 1931) were discovered inhabiting several rivers in Lithuania for the first time. A phylogenetic analysis based on mtDNA markers revealed that *L. fluviatilis* and *L. planeri* belong to the same evolutionary lineage. Additionally, the findings suggest that the Baltic Sea *Lampetra* spp. populations might experience the gene flow between two life strategies, which is supported by a successful *in situ* experimental hybridisation between *L. fluviatilis* and *L. planeri* spawning in communal redds. These findings are essential to the understanding of the population genetic structure of *L. fluviatilis* and *L. planeri* and contribute to the current knowledge of the speciation mechanism. From

a practical point of view, the findings can be used in lamprey conservation. The current lamprey conservation status is assessed, and recommendations for further lamprey research and monitoring in Lithuania are given.

**ALLELOPATHIC IMPACT OF DOMINANT SPECIES OF
PINETUM-VACCINIOSUM CLEAR-CUTS ON SCOTS PINE SEEDLING GROWTH
UNDER CHANGING CLIMATE CONDITIONS (Ecology and environmental science)**

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The dissertation defended:

20 November 2015

For the first time the allelopathic effect of dominant plant species of different-age clear-cuts of pine forests (*Vaccinia-Pinetum*) on Scots pine regeneration (seed germination, seedling growth, and initial seedling photosynthetic pigments parameters) in different environmental conditions was estimated. The findings of this dissertation demonstrate that increasing temperature reinforces the plant-donor allelochemical effect; consequently, it reduces Scots pine seed germination and inhibits seedling morphophysiological parameters. Compensatory effect of higher CO₂ concentration reduced the negative impact of increasing temperature. Therefore, the negative allelochemical impact of plant-donor extracts on Scots pine seed germination and seedling growth of plant-donor extracts decreased. It was found that not only dominant species had different responses to changing environmental conditions, but the activity of identified allelochemicals in plants also was changing. No less important is the fact that the adaptive capacity of the Scots pine as a plant-acceptor strongly decreases with high increase in environmental temperature and CO₂ concentration. These factors can reduce Scots pine resistance to the effects of dominant species and affect the migration of Scots pine habitats to more favourable environmental conditions.

HAZARDOUS SUBSTANCES DISTRIBUTION AND GEOCHEMICAL ANOMALIES IN THE SURFACE SEDIMENTS OF A HEAVILY MODIFIED WATER BODY (Ecology and environmental science)

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The dissertation defended:

26 November 2015

Shallow water zones of the south-eastern Baltic Sea, including semi-closed bays (lagoons), are zones of high bio-productivity, recreation potential and perspective for the mass development of tourism. The fragile environment is threatened by the intensive shipping activities, operations of ports, wastewater discharges from coastal cities and constant input of contaminants with waters of the Nemunas basin rivers. The Klaipėda Strait is an inlet connecting the freshwater Curonian Lagoon with the Baltic Sea. Due to the physical alterations by human activity, this object was substantially changed in character and recently was designated as a “heavily modified” water body, which cannot meet “good ecological status”. Even though contamination of this water body is relevant not only for the protection of the natural environment, but also for the implementation of economic activity, apart from several scarce studies addressing the geochemical issues, there was no extensive scientific research which could distinguish priority hazardous substances and evaluate the geochemical state of the heavily modified water body stressed by the joint impact of natural processes and anthropogenic activities. Considering constantly changing sedimentary environment, intensifying activities of port enterprises and a variety of potential pollution sources, this study clarifies the peculiarities of some hazardous substances distribution in the surface sediments, identifies groups of priority hazardous substances and distinguishes geochemical anomalies.

GEOLOGICAL STRUCTURE AND SPATIAL DISTRIBUTION OF PALEO-INCISIONS IN THE SOUTHEASTERN PART OF THE BALTIC SEA AND ADJACENT LAND (Ecology and environmental science)

Dmitrij Gerok

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The dissertation defended:

26 November 2015

A detailed analysis of the geological structure was carried out applying modern digital technique for interpretation of seismo-acoustic records in the southeastern part of the Baltic Sea and adjacent land. The work is focused on the palaeo-incisions. These geological objects are important from a geoecological point of view – palaeo-incisions could be associated with “hydro-geological windows” between different aquifers. The salt water from deep aquifers or polluted water from

surface might infiltrate into the horizons of drinking water via palaeo-incisions. Also, the discharge of salt or fresh water from the seafloor could affect the development of benthic ecosystems.

EFFECTS OF THE ALLELOPATHICALLY ACTIVE MACROPHYTE *Myriophyllum spicatum* ON THE POTENTIALLY TOXIC CYANOBACTERIUM *MICROCYSTIS AERUGINOSA* (Ecology and environmental science)

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The dissertation defended:

27 November 2015

One of the measures assumed to potentially help control *M. aeruginosa* blooms is the use of allelopathically active macrophytes. The aim of the study was to evaluate the effect of the allelopathically active macrophyte *M. spicatum* on *M. aeruginosa* and its toxicity. This dissertation evaluates how allelopathically active macrophyte *M. spicatum* affects MC-producing and non-MC-producing *M. aeruginosa* genotypes. The study evaluates the protective role of allelopathy of *M. spicatum* under *in situ*-like conditions. This study provide that the coexistence of MC-producing and non-MC-producing *M. aeruginosa* strains influences the effects of allelochemicals of *M. spicatum* on *M. aeruginosa* strains. The study evaluates the role of MCs in *M. aeruginosa* against allelochemicals of *M. spicatum* and gives indications that biochemical characteristics of phytoplankton to produce certain metabolites could be one of the reasons of differential sensitivities of phytoplankton organisms to allelochemicals of macrophytes.

EFFECTS OF ENVIRONMENTAL EXPOSURES ON PRESCHOOL CHILDREN ALLERGIES (Ecology and environmental science)

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The dissertation defended:

27 November 2015

Over the last decades, the prevalence of asthma has progressively increased in the world. The epidemiological findings shows that environmental pollution during pregnancy may affect newborns' development and later can increase the risk of the developing allergies, obesity and other chronic childhood diseases. The causes of childhood allergies are still unclear. Epidemiologic data on the influence of the natural environment and urban green spaces on allergy and asthma in children are limited and inconsistent. In this environmental

epidemiological study, using objective individual-level greenness exposure measures in the living environment (NDVI) data and the distance to the nearest city park and the physician-diagnosed asthma and allergies, we estimated the integrated impact during critical child development periods. Low greenness exposure during pregnancy statistically significantly increases the prevalence of low birth weight and term low birth weight. Exposure to tobacco smoke during pregnancy and early life, maternal chronic diseases affected foetus development, while low birth weight babies, term low birth weight and preterm delivery were the birth outcomes associated with higher allergies risk at 4–6 years of age. Increase in greenness (NDVI IQR) within a 100-m buffer of home associated with a 43% increase in the risk of asthma at the age of 4–6 years. The risk of asthma in children residing in low greenness exposure areas and spending more than 5 hours per week in green spaces was statistically significantly lower, compared to children who spent less time outdoors. Physical activities have health benefits and can significantly reduce the risk of childhood asthma in areas with lower exposure of greenness. A distance greater than 500 m to the nearest city park increases the probability that children will spend less than 5 hours per week in a park, compared with children residing close to a park. This research adds to the growing body of evidence that environmental factors and urban greenness may have a significant effect on children health. Elucidation of causal associations between environmental exposures and risk of allergies could provide preventive measures for allergy management and intervention programs.

EUROPEAN ASPEN (*Populus tremula* L.) IN LITHUANIA: GENETIC DIVERSITY OF PLUS TREES AND POPULATIONS ASSESSED USING MOLECULAR MARKERS (Ecology and environmental science)

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The dissertation defended:

18 December 2015

The European aspen (*Populus tremula* L.) is the only native poplar species in Lithuania. *P. tremula* is an ecologically important and widespread species with a broad distribution range, growing in temperate and boreal regions of Eurasia, and widely used for hybridization and plantation forestry. The main aim of the study was to assess the genetic diversity of the European aspen plus trees and populations in Lithuania and to reveal a possibility to identify hybridization between *P. tremula* and hybrid aspen using morphological traits of their leaves. Weak correlations between genetic and

geographic distances were obtained using both RAPD and SSR markers ($r = 0.178$ and 0.068 , respectively), indicating that the largest part of molecular variance can be attributed to a within-population variation. RAPD data allowed genotype clustering into groups that are geographically closer to each other compared to SSR-based cluster groups. Microsatellite analysis revealed that individuals of *P. tremula* should be grouped into three clusters, while RAPD analysis clearly showed only two clusters. The results obtained by both clustering methods (UPGMA and Bayesian) indicate that the existing *P. tremula* provenance regions in Lithuania may be revised, as both molecular markers revealed a latitudinal trend. Population, provenance region and individual heterozygosity have little or no influence on infection of *P. tremula* plus trees with a trunk rot fungus *Phellinus tremulae*. The phenological structure of *P. tremula* and hybrid aspen stands is helpful for initial prediction of possible gene flow among aspen populations: trees with the earliest flowering phenology produced the highest proportion of hybrid seedlings.

PHOSPHORUS POOL VARIATIONS IN THE CURONIAN LAGOON AND ITS IMPLICATION TO EUTROPHICATION (Ecology and environmental science)

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The dissertation defended:

18 December 2015

The increased availability of reactive phosphorus may favour cyanobacterial blooms, which is a phenomenon well known around the globe. Cyanobacteria are capable to proliferate intensively and form huge biomass in the water column, later settling to sediments in a form of rapidly degrading organic matter. Related microbial decomposition processes may lead to the formation of hypoxia/anoxia at the bottom water layers and, ultimately, to the release of dissolved inorganic phosphorus from sediments. Regenerated phosphorus supports nitrogen fixing cyanobacteria bloom forming a self-sustaining cycle. In this thesis, *P* cycling was investigated in the Curonian Lagoon by analysing external and internal phosphorus loads and the relationship between phosphorus release and cyanobacteria blooms. At two sites, representing dominant sediment types in the lagoon, we characterized *P* pools and mobility via combined pore water analysis, calculation of diffusive exchange and flux measurement in core incubations. Annual balance was also calculated to analyse the role of the whole lagoon as a net sink or source of phosphorus. Muddy sediments had higher *P* content as compared to sandy sediments, and most of phosphorus pool was reactive. Muddy sites had consequently

higher pore water DIP concentrations, maintaining higher diffusive and net fluxes across sediment-water interface. However, the measured fluxes suggested that both sediment types were mostly P sinks, except for a large regeneration of DIP (nearly 30 $\mu\text{mol m}^{-2}\text{h}^{-1}$) recorded at muddy sediments during the cyanobacteria bloom. On average, 69% of the total inflowing DIP from the Nemunas River is assimilated in the Curonian Lagoon; however, during the intensive river discharge events (as in June 2013), about 97% of inflowing DIP could be directly transported to the Baltic Sea. 76% of outflowing phosphorus from the Curonian Lagoon to the Baltic Sea is in particulate form.

NEPOVIRUS CAUSED PLANT DISEASES AND GENETIC VARIABILITY OF VIRUS ISOLATES IN LITHUANIA (Botany)

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The dissertation defended:

25 March 2015

Nepovirus is spread in a semi-persistent manner by parasitic soil nematodes belonging to *Longidorus* or *Xiphinema* genera. Nepoviruses cause epidemics of many plant species in many countries in all continents. The members of this genus cause diseases of grapevines, raspberries, blackberries, strawberries, wild strawberries, currants, tomatoes, cucumbers, potatoes, peppers, carrots, peas, beans, rice, olive trees, hops, apples, cherries, peaches and many other herbs and ornamental plants. The aim of this research was to investigate the spread, occurrence and genetic variability of five globally widespread *Nepovirus* species in Lithuania and to assess their plant host range and disease symptoms. For this study, 218 different plant samples were collected. In total, 22 *Nepovirus* isolates were detected and identified as members of five species: *Tomato black ring virus* (TBRV), *Arabic mosaic virus* (ArMV), *Raspberry ringspot virus* (RpRSV), *Tobacco ringspot virus* (TRSV), and *Tomato ringspot virus* (ToRSV). TBRV was detected and identified in many different plants and it seems to be the most common and widespread nepovirus in Lithuania. TBRV and ArMV are genetically variable; it is possible that there are several different strains of these species spread in Lithuania. ToRSV *cp* gene sequence differs significantly from other isolates of this species; virus isolate might be close to being separated as a new species.

***Equisetum variegatum* SCHLEICH. EX WEBER ET MOHR IN LITHUANIA: DISTRIBUTION AND STRUCTURE OF POPULATIONS (Botany)**

Mindaugas Rasimavičius**Scientific supervisor:**Prof. Dr. habil. Jonas Remigijus Naujalis,
Vilnius University**The dissertation defended:**

22 June 2015

The main research object of this dissertation was *Equisetum variegatum* Schleich. ex Weber et Mohr, a perennial, clone-forming horsetail. The aim of the research was determine the distribution and population structure of *E. variegatum* in Lithuania. It was found that the greater part of *E. variegatum* habitats in Lithuania are of anthropogenic origin. It was shown that assessment of *E. variegatum* ecological plasticity can be successfully performed by an indirect phytoindication method. The study of *E. variegatum* population structure revealed that the main reason for horsetail partial tuft degradation is the overgrowth of bushes and the creation of shadowed environment. Such environmental conditions decrease the number of *E. variegatum* sporificating shoots and increase the amount of senile partial tufts. Similar processes take place in *E. variegatum* populations thriving in aquatic environments. It was found that the majority of Lithuania's *E. variegatum* populations are stable, as maturity spectrums are complete, left-sided, one-peaked, and symmetrical.

POSSIBILITIES OF ECOLOGICAL RESTORATION OF RAISED BOG PLANT COMMUNITIES IN DEGRADED PARTS AND IN A CUTOVER PEATLAND OF AUKŠTUMALA RAISED BOG (Botany)

Leonas Jarašius**Scientific supervisor:**Dr. Romas Pakalnis,
Nature Research Centre**The dissertation defended:**

4 December 2015

Lithuania has lost about two thirds of its total peatland area during the 20th century. Due to the drainage and mineralization of the peat layer, disturbed peatlands produce huge amounts of greenhouse gasses. Large-scale land reclamation works were implemented in the Nemunas delta region, where the object of this study, Aukštumala raised bog, is located. The aim of the study was to assess the state of raised bog plant communities and hydrological regime in the Aukštumala Telmological Reserve and to study the potential of restoration of plant communities in the parts of the Aukštumala raised bog that were degraded by drainage and fire and in the cutover peatland. Specifically, we aimed to assess effectiveness of two different means of hydrological regime restoration: (i) bog isolation from peat harvesting fields using a polyethylene membrane and "peat lock" system, and (ii) blocking of ditches using peat dams. In order to restore the cutover part of the Aukštumala peatland, the fragments of typical raised

bog vegetation were spread in an experimental field. Aiming to restore the hydrological regime favourable to natural raised bog plant communities, the mean water table depth during the vegetation season should be upheld above -30 cm. A polyethylene membrane, due to significantly higher mean water table depth, is regarded as a rather effective means to restore the hydrological regime that creates unfavourable conditions for tree growth. The efficiency of “peat lock” was only observed on seasonal water level fluctuations. Although in the first year, 93% of the planted donor fragments of raised bog vegetation recovered successfully at the experimental site of cutover peatland, the further formation of Sphagnum cover stopped due to extremely dry subsequent years. The processes may only be re-established if favourable hydrological conditions are ensured by effective regulation measures.

BIOLOGICAL EFFECTS INCLUDED BY THE SUSPENSION OF COPPER OXIDE NANOPARTICLES IN THE CELLS OF *Nitellopsis obtusa* (DESV.) J. GROVES (Botany)

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The dissertation defended:

28 December 2015

Nanosized copper oxide particles (nCuO) are widely increasingly used in technological applications. Nanomaterials may be released to the environment with wastewater and activated sludge. The occurrence of these small size particles in the aquatic media may cause adverse effects on different organisms. nCuO are acutely toxic to many organisms, including crustaceans, algae, aquatic plants and fish; nevertheless, ionic copper is more toxic. Several studies have indicated that the acute toxicity of nCuO is due to the release of Cu^{2+} from NPs. Other studies state that observed effects could not be explained only by dissolved copper from NPs. However, strict differentiation between the main mechanisms of NPs toxicity seems to be hardly achieved under the current state of knowledge. The current study is assigned for the investigation of toxicity effects of nCuO suspensions on the cell of freshwater algae *Nitellopsis obtusa*. The data of experimentation demonstrated that: harophyte cell of *N. obtusa* exposed for several seconds in the suspension of CuO nanoparticles accumulates a sufficient amount of copper in the wall, which induces cell lethality within several days or months. If similar concentrations accumulate in the cells of *N. obtusa* during their exposure to copper salt solution or CuO nanosuspension, the rapid depolarization (within several minutes) of cell membrane occurs in case of copper solution only; however, both impacts lead to cell lethality.
