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

Compiled by Indrė LIPATOVA

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COMPLEX SINGLE-CELL ANALYSIS USING HYDROGEL MICROCAPSULES (Biochemistry)

Greta Leonavičienė

Scientific supervisor:
Prof. Dr Linas Mažutis
Vilnius University

Dissertation defended:
24 February 2023

Biological samples are intrinsically heterogeneous. Unravelling this heterogeneity is important for a better understanding of the biological functions of individual cells and their differences. Single-cell profiling often requires complex multi-step molecular biology techniques to isolate single cells and process their genetic material. However, despite recent progress in the single-cell analysis field, the high-throughput analysis remains limited due to numerous technological constraints. This doctoral thesis introduces a practical and innovative solution to enable a high-throughput and complex single-cell analysis. This achievement was made possible by the development of semi-permeable microcapsules. The microcapsules are picoliter-nanoliter volume droplets enveloped by a thin hydrogel shell that acts as a semi-permeable membrane. Isolated single cells and high molecular weight biomolecules (e.g., gDNA, mRNA) are retained, while smaller molecules (e.g., enzymes, oligonucleotides, reagents) transverse the hydrogel shell by diffusion. Due to this semi-permeability, various enzymatic reactions and assays can be performed on a massive scale by simply transferring the microcapsules from one reaction tube to another. The microcapsules were applied for single bacterial genome amplification, multiplex RT-PCR, RNA sequencing, and cell culture. The technology and results revealed in this thesis are likely to create a foundation for further development of high-throughput single-cell omics methods.

METHOD FOR HIGH-RESOLUTION GENOME-WIDE ANALYSIS OF 5-HYDROXYMETHYLCYTOSINE AND ITS APPLICATION FOR EPIGENETIC STUDIES OF HUMAN DISEASES (Biochemistry)

Milda Narmontė

Scientific supervisor:
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Dissertation defended:
9 March 2023

5-hydroxymethylcytosine (5hmC) is an important DNA modification that plays a gene regulatory role in human physiological and pathological states. The so-called 'gold standard' single-base resolution methods for 5hmC profiling are based on bisulfite treatment, which causes DNA loss and imposes challenges in data analysis due to altered base composition. Furthermore, the high cost of whole-genome sequencing makes these approaches prohibitive for the disease or population studies. This work describes the development of a novel cost-effective 5hmC profiling method based on the covalent DNA labeling – hmTOP-seq (5hmC-specific tethered oligonucleotide-primed sequencing). We demonstrated the main advantages of hmTOP-seq: its high resolution and high specificity for 5hmC, good reproducibility, high correlation with other methods, and ability to provide DNA strand-specific hydroxymethylation information. The developed hmTOP-seq method was successfully applied in the epigenomic studies of human diseases – neuroblastoma (NB) and trisomy of the 21st chromosome (Down syndrome). Using hmTOP-seq and uTOP-seq, we performed a detailed multi-omic (5hmC, unmodified CG and transcriptomic) analysis of different NB cell types, which allowed us to determine hypoxia-induced changes in gene hydroxymethylation and expression, as well as comprehensively investigate differences among various NB cells. We also demonstrated that hmTOP-seq method can be applied for the epigenetic non-invasive prenatal testing of the foetal trisomy of chromosome 21 from blood plasma cell-free DNA of pregnant women using quantitative PCR or DNA sequencing.

IDENTIFICATION OF OXIDIZED 5-METHYLCYTOSINE DERIVATIVES BY NOVEL TECHNOLOGIES, THEIR DISTRIBUTION AND FUNCTIONS (Biochemistry)

Janina Ličytė

Scientific supervisor:
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Dissertation defended:
20 April 2023

5-methylcytosine (5mC) is a DNA modification important for eukaryotic gene regulation. TET proteins produce oxidised forms of 5mC (oxi-mCs) – 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). To understand the functions of oxi-mCs, accurate and cost-efficient genomic profiling methods are needed. In this work we investigated the 5caC decarboxylation by DNA methyltransferases and combined this reaction with the economical nucleotide-resolution TOP-seq method to develop a sensitive and specific 5caC profiling method caCLEAR (5caC clearance). caCLEAR analysis showed differences in the genomic distribution of 5caC between two pluripotency states of mouse embryonic stem cells, grown in serum-2i and serum conditions. Furthermore, we found an asymmetric distribution of 5caC in the antisense strand of active protein-coding genes of these cells. We also studied the basidiomycete fungi *Laccaria bicolor* and *Coprinopsis cinerea*, which have many homologues of TET genes, and for the first time, identified oxi-mCs in DNA of *L. bicolor*. In these fungi, we mapped genomic 5hmC and 5fC with the high-resolution 5hmC-specific hmTOP-seq method and developed its novel modification for 5fC profiling, foTOP-seq. A detailed assessment of 5hmC, 5fC and 5mC influence on gene expression showed differences between these organisms. For the first time, we detected oxi-mCs in RNA of both fungi and created a method facilitating the detection of 5caC in RNA by mass spectrometry which allowed us to analyse the abundance of 5caC in different RNA fractions.

IDENTIFICATION OF THE ROLE OF TRANSCRIPTION FACTOR ETV7 IN BREAST CANCER AGGRESSIVENESS (Biochemistry)

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Dissertation defended:
22 June 2023

This study focuses on ETV7, a transcriptional repressor known to be up-regulated in breast cancer (BC). Firstly, we demonstrated a new role of ETV7 in promoting breast cancer stem-like cell (BCSC) plasticity and resistance to chemotherapy in BC cells. We observed that ETV7 repressed a large panel of interferon response genes and increased BCSC cell plasticity, leading to resistance to 5-fluorouracil. Then, we investigated the role of ETV7 in inflammatory and immune responses in BC cells. We identified TNFRSF1A, encoding for TNFR1, as one of the genes repressed by ETV7. We demonstrated that ETV7 directly bound to the intron I of this gene, and we showed that the ETV7-mediated downregulation of TNFRSF1A reduced the activation of NF-κB signaling. These results suggest that ETV7 can reduce inflammatory responses in BC cells by repressing the TNFR1/NF-κB axis. Moreover, we analysed the role of ETV7 in the regulation of antigen presentation and confirmed that ETV7 downregulated genes involved in the antigen-presenting pathway, potentially leading to cancer immune evasion We also analysed if the silencing of ETV7 affected the viability of cancer cells and observed that knock-down of ETV7 can induce p53-mediated apoptosis in cancer cells. Lastly, we analysed the pro-tumorigenic potential of ETV7 using in vivo model and we observed that mammary gland tumour cells overexpressing ETV7 formed bigger tumours with higher proliferation potential. Taken collectively, the data acquired during this project confirm the role of ETV7 as an important regulator of BC aggressiveness both in vitro and in vivo and propose ETV7 as a novel player in BC immunity, opening a new research direction and giving useful insights for more effective therapeutic strategies.

INVESTIGATION OF PROTEIN KINASE MAPK AND AKT CROSSTALK AND DEPENDENCIES ON EXTRACELLULAR CONTACTS IN LUNG CANCER DERIVED CELL MODELS (Biochemistry)

Aurimas Stulpinas

Scientific supervisor:
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Dissertation defended:
3 July 2023

The thesis focuses on the molecular signaling pathways that regulate cell functions. Mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling molecules are responsible for cell proliferation, regulation of cell death, and participate in signal transduction from extracellular contacts. MAPK and AKT are also involved in drug-induced signaling; disruption of these signaling pathways is common in cancer cells and is associated with resistance to chemotherapy. A promising strategy for cancer treatment is the combination of conventional chemotherapy with targeted drugs, such as protein kinase inhibitors, to manipulate the signals induced by conventional drugs. Therefore, another topic addressed in this work is the resistance of cancer cells to treatment. We chose several primary non-small cell lung cancer cell lines, a commercial A549 lung adenocarcinoma cell line, and a healthy, stem-like Myo cell line as the subjects of our study, as well as models of inhibition of the extracellular contacts, in combination with the use of various protein kinase inhibitors (PI3K–LY294002, MEK–PD98059, p38–SB203580, JNK–SP600125, etc.) and approved drugs (MEK inhibitor selumetinib, AKT inhibitor capivasertib, etc.). We investigated whether/how MAPK and AKT participate in the death of adherent cells in 'weightless condition', i.e., in the anoikis process, as well as the interdependence of MAPK and AKT activity when the cells are treated with kinase inhibitors and the conventional drug cisplatin. Our studies suggest that, irrespective of cell origin/cell type, the basal phosphorylation level of the protein kinases studied is dependent on extracellular contacts. Among other results, we also showed that despite differences in cell phenotype or genotype, inhibition of the PI3K/AKT pathway promoted activation of the MAP kinase ERK and, vice versa, inhibition of the MEK/ERK pathway increased the level of phosphorylated AKT. This phenomenon was confirmed in both control and cisplatin-treated cells, also using different inhibitors of these kinases. Importantly, the ERK-AKT interaction is dependent on cell-substratum contacts and/or the kinase FAK. Since the activity of signaling molecules regulating cell fate is a principal factor in predicting cell behaviour as well as the response to therapy, this work highlights the dependence of the self-regulation and the response to the targeted drugs on the cellular state, proposing different molecular strategies for compact (tumour) and circulating cancer cells.

STUDY OF BIOCATALYSTS IMMOBILIZED IN THREE-DIMENSIONAL STRUCTURES (Biochemistry)

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Scientific supervisor:
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Dissertation defended:
30 August 2023

After the introduction of the electrode with immobilised enzyme in 1962, a new branch of science, currently known as biosensors, was established. Over the years, this field continues to grow due to advancing nanotechnology and molecular biology, resulting in various biosensors and bioelectronic devices aimed not only at determining the concentration of various physiological and technologically relevant analytes but also at powering small-scale devices. The research presented in this doctoral dissertation is intended to demonstrate not only a new application of well-studied enzymes by creating mono-enzymatic biosensors of various transduction types but also to understand the catalytical behaviour of oxidoreductases when immobilized on nanostructures. The goals of this research were to create three mono-enzymatic biosensors to determine the concentration of glycerol and L-amino acids in human fluids and to measure pH in the media by the use of immobilised alcohol dehydrogenase, L-amino acids oxidase, and bilirubin oxidase using nanostructured electrodes. At the same time, the catalytical behaviour of glucose oxidase and bilirubin oxidase was studied when immobilised on gold nanoparticles and carbon nanotubes, respectively, trying to address and explain the changes in the rate of the glucose oxidation reaction catalysed by glucose oxidase and the mechanistic study of the oxygen reduction reaction catalysed by bilirubin oxidase with respect to pH.

MOLECULAR MECHANISMS SHAPING NEURONAL NETWORKS DURING BRAIN DEVELOPMENT AND IN NEUROPATHOLOGIES (Biochemistry)

Ugnė Kuliešiūtė

Scientific supervisor:
Dr Urtė Neniškytė
Vilnius University

Dissertation defended:
1 September 2023

The mature brain connectome emerges during development via the extension and pruning of neuronal connections. Glial cells have been identified as key players in the phagocytic elimination of neuronal synapses and projections. Neuron-microglia contacts are tightly regulated by microglial receptors and neuronal 'eat me' and 'spare me' signals that initiate or inhibit microglial phagocytic activity, respectively. Therefore, the aim of this study was to define the molecular mechanisms that shape neuronal networks during brain development and in neuropathologies. In particular, the focus was on the characterisation of the unknown role of Xkr8 scramblase and phosphatidylserine exposure in developmental axonal pruning, the importance of sialylation and desialylation in healthy brain development and in epileptic human brain circuitry and the investigation of the significance of sialic acid in the establishment of cellular networks of human glioblastoma. Using both mouse models and surgically resected human brain tissue, a plethora of techniques and tools were utilized to thoroughly investigate the 'eat me' and 'spare me' signals in the brain, to define the cascades required for their presence on neurons and their role in developing and aberrant circuitry. This thesis demonstrated that phospholipid scramblase Xkr8 is required for phosphatidylserine exposure on neuronal projections and the pruning of axons in the developing mouse brain. Neuronal sialylation was found to be dynamically regulated in the developing brain and is disturbed in epileptic tissue. Human organotypic brain slice cultures were successfully applied for functional investigations of neuropathologies recapitulating their local microenvironment. Finally, sialic acid metabolism was shown to orchestrate transcellular connectivity and signalling in glioblastoma.

IMPACT OF SEED TREATMENT WITH COLD PLASMA AND ELECTROMAGNETIC FIELD ON PLANT GROWTH, CONTENT OF SECONDARY METABOLITES, AND MICROBIOT (Biochemistry)

Anatolii Ivankov

Scientific supervisor:
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Vytautas Magnus University

Dissertation defended:
27 September 2023

This dissertation explores the ecological and agricultural implications of using cold plasma (CP) and electromagnetic fields (EMF) as environmentally-friendly alternatives for seed priming, focusing on their effects on plant growth, secondary metabolite (SM) production, and microbiota. Utilizing six economically important plant species – three legumes (red clover, blue-flowered alfalfa, broad bean), common buckwheat, industrial hemp, and Norway spruce – the study examines these effects across diverse species, cultivars, genders, and genotypes over short-term and long-term periods (up to eight vegetation seasons for perennials). Methodologically, the research integrates germination tests, seedling growth measurements, SM analysis, root nodulation assessments, and microbiota studies. Key findings reveal that CP and EMF treatments universally induce dynamic but enduring changes in SM synthesis, affecting plants' interactions with microorganisms such as nodulation in legumes and microbial communities. The study also finds that the effects of these treatments are modulated by several biological variables like plant gender, seed color, and genetic lineage. The research not only elucidates the complex interactions between seeds and physical stressors like CP and EMF but also advances the field by highlighting the multi-layered, variable-dependent nature of these innovative seed priming techniques. This nuanced understanding is critical for tailoring reliable agro-biotechnological applications aimed at enhancing plant resilience, growth, and ecological sustainability.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL CRISPR-Cas NUCLEASES (Biochemistry)

Tomas Urbaitis

Scientific supervisor:
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Dissertation defended:
27 September 2023

Prokaryotic CRISPR-Cas (clustered regularly interspaced short palindromic repeats – CRISPR associated) systems provide adaptive immunity to bacteria and archaea against foreign nucleic acids. Cas proteins are specific nucleases, which can be guided to any DNA target by changing the sequence of small RNA molecules which form complexes with these enzymes. This enabled the use of Cas nucleases in genome editing applications. However, the actual targeting space of Cas proteins is limited by the requirement of the recognition of a short nucleic acid sequence near the target, termed the PAM (protospacer adjacent motif), which is inherent to each individual Cas nuclease. Furthermore, the relatively large size of the most used genome editors CRISPR-Cas9 and Cas12a (~1300 amino acids) make the delivery of these systems into cells a challenge. We sought to harness the natural diversity of CRISPR-Cas systems to search for novel Cas nucleases which could help circumvent these issues. This thesis presents a collection of 79 type II CRISPR-Cas9 nucleases which recognize diverse PAM sequences, as well as exhibit varied physical and biochemical properties. Also, we discovered and evaluated experimentally a new family of compact (~860 amino acids) type V CRISPR-Cas12l effectors and their nuclease activity. In summary, the nucleases identified and characterised in this work expand on the diversity of potential genome editors.

CHARACTERISTICS OF MULTIDRUG EFFLUX PUMP INHIBITORS: THE INTERACTION WITH BACTERIA AND THE ROLE IN REDUCING ANTIBIOTIC RESISTANCE (Biochemistry)

Sandra Sakalauskaitė

Scientific supervisor:
Prof. Dr Rimantas Daugelavičius
Vytautas Magnus University

Dissertation defended:
24 November 2023

Bacterial infections are becoming more challenging to treat due to the development of bacterial resistance to many antibiotics. One of the main reasons for this is the multidrug resistance (MDR) efflux pumps, membrane proteins that extrude drugs from cells. The incorrect use of drugs or various chemicals in the household stimulates their activity in the cells. To reduce the problem of drug resistance, one of the ways to increase the intracellular concentrations of antibiotics is the use of inhibitors of MDR efflux pumps. In this work, much attention is paid to researching the activity of MDR efflux pumps, searching for ways to increase the sensitivity of bacteria to antibacterial substances by reducing their efficiency. Potentiometric and fluorescence analysis were used for MDR efflux pump activity studies, and microbiological methods were used for efficiency studies of substance combinations. The study is divided into three parts: (1) factors promoting the development of bacterial resistance, (2) MDR efflux pump activity indicators and interpretation of obtained results; (3) characteristics of the interaction of inhibitors of MDR efflux pumps with bacteria. It has been established that the substances in cosmetics are substrates of MDR efflux pumps, as a result of which the activity of MDR efflux pumps and the formation of antibiotic-resistant bacteria are activated. It has been proven that PAβN and NMP inhibit the MDR efflux pumps in gram-negative and gram-positive bacteria. It was found that the soxS gene, which is responsible for controlling oxidative stress in cells, can be a target of newly developed compounds for inhibiting the activity of MDR efflux pumps because the efficiency of the AcrAB-TolC system depends on soxS.

SOCIAL DEVELOPMENT AND CHANGES IN STEROID HORMONES DURING EARLY ONTOGENESIS OF GREY SEALS (*HALICHOERUS GRYPUS*) (Biology)

Vaida Survilienė

Scientific supervisor:
Prof. Dr Osvaldas Rukšėnas, Dr Kimberley Bennett
Vilnius University

Dissertation defended:
20 January 2023

This study focused on the social behaviour and steroid hormones, as social behaviour-affecting factors, during early ontogenesis of young grey seals (*Halichoerus grypus*), with a goal of contributing to a better understanding of demographic and physiological changes in this species. This work investigates the behavioural elements and factors affecting social play in grey seals. It then compares the suitability of saliva and blood-based samples for steroid hormone analysis using different analysis methods and assesses their compatibility. Finally, an attempt to investigate the interactions between the behaviour of grey seal pups and steroid concentrations during the suckling and post-weaning fast periods was made. Results showed that the majority of social play interactions on land were performed by subadult males and the behavioural repertoire consisted mainly of play-fight elements. Male predominated group size, availability of haul out space and temporal proximity to the breeding season were found to have significant positive effect on the number of play interactions. Saliva required a longer collection time and resulted in lower collection success rates compared to blood samples, and is therefore recommended for trained phocids in captivity. Commercially available enzyme-linked immunosorbent assays (ELISAs) performed well for oestradiol and cortisol analysis and can be used for both plasma and saliva samples, even in young grey seal pups, but saliva samples provided lower resolution for oestradiol than plasma samples. Concentrations of steroid hormones in suckling grey seal pups were determined for the first time. The strongest associations were found between glucocorticoids and pup behaviour.

CHANGES IN THE GENETIC DIVERSITY OF BOARS IN THE TERRITORY AFFECTED BY AFRICAN SWINE FEVER (Biology)

Žygimantas Janeliūnas

Scientific supervisor:
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Dissertation defended:
27 January 2023

Boar (*Sus scrofa*) is one of the most common large mammals. Boars are considered one of the species of greatest concern due to the damage they cause to agriculture and forestry and their large population. The spread of infectious diseases caused by wild boars, including African swine fever (ASF), can have harmful effects on the livestock industry. The first ASF outbreak was first detected in the eastern part of Lithuania, near the border with Belarus. In the following years, the disease also spread in a large part of the territory of Lithuania. Understanding any genetic effects that ASF outbreaks may have on wild boar populations is critical. ASF may contribute to variation in the spatial genetic structure of *S. scrofa* populations. The aim of the work is to study the genetic diversity of *S. scrofa* before African swine fever and during the outbreak of the disease by the method of microsatellite analysis. Also to assess the genetic diversity of *S. scrofa* by analysing the non-coding control region of the mitochondrial DNA D-loop. Research on the genetic structure of the wild boar population before the ASF outbreak revealed the presence of only one population in the entire study area. The widespread wild boar population in Lithuania, the high genetic variation of subpopulations and the low level of subgroup differences could indicate migration and gene flow between localities. The analysed wild boar population before ASF and during the ASF outbreak showed a high level of genetic variation. Before and during the outbreak, significant deviations from Hardy-Weinberg after Bonferroni correction were observed in all subpopulations, mainly due to lack of heterozygotes. The genetics of the wild boar population in Lithuania can be influenced by the selective hunting strategy implemented both before and during the outbreak. The study of boar mitochondrial DNA showed that about 48% of the analysed boars living in the territory of Lithuania can be attributed to one haplotype. The phylogenetic tree obtained by the maximum likelihood method showed 7 main clusters. The majority of Lithuanian boars belong to the same clusters as the boars of other European countries.

MORPHOLOGICAL AND GENETIC DIVERSITY OF *SARCOCYSTIS* SPECIES PARASITIZING GULLS (LARUS) AND CORVIDS (CORVIDAE) IN LITHUANIA (Biology)

Evelina Juozaitytė-Ngugu

Scientific supervisor:
Dr Petras Prakas
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Dissertation defended:
22 February 2023

Members of the genus *Sarcocystis* are parasitic protozoa widespread in reptiles, birds, and mammals distinguished by a two-host prey-predator life cycle. Asexual multiplication with sarcocyst formation occurs in the intermediate host, while sexual stages develop in the small intestine of the definitive host. Previous studies confirmed two species of *Sarcocystis* each in the muscles of gulls and corvids, but a greater diversity of these parasites is assumed in these hosts. The objective of this study was to determine the species richness of *Sarcocystis* parasites in muscle and intestine samples of Lithuanian gulls and corvids using morphological and molecular analysis. DNA sequence analysis revealed four morphologically indistinguishable *Sarcocystis* species in the muscle of Lithuanian gulls and three species – in the muscles of the investigated corvids. A new species of *Sarcocystis*, *S. kutkienae*, was described and confirmed in common raven, common magpie and hooded crow. The prevalence ($p < 0.001$) and density ($p < 0.05$) of *Sarcocystis* infection were reliably higher in the muscles of corvids than in the muscles of guls. During the study, *S. columbae* was identified for the first time in the muscles of gulls. For the first time, a pathogenic species *S. halieti* was identified in muscle samples of gulls and corvids. Also, during this study, for the first time, mixed *Sarcocystis* species infections were detected in the muscles of gulls and corvids. New intermediate hosts confirmed for five *Sarcocystis* species. For the first time, 11 *Sarcocystis* species were identified in the intestine mucosal scrapings of corvids.

THE EFFECT OF WHOLE-BODY COLD-WATER IMMERSION ON THERMOREGULATION, STRESS HORMONES AND CYTOKINES IL-1B, IL-6 AND TNF-A KINETICS (Biology)

Milda Eimontė

Scientific supervisor:
Prof. Dr Marius Brazaitis
Lithuanian Sports University

Dissertation defended:
9 June 2023

The general aim of the research was to investigate the acute whole-body cold water immersion (CWI) effects on thermoregulation, stress hormones, immunity and lipid status indicators within 48 h after cold exposure. No previous study has explored the residual (i.e., post cooling kinetics within 48 h) effects of whole-body CWI (14°C) on the thermoregulation, stress hormone, immune and circulating lipid status indicators responses in healthy adult males. The hypothermic response is related to pathologic conditions and it can also be induced by recreational or sport activities, as such it is relevant to understand the specific effect of low body temperature on related immune functions. We believe that the results of first and second studies could be of great importance for individuals who deal (i.e., treat and manage) with accidental or therapeutic hypothermia, or to those who freeze during recreational activities. Our findings provide the first evidence that not only the effects of stress hormones but also a low body temperature are a delayed factor in cytokine expression. To date, the effect of cold exposure on the predisposition to illness remains poorly understood, and better understanding of the immune responses during cold exposure and its residual (post-cooling kinetics within 48 h) effect may explain susceptibility of the organism to infection. The findings of our study suggest that even though CWI caused changes in stress and immune markers, there was no predisposition to symptoms of the common cold in the participants within 48 h after CWI. Third study results could be of great importance for individuals with limited mobility to deal with or prevent diseases associated with elevated lipid profiles and will be useful for practical implications in therapeutic protocols.

STUDY OF MOLECULAR MECHANISMS OF MYOCARDITIS AND DILATED CARDIOMYOPATHY (Biology)

Ieva Rinkūnaitė

Scientific supervisor:
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Vilnius University

Dissertation defended:
22 June 2023

Heart failure is a significant and growing public health problem worldwide that poses many challenges due to the vast diversity of etiologic factors. Myocarditis and dilated cardiomyopathy are among the leading causes of heart failure. Gaining new insights into the causal pathophysiological mechanisms of these diseases would contribute to developing more effective prevention and treatment strategies for heart failure. This dissertation investigated the possible molecular pathophysiological mechanisms underlying myocarditis and dilated cardiomyopathy associated with parvovirus B19 and ANKRD1 protein. Our *in vitro* study demonstrates the unique region of the parvovirus B19 capsid protein VP1 cannot be considered the sole determinant of cell and species tropism of parvovirus B19. Furthermore, exposure to the unique region of VP1 leads to activation and stress response of primary endothelial cells, irrespective of its internalisation potential. This novel mechanism may contribute to the pathophysiology of parvovirus B19-associated myocarditis. *In vivo* studies revealed that ANKRD1 protein does not play a major role during murine post-myocarditis cardiac remodeling leading to dilated cardiomyopathy. However, the genetic ablation of Ankrd1 mitigates cardiac tissue damage and remodeling. These findings suggest that timely pharmacological regulation of ANKRD1 expression could be a potential target for mitigating the outcome of myocarditis-induced DCM.

ANALYSIS OF GENETIC AND EPIGENETIC BIOMARKERS FOR BREAST CANCER DIAGNOSIS AND PROGNOSIS (Biology)

Ieva Sadzevičienė

Scientific supervisor:
Prof. Dr Sonata Jarmalaitė
Vilnius University

Dissertation defended:
23 June 2023

Despite improving diagnostic tools, breast cancer (BC) remains the leading oncological disease in women. Inherited BRCA1/2 gene mutations increase the risk of BC by 70%, and TP53 mutations are found in 20–50% of all BC cases. In cancer cells, hypermethylation of tumour suppressor genes results in the silencing of gene function. Our study analysed the influence of the inherited BRCA2 c.3847_3848delGT mutation on the progression of familial BC disease. TP53 mutations and hypermethylation of 17 genes with different functions in the cell were analysed in sporadic BC. Ten of the 17 tested DNA methylation markers statistically significantly distinguished BC from control samples. During analysis of the highly heterogeneous and aggressive triple-negative (TN) subtype, we found MT1E and FILIP1L biomarkers specific to it, which were associated with tumour proliferation, poor differentiation grade, and TP53 mutations. In the survival analysis, a statistically significant association was found between hypermethylation of RUNX3 with MT1E and FILIP1L or all three genes together and patient survival. FILIP1L hypermethylation along with TP53 mutations were associated with poor disease outcomes. The study identified diagnostic and prognostic BC biomarkers that could facilitate timely tumour identification and biological subtyping and enable the prediction of the progression of BC, prognosis of the response to treatment, and expand the possibilities of individualised BC therapy.

CARDIORESPIRATORY, COGNITIVE, AND NEUROMUSCULAR RESPONSE TO ACUTE NOXIOUS HEAT EXPOSURE (Biology)

Soneta Ivanovė

Scientific supervisor:
Prof. Dr Marius Brazaitis
Lithuanian Sports University

Dissertation defended:
23 June 2023

The aim of the research was to determine how a short period (5 min) of whole body noxious hot water immersion that does not induce whole body hyperthermia affects cardiorespiratory, cognitive, and neuromuscular functions. The results of our study presented in this thesis, will provide additional practical knowledge for future studies and will contribute to the advancement of knowledge on the effects of heat on motor, neuromuscular, and cognitive functions. The facts examined in this thesis will be used for a better understanding and could be useful for certain professions that engage in prolonged physical activity and are exposed to heat stimulus (i.e., athletes, soldiers, construction workers, firefighters, etc.). We hope the results will be useful for people who exercise in the heat as well as for people travelling to warm countries. When the nerve networks warm up, impulses are transmitted more quickly, reactions are quicker, and simple tasks can be performed more quickly. This can help to perform better exercise related tasks or cognitive tasks that are important for sport engaged individuals.

SARS-COV-2 GENETIC VARIABILITY IN LITHUANIA, 2020–2021 (Biology)

Lukas Žemaitis

Scientific supervisor:
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Lithuanian University of Health Sciences

Dissertation defended:
27 June 2023

The pandemic COVID-19 is a global crisis with unprecedented impact on humanity and challenges in health, social, economic, and other areas. Understanding the SARS-CoV-2 virus that triggered the pandemic has become a critical task. Whole genome sequencing of the SARS-CoV-2 virus was a major effort, supported by an open and continuously updated sequencing database of researchers from around the world. This enabled rapid vaccine development, molecular epidemiological studies, and analysis of viral evolution. Despite significant progress, many questions remain unanswered regarding the evolution of the virus, its virulence, and the effectiveness of control measures. Longer-term studies evaluating the evolution of the virus are lacking. The aim of this study was to identify the major SARS-CoV-2 lineages in Lithuania between 2020 and 2021, to assess their origins and patterns of change, and to highlight the reasons for their spread. Sequencing results and two case studies are presented: one exploring the B.1.1.523 lineage origin, mutations, their spread, and their impact on viral characteristics, and the other examining genetic mutations of the SARS-CoV-2 virus in mink farms and their impact on human morbidity.

THE ROLE OF CALRETICULIN IN THE PATHOGENESIS OF MYELOPROLIFERATIVE NEOPLASMS (Biology)

Roberta Vadeikienė

Scientific supervisor:
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Lithuanian University of Health Sciences

Dissertation defended:
7 July 2023

BCR-ABL1-negative myeloproliferative neoplasms (MPNs) are classified into polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). MPN is caused by genetic changes in hematopoietic stem cells. Calreticulin (CALR) mutations, a 52 bp deletion, and a 5 bp insertion, were identified in patients with ET and PMF. This study aims to investigate the impact of CALR 52 bp deletion and CALR 5 bp insertion on the pathogenesis of MPN. However, there are still no commercial cell lines with MPN-specific mutations in the CALR gene. In the first stage of this study, a 52 bp deletion and a 5 bp insertion were initiated in UT-7 cells using the CRISPR/Cas9 genome editing system. Further, it was found that the JAK/STAT and PI3K/Akt/mTOR signaling pathways were activated in CALR Del52 and CALR Ins5 cells. Functional analysis revealed that CALR Del52 and CALR Ins5 cells had impaired responses to oxidative stress induced by H2O2 treatment when compared to UT-7 cells. CALR-mutated cells presented with elevated levels of intracellular reactive oxygen species (ROS) and had a lower ability to reduce it. CALR Del52 and CALR Ins5 cells were characterised by higher levels of DNA damage and impaired DNA damage repair. Moreover, CALR Ins5 cells demonstrated higher levels of apoptosis. CALR-mutated cells showed increased cell cycle arrest at the G2/M phase. This study contributes to a deeper understanding of specific molecular mechanisms underlying CALR-mutated MPNs.

BREAST, CERVICAL, HEAD AND NECK CANCER: TFAM AND POLG VARIANTS, MITOCHONDRIAL DNA ALTERATIONS AND MIRNA-210-3P EXPRESSION EFFECT ON TUMOR PHENOTYPE AND DISEASE OUTCOME (Biology)

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Dissertation defended:
29 August 2023

High cancer prevalence and mortality rates indicate the need for more accurate disease biomarkers. Different cancer aetiology theories are proposed, and one suggests that cancer is a metabolic disease significantly influenced by mitochondria. Various elements are involved in this theory, including mitochondrial DNA germline and somatic variants, nuclear DNA encoding mitochondrial proteins or mitoepigenetics, which regulates mitochondrial gene expression. These alterations could be a significant factor in the development and progression of different cancer types. The aim of the study was to investigate the effect of TFAM and POLG variants, mitochondrial DNA alterations and miRNA expression on tumour phenotype and prognosis in breast (BC), cervical (CC), head and neck (HNSCC) cancers. Objectives: (1) To determine the effect of TFAM (rs11006132, rs11006129, rs1937, rs16912174, rs1692202, rs3900887) and POLG (rs3087374, rs2072267, rs976072, rs2307441) variants on tumour pathomorphological parameters and disease outcome in BC, CC and HNSCC; (2) To analyse mitochondrial DNA variations and differences between tumour and normal tissue adjacent to the tumour in BC and HNSCC patients and to determine their possible associations with tumour phenotype; (3) To select mitochondria-associated miRNAs from the literature, investigate their expression in the TCGA database and our BC and HNSCC patients, and determine possible associations with pathomorphological tumour parameters and prognosis.

THE IMPACT OF TICAGRELOR ANTIPLATELET EFFICACY, GENETIC AND CLINICAL FACTORS, AND DRUG METABOLITES ON ADVERSE EVENTS IN PATIENTS WITH MYOCARDIAL INFARCTION (Biology)

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The aim of this study was to determine the impact of clinical and most relevant genetic factors on the occurrence of dyspnoea during treatment with ticagrelor in patients with acute myocardial infarction, who underwent PTCA with stent implantation. Up to date, information on the causes of ticagrelor-related adverse events, pathogenesis, and possible tools for predicting adverse events in the literature is scarce. Moreover, the existing body of evidence on the effect of clinical and genetic factors on the pharmacodynamic and pharmacokinetic characteristics of ticagrelor is contradictory. Our study was the first to determine the value of platelet aggregation testing in predicting the development of ticagrelor-related dyspnoea. Determination of an exact value of platelet aggregation testing allows predicting the development of ticagrelor-related dyspnoea. After the experiments on the concentration of ticagrelor metabolites in blood plasma and urine, we confirmed a hypothesis that lower platelet aggregation is associated with a greater activity of ticagrelor and its active metabolite and a higher concentration. Moreover, we determined the associations of ticagrelor antiplatelet efficacy with CYP2C19 (rs12248560), FBG C148T (rs1800787), and ABCB1 (rs1045642) gene polymorphisms, which could allow predicting the occurrence of ticagrelor-related adverse events in the future and thus prevent the risk of life-threatening complications related to premature ticagrelor discontinuation.

MiRNA STUDIES OF TUMOR TISSUE AND BLOOD EXTRACELLULAR VESICLES FOR DIAGNOSIS AND PROGNOSIS OF GLIOMAS (Biology)

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Dissertation defended:
30 August 2023

Glioma is the most common type of primary malignant brain tumour. The most malignant form of glioma is glioblastoma, only 6.8% of glioblastoma patients survive for more than five years, and only 42.5%. patients reach one year survival. It is believed that earlier diagnosis of glioma would improve the effectiveness of treatment for patients. Determining the expression of micro-RNA from blood would be suitable for early diagnosis, but an accurate set of micro-RNA for glioma diagnosis and prognosis has not yet been determined. The aim of this study was to identify a set of diagnostic and prognostic microRNAs for glioma progression and outcome. Micro-RNA expression analysis was performed from glioma tumour tissues and blood serum exosomes from the same patients. The study included four non-cancerous brain samples, 15 grade II, six grade III, and 82 glioblastoma samples, and the expression of 14 microRNAs. It was found that: (1) the tumourt expression of miR-21-5p and miR-10b-3p increases consistently with the grade of glioma; (2) tumour expression of miR-10b-3p and miR-7-5p is significantly different between low- and high-grade gliomas; (3) the expression of miR-338-5p, miR-17-5p, miR-93-5p, and miR-193a-5p was most similar in tumour tissue and blood serum exosomes from the same patients; (4) A better prognosis was found in glioma patients who were younger, had lower miR-143-3p expression or higher miR-143-3p expression but at the same time lower miR-10b-3p expression.

GENETICAL BIOMARKERS OF OVARIAN CANCER (Biology)

Diana Žilovič

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Dissertation defended:
7 September 2023

The aim of the dissertation was to identify type II ovarian cancer and SEOC-specific mutations and develop a low-invasive test for early diagnosis of the disease. In this study, we sought to find biomarkers that could help diagnose OC in the early stages or even precancerous changes in the fallopian tube (serous tubal intraepithelial carcinoma -STIC) in the future. The data accumulated during the study will help to assess the suitability of uterine lavage as a test substance for the early diagnosis of OC. The study results showed that: (1) In type II OC uterine lavage samples mutation of selected genes using NGS were detected: TP53 (27.0%), BRCA1 (35.1%), BRCA2 (10.8%), PTEN, PIK3CA and KRAS (2.7%). The sensitivity of the 6 gene set (any gene) in the uterine lavage sample for type II OC was 64.9% and specificity – 66.0%; (2) Mutations in the TP53 gene in the type II OC were detected in 79% of ovarian tissue biopsy samples and only 41.6% of paired uterine lavage samples. TP53 mutation analysis-based uterine lavage test for type II OC showed specificity of 100% and sensitivity of 27%; (3) More mutations in uterine lavage samples were detected in FIGO stage IV II type OC (p = 0.002, Fisher test) than in FIGO stage II-III. CA-125 levels correlated with the frequency of mutations detected in uterine lavage samples; (4) Gene mutations found in the SEOC tumour tissue could not be detected in uterine lavage biopsy. The search for diagnostic markers for SEOC must be continued in larger cohorts.

DEVELOPMENT AND APPLICATION OF NEW METHODS TO ASSESS ARTICULAR CARTILAGE DAMAGE CAUSED BY MENISCAL LESIONS (Biology)

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Centre for Innovative Medicine

Dissertation defended:
29 September 2023

The aim of this study was to determine the cartilage resistance and extracellular matrix remodeling caused by mechanical stress-induced meniscal damage and the search of novel biomarkers and approaches. Murine study showed that anti-hypertensive drugs such as NIF may reduce degradation of the cartilage on the mechanical stress induced MMD models. These data confirm previous in vitro results about positive effect of NIF on the cartilage. Our data are good news for the aging patients receiving NIF treatment, because a large part of them have comorbidities, including arterial hypertension and OA lesions. Intraarticular injections of NIF showed a negative effect on healthy cartilage in this study, suggesting that application of NIF may be harmful for healthy cartilage and administered with caution. Multiplexed detection of minor collagens (COLIV and COLVI) using QDs provided us an opportunity to evaluate microstructural changes in the OA cartilage. Visualisation of few biomolecules in the same slide by using different QDs shows subtle microstructural changes of the cartilage, decreases number of samples. Methodological recommendations for QD detection protocol in cartilage tissues were described in this study. In future studies, a numerical three-dimensional model could be utilised to calculate the theoretical risk level of cartilage damage. Subsequently, this data could be applied in forthcoming rehabilitation programs or for planning knee treatment or surgical interventions. SHG microscopy may be used for testing efficacy of drugs for OA treatment in vitro on cartilage explants.

INVESTIGATING SALIVARY TESTOSTERONE AND CORTISOL AS MONITORING TOOLS IN BASKETBALL (Biology)

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Dissertation defended:
27 October 2023

The research described in this doctoral dissertation includes four scientific studies addressing differently designed but related research questions. The assessment of weekly fluctuations in hormonal responses in different basketball populations (i.e., semi-professional and professional, male players), during different phases of the season (i.e., pre-season and in-season) were investigated in study 1, study 3, and study 4. The second study question, a comparison of weekly changes in hormonal responses in relation to changes in load measures and well-being between European- and national-level professional, male basketball players was investigated in study 4. Finally, the last study question of this research, investigating relationships and associations between weekly changes in hormonal responses and weekly changes in load measures and players' well-being during different phases of the season in different basketball populations were investigated in study 1, study 2, and study 3. The main findings of study 1 showed non-significant, trivial-to-moderate relationships between weekly changes in hormonal responses and weekly changes in load and well-being variables during the in-season phase in semi-professional, male basketball players. Similarly, study 2 showed that changes considered either separately, or jointly in load measures are not influencing changes in weekly hormonal responses, during the pre-season phase in professional male basketball players. Additionally, study 3 showed only negative and weak associations between weekly changes in well-being and weekly changes in load measures, and no associations between weekly changes in well-being and weekly changes in hormonal responses, during the pre-season phase in professional male basketball players. The findings of all three studies suggest that other measures than those investigated in these studies or combination of them might be influencing weekly fluctuations in hormonal responses. Moreover, these findings suggest that all investigated measures might provide a unique insight about training and recovery process in basketball and should be separately used for the monitoring of basketball players. The main conclusion of the quantification of relationships and associations between weekly changes in hormonal responses, load measures, and well-being variables indicates that these measures are not influencing weekly changes in between each other and that weekly fluctuations in these variables might be induced by other physical, physiological, psychological measures or combination of factors.

INFLUENCE OF BIOLOGICAL RISK FACTORS ON THE DEVELOPMENT OF CARDIOVASCULAR EVENTS (Biology)

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Centre for Innovative Medicine

Dissertation defended:
8 December 2023

The aim of the research was to assess the prevalence and dynamics of laboratory biomarkers, their correlations, and associations with cardiovascular risk factors and cardiovascular events in a cohort of individuals with metabolic syndrome. The results presented in this thesis show that participants in a primary cardiovascular disease prevention programme diagnosed with metabolic syndrome show positive lipid dynamics over a 10-year period, with decreasing concentrations of total and low-density lipoprotein cholesterol and triglycerides and increasing concentrations of high-density lipoprotein cholesterol. In middle-aged individuals (males aged 40–55 and females aged 50–65) diagnosed with metabolic syndrome, elevated arterial blood pressure, waist circumference, and fasting glucose were the most common components of metabolic syndrome, and the most identified abnormal laboratory parameters were elevations in low-density lipoprotein cholesterol and high-sensitivity C-reactive protein. Independent of other cardiovascular risk factors, the strongest association was found between: (a) triglyceride levels and smoking, and triglyceride levels and hyperuricaemia, (b) high-sensitivity C-reactive protein levels and arterial hypertension and high-sensitivity C-reactive protein levels and obesity (BMI ≥ 30 kg/m^2), (c) serum uric acid levels and obesity (BMI ≥ 30 kg/m^2). Key biomarkers of future cardiovascular events in middle-aged Lithuanian residents with metabolic syndrome: (a) hyperglycaemia is significant for myocardial infarction, hypertriglyceridaemia for stroke, and elevated systolic blood pressure for cardiovascular death, (b) age and the ratio of total cholesterol to high-density lipoprotein cholesterol are the best predictors of cardiovascular events in females, whereas age and the albumin-to-creatinine ratio in urine are the best predictors in males.

FUNCTIONALISATION OF EXTRACELLULAR VESICLES FOR TARGETED DRUG DELIVERY TO GLIOBLASTOMA CELLS (Biology)

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Dissertation defended:
14 December 2023

Glioblastoma multiforme (GBM) remains the most common and aggressive brain tumour. Previous efforts to establish improved therapies resulted in a modest survival increase as the 5-year GBM survival rate held out below 10%. GBM characteristics, which determine poor prognosis, include its invasive nature, compounding of mutations, and heterogenicity of tumour cells. Extracellular vesicles (EVs) are attracting tremendous interest from the scientific community and biotechnology field. The early concept of functionalising EVs for drug delivery was based on the essential characteristics of these vesicles: nano size, biocompatibility, ability to cross the blood-brain barrier, and capacity to be loaded with exogenous materials. The present work aimed to develop an EV-based drug delivery system adapted for the treatment of brain tumour. The study investigated the therapeutic potential of genetically engineered EVs from HEK293ft cells with elevated levels of integrin binding peptide RGD (RGD-EVs), as well as tumour suppressive properties of adipocyte-derived mesenchymal stem cell EVs (ASC-EVs). Experiments revealed that RGD-EVs are well internalised in GBM cells and exert enhanced properties when loaded with doxorubicin hydrochloride or silencing RNA. In addition, data showed that natural ASC-EVs possess natural GBM inhibiting properties, suppressing tumour cell proliferation and invasiveness. Results demonstrate that EVs could be potential candidates for anti-cancer drug delivery.

MICROBIOTA OF SOUR AND SWEET CHERRIES AND THEIR BIOCONTROL COMPONENTS (Biology)

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Nature Research Centre

Dissertation defended:
20 December 2023

Cherries are important in the food industry, agriculture, and medicine. Microorganisms widespread on these berries can affect the structure and biodiversity of microbiota in the natural environment as well as plant productivity, berry quality, and human health. Microorganisms with biocontrol properties and their bioactive substances are promising for plant disease management, reduction of microbiological contamination, and improvement of food quality. The aim of this work was to evaluate the structure of the sour and sweet cherry microbiota and to study the effects of biocidal substances on the microbiota components to justify their applicability. This work presents new findings on the fungal communities occurring on the surface of sour and sweet cherry berries and a comparative analysis of the mycobiota composition of freshly picked and store-bought berries. Bioinformatic analysis was carried out to characterise the fungal communities and to assess the diversity and relative abundance of individual microorganisms. Cultivable prokaryotic and eukaryotic microorganisms present on the surface of sour and sweet cherries were isolated and identified. The occurrence of killer yeasts on the surface of the berries was studied and their effect on the survival of the microbiota components was analysed. The profiles and potential for biocontrol of volatile compounds synthesised by yeasts were evaluated. The effect of natural and encapsulated biocide peptide-nisin on the survival of microbiota components was investigated. Modulation of nisin efficacy by electric and magnetic fields and evaluation of its applicability were performed, and data confirming the efficacy of the nanostructured nisin in a real food system were presented.

INNOVATIVE IMAGING TECHNOLOGIES AND GENETIC TESTS FOR THE DETECTION OF CLINICALLY SIGNIFICANT PROSTATE CANCER (Biology)

Augustinas Matulevičius

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Dissertation defended:
29 December 2023

This study investigated the specificity and sensitivity of mp-MRI/US-guided targeted prostate biopsy for the detection of clinically significant prostate cancer. Based on the results of this study, clinical practice could reduce the detection rate of clinically insignificant prostate cancer by completely abandoning systematic prostate biopsy and replacing it with adaptive MRI/US-guided prostate biopsy. This would allow a reduction in overdiagnosis and associated adverse outcomes and maintain the same frequency of detection of clinically significant prostate cancer. The study also allowed us to evaluate the possibility of improving the prognostic value of MRI by predicting the risk of cancer progression, selecting treatment tactics, or performing active surveillance. Our study showed that the level of DNA methylation of all investigated genes was statistically significantly higher in mpMRI-recorded foci than in perifocal samples, and that hypermethylation decreased significantly when it reached a distance >10 mm from the tumour focus. Therefore, we can conclude that a safe distance from the tumour focus is at least 10 mm, and in clinical practice, focal hemiablation of the prostate would be considered as a radical treatment option for intermediate-risk prostate cancer. Non-invasive urine tests are very valuable for follow-up of patients after prostate cancer treatment, but larger prospective studies are needed.

THERANOSTIC NANOPARTICLES APPLICATION FOR CANCER DIAGNOSTICS AND TREATMENT (Biophysics)

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Dissertation defended:
15 March 2023

Recently, there has been a lot of efforts devoted to material research, which could be used for both cancer diagnosis and treatment. The field of such research is known as cancer theranostics. One of the main directions of cancer theranostics is the combination of optical biopsy methods and photodynamic tumour therapy. The aim of this work was to investigate the potential application of biocompatible gold nanoclusters and functionalized rare earth metal nanomaterials in cancer theranostics. By using model cell systems *in vitro*, it was investigated whether the nanoparticles could be used as photoluminescent markers and photoactive medicines. Research shows that gold nanoclusters stabilised by blood serum proteins accumulate in cancer cells and, under visible light irradiation, generate reactive oxygen species that cause death of cancer cells. Upon irradiation with infrared radiation, which enters the tissue transparency window, photosensitizer-functionalized rare earth metal nanoparticles in cell monolayers and spheroids generate singlet oxygen, which induces cell death. However, the penetration of nanoparticles into three-dimensional cell spheroids is limited. Mesenchymal stem cells can be used as a nanoparticle carrier: they accumulate photosensitizer-functionalized rare earth metal nanoparticles and ensure their transfer in a tumour model, 3D cellular spheroids.

EVALUATION OF ELECTRICAL BRAIN ACTIVITY OF THE RESTING STATE: RELATION WITH SUBJECTIVE EXPERIENCES (Biophysics)

Povilas Tarailis

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Dissertation defended:
4 July 2023

The resting state paradigm is frequently applied to study spontaneous activity of the brain in normal and clinical conditions. However, the relationship between the ongoing experience of mind wandering and the individual biological signal is still unclear. To quantify subjects' subjective experiences at rest, the Amsterdam Resting-State Questionnaire (ARSQ) was introduced covering several dimensions of mind wandering. The aim of this work was to estimate associations between subjective experiences during the resting state session and electrical signal of the brain, focusing on EEG power, phase synchronisation and topographical aspects. As it was shown in this work, different aspects of the same signal are related to both – different and the same – domains of ARSQ. The power aspect of EEG signal as evaluated with frequency principal component analysis showed that activity in theta frequency range was related to scores for Sleepiness, but not the phase, as evaluated with global field synchronisation. On the other hand, phase synchronisation of beta frequency range, but not the power aspect correlated with domain of Comfort. And both, power and phase parameters of alpha band activity, correlated with ARSQ domain of Comfort. Three different microstates – C, E, and G – displayed associations with domain of Comfort, while microstate B and D correlated with domain of Self, and microstate F correlated with Somatic Awareness.

STUDY OF FACTORS AFFECTING ELECTROTRANSFER OF NUCLEIC ACIDS INTO CELLS (Biophysics)

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Dissertation defended:
30 August 2023

Although electroporation is applied in many areas and the understanding of the effect of electric fields on cells has increased significantly, the relatively low efficiency of nucleic acid electrotransfer remains a limiting factor for the wider application in the clinic. In order to increase the applicability in therapy, the aim of this study was to evaluate the factors that may affect the efficiency of the electrotransfer of nucleic acids. To achieve this goal, the effects of cations and larger nucleic acids were evaluated using flow cytometry and confocal microscopy. During the study, it was shown for the first time that ≤1 mM Ca^{2+} concentrations reduce the efficiency of pDNA electrotransfer, the expression of the encoded gene, and cell viability. After carrying out *in vivo* studies, evaluating the effect of Ca^{2+} and pDNA transfer on the dynamics of tumour growth, the effectiveness in initiating cell death, and the possibility of activating the systemic immune response were determined. In the case of small nucleic acids (oligonucleotides and siRNA), a new transfer model based on a combination of electrophoresis and diffusion, entry through both sides of the permeabilised cell, and localisation in the nucleus and cytoplasm was demonstrated for the first time. During the study, the influence of pDNA on the electrotransfer of small nucleic acids into cells was evaluated for the first time, and a negative effect on the efficiency of siRNA electrotransfer was determined.

THE LINKS OF RESPIRATION WITH PHYSICAL FITNESS, MUSCLE TENSION, AND EXECUTIVE FUNCTIONING (Biophysics)

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Respiratory exercises have enormous therapeutic potential that warrants our exploration. Meanwhile, the unclear relationship between spontaneous respiratory patterns and physical fitness requires further investigation to provide additional information that can guide exercise interventions. Therefore, we conducted two experiments: the first to investigate the link between spontaneous respiratory patterns and physical fitness across various ages, and the second to examine the immediate impact of breathing exercises on muscle tension and executive function. We found that whether intentionally lowered or breathed spontaneously, a slower respiration rate is preferable for executive function. In addition, middle-aged males (aged 40–59) with a relatively lower frequency (13.8±2.75 reps/min) tend to have superior physical fitness compared to those with a higher frequency (18.3±2.27 reps/min). Furthermore, male abdominal contribution to total respiratory movements during spontaneous breathing was not substantially linked with physical fitness, and the abdominal contributions were larger in older males than in younger males. Thus, we recommend: (1) Healthcare professionals focus on improving thoracic mobility for aging-related health issues; (2) Maintaining normal thoracic expansion during abdominal breathing is vital; (3) Slow breathing could be an effective workplace exercise method.

COMPARATIVE STUDY OF ECOSYSTEM SERVICES PROVIDED BY TWO COASTAL LAGOONS IN RELATION TO THEIR HYDRODYNAMIC FEATURES (Ecology and environmental)

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Dissertation defended:
17 March 2023

All over the world, coastal lagoons can differ in size, geomorphology, hydrodynamics, climatic conditions, etc. while being productive ecosystems in common. Therefore, studying the behaviour of these ecosystems by assessing the ecosystem services (ES) they provide is a key to coastal lagoon management and decision-making. This research represents a comparative study of two different coastal lagoons located, respectively, in the Atlantic Ocean and in the Baltic Sea. The saline water body, the Oualidia Lagoon, despite its small size of only 4 km^2, provides life and sources of income to the surrounding communities, which makes it an attractive study site for the present research. The nearly freshwater Curonian lagoon, the largest one in Europe, covers an area of up to 1584 km^2 and is also rich in terms of ES. This comparative research is divided into three main parts, starting with (1) investigating the hydrodynamics of the study sites; (2) an expert-based scoring method was applied to assess the scores of ES in the coastal parts of the study sites for each of the CORINE land-cover classes (CLC), and (3) assessing and mapping the ES flow of 13 selected ESs in the aquatic areas of both study sites.

CONTAMINATION OF SHOOTING RANGES IN LITHUANIA: EXTENT, ECOTOXICOLOGICAL IMPACT AND POTENTIAL FOR PHYTOREMEDIATION (Ecology and environmental)

Jūratė Mankė

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Shooting ranges are a source of heavy metal pollution in soil. The pollution of shooting ranges has a negative impact on soil organisms and leads to the entry of heavy metals into higher levels of the food chain. To reduce heavy metal pollution in shooting ranges, various soil remediation methods can be used. During the research, it was found that shooting ranges of different types and activities exhibited varying degrees of heavy metal contamination, with the maximum lead concentration reaching 54 600 mg kg^{-1}. Laboratory studies revealed that the contamination of shooting range soil has a negative impact on the physiological indicators of plants like *Lactuca sativa* L. and on the growth and reproduction of soil organisms like *Eisenia fetida* L., causing a biochemical response. For the first time, lichens *Evernia prunastri* L. and *Ramalina farinacea* L. were used as bioindicators of air pollution in an indoor shooting range. It was determined that the air in shooting ranges is contaminated with heavy metal particles. A field study conducted in a shooting range polluted with heavy metals demonstrated the possibilities of using three different species, *Medicago sativa* L., *Festuca arundinacea* L., and *Trifolium pratense* L. for phytoremediation purposes. These selected species were not only able to grow biomass and tolerate increased levels of Pb, Sb, and Ni but also accumulate significant concentrations of Pb. Thus, this research not only revealed the complex impact of contaminated soil on soil organisms, described the extent and specifics of pollution, but also highlighted the possibilities for control and pollution management during shooting activities in outdoor shooting ranges.

NATURAL ENVIRONMENT AND WOOD USE IN LITHUANIAN TERRITORY DURING HOLOCENE ACCORDING TO ANATOMICAL ANALYSIS OF ARCHAEOLOGICAL WOOD (Ecology and environmental)

Kęstutis Peseckas

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Dissertation defended:
29 September 2023

Wood has always been an important natural resource that has been extensively and diversely exploited. Despite its organic nature, the artifacts and traces of woodwork and fires made in the distant past can be found in archaeological contexts. Such finds are witnesses to the technological skills and capabilities of people in the past and reflections of the past natural environment. This thesis explores the potential of anatomical analysis methods for the study of wooden finds discovered during archaeological excavations and presents four different studies focusing on different types of archaeological contexts and finds. The first of them is devoted to the Sub-Neolithic, Neolithic, and Bronze Age artefacts found in the ancient sites of Šventoji, studied by the author since 2014. The second one is devoted to wood charcoal collected during the archaeological investigations of the Neolithic sites in the Curonian Spit. The third study is deals with the wood of fishing structures dating from the Mesolithic to the modern period found in the Žeimena River. The fourth study concerns wooden dugout boats found in various places in Lithuania, mostly during underwater explorations, the oldest of which dates back to the Neolithic period and the youngest to the modern period. The methods used in this work are based on the analysis of anatomical microscopic features of wood: species identification, the determination of the harvesting season and the roundwood age and diameter analysis which is applied for the first time in the study of the wooden finds found in Lithuania. The work also introduces the discipline of anthracology, which has not yet found a place in Lithuanian science.

DISTRIBUTION, BIOACCUMULATION, AND ECOTOXICOLOGICAL EFFECTS OF EMERGING POLLUTANS IN AQUATIC ECOSYSTEMS (Ecology and environmental)

Reza Pashaei

Scientific supervisor:
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Dissertation defended:
27 October 2023

The alarming increase of emerging pollutants like pharmaceuticals, personal care products, nanoplastics, and microplastics in aquatic ecosystems is a rising threat to water quality and marine organisms. This study examines the occurrence, distribution, bioaccumulation, and ecotoxicological impact of pharmaceutical, personal care product, nanoplastic, and microplastic pollutants across diverse aquatic ecosystems. A range of techniques were used for analysis, including high-performance liquid chromatography-tandem mass spectrometry, thermogravimetric analysis, micro-Raman spectroscopy, asymmetrical flow field-flow fractionation, optical microscopy, and acute toxicity test. Particles of nanoplastics and microplastics were detected in Urmia Lake, one of the largest salt lakes worldwide, located in Iran. Moreover, a total of 15 human pharmaceuticals and personal care products were discovered in fish and shrimp samples from four distinct markets, and in wastewater from locations in Latvia and Lithuania. Besides, a high concentration of microplastic particles was found in mussels (*Mytilus* spp.) from Svalbard, Norway. Laboratory tests were conducted using Daphnia magna as a model organism to evaluate the ecotoxicological implications of triclosan, caffeine, nanoplastic, and microplastic pollutants. Acute toxicity tests demonstrated a high mortality level in Daphnia magna exposed to a mixture of triclosan, caffeine, nanoplastic, and microplastic contaminants, highlighting the intricate challenges of assessing ecotoxicological risks. Compelling evidence was provided by the findings, showing the pervasive presence, distribution, and bioaccumulation of these emerging pollutants, as well as their harmful effects on aquatic ecosystems. They underscore the urgent need for comprehensive strategies to mitigate the release of pharmaceutical, personal care product, nanoplastic and microplastic pollutants into aquatic ecosystems and safeguard the health of water bodies and marine organisms. This study also underscores the complexities of ecotoxicological risk assessment, indicating the necessity for more investigation that can account for interactions among triclosan, caffeine, and nanoplastic and microplastic pollutants.

PECULIARITIES OF SMALL MAMMAL ECOLOGY IN COMMERCIAL ORCHARDS, BERRY PLANTATIONS AND COMMENSAL HABITATS (Ecology and environmental)

Vitalijus Stirkė

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The main objective of this study was to assess the diversity and relative abundance of small mammals in commercial orchards and berry plantations in Lithuania, as well as the population structure and ecology of small mammal species. During the research, 13 small mammal species were trapped in commercial fruit and berry farms. The relative abundance of the dominant species common vole (*Microtus arvalis*), yellow-necked mouse (*Apodemus flavicollis*), striped field mouse (*A. agrarius*) and bank vole (*Clethrionomys glareolus*) and their proportion in the investigated community varied according to year, season, and habitat, with no outbreaks recorded during the study period. According to species proportions in the commercial fruit farms, *M. arvalis* should be the focal species for the evaluation of plant protection products. Trophic resources in commercial fruit farms are partitioned between trophic groups and between species within groups, so that more species can exploit the limited trophic space simultaneously. *Clethrionomys glareolus* in the apple orchards is omnivorous, its trophic niche being separated from granivores and herbivores according to δ13C, while being the closest to insectivores according to δ15N. The degree of omnivory of these voles in the orchards differed from that in surrounding meadows and forests. In our analysis of 21 elements, we found that the main sources of variability in elemental concentrations were animal species and age, crop and agricultural intensity, while location, animal sex, and crop age were not important. The higher concentrations of Cu, Mn, Bi, Co, Cr, Fe, Ni, Sr, and Pb in the muscle and bone of the dominant species of rodents from the crop are as in comparison to those from the control habitats supported the hypothesis that fertiliser and pesticide use in commercial gardens should account for the variation of concentrations of the elements in the muscles and bones of small mammals. *Sarcocystis* spp. were detected in *A. agrarius*, *A. flavicollis*, *C. glareolus*, *M. arvalis* and *M. oeconomus* in six out of 14 orchards and berry plantations. The overall prevalence of *Sarcocystis* spp. was 1.38%, that in voles 2.23%, in mice 0.79%.

RESIDENTS BEHAVIOUR AND ITS IMPACT ON GREENHOUSE GAS EMISSIONS IN LITHUANIA IN THE CONTEXT OF EU (Ecology and environmental)

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In 2021, with the approval of the Green Deal by the European Parliament, a climate-neutral and sustainable Europe has become the main objective of the EU's climate change policy. Behaviour has the greatest influence on household energy consumption compared to other factors and has a statistically significant effect on the sector's CO_2 emissions. Therefore, the aim of the study was to analyse climate-friendly behaviour and its changes in the EU countries (including Lithuania) and to determine the factors that determine greenhouse gas (GHG) emissions in the household sector. The results showed that during the research period, the levels of attitudes towards climate change and personal responsibility increased statistically significantly in the EU countries, but not all changes in the performance of climate-friendly behavioural actions were positive. The reduction of heating/cooling emissions was positively influenced by the choice of a more climate-friendly energy supplier, and the level of attitudes towards climate change was positively influenced by transport GHG emissions. Regression analysis showed that the respondents' use of electricity and the resulting GHG emissions depended on behaviour, house type, and gender, thermal energy consumption was determined by the area of the home and house type, and these GHG emissions by the method of home heating in Lithuania. A statistically significant effect of renovation on energy heat consumption and the effect of renovation and the choice of a green electricity supplier on the emissions caused by this energy consumption were also determined. The study revealed that in order to mitigate the impact of the household sector on climate change, it is necessary to apply complex measures that include the role of policy makers, government institutions (APVA support, etc.) and the residents themselves (applying knowledge in daily activities, reforming their habits).

COMPARATIVE ASSESSMENT OF THE BIOLOGICAL AND ECOLOGICAL TRAITS DETERMINING THE INVASIVENESS OF ALIEN *CORNUS* TAXA IN LITHUANIA (Ecology and environmental)

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The aim of this study was to assess and compare the biological and ecological traits of alien *Cornus* species and subspecies that determine their invasiveness in Lithuania. The study compares the performance of alien *Cornus* and native *Cornus sanguinea* subsp. *sanguinea* and the ecological and biological characteristics. The reproductive output of alien *Cornus* was found to be better than that of the native *Cornus sanguinea* subsp. *sanguinea* in terms of higher flower and fruit production. Alien *Cornus alba* and *Cornus sanguinea* subsp. *australis* form a denser soil seed bank than the native *Cornus sanguinea* subsp. *sanguinea*. A negative effect of the alien *Cornus sericea* and *Cornus sanguinea* subsp. *australis* was observed on the diversity of herbaceous plant species in communities. The surveys led to a revision of the diversity of plants of the genus *Cornus* in Lithuania and to the addition of one species to the list of European alien plants. According to the data of cryptic invasion of *Cornus sanguinea* subsp. *australis*, the results of invasion rate showed the necessity of paying close attention to the origin of propagating and planting material of woody plants for forest cultivation and to determine their precise taxonomic identity. Based on the results of this research, it is proposed to add *Cornus sericea* to the list of invasive species in Lithuania.

QUANTIFICATION OF WINTERING SEABIRD BYCATCH IN THE BALTIC SEA COASTAL FISHERIES AND INGESTION OF MARINE DEBRIS (Ecology and environmental)

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The objective of this research was to quantify the consequences of two human-induced activities that affect seabirds in the south-eastern Baltic Sea: the bycatch of seabirds in coastal fisheries and the ingestion of marine debris. Both of these threats have been identified as major worldwide issues that affect seabird populations and both are important in the Baltic Sea where wintering seabirds can spend six to seven months of the year. In Lithuania, bycatch in gillnets is one of the critical causes of human-induced mortality for several seabird species that have seen significant declines in their populations in recent decades. Using one-fifth of the total fishing effort and 909 bycaught birds of 15 species, this study estimated that the coastal fishery in Lithuania captures between 1500 and 3000 birds annually. As part of this study, gillnet modification trials using black-and-white panels and steady green or flashing white net lights were shown to be not effective at reducing bird bycatch in gillnet fishery. Contrary to official statistics based on inadequate data, the unintended capture of seabirds in gillnets remains high despite financial investments to reduce the impact of fisheries on biodiversity. Digestive track analysis of collected birds entangled into gillnets in this study revealed the first data on ingestion of marine debris by seabirds wintering in the Baltic Sea and the first global record of ingestion of plastic and nonplastic debris in the Long-tailed Duck (*Clangula hyemalis*). This species dominates in gillnet fishery bycatch and has the highest rate of marine debris ingestion among studied diving wintering seabirds, representing it as one of the most sensitive and conservation-demanding species in the Baltic Sea. Studied winter distribution of the Long-tailed Duck using implanted satellite transmitters and discrimination of important stopover sites in this work informs about possible effective conservation zones along the flyway and in the Baltic Sea.

EXO-ERYTHROCYTIC STAGES OF *HAEMOPROTEUS* (APICOMPLEXA, HAEMOSPORIDA) PARASITES IN WILD BIRDS: INSIGHTS INTO DEVELOPMENTAL PATTERNS (Zoology)

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Avian haemosporidian parasites of the genus *Haemoproteus* (Apicomplexa, Haemosporida) are widespread and can cause mortality in non-adaptive hosts. Over 170 species of these parasites were described but little is known about their complete life cycles, especially exo-erythrocytic stages, which develop in the organs of their bird host. The objective was to expand the knowledge about exo-erythrocytic stages of *Haemoproteus* parasites in naturally infected birds and to unravel some patterns of their development. The results showed that the parasites were species-specific in regard of their exo-erythrocytic development, and this character is valuable for future taxonomic research. *Haemoproteus majoris*, *H. pastoris*, and *H. hirundinis* developed megalomeronts (huge-size tissue stages) while *H. dumbbellus* and *H. attenuatus* developed only meronts (relatively small tissue stages). All found tissue stages were of different morphologies between the different parasites. The intensity of parasitaemia was found to be an unreliable indicator of avian host with exo-erythrocytic stages. Chromogenic in situ hybridisation technique was successfully used for the search of tissue stages and the confirmation of their generic identity. The obtained new data contribute to new knowledge about exo-erythrocytic development of *Haemoproteus* parasites, including the predictability of molecular phylogenies in investigation of tissue stages of avian haemoproteids.

TAXONOMY AND PHYLOGENY OF THE GENUS *ANTOCHA* OSTEN SACKEN, 1860 (Zoology)

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Antocha Osten Sacken, 1860 is the genus of the crane fly, which belongs to the family Limoniidae, subfamily Limoniinae; 162 species and four subspecies of these insects are known. This research aimed to accomplish a taxonomic and phylogenetic review of the genus *Antocha* and to gain new knowledge about this insufficiently investigated group of insects. During this research, type and non-type specimens were examined and illustrated. Also, phylogenetic analysis of this genus species was conducted for the first time. Twenty-five species groups were found after phylogenetic analysis, of which 22 are mentioned for the first time. According to morphological characters and phylogenetic analysis, it was figured out that *Antocha* (*Antocha*) *brevistyla* Alexander, 1924 belongs to the subgenus *Proantocha* Alexander, 1919. Two new species of *Antocha* were described and characterised molecularly: *Antocha* (*Antocha*) *bella* Markevičiūtė & Podenas, 2019 and *Antocha* (*Antocha*) *pulchra* Markevičiūtė & Podenas, 2021, and a new species for the Chinese fauna *Antocha* (*Antocha*) *quadrifurca* Alexander, 1971 was discovered. Moreover, sequences of 17 species of this genus mtDNA cytochrome c oxidase subunit I were supplied to the GenBank for the first time, and it was found after species delimitation analysis that *Antocha* (*Antocha*) fulvescens Lackschewitz, 1940 and *Antocha* (*Antocha*) vitripennis (Meigen, 1830) are different species. These results provide the basis for future research of the genus *Antocha* fauna in the world.