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

Compiled by Indrė LIPATOVA

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STUDY AND APPLICATION OF CRISPR-Cas SYSTEMS (Biochemistry)

Gediminas Drabavičius

Scientific supervisor:
Dr Giedrius Gasiūnas,
Vilnius University

Dissertation defended:
16 March 2021

The research objective of this work was to investigate and study details of the CRISPR adaptation process in several different CRISPR-Cas systems as well as use genome-wide CRISPR screening to elucidate cell-intermedilysin interactions. The results of this study showed that Cas1-Cas2 complex from *S. thermophilus* CRISPR4-Cas system forms a complex that integrates prespacers into the CRISPR array. DnaQ domain fused to Cas2 in this system is a 3'–5' DNA exonuclease. DnaQ domain is dispensable for spacer integration; however, it serves to trim back overextended 3' overhangs of the prespacer. Cas1, Cas2, and Csn2 proteins from *S. thermophilus* CRISPR3-Cas system form at least three different complexes, which interact with Cas9 from the same system via the DNA tether. The identified complexes from CRISPR3-Cas system represent a spacer capture step of the new spacer acquisition process, as they harbour spacer length DNA in their assemblies. Genome-wide CRISPR screening can reveal novel fundamental biological pathways in the membrane composition and lipid metabolism when used in concert with membrane targeting toxins. Intermedilysin has many more dependency factors than previously known CD59 and cholesterol. Among them are heparan sulfates, glucosylceramides, and many other protein or lipid glycosylation factors. ILY can be inhibited by heparin or the removal of heparan sulfates from cells using bacterial heparinases.

THE PREVALENCE STUDIES AND DIAGNOSTIC OF HEPATITIS E VIRUS (Biochemistry)

Martynas Simanavičius

Scientific supervisor:
Dr Indrė Kučinskaitė-Kodzė,
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Dissertation defended:
4 June 2021

Hepatitis E is an infectious disease caused by hepatitis E virus (HEV). HEV genotype 1 (HEV-1) and HEV-2 infect only humans, while HEV-3 and HEV-4 infect humans and other mammals such as pigs, wild boar, deer, and rabbits. In developing countries, HEV-1 and HEV-2 spread through contaminated water. These genotype infections are detected in Europe; however, they are mostly travel-associated. HEV-3 is a zoonotic virus spread in the developed countries. Uncooked or overcooked food is the main source of HEV-3 infections. Recently, rat HEV was discovered to cause hepatitis E in humans. Thus, the demand for specific rat HEV investigation tools should grow. In this thesis, the generation and characterisation of monoclonal antibodies (MAbs) against yeast-expressed virus-like particles-forming HEV-3 and rat HEV capsid proteins are described. Newly developed MAbs bear characteristics which are described for the first time. It enables an opportunity to model and test novel and broadly applicable HEV investigation techniques. In this thesis, wild rat, pig, and human samples were tested for the presence of anti-HEV antibodies by immunochemical methods based on recombinant HEV capsid proteins and monoclonal antibodies. For the first time, HEV prevalence was estimated in human and wild rat populations in Lithuania.

INVESTIGATION OF FORMATION OF PATHOLOGICAL TAU SPECIES AND THEIR NEUROTOXITY (Biochemistry)

Katryna Pampuščenko

Scientific supervisor:
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Dissertation defended:
29 June 2021

Tauopathies are neurodegenerative diseases, including Alzheimer's disease, which are associated with pathological changes of tau (tubulin associated protein) protein. These diseases are characterised by progressive neuronal and synaptic loss resulting in decline of cognitive and motor functions. Neuroinflammation is another common feature of tauopathies. The present study was designed to examine the effects of extracellular tau, which is one of the factors involved in pathogenesis of tauopathies. This study sought to evaluate the relationship between tau protein isoform, aggregation state, post-translational modifications, and cytotoxicity and to determine the molecular mechanism of neurotoxic effects. The experiments were carried out using

primary neuronal-glial co-cultures from rat cerebellum, which allow to determine the direct effect of tau protein on neurons and to investigate how brain immune cells – microglia – affect neuronal functions and survival. Results show that tau2N4R protein (the longest isoform) activates and stimulates microglia to phagocytose damaged but viable neurons, i.e., causes primary neuronal phagocytosis, and tau2N4R phosphorylation potentiates neurotoxic effects. Monomeric and pre-aggregated tau2N4R exerted similar effects, however, tau1N4R aggregates but not monomers were toxic to brain cells. New data on the molecular mechanisms of neurotoxicity of extracellular tau species may contribute to the development of pharmacologic treatments inhibiting neuronal loss.

INVESTIGATION OF THE CATABOLISM OF 7-HYDROXYCOUMARIN IN *PSEUDOMONAS MANDELII* 7HK4 BACTERIA (Biochemistry)

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Dissertation defended:
30 June 2021

Coumarins are well known secondary metabolites widely found in various plants. However, a degradation of those compounds in the environment is not studied in detail. A soil isolate *Pseudomonas mandelii* 7HK4 is able to degrade 7-hydroxycoumarin (umbelliferone), but the enzymes catalyzing its transformations have not been characterised. To elucidate the pathway of catabolism of 7-hydroxycoumarin, 7-hydroxycoumarin-inducible genes hcdA, hcdB, hcdC, hcdD, hcdE, hcdF, and hcdG were identified by an RT-qPCR analysis and protein MS-MS analysis. The DNA fragment encoding a putative alcohol dehydrogenase HcdE was cloned, and the recombinant protein catalyses the NADPH-dependent reduction of 7-hydroxycoumarin both *in vivo* and *in vitro*. The reaction product was isolated and characterised as 7-hydroxy-3,4-dihydrocoumarin, which proceeded through hydrolysis in aqueous solution resulting in formation of 3-(2,4-dihydroxyphenyl) propionic acid based on HPLC-MS and NMR analyses. Further, bioinformatic analysis showed that the hcdABC genes encodes a flavin-binding hydroxylase (HcdA), an extradiol dioxygenase (HcdB), and a putative hydroxymuconic semialdehyde hydrolase (HcdC). The analysis of the recombinant HcdA activity *in vitro* has confirmed that this enzyme belongs to the group of ipso-hydroxylases. The activity of the proteins HcdB and HcdC was analysed by using recombinant *E. coli* cells. Identification of intermediate metabolites allowed us to confirm the predicted enzyme functions and to describe

the downstream catabolic pathway of 7-hydroxycoumarin via 3-(2,4-dihydroxyphenyl) propionic acid pathway. HcdA catalyses the conversion of 3-(2,4-dihydroxyphenyl) propionic acid to 3-(2,3,5-trihydroxyphenyl) propionic acid through an ipso-hydroxylation followed by an internal (1,2-C,C)-shift of the alkyl moiety. Then, in the presence of HcdB, a subsequent oxidative meta-cleavage of the aromatic ring occurs, resulting in the corresponding linear product (2E,4E)-2,4-dihydroxy-6-oxonona-2,4-dienedioic acid. Here, we described a *Pseudomonas mandelii* strain 7HK4 capable of degrading 7-hydroxycoumarin via 3-(2,4-dihydroxyphenyl) propionic acid pathway.

VIRUS COMPATIBILITY IN *SACCHAROMYCES CEREVISIAE* LA AND M VIRUS SYSTEMS (Biochemistry)

Lina Aitmanaitė

Scientific supervisor:
Prof. Dr Saulius Serva,
Vilnius University

Dissertation defended:
2 July 2021

Saccharomyces cerevisiae and other yeast species of the *Saccharomyces* genus possess endogenous ssRNA and dsRNA viruses. The best-known are killer phenotype-encoding LA and M dsRNA viruses of the Totiviridae family. In nature, M viruses are detected altogether with their specific helper virus LA. The interplay between LA viruses and M viruses and the specificity determinants are not fully understood. The research into the mentioned aspects is essential for understanding of the biocidic phenotype of yeasts and revealing the ontogenesis mechanism of these viruses. This study evaluated the impact of recombinant LA viral protein expression on all known *S. cerevisiae* LA and M viral systems and related, belonging to the *S. paradoxus* viral system. It was found that the expression of truncated LA capsid protein Gag causes the elimination of native yeast viruses despite their type. For the first time, the interactions of LA-1, LA-lus, and LA-28 viral proteins with native viral systems were studied systematically. The results indicate that the specificity of interactions between recombinant LA proteins and endogenous yeast viral systems could depend on the specific type of M virus. The role of variable sequences of LA viral proteins in establishing interactions between recombinant proteins and endogenous LA and M viruses was assessed.

SYNTHESIS OF DNA AND MAGNESIUM PYROPHOSPHATE PARTICLES AND THEIR USE FOR PROTEIN EXPRESSION *IN VITRO* (Biochemistry)

Robertas Galinis

Scientific supervisor:
Dr Linas Mažutis,
Vilnius University

Dissertation defended:
31 August 2021

In this work, a new method was described to apply magnesium pyrophosphate and DNA particles as a matrix for *in vitro* protein synthesis in microfluidic droplets. Single DNA molecules were encapsulated and amplified in 4 pl droplets. Optimised DNA amplification conditions were established to synthetise ~1 μm, magnesium pyrophosphate and DNA containing particles from single molecules. Also, reaction conditions were adjusted to reduce DNA molecule loss due to nonspecific adsorption on surfaces. Obtained particles were separated from reaction mixture. Finally, these particles were used to synthetise green fluorescent protein and β-galactosidase in microtiter plate and microfluidic droplets.

CHARACTERISATION OF CHITOSAN, β-GLUCAN, AND LIGNIN FOR FOOD INDUSTRY AND BIOMEDICINE (Biochemistry)

Alona Geciene

Scientific supervisor:
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Dissertation defended:
17 September 2021

Chitosan, β-glucans, and lignin are considered suitable structural parts of edible biodegradable packaging for replacing petroleum-based plastics, where chitosan and lignin are possible anticancer agents. This study clearly revealed that fungal chitosan could be a part of edible films and biodegradable packaging because of its antibacterial properties, and Eucalyptus kraft lignins precipitated at pH 2 and pH 6 could be a part of edible films and biodegradable packaging because of its antioxidant properties. Low-molecular-weight chitosan films and solutions with a high degree of deacetylation can act cytotoxically on both tumour mouse hepatoma MH-22A and normal Chinese hamster ovary (CHO) cells in vitro. Kraft lignins act cytotoxically inducing apoptosis- and necrosis-like processes on both on tumour and normal cells. However, the results evidenced that cancerous hepatoma MH-22A cells showed greater sensitive behaviour than B16 melanoma and non-cancerous CHO cells, which were more tolerant of kraft lignin. Consequently, this work may be useful for the future development of bio-based environmentally friendly polymers and for further investigations of natural anticancer products in medical areas.

STUDY OF MOLECULAR MECHANISMS INDUCED BY CELL-SCAFFOLD INTERACTION (Biochemistry)

Egidijus Šimoliūnas

Scientific supervisor:
Dr Daiva Baltriukienė,
Vilnius University

Dissertation defended:
28 September 2021

Cells in different tissues have unique environments, which are important in maintaining tissue homeostasis. Even slight changes in this system can cause irreversible changes and induce the development of various diseases. Thus, in this work, we evaluated how mechanical properties of the growing surface impacts healthy and cancerous tissue cells. Gingiva mesenchymal stem cells (MSC) were chosen as representative of healthy tissue cells. Due to wide range of their differentiation potential, immunomodulation functions and alleviation of ongoing oxidative stress (OS), these cells have gained a lot of attention in the field of regenerative medicine. For this reason, we evaluated how mechanical environment properties impacted MSC resistance to OS. We observed that mechanical environment properties influenced many processes of these cells, including their resistance to OS. The stiffer the surface, the more resistant cells were. Breast cancer cell line MCF-7 was chosen as a model of cancerous tissue cells. Breast cancer is the most commonly diagnosed cancer and MCF-7 cell line represents the 70% of all breast cancer cases phenotype. During the progression of cancer, its environment gets increasingly stiffer. Thus we investigated if mechanical properties of the surface had an impact on MCF-7 resistance to the chemotherapy drug doxorubicin. In this case, mechanical properties of the surface had no pronounced impact on MCF-7 sensitivity to doxorubicin; nevertheless, other tested properties of the cells depended to the stiffness of the surface, e.g., morphology, adhesion, proliferation, and even the expression and activation of signaling molecules. In addition to the mechanobiological research, a new method to evaluate cell number in 2D, 2.5D, and 3D environment was developed.

BACTERIAL DEFENSE ISLANDS: CRISPR-Cas AND TOXIN-ANTITOXIN SYSTEMS (Biochemistry)

Inga Songailienė

Scientific supervisor:
Prof. Dr Virginijus Šikšnys,
Vilnius University

Dissertation defended:
15 November 2021

Under constant evolutionary pressure, bacteria developed a set of DNA and RNA-targeting systems to combat their invaders – bacteriophages. Fundamental research on these resistance mechanisms led to the discovery of molecular tools for genetic engineering, such as restriction enzymes, bacterial argonautes and CRISPR-Cas systems. CRISPR-Cas systems are located in the genome part called 'defense islands' where genes encoding different antiviral systems are clustered together. The goal of this thesis is to explore the mechanisms of action of type I CRISPR-Cas and CRISPR-Cas associated toxin-antitoxin systems. The first part of this thesis focuses on fundamental aspects of multiprotein class 1 type I-E and I-F CRISPR-Cas effector complexes aiming to understand molecular mechanisms of target recognition and degradation using a combination of biochemical and single molecule assays. The findings presented here show that I E and I F type effectors recognize their dsDNA targets by unidirectional unwinding starting from PAM sequence motif. Furthermore, the complexes can assemble on different length of crRNA and enable dsDNA targeting in a WT-like manner. The second part of the thesis is dedicated to the biochemical and structural characterisation of the toxin-antitoxin system HEPN-MNT that is present in the operon of the type I-D CRISPR-Cas system. It is shown that HEPN toxin ribonuclease activity is neutralised by covalent di AMPylation performed by MNT antitoxin. In addition, the data reveals that the unmodified active HEPN toxin cleaves 4 nt from 3′-stem of a range of tRNA, showing a new mechanism of toxin action on tRNA.

CONSTRUCTION OF INDUCIBLE EXPRESSION SYSTEMS FOR CONTROLLED TRANSFER OF *AGROBACTERIUM TUMEFACIENS* T-DNA IN PLANT TRANSIENT EXPRESSION SYSTEMS (Biochemistry)

Erna Denkovskienė

Scientific supervisor:
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Vilnius University

Dissertation defended:
25 November 2021

Recombinant proteins are already successfully produced in plants in contained facilities using *Agrobacterium*-mediated transient transfection of plants. However, transfection methods suitable for open field applications are still desirable as a cheaper alternative. Biosafety concerns related to the use of recombinant agrobacteria in an industrial transfection process include possible transformation/transfection of unintended hosts or spread of the genetically modified agrobacteria in the environment. In this work, we explored novel biocontrol approaches resulting in higher biosafety of the transient expression in plants. Our proposed solutions involve an inducible expression of essential *Agrobacterium tumefaciens* virulence factor virE2 and expression of toxin pemK and antitoxin pemI from bacterial toxin-antitoxin (TA) module pemIK in Agrobacterium that provides for strictly regulated T-DNA transfer from agrobacteria to model plants Nicotiana benthamiana. The study provides detailed information about the relative activity and regulation of several IPTG and cumic acid inducible promoters in *A. tumefaciens*. Additionally, new efficient cumic acid inducible promoters were constructed. Also, several *A. tumefaciens* toxins were identified from putative TA systems, and their activity was evaluated. Toxins Dead, PIN, VapC, IetS and PemK showed high efficiency against *A. tumefaciens*.

THE APPLICATION OF ANIMAL HEN1 METHYLTRANSFERASES FOR LABELLING AND SEQUENCING OF SINGLE-STRANDED RNA (Biochemistry)

Milda Mickutė

Scientific supervisor:
Prof. Dr Giedrius Vilkaitis,
Vilnius University

Dissertation defended:
10 December 2021

Animal Hen1 methyltransferases are composed of the N-terminal methyltransferase domain and the variable-length C-terminal region, and catalyze the S-adenosyl-L-methionine (AdoMet) dependent methylation of the 2'-O-ribose of the 3'-terminal nucleotide in a subset of small non-coding RNAs. This modification protects small RNAs from degradation, meanwhile the deficiency of the Hen1 protein causes detrimental phenotypic changes to particular organisms. Current work presents the first detailed characterisation of the enzymatic reactions catalysed by animal Hen1 methyltransferases: we have discovered that the enzymatic activity

of *Drosophila melanogaster* DmHen1 and *Homo sapiens* HsHen1 depends on Co^{2+} and Co^{3+} ions; we found that *in vitro* these proteins can transfer not only methyl group, but also larger side chains from synthetic AdoMet analogues on both natural 22–28 nt and longer RNA substrates; we have shown that the C-terminal region of DmHen1 is unnecessary and even inhibits the enzymatic activity of the protein. In the current study, we have applied DmHen1 and its variant without the C-terminal sequence, DmHen1ΔC, for the one-step and two-step labeling of single-stranded RNA and its subsequent visualization, enrichment, and synthesis of copy DNA (cDNA). Based on the latter reaction, we have developed the mDOT-seq (methyltransferase-Directed Orthogonal Tagging and RNA sequencing), the method for the cDNA library preparation for single-stranded RNA sequencing. With mDOT-seq in hand we have not only demonstrated that it can be applied for the sequencing of small eukaryotic RNAs (such as microRNA or siRNA), but also for the first time characterised the 50–500 nt small regulatory RNAs from probiotic *Lactobacillus casei* BL23.

INVESTIGATION OF MESENCHYMAL STEM CELL PROPERTIES IN DEGENERATIVE AND INFLAMMATORY DISEASES (Biology)

Jaroslav Denkovskij

Scientific supervisor:
Dr Eiva Bernotienė,
Centre for Innovative Medicine

Dissertation defended:
14 January 2021

The study shows that adipose tissue-derived stem cells (ASC) could become a potential and safe cell source for the intraarticular treatment of cartilage tissue damages in osteoarthritis (OA). It has been shown that ASC inhibit OA cartilage degradation process and hypertrophy. Also, by modulating secretion and activity of extracellular matrix enzymes, the ASC may restore the balance between extracellular matrix metalloproteinases (MMP) and their inhibitors (tissue inhibitors of matrix metalloproteinases (TIMP). This ASC effect on human OA cartilage has been shown for the first time. The present study has shown that an acute inflammatory environment in rheumatoid arthritis (RA) can induce long-term changes in the expression of surface markers of the of synovial mesenchymal stem cells (SMSC). A comparison analysis of OA and RA SMSC surface markers showed a significantly higher expression of Neprilysine (CD10) and lower expression levels of CD47 and CD271 in the RA SMSC group. In autoimmune as well as cancer diseases, the mitochondrial cell functions are disturbed, including the heme biosynthesis process, which is often associated with increased malignant cell activity. Therefore, based on the similarity of RA synoviocytes to cancer

cells, PpIX accumulation measurements were performed. It has been demonstrated that accumulation of PpIX is typical for SMSC. A higher PpIX accumulation trend was observed in the RA SMSC group, which, to the best of our knowledge, was shown for the first time.

EFFECTS OF GENDER, AGE, AND PHYSICAL FITNESS ON RESPONSIVENESS TO INTERVAL TRAINING (Biology)

Raulas Krušnauskas

Scientific supervisor:
Prof. Dr Sigitas Kamandulis,
Lithuanian Sports University

Dissertation defended:
5 March 2021

This thesis was directed towards assessing the minimal or optimal volume of HIIT/ SIT to gain benefits in various physical condition, gender, and age groups. We were able to provide a much-needed insight into high intensity interval training/ sprint interval training (HIIT/SIT) volume relationship with enjoyability, responsiveness, and physiological stress. It is of high importance as these findings can be given to physicians and then passed on to wide range of population. Our research illustrated differential responsiveness to continued HIIT/SIT training within group. Unfortunately, we were not able to identify specific markers for higher responsiveness to HIIT/SIT training. Therefore, we highlight the need for future studies to build on our findings and determine responsible elements for differential responsiveness. All in all, this study investigated the importance of gender, age, and physical status effects in HIIT/SIT responsiveness thus increasing the likelihood of successfully prescribed personalised training. This could potentially correlate with better exercise adherence and, on a broader scale, reduce health care cost and improve life quality, especially for older individuals.

PREVALENCE OF TICK-BORNE ENCEPHALITIS VIRUS IN IXODIDAE TICKS AND THEIR RESERVOIRS (RODENTIA) (Biology)

Marina Sidorenko

Scientific supervisor:
Prof. Dr Algimantas Paulauskas,
Vytautas Magnus University

Dissertation defended:
19 March 2021

The aim of the research was to determine the prevalence of TBE virus (TBEV) in Ixodidae ticks and small rodents (Rodentia), to identify virus subtypes and strains. Out of all ticks collected and tested in Lithuania, TBEV was found in 34 of 969 pools. The overall minimum infection rate was 0.37%. It has been confirmed that *Ixodes ricinus* and *Dermacentor reticulatus* ticks, common to Lithuania, participate in TBEV transmission. The virus is found at all stages of development

of *I. ricinus*. The prevalence of TBEV in *I. ricinus* adults was 0.6%, which was 2.4 times higher than in nymphs (0.25%) and larvae (0.26%). The percentage of TBEV among adult ticks was higher in *I. ricinus* (0.6%) ticks than in *D. reticulatus* (0.4%). Of the five small rodent species studied, the virus was found only in yellow-necked mice (*Apodemus flavicollis*). Based on the results of this and other studies, it can be stated that *A. flavicollis* is the dominant species in Lithuania and can be considered the main reservoir host of TBEV. The results show the diversity of TBEV European genotypes in the territory of Lithuania and the links to geographical areas. This study also describes an investigation carried out in Norway. The results of that survey can confirm only the presence of *I. ricinus* and the presence of TBEV in ticks in nymph and adult stages in southern Norway.

MONITORING WORKLOAD AND READINESS TO PERFORM DURING INTENSIFIED PERIODS IN BASKETBALL (Biology)

Inga Lukonaitienė

Scientific supervisor:
Prof. Dr Sigitas Kamandulis,
Lithuanian Sports University

Dissertation defended:
1 April 2021

The aim of the research was to evaluate the workload and readiness to perform during short intensified training and match periods in basketball. A total of sixty-two female and male basketball players participated in the study. The main findings indicate that a constant adequate workload positively benefits the players' readiness and physical performance during short intensified preparation periods. Conversely, the use of high workload with a periodisation strategy encompassing short overload and taper phases induced positive changes on the players' aerobic performance, lower readiness values, and no changes in anaerobic performances. These findings seem fundamental for youth female basketball coaches and practitioners to optimise their training periodisation during short intensified preparation periods. This study showed that a schedule of seven matches in nine days was suitable loading for junior female basketball players irrespective of the competition context or individual differences in workload. This might reflect a high level of player preparedness, optimal player rotation, or adequate intensity demands during basketball matches. These factors should be considered to avoid performance deterioration during tournaments with a congested match agenda. Moreover, the use of a combination of objective and subjective methods should be recommended because it provides different information about load and readiness. A congested match schedule with two games

played on consecutive days elicited similar game workloads with higher perceived fatigue and lower well-being status prior to the second game compared to the first game. These findings suggest basketball coaches and practitioners using recovery strategies to optimise player well-being during congested game schedules.

VIABILITY AND EFFECT OF EOSINOPHILS ON PROLIFERATION OF AIRWAY SMOOTH MUSCLE CELLS AND PULMONARY FIBROBLASTS IN ASTHMA (Biology)

Andrius Januškevičius

Scientific supervisor:
Prof. Dr Kęstutis Malakauskas,
Lithuanian University of Health Sciences

Dissertation defended:
22 April 2021

Asthma is a heterogeneous disease characterised by chronic airway inflammation and the development of remodeling. This research aimed to investigate the viability of the main inflammatory cells, eosinophils, and their effect on airway smooth muscle cells and pulmonary fibroblast proliferation, which is one of the remodeling components. Our results demonstrate that asthmatic eosinophils have more intensive adhesion that enhances their viability. Moreover, asthmatic eosinophils promote airway smooth muscle cells and pulmonary fibroblasts proliferation and inhibit their apoptosis. Suppression of eosinophil integrins decreased their adhesion and pro-proliferative effect. This research is the first to describe the differences of biological properties of the subtypes of human blood eosinophils. We found that inflammatory eosinophils are the predominant subtype in the blood of allergic asthma patients, while lung-resident eosinophils prevailed in severe non-allergic eosinophilic asthma patients. We proved that lung-resident eosinophils demonstrate more intensive adhesion, and their viability depends directly on their interaction with airway smooth muscle cells. Hereby, the management of eosinophil viability and pro-proliferative effect expand the options of asthma pathogenetic treatment.

DIURNAL PECULARITIES OF HEART RATE VARIABILITY AND BLOOD PRESSURE FLUCTUATIONS IN SUBJECTS WITH ARTERIAL HYPERTENSION AND CONCOMITANT METABOLIC SYNDROME (Biology)

Anžela Slušnienė

Scientific supervisor:
Prof. Habil. Dr Aleksandras Laucevičius,
Centre for Innovative Medicine

Dissertation defended:
14 May 2021

The primary purpose of this study was to evaluate the dynamics of diurnal heart rate variability, arterial blood pressure, and autonomic nervous system in patients with arterial hypertension and concomitant metabolic syndrome. Altogether, 106 middle-aged 50–55 year-old subjects were included into study. The investigation group consisted of 69 subjects with confirmed MS, all of whom had arterial blood pressure (BP) of more than 130/85 mmHg or they were treated with antihypertensive drugs (MetS group). The control group consisted of 37 subjects of the same age without MS and whose BP was lower than 130/85 mmHg. In this study, a new methodology was developed to enable us to evaluate objectively the influence of physical activity (PhA) on HRV dynamics while performing 24-hour electrocardiogram (ECG) monitoring. This methodology is based on a provision, that fluctuations of HRV in all cases depend on physical effects on HR that are realised via autonomic nervous system (ANS). The essence of this innovation consisted of integration of actigraph data with every ECG RR interval (RRI) and elimination of the impact of PhA, while using mathematical modeling. Thus, by means of calculation the parameters of HRV before and after elimination of PhA, it became feasible to evaluate the level of HRV in patients with arterial hypertension (AH) and concomitant metabolic syndrome. Another innovation in this study consisted of synchronisation of 24-hour arterial blood pressure monitoring (ABPM) measurements with PhA vectors, using mathematical modeling. ABPM parameters before and after elimination of impact of PhA were refined.

MOLECULAR CHARACTERISTICS OF AFRICAN SWINE FEVER VIRUS OF LITHUANIAN DOMESTIC PIGS AND WILD BOAR POPULATION (2014–2019) (Biology)

Simona Pilevičienė

Scientific supervisor:
Prof. Dr Vaclovas Jurgelevičius,
Vytautas Magnus University

Dissertation defended:
28 May 2021

African swine fever is a highly contagious viral disease of domestic pigs and wild boars (*Sus scrofa*) with a high mortality rate in infected animals. Monitoring of African swine fever in Lithuania has been carried out since 2003, but active data collection started only in 2011. ASF was first registered in Lithuania in early 2014. In this work, for the first time in Lithuania, the prevalence of ASF virus in the period from 2014 to 2019 was assessed using molecular methods and the number of positive cases in relation with age and sex of infected animals was analysed, validation of the multiple ASF-IC method was performed, and the results were compared with the conventional ASF King Real-time PCR method, and the detection of ASF virus in different organs was evaluated. However, not only detection and identification of the disease is challenging, it is also important to elucidate the dynamics of the ASF and changes in the sequence of the virus strains in different regions as the disease spreads. For the first time, a sequence analysis of the MGF505-10R gene circulating in Lithuania was performed and unique nucleotide substitutions were detected in comparison with ASF viruses circulating in other countries. During the study, for the first time, a partial sequence analysis of the B646L gene circulating in Lithuania was performed, and the differences in the sequences of circulating viruses detected in different years in Lithuania were compared. It was established that ASF virus circulating in Lithuania belongs to genotype II.

EFFECTS OF LOW CITRATE SYNTHASE ACTIVITY, MYOSTATIN DYSFUNCTION, AND CALORIC RESTRICTION ON ENERGY METABOLISM AND BODY COMPOSITION IN MICE (Biology)

Andrej Fokin

Scientific supervisor:
Prof. Dr Aivaras Ratkevičius,
Lithuanian Sports University

Dissertation defended:
1 June 2021

The main aim of our study was to examine the effects of low citrate synthase activity, myostatin dysfunction, and caloric restriction on energy metabolism and body composition of laboratory mice. The results show that mitochondrial respiration did not differ between mice with normal and low CS activity. There were no major differences in energy expenditure, respiratory quotient, or physical activity between mice with normal and low CS activity, with the exception of female mice, in which low CS activity is associated with reduced

energy expenditure. After 48-hour food deprivation, energy expenditure and respiratory quotient were lower in congenic mice with low CS activity compared to control mice with normal CS activity. Myostatin dysfunction did not protect from skeletal muscle wasting during food deprivation. 48-hour food deprivation was also associated with a significant decrease in physical activity, energy expenditure, and fat reserves of mice with normal with no impact of myostatin dysfunction. Low-Fat and Low-Carb diets had similar effect on body composition, energy metabolism, physical activity, and glucose tolerance during caloric restriction. Fixed energy and protein intake rather than a distribution of dietary carbohydrate and fat were the main factor for improvement in body composition and metabolic health of obese mice. Similarly, improvements in glucose tolerance of obese mice were due to the reduction in body fat rather than dietary carbohydrate and fat content of the diets.

DILATATIVE PATHOLOGY OF ASCENDING AORTA: SEARCH FOR EPIGENETIC BIOMARKERS (Biology)

Vaiva Patamsytė

Scientific supervisor:
Prof. Habil. Dr Vaiva Lesauskaitė,
Lithuanian University of Health Sciences

Dissertation defended:
23 June 2021

Most ascending thoracic aortic aneurysms (TAAs) are clinically silent and result from structural alterations in the aortic wall. Aortic media related changes in vascular smooth muscle cell (VSMC) phenotype lead to the development of dilatative pathology of ascending aorta. In the expanding aorta, VSMCs lose contractile function and begin to synthesise various proteases and their inhibitors, destroying the integrity of vascular wall and weakening the aorta. Dilated aorta is prone to rupture, thus surgical intervention is often required to prevent a sporadic dissection. Researchers around the world are working to identify biomarkers of TAA formation that could be used for early diagnosis and prognosis of the disease as well as clinical indicators for surgical treatment. Epigenetic modifications show a significant effect on the development and progression of cardiovascular diseases. These mechanisms target DNA molecules, transcriptional machinery, and transcription products influencing gene expression and protein synthesis. Micro-RNAs (miRNAs) and long ncRNAs (lncRNAs) are involved in the pathology of various disorders, can be detected in tissue biopsies and blood, show high stability and sensitivity. The aim of this study was to evaluate changes in selected ncRNA profiles during formation of TAA and to assess their value as clinical biomarkers in TAA diagnosis and progression.

INVESTIGATION OF ASPIRIN EFFECT ON mTORC1 SIGNALING IN HUMAN BREAST CANCER CELL LINES (Biology)

Aistė Savukaitytė

Scientific supervisor:
Prof. Dr Elona Juozaitytė,
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Dissertation defended:
29 June 2021

A number of studies suggested that regular aspirin use reduces the risk of development and progression of several cancers, including breast cancer. The mechanistic basis for the antitumor activity of aspirin needs to be defined to identify specific biomarkers for selection of patients who would benefit most from aspirin intake. The aim of this study was to investigate the effect of aspirin on mTORC1 signaling in human breast cancer cell lines. The effect of aspirin and its primary metabolite salicylic acid on breast cancer cell viability was analysed in the present study. Gene, encoding proteins involved in PI3K/AKT/mTOR signaling, expression profiles were determined in aspirin and salicylic acid-treated and untreated breast cancer cells. Modulation of phoshorylation of mTORC1 substrates by aspirin and salicylic acid treatment was assessed. The involvement of REDD1 in aspirin-mediated effect on phosphorylation of 4E-BP1 was addressed.

IMMUNE RESPONSE ASSESSMENT IN A COLORECTAL CANCER MICROENVIRONMENT USING DIGITAL PATHOLOGY ANALYTICS (Biology)

Aušrinė Nestarenkaitė

Scientific supervisor:
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Dissertation defended:
1 July 2021

Tumour infiltrating lymphocytes (TIL) have been associated with patients' clinical course and survival in different types of cancer. Digital immunohistochemistry (IHC) methods are used for automated quantitative assessment of TIL distribution in the tissue. This study presents a new method based on the principles of spatial hexagonal grid analytics by processing digital image analysis data to detect the tumour-stroma interface zone and to measure TIL density profiles within. Novel immune response indicators to quantify an absolute TIL density and its density gradient (immunogradient) in the direction from stroma to tumour in the interface zone were developed. The prognostic value of the indicators was tested in two cohorts of independent colorectal cancer (CRC) patient treated in health care institutions in Vilnius (Lithuania) and Nottingham (United Kingdom). The cytotoxic T cell (CD8) immunogradient (in both cohorts), the B cell (CD20) immunogradient, and the histological tumour growth pattern (in the Nottingham cohort) were independent prognostic features of overall

patient survival and were more informative than the absolute TIL density in the tumour as well as conventional clinicopathological and molecular indicators. Combined prognostic scores were proposed: (1) the CD8–CD20 immunogradient score based on CD8 and CD20 IHC markers alone, and (2) the immuno-interface score which combines CD8 and CD20 IHC markers and the histological tumour growth pattern. These scores were strong prognostic factors for overall CRC patient survival, independently of the tumour microsatellite instability status.

INVESTIGATION OF BLOOD-CIRCULATING PROTEINS AS BIOMARKERS FOR DIAGNOSIS AND PROGNOSIS OF ASTROCYTIC GLIOMA TUMOURS (Biology)

Rūta Urbanavičiūtė

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Dissertation defended:
30 August 2021

Brain tumour astrocytoma is diagnosed by complex and expensive methods, usually when tumour spread is advanced. Non-invasive or less-invasive diagnostic would allow us to monitor tumours earlier and improve patient's quality of life. Several single promising astrocytic glioma blood markers have been reported by different groups, but none of them were powerful enough for clinical applications. Regrettably, protein databases information about protein expression data in tumour tissue and serum are very limited too, especially when talking about multiple protein expressions in the same sample. The aim of this study was to determine the quantitative profiles of blood serum proteins, specific for astrocytic glioma tumours, which would allow non-invasive monitoring of cancerous processes in brain tissue. The novelty of this study was the simultaneous assessment of multiple serum proteins and determination of clinically applicable combination of serum markers for astrocytoma screening. Moreover, it is one of only a few up-to-date studies which confirmed a list of blood-circulating protein complexes for astrocytic glioma diagnosis and prognosis. Finally, we proved that brain cancer detection and prediction from patient serum using cancer associated proteins and applying mathematical classification and calculation tools is an achievable goal and should be developed in further studies.

REGENERATION POTENTIAL OF HUMAN DILATED MYOCARDIUM-DERIVED MESENCHYMAL STROMAL CELLS *IN VITRO*: THE IMPACT OF HISTONE DEACETYLASE INHIBITORS AND BIOMATRICES (Biology)

Rokas Mikšiūnas

Scientific supervisor:
Dr Daiva Bironaitė,
Centre for Innovative Medicine

Dissertation defended:
24 September 2021

The aim of the study was to investigate regeneration mechanism of human healthy and dilated myocardium-derived primary mesenchymal stem/stromal cells *in vitro* and to evaluate the impact of histone deacetylase inhibitors and biomatrices. The data of this study suggest that choosing the right composition or surface geometry biomatrices, cardiomyogenic differentiation inducers, or their combination is very important for the successful cardiomyogenic differentiation studies *in vitro*. In this study, we showed that dilated myocardium-derived hmMSC had a significantly higher HDAC level but decreased energetic potential compared to the healthy myocardium hmMSC. HDAC inhibitors suppressed HDAC level and increased mitochondrial activity, oxygen consumption and ATP production in healthy as well as dilated myocardium hmMSCs. Increased mitochondrial activity contributed to cardiac differentiation *in vitro* by upregulation of GATA4, NKX2-5, HOPX, TNNT2, ACTC1 genes and one of the main structural heart proteins, cardiac alpha-actin. Thus, the findings of this study show that dilated myocardium mesenchymal stem/stromal cells still retain cardiomyogenic differentiation potential, which can be stimulated by the targeted combination of HDAC inhibitors and ECM-based biomatrices. Hopefully, these results will serve as a molecular background for the further more detailed DCM studies searching for new therapeutic means.

THE EFFECT OF ACTIVE WEIGHT LOSS STRATEGIES ON HEALTH INDICATORS, MOTOR, AND COGNITIVE FUNCTIONING IN MIDDLE-AGED OVERWEIGHT AND OBESE WOMEN (Biology)

Laura Žlibinaitė

Scientific supervisor:
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Dissertation defended:
27 September 2021

Due to a sedentary lifestyle, more and more people are becoming obese nowadays. In addition to health-related problems, obesity can also impair cognition and motor performance. Substantial evidence has suggested exercise to be an effective way to fight obesity and related cognitive and motor dysfunctions. The main aim of the research was to determine the effect of active weight loss strategies (exercise and calorie restriction) on health indicators, autonomic

nervous function, and motor and cognitive functioning in middle-aged overweight and obese women. The findings of the study specify globally recommended levels of optimised physical activity volume and its benefits for physiological and psychological parameters in middle-aged overweight and obese women. The present study indicated that a healthy weight loss strategy (when body mass is reduced, metabolic syndrome parameters improve, aerobic fitness improve, and psychosocial state improves) is capable to significantly reduce excessive body weight either with 150 or 300 minutes of aerobic training per week. However, with the first option of training (150 min/week) there is still a need to restrict calorie intake. Notwithstanding, reducing weight with these two exercise programmes does not improve either cognitive function, motor control, or muscle strength. Therefore, those qualities need to be developed by specific strategies. Overall, these results may lead to future studies examining specific influence of age and gender on motor and cognitive behaviour alterations and investigating whether alterations persist after a period of detraining. Also, longer interventions favouring brain changes in overweight and obese population with more detailed analysis of myokines and metabolic health markers should be investigated.

THE EFFECT OF AGE AND PHYSICAL ACTIVITY ON OXYGEN UPTAKE KINETICS AND COGNITIVE FUNCTION (Biology)

Ligita Šilinė

Scientific supervisor:
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Dissertation defended:
28 September 2021

The oxygen uptake (VO_2) kinetics is one of the key parameters of aerobic fitness. The aim of the research was to determine the effect of age and physical activity on oxygen uptake, muscle deoxygenation kinetics, and cognitive function. Healthy non-smokers females and males without previously diagnosed respiratory, cardiovascular, metabolic, or musculoskeletal disease volunteered to participate in this study. Additionally, participants were not taking any medications that could affect their cardiorespiratory or metabolic response to exercise. The results showed that physical activity volume is associated with higher ventilatory threshold and faster oxygen uptake kinetics, while sedentary time is related to lower ventilatory threshold but is not associated with oxygen uptake kinetics. Heart rate kinetics during on – and heart rate and oxygen uptake kinetics during off – transition did not yield any significant correlations with the physical activity variables in elderly

women. Better executive function is associated with faster on-transition aerobic metabolism and higher aerobic fitness among older adults. Untrained women have significant slower oxygen uptake kinetics than recreationally trained women. However, oxygen uptake kinetics were not slower in older adults compared to their training-matched younger counterparts.

GENETIC DIVERSITY OF LITHUANIAN POPULATIONS OF COMMON REED GRASS (*PHALARIS ARUNDINACEA* L.) AND RELATED FACTORS (Biology)

Edvina Krokaitė

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Dissertation defended:
30 September 2021

Aquatic ecosystems are of big economic and ecological importance, so there is a great need for data on their status, spatial and temporal changes. One of the most common riparian species of Lithuania is the reed canary grass (*Phalaris arundinacea* L.). The plant is grown in various countries for the production of fodder and biofuels also for phytoremediation. Our work on the genetic structure of riparian populations of *P. arundinacea* is the first among the Baltic States. The aim of the study was to evaluate the molecular and physiological diversity of Lithuanian populations of *P. arundinacea*, linking it with the environment. The genetic diversity of the Merkys Basin populations was assessed using 14 microsatellite markers. The genetic diversity of Lithuanian populations of *P. arundinacea* was compared with populations of the other Baltic States, Luxembourg and Russia Far East. The dependence of the genetic traits of populations of *P. arundinacea* on various environmental factors – river basins, land cover and use class, water state in the rivers, nitrogen pollution by agriculture, river size and river bed type is described. Genetic differentiation has been determined between individuals of the variegated form grown in Lithuania and the naturally growing individuals of *P. arundinacea*. Nitrogen concentrations in the leaves of Lithuanian populations were analyzed and compared with populations of the other riparian species.

DIVERSITY, MOLECULAR CHARACTERISATION, AND ASSOCIATED PATHOGENS OF LAELAPIDAE (ACARI: MESOSTIGMATA) MITES FROM SMALL RODENTS (Biology)

Evelina Kaminskienė

Scientific supervisor:
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Vytautas Magnus University

Dissertation defended:
30 September 2021

The aim of the research was to investigate the diversity, abundance, and prevalence of Laelapidae mite species parasitising small rodents, to molecularly characterise mite species, and to determine the occurrence and prevalence of vector-borne bacterial pathogens in mites. A total of 748 small rodents were snap- and live-trapped in various locations in Lithuania, Slovakia, the Czech Republic, and Norway from 2013 to 2017. A total of 343 (47.1%) rodents in Lithuania were found to be infested with eight species of mites (n = 1363): *Laelaps agilis*, *Laelaps hilaris*, *Hyperlaelaps microti*, *Haemogamasus nidi*, *Haemogamasus hirsutus*, *Eulaelaps stabularis*, *Hirstionyssus sunci*, and *Myonyssus gigas*. The dominant species of mite found on 43.4% rodents was *L. agilis* (89.1%). Phylogenetic relationships of Laelapidae mites inferred from the 28S rRNA and COI were similar to those derived based on their morphological classification and could be used for molecular identification of Laelapidae mite species. Phylogenetic analysis based on COI showed sequences variability from different regions of Europe and host species. Mites were infected with *Rickettsia helvetica*, *Rickettsia felis*, *Rickettsia* sp., *Bartonella grahamii*, and *Bartonella taylorii* pathogens. This study provides new geographical and host records for laelapid mites and new molecular information for the identification and phylogenetic relationship of Laelapidae mites. This is the first detection of *R. felis* in *L. agilis* and *H. microti* mites, *R. helvetica* in *M. gigas* mites, *B. taylorii* in *L. agilis*, *Hg. Nidi*, and *M. gigas* and *B. grahamii* in *L. agilis* mites.

THE ROLE OF THE MICROCIRCULATION IN SKELETAL MUSCLE FUNCTION AND PLASTICITY (Biology)

Paul Hendrickse

Scientific supervisor:
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Dissertation defended:
30 September 2021

The aim of the study was to investigate, in rodent models and human subjects, the function of capillarisation in skeletal muscle fatigue resistance and hypertrophy in health, disease (CHF in particular), ageing, and at different baseline muscle mass. The results of the study showed that loss of functional capillaries is detrimental to muscle function in rats, even in otherwise healthy tissue, independent of arterial perfusion. Impairment of muscle performance through

capillary blockage can be restored through mechanical overload stimulus, indicating that angiogenic treatments can restore exercise tolerance. Compensatory cardiac hypertrophy in rats results in reduced functional CD which is associated with a reduction in muscle performance. This reduction in muscle performance can be countered with the proangiogenic interventions of wheel running exercise and overload. Adding endurance training to resistance training in highly resistance-trained individuals can lead to increases in muscle capillarisation and oxidative capacity without negative consequences for muscle size and strength. This occurs in both younger and older highly resistance trained men. Overload induced hypertrophy was not blunted by endurance exercise and endurance exercise increases in fatigue resistance were not impaired by overload. Therefore combining endurance exercise and overload in young and old mice induces the benefits of endurance and hypertrophic stimuli without compromising adaptations to either. Old mice experienced an attenuated hypertrophic response to overload which may be due to a diminished capacity for angiogenesis. BEH, C57, and BEL mice strains demonstrated similar absolute increases in FCSA, although BEH mice with more fibres demonstrated the largest increase in the muscle mass and BEL with the fewest fibres demonstrated the smallest increase in the muscle mass.

EVALUATION OF PATTERN OF CHEMOTHERAPY RESISTANCE AND TUMOUR RELAPSE RELATED TO CYTOTOXIC DRUG UPTAKE AND RETENTION (Biology)

Sima Garberytė

Scientific supervisor:
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Centre for Innovative Medicine

Dissertation defended:
13 October 2021

In this work, the influence of the AUC (area under the concentration curve) of the chemotherapeutic drug doxorubicin on tissue chemotherapeutic efficacy was investigated for the first time. Previous studies have been limited to detecting changes in plasma. According to many authors, high levels of doxorubicin may accumulate in tissues and persist there for a long time, even 8–240 days, after the cessation of treatment. Tissue-bound doxorubicin levels may reach up to 550-fold drug amount compared to plasma. However, to the author's knowledge, the effect of this drug on tissue AUC to therapeutic efficacy or side effects has not been previously studied. The stability of the chemotherapeutic drug doxorubicin has been studied under a variety of conditions: hydrolysis, peroxide oxidation at 80°C, photolysis, microwave irradiation. To the author's knowledge, the long-term

(365 days) effect of human body temperature on doxorubicin stability has not been evaluated. A special *in vitro → in vivo* TSDR (two-step dormancy/recurrence) model was developed to evaluate the association of doxorubicin accumulation in cells with tumour recurrence. In contrast to other authors, our model is based on short-term (up to 30 min) incubation of tumour cells with the chemotherapeutic drug and further evaluation of these cells not *in vitro*, but *in vivo* in the peritoneal cavity of mice.

CHANGES IN KYNURENINE METABOLITE CONCENTRATION FOLLOWING PHYSICAL ACTIVITY (Biology)

Ada Trepci

Scientific supervisor:
Prof. Dr Sophie Erhardt,
Lithuanian Sports University

Dissertation defended:
27 October 2021

The main aim of this study was to establish a sensitive bioanalytical method for simultaneous quantification of kynurenine pathway metabolite concentrations in human CSF and plasma and investigate how different physical activity paradigms and stress conditions affect the concentration of these metabolites in brain and in the periphery of healthy subjects. A sensitive LC-MS/MS method for quantification of kynurenine metabolites in human CSF and plasma was developed and validated. Kynurenines show good stability both in CSF and plasma. Ratio PIC/QUIN is decreased in CSF of suicide attempters. One bout of sprint interval exercise (SIE) affected kynurenine and KYNA plasma levels only in older healthy subjects, by decreasing them, but did not affect any of the kynurenine metabolites in younger healthy subjects. In elderly healthy subjects, after three weeks of SIE plasma tryptophan levels increase for subjects taking antioxidant supplementation and plasma QUIN levels decrease with or without antioxidant supplementation. Intensive training decreases plasma tryptophan and kynurenine and increases CSF KYNA, 3-HK, and PIC. Acute cold stress does not affect any of the kynurenine metabolites in healthy male subjects, but prolonged cold stress increases plasma NAA levels in healthy male subjects.

THE EFFECT OF EXERCISE AT SCHOOL ON COGNITIVE AND FINE MOTOR PERFORMANCE IN ADOLESCENTS: ASSOCIATION WITH STEROID HORMONES (Biology)

Justė Knatauskaitė

Scientific supervisor:
Prof. Habil. Dr Henning Budde,
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Dissertation defended:
29 October 2021

The aim of this study was to evaluate the effect of exercise on cognitive and fine motor performance and to find out if this effect relates to steroid hormones in adolescents. The results of the study showed that cortisol concentration increased after vigorous intensity exercise and decreased after light intensity exercise 20 min after acute intervention. There were no differences between the experimental groups and the controls in cognitive performance and testosterone concentration after acute game-based exercise of light vs. vigorous intensity. However, changes in testosterone concentration were adversely associated with the changes in cognitive performance after the vigorous intensity game-based exercise and control condition. Accuracy of the attentional control was improved in the coordinative exercise training group, while accuracy of the visuospatial processing did not change in any of the two experimental groups. Mathematical skills were improved in the coordinative exercise training group, while language skills improved in both coordinative and cardiovascular exercise training groups. There were no changes in fine motor skills and concentration of steroid hormones after both coordinative and cardiovascular exercise training. Changes in accuracy of the visuospatial processing were negatively associated with the changes in testosterone concentration independent of the group. Changes in fine motor skills were not associated with the changes in concentration of steroid hormones after the coordinative vs. cardiovascular exercise training.

THE UTILITY OF MODIFIED NUCLEOTIDES FOR HIGH-THROUGHPUT NUCLEIC ACID ANALYSIS (Biology)

Žana Kapustina

Scientific supervisor:
Prof. Dr Arvydas Lubys,
Vilnius University

Dissertation defended:
22 November 2021

Synthetic base-modified nucleic acids are widely used in various fields. The aim of this dissertation was to investigate the properties and utility of oligonucleotide-tethered 2′,3′-dideoxyribonucleoside 5′-triphosphates (OTDDNs), with emphasis on labeling of DNA and cDNA molecules for high-throughput sequencing applications. It was demonstrated that OTDDNs are substrates for a range of DNA polymerases of families A, B, X, and RT. By means of *in vitro*

evolution, we selected T7 RNA polymerase mutant with relaxed substrate discrimination that is able to synthesise chimeric nucleic acids labeled by OTDDNs. Moreover, DNA polymerases that perform synthesis through the unnatural linkage within OTDDN were identified. Several known NGS library preparation methods rely on chemoenzymatic approaches; however, such techniques suffer from poor efficiency and laborious protocols. In this work, modified terminators enabled the integration of fragmentation and sequencing adapter addition into a single enzymatic step. This in turn opened doors for the development of new DNA and RNA sequencing methods which have rapid and simple protocols and generate high-quality data. Two new OTDDN-based methods described in the dissertation include novel microbiome analysis technique st16S-seq and novel gene expression analysis method MTAS-seq. Taken together, this work suggests OTDDNs as a promising tool for high-throughput nucleic acid analysis.

ROLE OF SECRETED CLASS 3 SEMAPHORINS IN PROCESSES OF GLIOMAGENESIS AND ANGIOGENESIS (Biology)

Indrė Valiulytė

Scientific supervisor:
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Dissertation defended:
6 December 2021

Astrocytomas are central nervous system tumours characterised by molecular heterogeneity, resistance to therapy, and the ability to regrowth. Despite complex therapy, the survival time of patients is generally short (approx. 14 months). Therefore, new astrocytoma-specific molecules are being sought that would contribute to the more accurate diagnosis, prognosis, and more effective treatment of the disease. Secreted class 3 semaphorin proteins (SEMA3s), consisting of seven members (A-G), play an important role in cancer biology and angiogenesis. Depending on the interaction of SEMA3s with receptors or protein proteolytic cleavage, they can mediate either tumour-promoting or -suppressing functions. Thus, SEMA3s could be promising molecules used for the treatment of astrocytomas. The aim of this research was to explore the potential of SEMA3/VEGF signaling molecules in the prognosis of astrocytomas and evaluate the biological effects of SEMA3 proteins on gliomagenesis and angiogenesis processes. During the study, the expression of the set of key genes of the SEMA3/VEGF signaling pathway was analysed in different malignancy astrocytoma tumours and a novel multibiomarker created for

patient survival prognosis. Also, the role of the SEMA3C protein in angiogenesis as well as the functional importance of the furin cleavage site 742RNRR745 at the basic domain of SEMA3C was revealed. Finally, hybrid SEMA3A/VEGF derivatives were created and their effects were analysed on angiogenesis *in vitro*.

THE STRUCTURE OF FRUIT MYCOBIOTA AND FUNCTIONING MECHANISMS OF ITS COMPONENTS – KILLER SYSTEMS OF *SACCHAROMYCES* GENUS YEASTS (Biology)

Iglė Vepštaitė-Monstavičė

Scientific supervisor:
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Nature Research Centre

Dissertation defended:
10 December 2021

Yeasts are widespread unicellular eukaryotic fungal microorganisms, abundant in the air, water, soil, and on various parts of plants, especially on fruits and berries. Certain yeasts possess killer trait and produce toxins that are killing sensitive yeasts, and in this manner killer yeasts can regulate the structure of microbiota. This work extends the knowledge about mycobiota of widely consumed fruits and berries: composition of fungal microorganism communities of white, black, and red currants, chokeberries, sea buckthorn berries, and apples, depending on the plant, fruit ripening stage, and external environment, as well as prevalence of yeast viral killer systems. The aim of this thesis was to evaluate the structure of mycobiota present on fruits and berries, and to perform the screening for killer systems of *Saccharomyces* spp. yeasts, and investigate their mechanisms of functioning. Investigation of the abundance of yeasts on the surface of fruits and berries led to the identification of *S. paradoxus* AML-15-66 strain, producing double stranded RNA-encoded K66 toxin. A novel viral dsRNA killer system is described, genetic factors involved in the resistance to K66 toxin are identified. Based on the action and cellular response mechanisms, *S. paradoxus* K66 and *S. cerevisiae* K2 killer systems were compared.

DIAGNOSTIC AND PROGNOSTIC DNA METHYLATION BIOMARKERS OF RENAL CLEAR CELL CARCINOMA (Biology)

Raimonda Kubiliūtė

Scientific supervisor:
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Dissertation defended:
22 December 2021

Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney tumour with the highest incidence rates in Lithuania. CcRCC is characterised by the highest mortality rate of genitourinary cancers, therefore new diagnostic and/or prognostic biomarkers are urgently needed. Considering this, the current study aimed to develop a gene-specific DNA methylation tool for non-invasive and early kidney cancer diagnosis and follow-up. Genome-wide DNA methylation profiling utilising microarrays allowed the identification of a set of novel presumable ccRCC-specific DNA methylation biomarkers, having moderate to high diagnostic and/or prognostic potential. Moreover, identified DNA methylation alterations at the regulatory regions of selected genes appeared to be amenable for non-invasive detection in the urine samples of ccRCC patients, where they outperformed the diagnostic and prognostic value of previously described biomarkers and even some other parameters currently used in the clinical practice. Thus, the results showed a promising potential of the chosen genes as candidates for further development of non-invasive tools for kidney cancer patients testing. Although further comprehensive verification is mandatory to prove the clinical significance of the provided DNA methylation biomarkers for ccRCC, the obtained results seem to be rather promising and will definitely encourage these validation studies.

INFLUENCE OF CALCIUM IONS ON MOLECULAR TRANSPORT THROUGH PLASMA MEMBRANE AND CELL VIABILITY AFTER ELECTROPORATION AND SONOPORATION (Biophysics)

Diana Navickaitė

Scientific supervisor:
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Vytautas Magnus University

Dissertation defended:
30 August 2021

Although calcium electroporation/sonoporation has recently begun to be used as an effective method for inducing cancer cell death, much is still unknown about the fundamental mechanisms involved in molecular electrotransfer/release into/from cells or cell death following the application of electrical pulses or ultrasound. One of the main phenomena identified in this work is the influence of Ca^{2+} on the transmembrane transport of small molecules after cell

electroporation. In this work, for the first time, a decrease in the electrotransfer efficiency of small molecules into/from cells was observed when the extracellular medium was supplemented with Ca^{2+}. Another phenomenon investigated in this work was the interdependence of simultaneous electrotransfer of Ca^{2+} and small molecules into cells. This work reveals that increasing extracellular Ca^{2+} concentration inhibits the electrotransfer efficiency of small molecules into cells. It shows for the first time that at concentrations of <1 mM Ca^{2+} in the extracellular medium after electroporation, the cell is able to pump out the excess of intracellular Ca^{2+}, but at ≥1 mM in the extracellular medium, the cells are unable to pump out the excess of intracellular Ca^{2+} after electroporation. This work also shows for the first time that the application of Ca^{2+} to the treatment of cancer offers new possibilities, causing rapid cell death (within 20 min). Results of experiments performed in this work provide fundamental insight on the mechanisms of Ca^{2+} electroporation and sonoporation that might lead to improved therapeutic outcomes.

MESENCHYMAL STEM CELL AND CANCER CELL RESPONSE TO TREATMENT WITH THERANOSTIC NANOPARTICLES: TOWARDS CELL THERAPY (Biophysics)

Dominyka Dapkutė

Scientific supervisor:
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Dissertation defended:
30 September 2021

The aim of this work was to investigate the ability of skin MSCs to selectively transport theranostic nanoparticles to tumours, ensuring therapeutic effects and diagnostic potential. The theranostic nanocomplex is composed of photoluminescent nanoparticles quantum dots (QDs) and photosensitiser chlorin e6 (Ce6). In this dissertation, passive delivery of nanoparticles to tumours was found to be non-specific and inefficient, thus justifying the need to use MSCs. It was also found that amphiphilic polymer coated and carboxyl functionalized QDs were the most suitable for complex formation with Ce6. Our research shows that such a complex effectively generates singlet oxygen when excited by a specific 470 nm wavelength light, but we demonstrated for the first time that serum proteins quickly destabilise the complex and significantly reduce its efficiency. In *in vitro* studies, we show that nanoparticles are biocompatible and accumulate in the MSCs in endocytic vesicles, where the QD-Ce6 complex is protected from the negative effects of serum proteins and remains stable. *In vitro* and *in vivo*

studies have proven that MSCs are able to transport theranostic nanoparticles selectively to cancer cells, to label tumours with visible photoluminescence, and cause cancer cell death during two-step irradiation procedure. The combined therapy of MSCs, QD-Ce6 complex, and non-toxic light significantly increases survival, reduces tumour growth, metastatic potential and overall cancer progression. Our results confirm that MSCs with QD-Ce6 complex can be applied in cancer theranostics.

CLIMATE CHANGE IMPACT ON WINTER WHEAT (*TRITICUM AESTIVUM* L.) SEASONAL DEVELOPMENT (Ecology and environmental)

Martynas Klepeckas

Scientific supervisor:
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Vytautas Magnus University

Dissertation defended:
22 January 2021

Wheat (*Triticum aestivum* L.) is the most widespread and economically significant cereal in the world. However, its cultivation requires a lot of energy and emits a lot of greenhouse gases. In this dissertation, it was found that the increased duration of vernalization had a positive effect on photosynthesis and hormone parameters in many cases. The effect of long-term global warming on changes in the phenological stages and seasonal development of winter wheat between 1961 and 2015, when the phenological phases of winter wheat changed due to the changing climate during 55 years, was analysed. Using mathematical models, developmental predictions were calculated for the onset of dormancy, vegetation regeneration, and maturation at phenological stages when even more phenomenal changes might be encountered due to the changing climate. After field trials, a plant growth model DSSAT was prepared and calibrated to determine the basic genetic coefficients of winter wheat cultivars 'Skagen'. The RCP 2.6 and RCP 8.5 climate change scenarios also calculate phenological stages and yield forecasts for winter wheat germination, flowering and maturity up to 2100, which seem to delay the germination and flowering stages of winter wheat and lead to early maturation. The estimated forecasts for the development of winter wheat need to be better understood between climate change and the seasonal development of crops and will also serve as a useful tool for the development of new crop production technology.

STATISTICAL ASSOCIATIONS OF GEOMAGNETIC ACTIVITY AND HIGH-SPEED SOLAR WIND WITH WEATHER PATTERN AND PATIENTS' CARDIOVASCULAR CHARACTERISTICS (Ecology and environmental)

Deivydas Kiznys

Scientific supervisor:
Prof. Dr Jonė Venclovienė,
Vytautas Magnus University

Dissertation defended:
5 February 2021

The research is designed to evaluate the relationships between geomagnetic storms, solar wind and stream interaction regions, and patient health, meteorological indicators. Data on health indicators were collected at the Institute of Cardiology of the Lithuanian University of Health Sciences. Daily meteorological and heliogeophysical indicators were collected from publicly available websites, databases, or scientific publications. For the first time, the study identified health effects of stream interaction regions and identified a new factor, the days of intersection (days that can be attributed to the days before and after the heliogeophysical event), and their impact on the risk of the acute coronary syndrome. During the analysis, the complex and intersection effects of high-speed solar wind and stream interaction regions on winter and spring meteorological indicators in the Baltic region were determined. In the winter and spring seasons, the high-speed solar wind increased the air temperature and reduced the atmospheric pressure. Due to the combined effects of geomagnetic storms, high-speed solar wind, stream interaction regions, the risk of myocardial infarction was higher, and the impact on meteorological indicators in the Baltic region in winter and spring was stronger than individual factors.

MIGRATORY BEHAVIOUR IN A LONG-DISTANCE MIGRANT SONGBIRD, THE GREAT REED WARBLER (Ecology and environmental)

Gintaras Malmiga

Scientific supervisor:
Dr Rimgaudas Treinys, Prof. Dr Dennis Hasselquist,
Nature Research Centre

Dissertation defended:
5 May 2021

In this study, migratory behaviour of the great reed warblers (*Acrocephalus arundinaceus*) breeding in south-central Sweden were analysed. Data on their location, time, flight duration, and altitude were collected using geolocators and multisensory data loggers mounted on their backs. The study shows that total active flight time between breeding and wintering grounds was larger in females than males, despite no significant difference in flight distance between the sexes. The great reed warblers were flying higher over Africa and their nocturnal flights lasted longer than when

they migrated over Europe. The great reed warblers often extended their nocturnal flights into the day when flying over the Sahara Desert and the Mediterranean Sea at the same time reaching higher flight altitudes during day than night. Moreover, males departed from the wintering grounds and reached breeding grounds earlier than females. Similarly, older great reed warblers departed earlier from the wintering grounds than younger individuals.

ENVIRONMENTAL GENOTOXICITY AND CYTOTOXICITY STUDIES IN FISH BLOOD ERYTHROCYTES AND GENOTOXICITY RISK ASSESSMENT IN THE GOTLAND BASIN OF THE BALTIC SEA (Ecology and environmental)

Janina Pažusienė

Scientific supervisor:
Habil. Dr Janina Baršienė,
Nature Research Centre

Dissertation defended:
18 June 2021

This thesis aims to assess the peculiarities of genotoxicity and cytotoxicity effects in fish species inhabiting the southern and eastern parts of the Gotland Basin of the Baltic Sea. *In situ* and *in vivo* studies results revealed cytogenetic effects on aquatic species caused by anthropogenic chemical and physical pollution. For the first time, genotoxicity and cytotoxicity levels were assessed in peripheral blood erythrocytes of herring (*Clupea harengus membras*), flounder (*Platichtys flesus*), and cod (*Gadus morhua callarias*) inhabiting the dumping site of chemical weapons (CW) located in the eastern part of the Gotland Basin. The highest genotoxicity level was recorded in herring from the CW dumping zone. Exceptionally high and high genotoxicity risks to herring and flounder were determined at most of the study stations located in the CW dumping zone and its vicinity. *In situ* studies provide new data about the ecological status of the southern part of the Gotland Basin. Exceptionally high and high genotoxicity risks were found to herring, flounder and cod at all the study stations located in the oil and gas platform zone. According to the obtained genotoxicity risk results, the southern part of the Gotland Basin cannot be qualified for the Good Environmental Status. For the first time, the potential *in vivo* genotoxic and cytotoxic effects of 50 Hz 1 mT electromagnetic field (EMF) were assessed in rainbow trout (*Oncorhynchus mykiss*) larvae and Baltic clam (*Limecola balthica*). These findings suggest that exposure to the EMF of the intensity typically generated by submarine cables affects molluscs and early life stages of typical salmonids significantly negatively.

THE INTERACTION BETWEEN MESIC AND STEPPE GRASSLANDS: SYNTAXONOMICAL, ECOLOGICAL, AND PHYTOGEOGRAPHICAL ASPECTS (Ecology and environmental)

Domas Uogintas

Scientific supervisor:
Dr Miglė Stančikaitė, Dr Valerijus Rašomavičius,
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23 June 2021

The object of the study is mesic and steppe grasslands and their syntaxonomical, ecological and phytogeographical interactions. The territory of Lithuania is in an ecotone between two biomes. The vegetation formed in such geographical coverage should be characterised by a higher diversity of plant species in the communities and a mosaic of different combinations of species. Research on the diversity and structure of such communities is important at the continental scale. Our results showed that mesic and steppe grasslands could belong to two vegetation classes (Molinio-Arrhenatheretea and Festuco-Brometea), four alliances (Cynosurion, Deschampsion, Arrhenatherion, and Bromion), and ten associations. Elenberg indicator values were used to identify the most important environmental gradients. We discovered out that the occurrence of steppe grasslands in the area is determined by local thermal conditions. Average annual air temperatures are 2–2.5°C higher than values typical of the Lithuanian climate. Short-term abandonment resulted in the loss of plant species and functional diversity. According to one of the phytogeographical features, the composition of phytocoenons is supported by a degree of oceanicity. The developed expert system of mesic and steppe grasslands classifies about 99% of vegetation plots.

MICROBIAL WATER QUALITY AND POTENTIAL RISKS FOR HUMAN HEALTH AT THE SOUTH-EASTERN BALTIC SEA BATHING WATERS (Ecology and environmental)

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Dissertation defended:
30 June 2021

Good bathing water quality is essential for recreational tourism and plays a crucial role in public health; therefore, it is monitored according to the Bathing Water Directive (BWD) (2006/7/EC). Based on the monitoring data of faecal indicator bacteria (FIB) of the past 15 years, pollution hot spots were identified. The case study of Lithuanian coastal waters is devoted to the first investigation of *Vibrio* presence, abundance, and dependency on environmental conditions. For the first time in the BSR, quantitative microbial risk assessment (QMRA) was conducted to identify potential risks of *Vibrio* infections for bathers. An integrated microbial risk

assessment scheme was developed and applied to investigate FIB, cyanobacteria, *Vibrio* bacteria and their coexistence based on the findings of this study. The results show that FIB do not exceed the acceptable 'low' risk threshold, while cyanobacteria and *Vibrio cholerae* abundance reveal a 'high' probability of adverse health effects. A positive correlation found between *E. coli*, cyanobacteria, and *V. cholerae* indicates that all target microorganisms may occur at the same time with consequently high risks for the health of bathers. Furthermore, *Vibrio* is known to cause mass bivalve mortalities and numbers of infections through undercooked seafood consumption. The pilot mussel farm established as a mitigation measure to improve good ecological status (GES) in the low-salinity BSR waters shows the biodeposition of cultivable *Vibrio*. Thus coastal recreational sites near the mussel farm could be affected by the higher concentrations of potentially pathogenic *Vibrio*. This study proves the need to monitor emerging pathogens in the coastal waters of BSR and provides essential tools for investigating risks for bathers.

ASSESSMENT AND IMPACT OF ICE COVER AND FUTURE PROJECTIONS FOR THE BALTIC CURONIAN LAGOON (Ecology and environmental)

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24 September 2021

This study, for the first time, presents comprehensive analysis of ice cover phenology, thickness distribution, as well as impact on the aquatic environment of the Curonian Lagoon, the largest lagoon in Europe. A combination of advanced remote sensing imaging techniques, conventional *in situ* observations, and numerical modelling techniques enabled the exploration of new approaches in the monitoring and forecasting of the ice cover. Using satellite data in the numerical modelling framework SHYFEM revealed the importance of high resolution ice observations for describing the physical processes during the ice cover season, such as circulation, water exchange capabilities, saltwater intrusions, water residence time, and suspended sediment dynamics. The incorporation of an advanced sea ice thermodynamic model in the modelling framework SHYFEM, together with remote sensing and ground observations, serve as an important tool for the analysis and representation of ice thickness over the whole lagoon surface, testing the impact of different climate change scenarios on the ice cover, and further improve the numerical description of this freshwater lagoon.

MESO- AND MICROPLASTIC IN THE BALTIC COASTAL ENVIRONMENT (Ecology and environmental)

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8 October 2021

It is important to implement harmonised beach litter monitoring methods to allow comparability of results. However, frequently used methods, such as the OSPAR method for beach macro-litter (>25 mm) monitoring, were shown to be less suitable for the Baltic Sea region due to its focus on rural and undisturbed beaches, which are rare along the Baltic shore, and its tendency to underestimate smaller litter (<25 mm). To overcome these weaknesses, six methods to examine meso-litter (5–25 mm) and large micro-litter (2–5 mm) were developed and tested at the beaches of the Baltic Sea. Four of these methods, investigating flood accumulation zones and beach wrack were less practical because they were not regularly repeatable. Further, pollution in the flood accumulation zone was highly irregular. Therefore, results were difficult to compare and to extrapolate, and this is hampering long-term trend analysis. A developed frame method was discarded as it only partly investigated the backshore, and surveys were too time intensive. A tested sand rake method, investigating the whole backshore of the beach, turned out to be useful, reliable, and fitting within monitoring requirements of the Marine Strategy Framework Directive. In more than 200 sand rake surveys carried out around the Baltic Sea, a total of 9345 litter pieces were found on 10,271 m^2, of which 69.9% were 2–25 mm in size. Plastic (4921 pieces) was the predominant material found (mean 52.7% ± 13.3). Abundance of litter was 0.91 pieces/m^2 ± 1.50 (median 0.40 pieces/m^2). Results of the Baltic-wide campaign can be used for calculating a pollution baseline, defining the Good Environmental Status for smaller litter (2–25 mm) and can serve for assessing the effectiveness of marine litter mitigation measures in the Baltic Sea region.

EVALUATION OF WILDLIFE–VEHICLE COLLISION PATTERNS AND ASSESSMENT OF MITIGATION MEASURES (Ecology and environmental)

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Dissertation defended:
19 October 2021

The aim of research was to evaluate long-term wildlife-vehicle collision (WVC) patterns and assess mitigation measures in Lithuania. Roadkill data was evaluated, habitat suitability based wildlife movement models were developed, WVC hotspot locations and temporal roadkill patterns were identified, effectiveness of mitigation measures was assessed, roadkill-data-based habitats were identified and ranked by attractiveness to wildlife and risk severity to the drivers. Road traffic accident database underestimates the impact of WVC on wild mammal diversity and roadkill of smaller species. The wildlife movement models accurately represented the most probable pathways and road crossings. Large part of wildlife pathways and WVC hotspots are not permanent. The highest risk of WVC occurs during twilight hours within the WVC hotspots and in the vicinity of habitat patches that are most attractive to wildlife and pose the highest risk for the drivers. Increased amounts of wildlife fencing reduce the number of WVC on main roads with highest traffic and shift WVC towards the lower category roads. Efforts to reduce WVC on higher category roads shall focus on fencing with permanent crossing structures. Efforts to reduce WVC on lower category roads should focus on driver trainings and dynamic warning systems. Ex-Post mitigating WVC without Ex-Ante analysis of WVC, the habitat isolation and roadkill mitigation costs may be amplified without any positive effect to the drivers and wildlife.

FACTORS OF ENVIRONMENTALLY FRIENDLY BEHAVIOUR IN THE EU AND CHANGE IN THE ENVIRONMENTALLY FRIENDLLY BEHAVIOUR OF THE LITHUANIAN POPULATION IN THE PERIOD OF 2011–2020 (Ecology and Environmental)

Audronė Minelgaitė

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Dissertation defended:
12 November 2021

The research aim of this thesis was to assess the factors at the European Union level influencing environmentally friendly behaviour and to compare the changes in the environmentally friendly behaviour of the Lithuanian population between 2011 and 2020 and to identify the main factors that contributed most to the changes in the environmentally friendly behaviour. According to the surveys already carried out by the Eurobarometer, this study analyses environmentally friendly behaviours such as the use of public transport,

waste sorting, the use of natural resources, their trends and factors throughout the European Union, including Lithuania. It is also important to analyse not only the factors that determine environmental friendliness but also to assess the effectiveness of the measures taken in implementing sustainable development policies. Therefore, the following thesis, based on a representative survey of environmental determinants of behaviour in Lithuania conducted in 2011, analyses changes in people's behaviour in respect of the environment and environmental attitudes from 2011 to 2020. This research is of great importance as it shows whether the measures recommended and implemented really contribute to increasing environmental friendliness and reducing the environmental impact. Also, based on the results obtained, recommendations will be prepared on what needs to be changed so that people in Lithuania become more conscious and behave as environmentally friendly as possible. Thus, this thesis is unique because (a) it includes research in respect of the place through the analysis of environmentally friendly behaviour in all European Union countries, and (b) research in respect of time through the analysis of changes in behaviour and attitudes of Lithuanian people from 2011 to the present, when special attention was paid to eco-education during this period.

CHANGES IN THE STATUS OF WOODLAND KEY HABITATS UNDER VOLUNTARY PROTECTION CONDITIONS (Ecology and environmental)

Indrė Ruškytė

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Dissertation defended:
25 November 2021

The aim of this research was to assess the contribution of the potential and woodland key habitats (WKH) initiative to conserve biodiversity in Lithuania's forests and relevant additional measures to continue this initiative. The analysis of the Lithuanian WKH database helped to assess the changed tendencies in the condition of woodland key and potential key habitats in Lithuania and the dependence relations between habitat elements and to evaluate the viability and the potential of survival for giant trees – old common oaks – in woodland key habitats. Over the period of 10–15 years, the condition of the majority (90.5%) of real woodland key habitats (rWKH) remained stable or improved, and the condition of 9.5% of rWKHs decreased and became potential. Meanwhile, the condition of 60.3% potential woodland key habitats (pWKH) remained stable or improved and even 39.7% of pWKHs became rWKHs. The ability of most WKHs to maintain the abundance and diversity of rare species as well as the significant increase in habitat values over a period of 10–15 years shows that

the WKH network provides opportunities to successfully increase high conservation value forest areas in Lithuania in order to implement international biodiversity commitments. The WKH initiative contributed to the conservation of forest habitats on the ground. However, under its current voluntary protection system and lack of funding, its continued contribution to the biodiversity conservation is uncertain. The study emphasises the official legal need for the conservation of WKHs by including them in the system of protected areas as one of its elements.

PROCESSES OF NATURAL HYBRIDISATION IN NATIVE SPECIES OF *ALNUS* AND *ULMUS* GENUS (Ecology and environmental)

Sigitas Tamošaitis

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15 December 2021

This study presents the results on the relationships between morphometric and molecular features of elms (*Ulmus* spp.) and alders (*Alnus* spp.). Based on the molecular results, 29% of the elm hybrids were classified as F2. Two microsatellite loci (Ulm198 and Ulm19) were identified that distinguish the European white elm from other species of the elm genus. For the identification of hybrids of *Ulmus* species and the study of genetic diversity, a set of eight nuclear microsatellite markers was developed: Ulm2, Ulm3, Ulmi1–21, Ulmi1–98, Ulmi1–165, Ulm19, Ulm6, and UR158. The leaf morphology and genetic analyses of putative elm hybrids indicated low genetic exchange between *U. laevis* and the other *Ulmus* species complex. This leads to a statement that *U. laevis* unlikely creates spontaneous hybrids among the *Ulmus* species. The genetic profile allows to identify *U. glabra* (female) × *U. minor* (male) spontaneous hybrids using leaf morphology. Molecular studies of species of the genus *Alnus* confirmed only 23% of interspecific hybrids identified by leaf morphological traits. Of the hybrids, 65% were classified as F1, the rest were subsequent generations of hybrids. All genetic diversity parameters were the lowest for grey alder, while the inbreeding coefficient was the highest. The group of hybrids had significantly higher values of genetic diversity indices. Thirty-nine private alleles were identified, and they were distributed differently in three groups of alder species, with most private alleles observed in the black alder group. This study provides a strong evidence for spontaneous hybridisation between sympatric species of *A. incana* and *A. glutinosa* in natural forests of northern Europe. The hybrid alders seem to be genetically closer to *A. incana* and are of a markedly greater genetic diversity than the corresponding parental species.