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

Compiled by Indrė LIPATOVA

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STATISTICAL AND COMPUTATIONAL APPROACHES FOR THE ANALYSIS OF HIGH-THROUGHPUT EPIGENOMIC DATA (Biochemistry)

Povilas Gibas

Scientific supervisor:
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Dissertation defended:
14 February 2022

Epigenetic mechanisms, such as DNA modifications, are of great importance in all living things. Despite the importance of DNA modifications, many of the difficulties involved in establishing and characterising epigenetic profiles discourage researchers from following this path of research. TOP-seq is the first DNA sequencing method which uses covalent labelling of unmodified individual CG sites followed by the synthesis of DNA polymerase. This DNA sequencing method combines the enrichment of the unmodified DNA fraction, the resolution of a single base, and the strand specificity. Several other TOP-seq-based sequencing methods that can identify other DNA modifications are currently being developed. However, to make full use of these methods, a set of appropriate statistical and computational methods is required. This study presents strategies to solve obstacles that arises from a very specific type of TOP-seq data. This study is based on three main stages: design, development, application. During the design stage, we present a data processing strategy that converts primary TOP-seq epigenomic data into a CG position coverage signal. In the development stage, we propose and integrate three signal transformations that enhance the signaling of enrichment-based epigenomic methods. Finally, we present several cases in which the TOP-seq signal can be used to obtain biological information.

MOLECULAR STUDIES OF DIFFERENTIATION IN AMNIOTIC FLUID STEM CELLS OF HEALTHY AND FETUS AFFECTED GESTATIONS (Biochemistry)

Aistė Zentelytė

Scientific supervisor:
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Dissertation defended:
5 February 2022

As the maternal age increases, so does the risk of chromosomal, genetic diseases and miscarriages. Stem cells, including amniotic fluid stem cells (AFSCs), could be used for therapeutic purposes during the prenatal or neonatal period. During the work presented in the dissertation, the characteristics and the adipogenic, osteogenic, myogenic and neurogenic differentiation potential were investigated and compared in AFSCs of healthy and fetus affected gestations. We have demonstrated that AFSCs of healthy gestations (2nd trimester) and early gestations with fetal abnormalities (2nd trimester) are similar in their stem cell properties and differentiation potential towards all investigated lineages. Meanwhile, after examining AFSCs of healthy (second trimester) and polyhydramnios (foetal pathology, third trimester) gestations, differences in stem cell gene and protein expression, metabolic and energetic cell phenotype, and the neural differentiation potential were established between the two sources of AFSCs. We also determined that small molecules and their combinations could be used to improve the efficiency of neural differentiation in AFSCs. These studies are particularly relevant, since the amniotic fluid is considered a very promising source of stem cells, and little is known about the potential of AFSCs derived from foetus affected gestations and their applicability as an autogenous source of stem cells in regenerative medicine.

INVESTIGATION OF PRIMARY KERATINOCYTES AND EPIDERMOID CARCINOMA CELLS RESPONSE TO PHOTODYNAMIC TREATMENT (Biochemistry)

Neringa Daugelavičienė

Scientific supervisor:
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Dissertation defended:
27 April 2022

Photodynamic therapy (PDT) is a treatment modality for the precancerous lesions of the skin and skin cancer. The effects of PDT on cancer cells have already been thoroughly studied. However, healthy cells surrounding the tumour are also affected during the PDT. In this work, the effect of PDT on human primary keratinocytes HEKa was investigated through their exposure to various localised photosensitizers: aluminium phthalocyanine disulfonate (AlPcS2a), which accumulates to lysosomal membranes, aluminium phthalocyanine tetrasulfonate (AlPcS4), which concentrates in the lumen of lysosomes, and m-tetra(3-hydroxyphenyl)-chlorin

(mTHPC), which diffusely localizes to cellular membranes. We demonstrated that PDT effects, mediated by AlPcS2a and AlPcS4, induce primary keratinocyte differentiation and apoptosis, while PDT effects, mediated by mTHPC, induce apoptosis and autophagy. We also evaluated the effect of AlPcS2a-PDT on human epidermoid carcinoma A-431 cancer cells. We found that apoptosis is initiated, and the number of autophagosomes increases after lysosomal membrane photodamage. However, the final stages of autophagy do not occur. In response to PDT, not only cell death can occur. Also, the expression of pro-inflammatory and proangiogenic cytokines, followed by tumour development, can increase. After the exposure of A-431cells to PDT, we established the increase of interleukin 1 α (IL-1α) and vascular endothelial growth factor A (VEGF-A) expression. It should be noted that the expression of these cytokines in primary keratinocytes remained unchanged. We demonstrated that IL-1α stimulates VEGF-A expression after mTHPC-mediated PDT in human epidermoid carcinoma A-431 cells.

INVESTIGATION OF ADSORBED BIOMOLECULES USING NEW RAMAN SCATTERING-ENHANCING SUBSTRATES (Biochemistry)

Indrė Aleknavičienė

Scientific supervisor:
Prof. Dr Gintaras Valinčius
Vilnius University

Dissertation defended:
8 June 2022

This dissertation aimed to develop silver SERS substrates suitable for biomolecular analysis and to use them to investigate the applicability of mixed anchor monolayers for the construction of functional tethered bilayer lipid membrane (tBLM) models on silver. In this work, we developed a new fast and scalable manufacturing method of low-cost SERS substrates using inexpensive raw material, soda-lime glass. This method allows rapid production of large quantities of uniform repetitive nanostructured surfaces that produce substrates suitable for SERS measurements when coated with *a* silver coating. The optimal SERS signal amplification factor, which averages ~3×10^5 over the entire active area, has been achieved. Using these SERS substrates, we developed and characterised new different mixed self-assembled monolayers that could be applied to the construction of tBLM models on silver without causing corrosion and ensuring stability over time. In addition, using the EIS method, we demonstrated that using our selected mixed self-assembled monolayers, we could generate functional and biologically relevant models of biological membranes on silver that could be applied to analytical biochemical studies.

INVESTIGATING pre-mRNA SPLICING AND ITS REGULATORY FACTORS IN A HYPOXIC CELL MICROENVIRONMENT (Biochemistry)

Laurynas Vilys

Scientific supervisor:
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Vilnius University

Dissertation defended:
17 June 2022

Hypoxia is the term used to describe a reduction in the amount of oxygen in the cell microenvironment, so that it is no longer sufficient to support normal cellular functions. Hypoxia is the most common pathological condition in ischaemic diseases and cancer and affects the progression of neurodegenerative diseases. One of the pathways in response to hypoxia is the altered expression of protein isoforms generated by alternative pre-mRNA splicing. Pre-mRNA splicing is one of the steps in mRNA maturation in which introns are removed from pre-mRNAs and exons are joined. Alternative splicing is a process where a single pre-mRNA can give rise to proteins with different or even opposing functions. Alternative splicing creates protein diversity, but changes in splicing can also lead to disease. In this work, we investigated the alternative splicing of the neurodegenerative disease-associated genes APP and TAU and its dependence on the hypoxic cell microenvironment. It is also shown that it is not only changes in the expression of splicing factors that regulate the hypoxia-dependent inclusion or omission of exon 6 of the FAS gene in the maturing mRNA. The level of modification of splicing factors also plays a crucial role in the regulation of alternative splicing. In this work, we discuss the discovery of a mechanism for hypoxia-dependent regulation of pre-mRNA splicing, which may be one of the key mechanisms regulating this process.

MINIATURE CRISPR-Cas NUCLEASES: FROM CHARACTERISATION TO APPLICATION (Biochemistry)

Greta Bigelytė

Scientific supervisors:
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Dissertation defended:
23 June 2022

Prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) systems provide adaptive immunity against foreign nucleic acids. Guided by RNAs, CRISPR-Cas nucleases theoretically can cleave any double-stranded (ds) DNA target of interest and this flexibility was utilised to adopt these nucleases as novel tools for genome editing. However, the size of commonly used Cas9 and Cas12a proteins (1300–1500 amino acids) remains one of the biggest obstacles limiting their cellular delivery. Therefore, recently discovered Cas12f nucleases, which are half the size of Cas9 and Cas12a, could provide an attractive

alternative in these applications. This dissertation presents a collection of ten exceptionally compact (422–603 amino acids) CRISPR–Cas12f nucleases. In this work, we show for the first time that Cas12f proteins cleave dsDNA in the protospacer adjacent motif (PAM) dependent manner. Additionally, we demonstrated that CRISPR-Cas12f1 systems from *Syntrophomonas palmitatica* (Sp) and *Acidibacillus sulfuroxidans* (As) provide robust dsDNA interference activity in heterologous *Escherichia coli* cells. Therefore, SpCas12f1 and AsCas12f1 nucleases were selected for a thorough biochemical characterisation which allowed us to demonstrate that SpCas12f1 is able to cleave genomic DNA in human and plant cells. Altogether, these findings pave the way for the development of miniature Cas12f-based genome editing tools.

INVESTIGATION OF MOLECULAR MECHANISMS OF MACROPHAGE ACTIVATION (Biochemistry)

Asta Lučiūnaitė

Scientific supervisor:
Prof. Dr Aurelija Žvirblienė
Vilnius University

Dissertation defended:
1 July 2022

Macrophages are innate immune cells responsible for defence and homeostasis. One of the innate immunity defence mechanisms is inflammasome activation, which is also related to many inflammatory diseases. The NLRP3 inflammasome can be triggered by a variety of factors. However, inflammasome activation by protein oligomers is less investigated. Our goal was to investigate macrophage activation by oligomeric proteins of various structures. We treated macrophages by the following proteins: amyloid beta (Aβ) small oligomers and protofibrils; filamentous N proteins of paramyxoviruses; spherical virus-like particles (VLP) of polyomaviruses. We measured inflammatory cytokine secretion and NLRP3 inflammasome activation by assessing IL-1β secretion, caspase-1 activation, and formation of ASC specks. We found that both Aβ structures activated the inflammasome. This shows the probability that immune response arises before accumulation of Aβ fibrils during Alzheimer's disease. Therefore, primary Aβ structures could be a source of neuroinflammation. Investigation of viral proteins showed that only VLP induced the inflammatory response and activated the inflammasome. VLP induced lysosomal damage and K^+ ion efflux mediating inflammasome activation. Our investigation demonstrates that the structure of oligomeric proteins is one of the factors defining the inflammatory response. Moreover, our results highlight the role of the NLRP3 inflammasome in macrophage response to various molecular structures.

REDUCTION MECHANISMS OF PROOXIDANT XENOBIOTICS BY FLAVIN-CONTAINING DEHYDROGENASES-ELECTRONTRANSFERASES (Biochemistry)

Mindaugas Lesanavičius

Scientific supervisor:
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Vilnius University

Dissertation defended:
29 September 2022

Flavin-containing dehydrogenases-electrontransferases can transform between two- and one-electron transfer in redox reactions with various electron acceptors via a ping-pong mechanism owing to the chemical activity conferred to the flavin by the isoalloxazine ring. Some of these electron acceptors, namely, quinones, nitroaromatics, and aromatic N-oxides, can be considered prooxidant xenobiotics, i.e., substances that can be found in a given organism yet not naturally produced by it. These xenobiotics can be reduced in a single-electron way upon catalysis by the three representative flavoenzymes that are presented in this work: ferredoxin:NADP+oxidoreductases from *Plasmodium falciparum* (PfFNR) and *Rhodopseudomonas palustris* (RpFNR) and rat neuronal NO synthase (nNOS). By combining steady- and presteady-state kinetics methods, comprehensive mechanistic studies on the catalysed reduction of xenobiotics of the aforementioned enzymes – were performed with the results in line with those obtained for homologous enzymes and reported earlier. Moreover, the photoreduction studies of RpFNR enabled a potentiometric characterisation of this novel thioredoxin reductase-like FNR. Finally, it was shown that dehydrogenases- electrontransferases can be employed for the approximate determination of single-electron reduction potentials of nitroaromatics and aromatic N-oxides, and the calculated electron transfer distances in these enzymes are in line with the outer sphere electron transfer model.

MOLECULAR MECHANISMS OF FERROPTOSIS RESISTANCE IN PANCREATIC CANCER CELLS (Biochemistry)

Eglė Žalytė

Scientific supervisor:
Dr Jonas Cicėnas
Vilnius University

Dissertation defended:
30 September 2022

Pancreatic cancer is the seventh most common cancer in the world. However, it ranks the fourth according to mortality rates. Delayed diagnosis and resistance to chemotherapy are the main causes of such a poor outcome. Thus, new combination therapies and new ways to induce cell death are constantly under development. The research of this dissertation aimed to find the anticancer potential and to elucidate the mechanisms of two strategies: iron-dependent cell death ferroptosis and starvation induction. The results show that sensitivity to ferroptosis can be mediated by growth

factor, amino acid L-glutamine, L-lysine and L-arginine deprivation and mTORC1 inhibition with rapamycin. It is shown that in starved pancreatic cancer cells, sensitivity to ferroptosis is mediated by kinases ERK1/2 and JNK. Moreover, in this dissertation, a new pancreatic cancer cell line Capan-26 was established and characterised *in vitro*. This is the first pancreatic cancer cell line derived from a tumour of a Lithuanian patient. In this unique and a commercial cell line, growth factor deprivation correlated with erastin, the inducer of sensitivity to ferroptosis. With this in mind, several EMT targeting compounds were evaluated for their effects on cell sensitivity to erastin and new effective combinations of erastin and EMT-modulating compounds were discovered.

PROTEOMIC ANALYSIS OF RECOMBINANT PROTEIN SYNTHESIS IN THE YEAST *SACCHAROMYCES CEREVISIAE* (Biochemistry)

Rūta Zinkevičiūtė

Scientific supervisor:
Dr Rimantas Slibinskas
Vilnius University

Dissertation defended:
18 November 2022

Due to various factors, the production of some recombinant proteins in the yeast *Saccharomyces cerevisiae* is inefficient. Two-dimensional electrophoresis (2DE) and mass spectrometry (MS)-based proteomic analysis is a powerful tool which may help reveal the reasons for the inefficient synthesis of these proteins. To find the most suitable 2DE method for yeast proteome analysis, we compared two 2DE methods to study the inefficient translocation of the measles virus hemagglutinin (MeH) protein to the endoplasmic reticulum (ER). We found that in a wide pH range, non-equilibrium pH gradient electrophoresis (NEPHGE)-based 2DE is the preferred method for yeast proteome analysis over the immobilised pH gradient (IPG)-based 2DE. Also, we showed that the application of heat shock to high-density cell cultures increases the efficiency of MeH translocation and the amount of glycosylated protein by ~3 times. After analysing the proteomes of cells grown under conditions of more efficient translocation using 2DE methods, we identified 15 protein targets possibly related to improved MeH translocation. We restored the gel solution and carrier ampholyte composition for NEPHGE-2DE that is suitable for use in place of commercially no longer available solutions. Furthermore, we performed a comparative, quantitative, NEPHGE-2DE and LC-MSE-based proteomic analysis of human calreticulin protein (CALR)-secreting and control yeast cells. Our results showed that the efficient

secretion of CALR in the yeast *S. cerevisiae* does not burden the yeast secretory machinery and does not induce any apparent cellular stress. These results show that NEPHGE-based 2DE combined with MS is a suitable method for proteomic analysis of the recombinant protein-synthesising yeast *S. cerevisiae*.

APPLICATION OF ENDOPHYTIC BACTERIA TO IMPROVE THE GROWTH AND ADAPTATION OF ANTIBIOTIC-TREATED TOBACCO SHOOTS *IN VITRO* (Biochemistry)

Elena Andriūnaitė

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Dissertation defended:
21 December 2022

The aim of the study was to evaluate the long-term effects of the timentin on tobacco shoot culture in vitro and to use endophytic bacterial isolates to improve adaptation and growth of antibiotic-treated transgenic tobacco shoot culture following the genetic transformation procedure. The results revealed that timentin treatment had an enduring residual negative effect on tobacco in vitro shoot culture which was related to an increased level of oxidative stress. The microbiome analysis revealed a diverse microbial community in the tobacco in vitro shoots, including 59 bacterial families, mainly dominated by the Mycobacteriaceae family. The timentin treatment resulted in a decline in microbial diversity reducing the number of identified families 4.5-fold, as well as increased dominance of Mycobacteriaceae and several other families which was likely associated with antibiotic resistance traits of the bacteria. Additionally, the potential of co-cultivation with growth-promoting endophytes to improve the efficacy of in vitro tissue culture following antibiotic treatment was investigated. It was established that pure isolates of endophytic bacteria of the Bacillus cereus group obtained from tobacco leaves could promote biomass accumulation in tobacco in vitro shoot culture. Furthermore, the endophytic isolates showed a capability to mitigate the reduced growth vigour in the transgenic tobacco shoots. Co-cultivation with endophytes reduced oxidative stress symptoms and enhanced the accumulation of shoot biomass of the transgenic tobacco shoots without interfering with the recombinant protein accumulation.

INTERSPECIFIC EFFECT OF MYCOBACTERIAL PROTEINS ON BACTERIA GROWTH AND QUALITATIVE CHANGES IN MICE LEUKOCYTES (Biology)

Joana Korabliovienė

Scientific supervisor:
Dr Mykolas Mauricas
Centre for Innovative Medicine

Dissertation defended:
20 January 2022

Despite the availability of tuberculosis (TB) vaccine, TB remains one of the leading causes of death among infectious diseases in the world. The BCG vaccine is considered to have different protective efficacy due to the exposure of individuals with non-TB mycobacteria (NTM), the environmental mycobacteria that interfere with the effectiveness of the BCG vaccine by masking or blocking. In order to better understand the causes limiting the effectiveness of the BCG vaccine, the research work selected bacteria belonging to different mycobacterial complexes, examined the interspecific influence of mycobacterial proteins on immune system cells after exposure to NTM, and assessed the differences in immune responses to mycobacteria proteins depending on the area of RD-1. Strains of *Mycobacterium avium* subsp. *avium* (ATCC 15769) and *M. terrae* (ATCC 15755) of mycobacteria ATCC were used in this study. Mycobacterial proteins were extracted from wild type *M. bovis* (MBP). In the MBP sample, we identified 20 proteins. Most of the proteins detected in MBP are associated with type VII secretion (T7S) pathway, metabolic processes, or signal peptides. Proteins isolated from *M. avium* subsp. *avium* (MAP), which has no RD-1 area, and from *M. bovis*, which has this area, were distinguished for their immunogenicity. We found that mycobacterial proteins have effect on immune cells. The most distinct changes in immune cells were in mice, which had been immunised with MAP and had exposure to *M. avium* subsp. *avium*. According to the data of the research, previous sensitisation with NTM antigens causes different immune reactions after the contact with different NTM, suggested that the phenomenon of masking the effectiveness of the BCG vaccine may depend on the type of mycobacteria. When developing new tuberculosis vaccines, it would be useful to consider the prevalence of NTM in the region in which new TB vaccines would be applied.

AGE-RELATED MACULAR DEGENERATION: NEW IMMUNOGENETIC BIOMARKERS AND ASSOCIATIONS WITH TREATMENT EFFICACY (Biology)

Alvita Vilkevičiūtė

Scientific supervisor:
Prof. Dr Rasa Liutkevičienė
Lithuanian University of Health Sciences

Dissertation defended:
16 June 2022

The doctoral dissertation aimed to identify new immunogenetic blood-based molecular markers in patients with age-related macular degeneration (AMD) and to evaluate the associations of the biomarkers with exudative AMD treatment efficacy. The study was performed using the methods of the real-time polymerase chain reaction and enzyme-linked immunosorbent assay. Considering the inflammatory pathogenesis in AMD development, we identified the protective role of IL-9 rs1859430, rs2069870, rs11741137, rs2069885, rs2069884 haplotypes A-G-C-G-G and G-A-T-A-T for early AMD development. VEGFA rs3025033 and VEGFA rs1570360, rs699947, rs3025033, rs2146323 haplotype A-A-G-A also showed a protective role but only for exudative AMD. Moreover, it was determined that VEGFA rs3025033 associations remain significant only in females with exudative AMD. The other genetic variant, RAD51B rs2588809, was found to be associated with an increased risk of early AMD development in females only. According to the databases, our study shows for the first time that the T allele in IL-10 rs1800896 is associated with lower IL-10 serum levels, and the C allele in VEGFA rs699947 is associated with elevated VEGF-A serum levels in patients with exudative AMD. Also, our study showed significant associations between genetic variants RAD51B rs8017304, TRIB1 rs4351379, IL-9 rs1859430, rs2069870, rs2069884, rs2069885 and VEGFA rs699947 and exudative AMD treatment with anti-VEGF injections efficacy.

ASSOCIATIONS BETWEEN BONE MINERAL CHARACTERISTICS, BODY COMPOSITION AND BLOOD BIOMARKERS IN ADOLESCENT FEMALE ATHLETES WITH DIFFERENT TRAINING PATTERNS (Biology)

Vita Tamolienė

Scientific supervisor:
Prof. Dr Jaak Jürimäe
Lithuanian Sports University

Dissertation defended:
17 June 2022

The general aim of the dissertation was to investigate the relationships between bone mineral characteristics and blood biomarkers, body composition and training load variables in adolescent female athletes with different loading patterns. It was found during this research that rhythmic gymnasts undertaking specific weight-bearing athletic activity present higher bone mineral values in comparison with swimmers and untrained controls, while non-weight-bearing activity of swimming training has a neutral effect on bone

mineral values in swimmers. Adolescent athletes with different training patterns have higher serum sclerostin concentrations than untrained controls. Circulating sclerostin concentration influenced bone mineral characteristics only in high-impact training group of rhythmic gymnasts, suggesting that the specific training pattern seen in rhythmic gymnasts has a beneficial effect on bone mineral values despite higher sclerostin that inhibits bone formation. The circulating sclerostin level is associated with leptin, irisin, and C-terminal telopeptide of type I collagen, suggesting a possible interaction between specific adipokine, myokine, and osteokine values in lean adolescent females with increased physical activity. Specific training volume together with appendicular muscle mass influenced cortical bone development at the femoral neck site of the skeleton in rhythmic gymnasts, while hormonal values influenced trabecular bone development at the lumbar spine site of the skeleton in both athletic groups with different loading patterns. The appendicular muscle mass was the only studied predictor of all measured bone mineral values in untrained controls.

IDENTIFICATION OF TRPM6 AND TRPM7 CHANNELS AND ANALYSIS OF THEIR EXPRESSION IN CARDIOMYOCYTES (Biology)

Inga Andriulė

Scientific supervisor:
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Lithuanian University of Health Sciences

Dissertation defended:
30 June 2022

TRPM7 and its homologue TRPM6 belong to the melastatin subfamily of TRP channels. Both are regulated by intracellular magnesium and are distinguished from other ion channels by unusual bifunctional activities, the ion channel, and protein kinase. Both are permeable to Ca^{2+} and Mg^{2+} and have now been identified as critical regulators of Mg^{2+} homeostasis. Current evidence shows that TRPM7 is ubiquitously expressed in all mammalian cells, with the highest expression in the heart and the kidney, whereas TRPM6 expression is restricted to epithelial cells of the kidney, placenta, and intestine. To date, studies in TRPM6 and TRPM7 channel expression in either un-diseased or diseased human heart have mainly relied on the genomic approach. Thus, the presence of TRPM6 in the heart remains controversial and, more specifically, it is an open question whether TRPM6 and TRPM7 are expressed in all chamber walls of the heart. By using different molecular approaches, in this work we examined the expression of TRPM6 and TRPM7 channel proteins in human and porcine cardiomyocytes and evaluated possible changes in the expression of these channels in cardiac pathology as well as their role in cardiac electrophysiology.

COMPREHENSIVE ANALYSIS OF CIRCULATING NUCLEIC ACIDS IN THE BLOOD PLASMA OF GASTRIC CANCER PATIENTS (Biology)

Greta Varkalaitė

Scientific supervisor:
Prof. Dr Jurgita Skiecevičienė
Lithuanian University of Health Sciences

Dissertation defended:
4 July 2022

This study aimed to analyse the plasma cell-free nucleic acids (cfNA) in patients with gastric cancer and to evaluate the function and suitability of these markers for minimally invasive diagnosis and prognosis. This study provides: (1) differential miRNA expression profiles in gastric premalignant (atrophic gastritis), malignant (gastric cancer), and control cases and analysis of the most deregulated miRNAs in plasma for liquid biopsy approach; (2) novel findings that reveal the important role of hsa-miR-20b-5p and hsa-miR-451a-5p in gastric carcinogenesis, which may be used to develop a beneficial strategy for future cancer therapy; (3) insights regarding urine collection as a liquid biopsy source for cell-free DNA (cfDNA) analysis; (4) optimised cfNA isolation protocols and detailed description of pre-processing variables (storage, haemolysis, and isolation methods) that could affect cfNA quality; (5) comparison between two plasma cfDNA sequencing approaches for the reliable detection of low-frequency alleles; (6) analysis of concordance of gastric cancer tissue and plasma cfDNA mutational profiles and potential application of liquid biopsy for disease monitoring and survival. Taking as a whole, this extensive research provides useful observations from the processing of samples and selection of sequencing approach to the applicability of liquid biopsy for gastric cancer diagnosis and prognosis.

THE EFFECT OF EOSINOPHILS ON GENE EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS, CONTRACTILITY AND MIGRATION OF AIRWAY SMOOTH MUSCLE CELLS AND PULMONARY FIBROBLASTS IN ASTHMA (Biology)

Ieva Janulaitytė

Scientific supervisor:
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Lithuanian University of Health Sciences

Dissertation defended:
24 August 2022

The aim of this study was to evaluate the effect of eosinophils on gene expression of extracellular matrix proteins, contractility, and migration of airway smooth muscle cells (ASMC) and pulmonary fibroblasts (PF) in asthma. To achieve this aim, we formulated three objectives: (1) to evaluate the effect of eosinophils on gene expression of extracellular matrix proteins, MMPs, and TIMPs in ASMC and PF in asthma; (2) to determine the effect of eosinophils on gene expression of canonical and non-canonical TGF-β signaling pathway molecules in ASMC and PF; (3) to investigate

the influence of eosinophils on the contractility, migration, and gene expression of contractile apparatus proteins in ASMC and PF. To achieve these objectives, we used combined cultures methods between subjects' eosinophils and ASMC or PF cell lines. We used real-time PCR method for the gene expression of ECM, MMP, TIMP, TGF-β signaling pathway molecules and markers of differentiation; the collagen gel method for contractility and wound healing assay for migration. We found that the asthmatic eosinophils promoted the expression of ECM, MMP, TIMP and differentiation marker genes in ASMC and PF. Eosinophils from patients with allergic asthma stimulated gene expression of the non-canonical TGF-β signaling pathway in ASMC and gene expression of molecules of the canonical signaling pathway in PF, while eosinophils from patients with severe non-allergic eosinophilic asthma stimulated the expression of genes of both canonical and non-canonical pathway molecules in both cell lines. Asthmatic eosinophils promoted the contractility, migration and differentiation of bronchial smooth muscle cells and lung fibroblasts.

APPLICATION OF SYNTHETIC HYDROGELS FOR NEURAL TISSUE MODELLING *IN VITRO* (Biology)

Zbigniev Balion

Scientific supervisors:
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Dissertation defended:
30 August 2022

The difference between environment of monolayer cell cultures on plastic and natural environment determines changes in biology, physiology, and functionality of these cells compared to *in vivo*. Drug discovery research based on such cultures is inefficient and inaccurate. New cell culture methods are developed using hydrogel materials that closely replicate the microenvironment of natural tissues. Synthetic hydrogels and small peptides imitating the activity of extracellular matrix proteins are used for standardising the cellular microenvironment design. Although this method restores successfully the functionality of various tissues *in vitro*, there is still no standardised functional network-forming neural cell cultures characterised by spontaneous neuronal signals. This work aims to characterise synthetic extracellular matrix-mimicking collagen peptide hydrogels as matrices for *in vivo*-like functional neural tissue engineering. The work investigates how these hydrogels promote the organisation, composition, motility, and functionality of primary neuronal and glial cell cultures *in vivo*. In addition, it reveals the importance of extracellular matrix peptide-induced focal adhesions in forming functional

neural tissue structures. Such synthetic hydrogel-based neural tissue models enable standardised high throughput scientific and drug selection screening studies. In addition, the studied synthetic hydrogel materials may be promising in the field of regenerative medicine.

CHEMICAL PHENOTYPES, MORPHOLOGY, ATRIAL DISTRIBUTION, DEVELOPMENT, AND AGE-RELATED CHANGES OF PORCINE INTRACARDIAC NEURONS (Biology)

Tomas Ragauskas

Scientific supervisor:
Dr Kristina Rysevaitė-Kyguolienė
Lithuanian University of Health Sciences

Dissertation defended:
31 August 2022

These studies aimed to deepen our understanding of the development and structure of the intracardiac nervous system. Given anatomical similarities between the hearts of the human and the pig as well as potential application of the porcine heart in xenotransplantation and electrophysiological research, the atria of new-born piglets and adult pigs were investigated. Immunohistochemical staining methodology of whole-mount preparations from two age groups allowed, for the first time, to characterise phenotypes and size of intracardiac neurons within distinct atrial ganglionated subplexi and also age-related developmental processes. These studies encourage the idea that intracardiac neurons of new-born animals are not fully developed and their phenotypical and morphological development and, likely, the process of neurogenesis, proceed postnatally. These age-related changes include a decreased proportion of sympathetic and increased proportion of parasympathetic neurons and also increased abundance of sympathetic neurons within the right atria of adult pigs. Also, putative mutual contacts between sympathetic and parasympathetic neurons as well as between peptidergic nerve fibres and parasympathetic neurons were revealed. Thus, present morphological results expand our knowledge of the development and age-related changes in the nervous system of the mammalian heart.

APPLICATION OF MITOCHONDRIA-TARGETED PHARMACOLOGICAL COMPOUNDS IN PREVENTION OF ISCHEMIC BRAIN DAMAGE (Biology)

Danielius Umbrasas

Scientific supervisor:
Prof. Dr Vilmantė Borutaitė
Lithuanian University of Health Sciences

Dissertation defended:
31 August 2022

Currently, treatment options for ischemic stroke are limited, therefore it is important to search for new compounds that can protect the brain from ischemic injury. As mitochondria are closely related to the pathological processes occurring during ischemic stroke, in this work we investigated the application of mitochondria-targeted pharmacological compounds in protecting brain tissue from ischemia-induced damage. The aim of this study was to investigate the molecular mechanisms of mitochondria-targeted pharmacological compounds that may protect against ischemia-reperfusion injury. Experiments were carried out using rat models of brain and cardiac ischemia. To investigate the protective mechanisms of the pharmacological compounds, mitochondria were isolated from brains and hearts, and functional studies were performed. Results show that nitric oxide donors induce changes in the phosphorylation of mitochondrial proteins, and some of the targets are implicated in the structure and regulation of the mitochondrial permeability transition pore. The ATP synthase inhibitor bedaquiline given to the animals prior to ischemia reduces the area of cerebral infarction and improves the neurological state of the animals. The endogenous metabolite itaconic acid inhibits mitochondrial respiration and the production of reactive oxygen species in mitochondria. The new data obtained may contribute to the use of the tested pharmacological compounds in the clinical treatment of ischemic stroke.

INFLUENCE OF SODIUM VALPROATE ON EXPERIMENTAL LUNG TUMORS (Biology)

Raminta Diržiuvienė

Scientific supervisor:
Prof. Dr Habil. Angelija Valančiūtė
Lithuanian University of Health Sciences

Dissertation defended:
31 August 2022

The most common cause of death from cancer in the world is lung cancer. In our work, we studied one of the histone deacetylases inhibitor, sodium valproate (NaVP), which is recognised as an anticancer drug. Our hypothesis about the potential effect of NaVP on experimental lung tumours was based on clinical investigations targeting overexpression of histone deacetylase which has been observed in clinical trials of various cancers, including non-small cell lung cancer. In our research, two experimental models of

lung cancer were applied for the investigation of NaVP action: (1) Chemically induced experimental lung cancer in mice; (2) Tumours formed from lung cancer cell lines on the chicken embryo chorioallantoic membrane (CAM). For the first time we have established a synergistic effect of urethane, NaVP, and possibly testosterone on the initiation of experimental lung tumours. We did not observe such a synergistic effect in the groups of castrated males and females. In our study, we found for the first time that the malignant transformation of tumours (transformation of adenomas into adenocarcinomas) and the number of Ki-67(+) positive cells decreased ($p < 0.001$) in groups of sterilised females due to exposure to NaVP. We did not observe changes in malignant transformation dependent on NaVP in the groups of males and unsterilised females. In our research for the first time, we described that influence of NaVP to the tumour initiation and progression is sex dependent. Our investigation showed that effect of NaVP can be cell line specific. This fact should be considered in personalised medicine applying treatment with NaVP as an additional therapeutic measure.

MOTOR AND MENTAL FATIGUE DEVELOPMENT DURING PERFORMANCE OF PROLONGED UNILATERAL AND BILATERAL MOTOR CONTROL AND COORDINATION TASKS (Biology)

Andrius Šatas

Scientific supervisor:
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Dissertation defended:
16 September 2022

Movement is a critical aspect of life. It is well known that the performance of prolonged motor and/or cognitive tasks leads to decreased performance of daily activities. Muscle fatigue has been studied for over a century, but almost no data are available to indicate how the brain perceives fatigue and modulates its signals to the fatiguing muscle. Development of fatigue is important in the context of work-related safety. Occupational fatigue is a concern for both individuals and organizations. Fatigue may lead to a loss of productivity, but, more dramatically, to an increased risk of accidents to workers, and to the public. We conducted three studies in order to investigate the manifestations of motor and cognitive fatigue during prolonged (first and second studies) and acute (third study) motor control and coordination tasks. The aim of the research was to investigate time-varying physiological signals reflecting changes in motor and cognitive functions during the performance of prolonged motor control and coordination tasks. We employed three novel unilateral and bilateral motor control and coordination tasks performed with both lower and upper limbs to investigate the manifestations

and significance of time-varying physiological signals associated with motor and mental fatigue. The results of our studies could help improve the general understanding of fatigue. In addition, the results of these studies could help unravel compensatory strategies against the adverse effects of motor and cognitive fatigue during the performance of motor control and coordination tasks. The occurrence of these compensatory strategies could serve as a potential early indicator of fatigue during motor control and coordination tasks.

ANALYSIS OF CLINICALLY SIGNIFICANT AND NEW GENETIC ALTERATIONS OF ACUTE LYMPHOBLASTIC LEUKEMIA TRANSCRIPTOME (Biology)

Rimvydas Norvilas

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Dissertation defended:
22 September 2022

Acute lymphoblastic leukemia (ALL) is an aggressive and heterogeneous disease of the hematopoietic system characterised by the accumulation of immature cells in the bone marrow. Depending on the immunophenotype of the affected cells, B-cell (B-ALL) and T-cell (T-ALL) disease types are distinguished. Different prognosis is often associated with specific genetic alterations detected at the time of diagnosis. B-ALL and T-ALL subtypes are often characterised by a different composition of these alterations. The aim of the research was to evaluate clinically significant and new genetic alterations of acute lymphoblastic leukemia transcriptome, determine their frequency, impact on survival and disease pathogenesis. Due to the relatively small size of the country's population and a centralized treatment centre, samples of B-ALL and T-ALL cases corresponding to the majority of Lithuanian population were selected and studied. This is the first study of its kind in Lithuania, during which a detailed analysis of the ALL transcriptome was performed using the RNA sequencing method. During this research, a genetic makeup of Lithuanian B-ALL and T-ALL was evaluated and the frequencies of these changes were compared with research data from other countries. Statistical analysis of clinical and genetic data of B-ALL patients have associated a certain group of genetic alterations with a poor disease prognosis. A number of gene fusions were identified for the first time in TALL cases and were associated with the disease pathogenesis.

PHYSIOLOGY AND PATHOLOGY OF THE BLOOD-BRAIN BARRIER: IMPLICATIONS FOR ASTROCYTE-SECRETED FACTORS (Biology)

Karolina Kriaučiūnaitė

Scientific supervisor:
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Dissertation defended:
23 September 2022

The primary goal of this study was to investigate the influence of factors secreted by healthy and Alzheimer's disease-affected astrocytes on the barrier properties of human brain endothelial cells. During the implementation of the dissertation project, a new *in vitro* blood brain barrier (BBB) model was developed. We co-cultured human immortalised BEC line hCMEC/D3 with immortalised astrocytes derived from the hippocampi of wild type (WT-iAstro) and triple AD mutation APP/MAPT/PS1 (3Tg-iAstro) carrying mice. Using this model, we demonstrated for the first time that 3Tg-iAstro exhibit impaired capacity to support BBB integrity *in vitro* through paracrine mechanisms. We found that WT-iAstro-secreted factors, including EVs, supported BEC barrier function by increasing trans-endothelial electric resistance (TEER) and expression of tight junction (TJ) proteins, whereas factors secreted by AD mutation-carrying astrocytes did not affect BBB *in vitro*. We employed a defined protocol for producing BECs from human inducible pluripotent stem cells (iPSCs) and established a BBB *in vitro* model. We demonstrated for the first time that autocrine secretion of basic fibroblast growth factor (bFGF) is necessary for the establishment a tight BECs barrier, as revealed by measurements of TEER. In contrast, addition of exogenous bFGF inhibited TEER in a concentration-dependent manner. We further demonstrated that MAPK/ERK signalling pathway does not affect autocrine bFGF signalling-dependent BECs barrier function, but is largely responsible for the inhibitory effects of the exogenous bFGF. Our findings for the first time demonstrate a dual role for bFGF in the regulation of BEC barrier function and could be useful for the development of a novel diagnostic and therapeutic strategies.

EVALUATION OF CHEMOIMMUNOTHERAPY EFFICACY IN MURINE TUMOUR MODELS WITH DIFFERENT ANTIGEN PROCESSING AND PRESENTATION MECHANISM FUNCTIONALITY (Biology)

Karolina Žilionytė

Scientific supervisor:
Prof. Dr Vita Pašukonienė
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Dissertation defended:
27 September 2022

Immunotherapy is a promising cancer treatment that stimulates patient's immune system to fight against tumour cells. Clinical results of immunotherapy reveal that patient response to immunotherapy is heterogeneous and often low. To improve the effectiveness of immunotherapy, it is necessary to identify possible resistance mechanisms, predictive biomarkers for treatment personalization and effective combinatorial treatment strategies. In this thesis, using mouse tumour models, the influence of antigen processing and presentation mechanism functionality in tumour cells on the efficacy of T-lymphocyte-based immunotherapy is demonstrated. The predictive value of the expression of genes related to the mechanism of antigen processing and presentation in the individualization of treatment with dendritic cell vaccines and anti-PD-1 is also revealed. The results obtained during the study show that the functionality of the antigen processing and presentation mechanism can be modulated by the chemotherapeutic drug cyclophosphamide. The activating effect of cyclophosphamide on the antigen processing and presentation mechanism may make tumour cells more immunogenic and thus pave the way for T-lymphocyte-based immunotherapy.

GENETIC DIVERSITY OF *LYTHRUM SALICARIA* L. POPULATIONS AND RELATED FACTORS OF ENVIRONMENT (Biology)

Dinara Shakeneva

Scientific supervisor:
Prof. Dr Eugenija Kupčinskienė
Vytautas Magnus University

Dissertation defended:
30 September 2022

This work was the first study devoted to elucidating the relationship between the genetic structure of *Lythrum salicaria* populations, the physiology of nutrition, and features of natural and human-altered environment. Our data on the molecular diversity of *Lythrum salicaria* may help to compare in more detail the genetic characteristics of natural and invasive *Lythrum salicaria*, the interpretation of which is important in the search of high invasiveness mechanisms of the species in N. America, and development of measures of limitation and eradication of the species within invasive areas. Genetic and physiological studies of Lithuanian populations of *Lythrum salicaria* supplement the data on the condition of aquatic plants in our country and are

important for the entire Baltic region, as similar studies of this type in neighbouring countries have not been described in the scientific literature so far. For the first time in the Baltic region, the population diversity of *Lythrum salicaria* was studied using the method of molecular markers and elemental analysis. The obtained results revealed that the populations of this species in Lithuania were formed by mixing with ancestors with 2 different gene pools. The data on the elemental composition of *Lythrum salicaria* might provide evidence that the pollution of Lithuanian rivers with nitrogen compounds and heavy metals is not high. The paper shows that the river regulation causes changes in the genetic structure of populations – a statistically significant genetic differentiation at AFLP loci between populations of *Lythrum salicaria* growing along natural and regulated riverbeds has been identified.

THE EFFECT OF ALTERNATIVE EXERCISES PROGRAMMES ON MOTOR AND COGNITIVE FUNCTIONS IN OLDER ADULTS (Biology)

Agnė Čekanauskaitė

Scientific supervisor:
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Dissertation defended:
30 September 2022

According to a 2018 report of the World Health Organization, the proportion of older adults will nearly double from 12% to 22% between 2015 and 2050 and is projected to reach two billion. As a result, prevalence of individuals with age associated cognitive deficits, motor declines, and decrements of dual-task performance will increase. We decided to evaluate yoga and tai-chi effects on cognitive and motor performance, psycho-emotional state and balance as a novel life-style/training strategy for elderly people. Based on existing evidence, we hypothesized that: I. Yoga would increase BDNF levels, improve cognition, and result in improved motor learning, and improve dual balance–cognitive task performance in older adults; II. Tai chi may increase BDNF and irisin levels, improve psycho-emotional state, autonomic control, and cognition, and result in improved motor performance, balance and learning in older adults during a pandemic. The aim of the research was to investigate and determine the effect of a 10- week alternative exercises programmes on motor and cognitive functions, and potential mechanisms in older adults. Results showed that 10 weeks of regular yoga practice resulted in improved balance under single and dual-task conditions and learning of motor tasks that were mediated by an increased BDNF level; however, yoga practice had no effect on cognition in older adults. The ten weeks of tai chi induced improvements in

inhibitory control, mental switching, and visuospatial processing, which were possibly mediated by decreased depressive symptoms and increased BDNF levels. Furthermore, improvement in visuospatial processing was associated with improvements in motor learning, induced improvements in balance under single- and dual-task conditions, which were linked to improved sustained attention and increased BDNF and irisin levels.

ASSESSMENT OF SOURSES OF VARIATION IN HER2 ONCOGENE AMPLIFICATION AND EXPRESSION (Biology)

Gedmantė Radžiuvienė

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Dissertation defended:
30 September 2022

Human epidermal growth factor receptor 2 (HER2) is an important predictive and prognostic biomarker in invasive breast cancer (BC). The heterogeneity of HER2, at both protein expression and gene amplification levels, is one of the main reasons of inaccurate assessment of HER2 status and non-effective response to therapy. The presence of tumour infiltrating lymphocytes and their distribution in the tumour microenvironment are associated with prognostic value in BC; however, the data are conflicting in different BC subtypes. In this dissertation, automated fluorescence in situ hybridization analysis algorithms were applied to investigate HER2 gene expression heterogeneity in HER2 borderline (by immunohistochemistry, IHC) BC patients. Novel quantifiable measures of HER2 intratumoural heterogeneity (ITH), based on HER2 signal variance in BC cells were developed. The ITH of HER2 protein and other BC IHC biomarker expression and its prognostic value was investigated using digital image analysis with subsequent hexagonal grid analytics. We established the independent prognostic indicators, representing ITH of HER2 and ER IHC expression, that supplemented the clinical and pathological parameters of BC and outperformed other quantitative indicators used for the assessment of the IHC biomarkers. Indicators of CD^{8+} lymphocyte distribution in the tumour microenvironment and their prognostic value were explored: in HER2 non-amplified tumours, antitumour immune response, assessed by the CD^{8+} interface zone Immunogradient indicators, provided prognostic stratification independent and superior to other pathology and IHC variables.

IMPACT OF METHIONINE RESTRICTION DURING A HIGH FAT DIET ON MUSCLE STRUCTURE, FUNCTION AND PLASTICITY IN AGEING (Biology)

Anandini Swaminathan

Scientific supervisor:
Prof. Dr Tomas Venckūnas
Lithuanian Sports University

Dissertation defended:
14 October 2022

The focus of this thesis was to assess the double burden of obesity and ageing on skeletal muscle mass, metabolism, and plasticity, and the potential of methionine restriction to slow or reverse these changes that in turn would improve the quality of life for older adults. The results presented in this thesis show that methionine restriction promotes glucose tolerance and results in a decrease in the body mass and the body mass index, even in the presence of a high fat diet. It also enhanced the hypertrophic response, but unexpectedly methionine restriction aggravated the denervation-induced atrophy. These effects of methionine restriction applied similarly to young and old mice. The data suggest that methionine restriction may be beneficial to combat the adverse effects of a high fat diet on sarcopenia but may be detrimental during periods of disuse such as during spaceflight, hospitalisation, and prolonged bed rest, especially in older adults. Methionine restriction enhanced the skeletal muscle hypertrophic response in old age that was accompanied with an increase in p-Akt without significant changes in muscle Akt, nicotinamide adenine dinucleotide, vascular endothelial growth factor levels, oxidative capacity, or low-grade systemic inflammation. Methionine restriction did not rescue the blunted hypertrophic response in old mice on a high fat diet. Since glucose tolerance tests are widely used to study glucose metabolism in mice, it is important to choose the right route of glucose administration to get reliable results. Our data suggests that oral gavage, intraperitoneal and intravenous administration of glucose can all be used to measure glucose tolerance in both old and young mice. Secondly, the glucose tolerance was similar in young (2 months old) and old (23 to 27 months old) mice.

THE EFFECT OF GENETIC BACKGROUND AND INTERMITTENT FASTING ON BODY TISSUE MORPHOMETRICS AND EXPRESSION OF PROTEOLYSIS MARKERS IN MUSCLE AND LIVER OF MICE (Biology)

Edgaras Lapinskas

Scientific supervisor:
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Dissertation defended:
14 November 2022

The skeletal muscle mass is one of the major components of human body composition. Muscle mass plays a key role in recovery from critical illness or severe trauma. Sarcopenia, low muscle mass and function, is an increasing problem in our ageing society. The first study aimed to investigate if gene variants residing on chromosome 10 of the A/J mice strain could affect muscle properties of the C57BL/6J mouse strain. Based on Lionikas et al. (2013) study results, muscle size and its underlying indices (fibre number and size) substantially differed among the six studied strains, implying a significant contribution of genetic variability to this difference. We hypothesised that chromosome 10 of the A/J strain may affect soleus muscle mass and morphometric characteristics of the C57BL/6J mouse strain. Our second study aimed to compare the effects of single and eight bouts of 48-h fasting with 120-h refeeding in C57BL/6J mice with a particular focus on body composition and proteolysis gene expression in skeletal muscles and liver. As metabolically active tissues, liver and skeletal muscles contribute significantly to changes in the lean body mass of mice exposed to fasting. We hypothesised that repeated bouts of fasting would lead to progressive loss of skeletal muscle mass and gain in fat as an adaptation for increase energy reserves. The third study aimed to compare the effects of the first and the second bout of two-day acute fasting followed by five-day refeeding in BEH+/+ and BEL mice. We focused on body composition and proteolysis gene expression in skeletal muscles and liver as tissues, which contribute significantly to changes in lean body mass after fasting (Fokin et al., 2019). We hypothesised that higher muscle mass of the BEH+/+ mouse strain would ameliorate proteolysis gene expression in skeletal muscles and liver as a reserved store of body fat will lead to a reduction in the loss of lean body mass.

THE INTEGRATED ASSESSMENT OF THE ISHIM RIVER WATER POLLUTION OF THE NORTH-KAZAKHSTAN REGION AND THE IMPACT ON POPULATION HEALTH (Biology)

Nazim Nikiforov

Scientific supervisor:
Prof. Dr Audrius Dėdelė
Vytautas Magnus University

Dissertation defended:
18 November 2022

Chlorine (Cl-) is used as a disinfectant of drinking water and drinking water distribution networks in Petropavlovsk, Kazakhstan. Interacting with fulvic and humic acids, the disinfectant forms disinfection by-products (DBPs), particularly, trihalomethanes (THMs). THMs might be harmful to the population as it may cause cancer and non-cancerous diseases. Also, drinking water may be polluted with heavy metals (HMs), appearing naturally or by pipelines containing heavy metals in their content, which also has a negative impact on human health by promoting cancer and non-cancerous health outcomes. The current study was focused on the assessments of the lifetime and current risks of cancer and non-cancerous diseases associated with THMs and HMs in Petropavlovsk community. The mean concentrations of $CHCl_3$, $CHBr_3$, CHCl2Br, TTHMs, Pb^{2+}, and Cr^{6+} in drinking water were 18.41 μg/L, 48.7 μg/L, 6.15 μg/L, 73.25 μg/L, 0.38 μg/L, and 3.4 μg/L, respectively. The mean lifetime risk of cancer associated with THMs and HMs in drinking water was 3.83×10^{-5} and 6.53×10^{-3}, respectively. The total lifetime risk of non-cancerous diseases associated with THMs and HMs was 2.13×10^{-1} and 1.08×10^{-7}, respectively. The total lifetime risks for cancer from the three routes for the three THMs and the two HMs at 50th and 95th percentile scenarios were higher than the risk of the US EPA recommendation of 1.00×10^{-6}, while the total lifetime risks of non-cancerous diseases associated with THMs were lower than the US EPA recommendation of 1. The risk of arrhythmia in the crude logistic regression model was found to be 2.2-fold higher in the higher-exposed group. The risk of arrhythmia for the participants in the higher-exposed group was increased to 2.6-fold in the adjusted logistic regression model (aOR = 2.57, 95 CI 1.08–6.13).

INVESTIGATION OF CHARACTERISTICS AND TRANSMISSION OF THE VIRULENT STRAINS OF PATHOGENIC MICROORGANISMS IN THE VIEW OF MOLECULAR EPIDEMIOLOGY (Biology)

Tatjana Kirtiklienė

Scientific supervisor:
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Dissertation defended:
15 December 2022

The present study is the first molecular epidemiological study in Lithuania that covers the evaluation of resistance of both *Acinetobacter* spp. and *E. coli* to various antibiotic groups, as well as virulence gene identification, phylogenetic isolation, application, and analysis of different genotyping methods. This study describes 194 *Acinetobacter* spp. and 256 multidrug-resistant *E. coli* isolates from hospitalised patients diagnosed with sepsis. Moreover, it was determined that 45.9% of all *Acinetobacter* spp. isolates exhibited an β-lactams resistance gene combination of blaOXA subgroup-3-blaOXA subgroup-1-blaOXA51-blaOXA sugroup-2-blaOXA-subgroup-4-blaVIM-1-blaTEM-92. Moreover, the most common resistance gene combination in *E. coli* isolates was tetA-strB-sul2-blaTEM-blaNDM-strA-fosA-blaAIM-sul3-aadA-blaCTX-M-9, which caused resistance to β-lactams, aminoglycosides, sulphonamides, fosfomycin, and tetracyclines. The most common virulence gene combination was fuyA-fimH-iroN, where fuyA and iroN encode siderophores, and fimH is responsible for bacterial adhesion to host cells. Phylogenetic group determination was performed for all *E. coli* isolates. The isolates belonged to four phylogenetic groups: A, B1, B2, and F. Group A isolates were detected at a significantly higher frequency (79.3% of all isolates) than isolates of groups B1 (0.8%), B2 (15.6%), and F (4.3%). BOX-PCR genotyping analysis was performed on all 194 *Acinetobacter* spp. isolates, a total of 191 BOX-PCR profiles were identified, which were separated into six clusters. In total, 235 BOX-PCR profiles of *E. coli* isolates were obtained where all profiles were separated into 14 genotypic clusters. Moreover, determination of resistance and virulence genes to genotyping profiles was observed and results showed potential changes in these genes.

ASSOCIATION OF PHYSICAL ACTIVITY AND OVERWEIGHT WITH AEROBIC CAPACITY AND COGNITIVE FUNCTION AMONG 6–19-YEAR-OLD PUPILS (Biology)

Vaida Borkertienė

Scientific supervisor:
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Dissertation defended:
16 December 2022

According to the World Health Organization, more than 40 million children over the age of five were overweight in 2011. Over 30 million overweight children live in the developing countries. It is known that there is a relationship between the body mass index and cognitive function in adults, but it is still unclear whether overweight has the impact for the development of cognitive functions of children and adolescents. The aim of the study was to determine the impact of overweight and physical activity on the aerobic capacity and cognitive functions of 6–19-year-old pupils. Results showed that overweight children demonstrate a slower time constant of oxygen uptake, lower VO2peak, and slower muscle oxygenation. Weight status is significantly associated with oxygen uptake dynamics and muscle oxygenation among 6- to 19-year-olds. Overweight children demonstrate poorer cognitive functions. Weight status is significantly associated with changes in the cognitive function among the groups of 6- to 19-year-old pupils. Sport-trained 6-to 19 year-old pupils demonstrate higher aerobic capacity (faster VO2kinetics and higher VO2peak) and faster muscle oxygenation as compared with untrained and overweight peers. Sport-trained 6–19-year-old pupils demonstrate better cognitive functions as compared with untrained and overweight peers. Aerobic capacity parameters are significantly and positively correlated with cognitive function tests results among 6–19-year-old pupils.

APPLICATION OF THE OPTICAL MAPPING ON LARGE ANIMAL HEART *IN SITU* AND EFFECT OF *ELSHOLTZIA CILIATA* ON CARDIOVASCULAR SYSTEM (Biology)

Vilma Zigmantaitė

Scientific supervisor:
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Dissertation defended:
20 December 2022

Cardiovascular diseases are one of the most common causes of death worldwide. There is a great need for improving the diagnostics of these diseases, and the development of innovative and minimally invasive diagnostic methods is a very important and necessary step. The aim of this thesis was to apply the optical mapping method in large animal model *in situ* under physiological and pathological conditions. This optical method is based on staining the heart tissue with

voltage-sensitive fluorescent dyes, this allows to analyse the propagation of the signal on the surface of the myocardium and to precisely determine the location and causes of disorders. As the diagnostics of cardiovascular diseases improves, new drugs with fewer side effects are being developed and improved. Therefore, plant extracts are becoming very important substances due to bioactive compounds, which are characterised by a broad-spectrum activity and a lower number of side effects. The object of the study was the annual plant *Elsholtzia ciliata* of the Lamiaceae family. It is native to Asia and also grows in Lithuania. The purpose of the study was to determine the effects of the *E. ciliata* essential oil on electrophysiological and haemodynamic parameters.

INTRODUCED *ARTEMISIA ABROTANUM* (L.), *ARTEMISIA VULGARIS* (L.), *ARTEMISIA ABSINTHIUM* (L.), *ARTEMISIA PONTICA* (L.) MORPHOLOGICAL PROPERTIES AND THE DYNAMICS OF THEIR VOLATILE AND NON-VOLATILE COMPOUNDS DURING VEGETATION PERIOD IN LITHUANIA (Biology)

Sandra Saunoriūtė

Scientific supervisor:
Prof. Dr Habil. Ona Ragažinskienė
Vytautas Magnus University

Dissertation defended:
20 December 2022

Human health promotion, its protection, and biodiversity conservation are the main problems in the twenty-first century. In recent years, an important role in solving these problems has been played by medicinal and spice (aromatic) plants of *Artemisia* (L.) genus of the Asteraceae (A. Bercht. & J. Presl) family, their medicinal raw material and various classes of biologically active compounds characterised by different pharmacological effects. The aim of the study was to investigate morphological properties, medicinal raw material productivity, and dynamics of volatile and non-volatile compounds during different vegetation stages of *Artemisia abrotanum* (L.), *Artemisia vulgaris* (L.), *Artemisia absinthium* (L.), *Artemisia pontica* (L.) species introduced in Lithuania. During the research period, investigations into the morphological characteristics and the influence of meteorological conditions to the growth and medicinal raw material productivity of the introduced species were caried out in Central Lithuania. Three new chemotypes were identified: *A. abrotanum* – (+)-piperitone, *A. vulgaris* – isogermacrene D, and *A. pontica* – 1.8-cineole. Using different methods, *in vitro* antioxidant activity of the extracts of medicinal raw material and qualitative and quantitative dynamics of phenolic compounds of four *Artemisia* (L.) species were determined during different vegetation stages.

SEX DIFFERENCES IN AUDITORY-EVOKED ELECTRICAL BRAIN ACTIVITY: A CASE OF N2 AND P3 WAVES (Biophysics)

Sigita Mėlynytė

Scientific supervisor:
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Dissertation defended:
14 April 2022

Auditory event-related potentials (ERPs) are electrical brain responses to a certain type of auditory stimuli during a precise task that are captured with the help of electroencephalography. ERP waves P3 and N2 can evaluate cognitive skills such as attention, working memory, and response inhibition. They are found to be impaired in psychiatric disorders, and despite sex related differences that are found in prevalence of the disorders and in the auditory system and functioning, sex-effect is still being ignored in scientific studies. We wanted to evaluate the sex factor in auditory ERPs evoked with simple tasks (that are promising for application in psychiatry) in healthy subjects. An experiment and a systematic review were conducted. In the experiment with an auditory equiprobable Go-NoGo task, we found higher P3 amplitudes in females as opposed to males in Go condition (that requires a response initiation), but no sex differences in NoGo (when response inhibition was needed). N2 amplitudes were comparable between sexes, and N2 and P3 latencies were found to be longer in females. The systematic review provided support for potential sex-related differences. However, the results are inconclusive: higher P3 amplitudes in females were reported in half of the included studies, while the other half found no sex effect. P3 latencies were found to be indifferent between sexes.

FEATURES OF MISPERCEPTION OF SPATIAL PARAMETERS OF VISUAL STIMULUS (Biophysics)

Vilius Marma

Scientific supervisor:
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Dissertation defended:
30 August 2022

The purpose of the doctoral thesis was to study the properties of geometric optical illusions and thus to clarify the peculiarities of perception of visual spatial metrics and relative sizes of visual objects. Using the method of adjustment in psychophysical experiments, the dependence of the strength of the filled-space illusion on the position of the distracting dot was determined. Illusion dependences on the length, orientation, and position of the distracting line-segment were also determined. The mathematical model explaining the filled-space illusion was improved by including parameters for observer's gaze fixation. For the first time, based on the results of studies with displaced line distractors, the spatial

structure of the hypothetical area of weighted summation of visual neural activity was established. The spatial profile of the area can be described as the product of two functions: the absolute value of the first-order derivative of a Gaussian along the radial direction in the visual field and the Gaussian function along the tangential direction. Successful approximation of experimental curves by theoretical functions confirmed the assumption that the asymmetry of the Müller-Lyer illusion was due to its interaction with the filled-space illusion. We hope that the results of the dissertation may contribute to a better understanding of the multifactorial interactions between the neural mechanisms underlying various geometric illusions.

GAMMA RANGE AUDITORY STEADY-STATE RESPONSES AS CORRELATES OF INDIVIDUAL COGNITIVE PERFORMANCE (Biophysics)

Vykinta Parčiauskaitė

Scientific supervisor:
Dr Inga Griškova-Bulanova
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Dissertation defended:
2 September 2022

The properties of the gamma range (30–80 Hz) of the electroencephalogram (EEG) can be investigated by analysing the auditory steady state responses (ASSRs) caused by periodic auditory stimulation. Cognitive impairment or the risk of cognitive impairment is associated with changes in ASSR. However, whether ASSRs can be used as a biomarker for cognitive impairment requires detailed research into their links. To investigate this, we conducted a systematic literature review. In addition, we conducted a study in which we analysed in detail the associations between a 40 Hz ASSR and different cognitive abilities involving simple and complex information processing in a homogeneous sample of healthy subjects. As individual gamma frequency (IGF) ASSRs can more accurately reflect the properties of an individual's neural network, we also conducted a study of the correlation between cognitive abilities and IGF-ASSRs in a sample of healthy subjects. A systematic review of the literature revealed that differences in gamma-band ASSRs may reflect abilities to manage attention and temporarily store and process information. The results showed that a 40 Hz ASSR is positively correlated with the average number of moves in the Tower of London task, and that 40 Hz and IGD ASSRs are positively correlated with the average move time in the Tower of London task. These results suggest that gamma-band ASSRs correlate with individual cognitive abilities and may reflect aspects of planning and working memory functioning.

STUDIES ON THE EFFECTS OF IONIZING RADIATION ON HEALTHY TISSUES (Biophysics)

Juras Kišonas

Scientific supervisor:
Prof. Dr Habil. Ričardas Rotomskis
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Dissertation defended:
27 September 2022

The aim of the dissertation was to determine the correlations between the signs of skin damage observed by reflectance confocal microscopy (RCM) and the clinical course of acute radiation dermatitis (ARD) and individual patient parameters, as well as to evaluate the predictive value of RCM. RCM is a widely used non-invasive skin investigation technique in order to diagnose malignant and benign diseases and evaluate the effectiveness of treatment. RCM was also used to assess ARD, but only in six patients. This is the first prospective clinical trial to analyse ionizing radiation (IR) induced skin lesions using RCM and dermoscopy involving more than a hundred patients. During this study, more than 600 measurements using RCM and the same number of digital dermoscopy (DD) were performed, so this is the largest study examining the use of non-invasive skin examination methods during radiotherapy (RT). This was the first large-scale clinical trial to show that using RCM and DD, the IR-induced skin lesions can be detected before the onset of clinical ARD symptoms. This was the first study to show that IR-induced skin lesions revealed by RCM have a typical course and to show that the severity of ARD at the end RT of can be predicted by RCM at the beginning of treatment. This was the first clinical study of this magnitude to reveal that IRS determined by the G2 method before the RT has no predictive value for the development of ARD.

INFLUENCE OF CONNEXIN-36 N-TERMINAL AMINO ACIDS ON BIOPHYSICAL PROPERTIES OF GAP JUNCTION CHANNELS (Biophysics)

Lukas Gudaitis

Scientific supervisor:
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Dissertation defended:
28 December 2022

This thesis aimed to evaluate the influence of the N-terminal amino acids on the biophysical properties of gap junction channels (GJCs) formed by connexin-36. The results showed that substitutions of amino acids at the thirteenth and eighteenth positions to ones containing positive charge substantially altered the effect of $[Mg^{2+}]i$ on junctional conductance and Mg^{2+} ion permeability. These results suggest that the N-terminus may play an important role in regulating the effect of Mg^{2+} ions on Cx36 channel function. In addition, the mathematical modelling of these data provided possible mechanisms of Mg^{2+} action. By using a mathematical model,

shown capable of assessing biophysical properties of GJCs, we modelled the data on wild type Cx36 and mutant N-terminal variants to extract parameters describing sensitivities to transjunctional voltage. Based on these data, the gating polarity of Vj-sensitive fast gate of Cx36 was determined. The results of this study significantly expand our knowledge of the biophysical properties of Cx36 GJCs, particularly their modulation by intracellular Mg^{2+} ions, and provide insights into the possible mechanisms of action. These findings are important given that Cx36 is the main connexin forming electrical synapses between neurons in the mammalian central nervous system. Changes in GJ-mediated communication between neurons under physiological and pathological conditions can affect neuronal excitability. Intracellular Mg^{2+} ion concentrations which can directly and robustly influence electrical synapses can, therefore, play an important role in governing neural activity and the coordination of activity within neural networks.

CYANOBACTERIA AS A SOURCE OF BIOACTIVE METABOLITES: THEIR POTENTIAL APPLICATION IN BIOTECHNOLOGY AND ENVIRONMENTAL IMPACT (Ecology and environmental)

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Dissertation defended:
6 May 2022

This work presents a comprehensive study on the diversity and occurrence of cyanobacteria and their secondary metabolites in the Curonian Lagoon and the coastal Baltic Sea. In the study, the ecological and socioecological significance of toxic cyanobacteria blooms and the biotechnological potential of cyanometabolites were explored. Phytoplankton analyses of samples collected in the Curonian Lagoon showed frequent occurrence of *Aphanizomenon*, *Dolichospermum/Anabaena*, *Microcystis*, *Planktothrix*, and *Woronichinia* genera. Of these, *Dolichospermum/Anabaena*, *Microcystis*, and *Planktothrix agardhii* were confirmed by genetic methods as potential microcystin (MC) producers. The assessment of water quality based on cyanobacteria parameters in the recreational areas of the Lithuanian Baltic Sea coast and the Curonian Lagoon indicated a low probability of adverse health effects, with a higher risk in the southernmost part of the Curonian Lagoon. As these two systems are interconnected, the dynamics and structure of cyanobacteria in the Curonian Lagoon have a significant impact on the diversity and concentrations of cyanotoxins in the coastal areas of the sea. During the comprehensive studies of field samples collected in the Curonian Lagoon, 119 cyanometabolites representing eight different classes of the compounds

were detected. Cyanopeptolins and microcystins were found to be the most structurally diverse class of cyanopeptides. The observed diversity and considerable variation in rare and potentially new microcystin variants may indicate the presence of different cyanobacteria chemotypes in the lagoon. Bioactivity screening of phytoplankton samples from the Curonian Lagoon confirmed pharmaceutical potential of aquatic microorganisms. The samples were active against antibiotic resistant clinical and environmental bacteria strains, they inhibited serine proteases and reduced the viability of the T47D human breast adenocarcinoma cells.

NEW EXPERIMENTAL DATA TO IMPLEMENT ECOLOGICAL NETWORK ANALYSIS OF NITROGEN AND PHOSPHORUS IN TWO EUTROPHIC LAGOONS (Ecology and environmental)

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Dissertation defended:
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The biogeochemical functioning of coastal lagoons is threatened by the interplay between eutrophication and climate change. In this study, the nitrogen and phosphorus dynamics were analysed in two eutrophic lagoons (the Goro Lagoon, the North Adriatic Sea, and the Curonian Lagoon, the southeastern Baltic Sea) by combining experimental and modelling approaches aimed at investigating variations in their nutrient retention or removal capacity. The effects of climatic extremes, anoxia events, and the presence of different bioturbating organisms were tested with experimental activities carried out at the microscale. Detailed measurements on benthic processes were implemented to construct networks depicting *N* and *P* circulation at the whole lagoon scale that were analysed via an integrative modelling tool. The results underline the high vulnerability of the analysed systems, mostly in summer and under heat waves and dry periods. Under these conditions the extent of internal recycling processes increase, largely exceeding external inputs and removal processes, with positive feedbacks on primary producers' activity. Vulnerable areas were identified, and they were characterised by low water circulation, muddy, organic-rich, and chemically reduced sediments where the low oxygen concentration and the accumulation of toxic compounds limit the presence of a biodiverse benthic community. In the perspective of nutrient stoichiometry, *N* and *P* cycling diverges in these vulnerable areas, especially during critical oxic-anoxic transitions, resulting in unbalanced regeneration and large *P* excess.

BIOGEOCHEMICAL INTERACTIONS AMONG BENTHIC MACROFAUNA, MICROBIAL COMMUNITIES AND MACROPHYTES IN EUTROPHIC COASTAL LAGOONS (Ecology and environmental)

Tobia Politi

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Dissertation defended:
3 June 2022

In this study, the relationship between diversity and ecosystem functioning was analysed at different spatial scales in the benthic compartment of two shallow eutrophic lagoons, the Curonian Lagoon (Lithuania) and the Sacca di Goro (Italy). Results suggest that macrofauna play an important role in the Sacca di Goro lagoon in regulating N transformations. However, its importance also depends on the prevailing environmental factors (i.e., salinity, hydrodynamics, and background nutrient concentrations). In the Curonian Lagoon, bioturbation did not significantly affect the nutrient metabolism and the stability of reductive-oxidative reactions during anoxia events. Molecular studies revealed that Chironomid larvae burrows are hot-spots of microbial communities involved in N cycling and that these organisms, via bioirrigation, significantly enhance both the recycling of ammonium and N removal via denitrification. Mussels primarily enhance the recycling of N to the water column, both via direct excretion and by stimulating dissimilatory nitrate reduction to ammonium. The latter is likely an effect of mussel biodeposits. For these two organisms, the quantification of functional genes showed a significantly higher potential for microbial denitrification, nitrate ammonification and N2-fixation in macrofauna as compared to the surrounding environment. As chironomid and dreissenid densities in eutrophic lagoons are large, animal-associated microbes may account for a substantial (and so far, overlooked) N import and recycling. *Pontogammarus robustoides* was finally demonstrated to have an important role in the survival of *Chara contraria* in the eutrophic Curonian Lagoon. The gammarid facilitates *C. contraria* via active grazing on the macroalgae-associated epiphytes combined with ammonium excretion, thus supporting the growth of the characeans.

LEGUMES AND GRASSES INTERSPECIES COMPATIBILITY FOR PERENNIAL SWARDS PRODUCTIVITY AND FOR SUSTAINABILITY OF THE AGROECOSYSTEM FUNCTIONS (Ecology and environmental)

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Dissertation defended:
22 June 2022

This thesis aimed to investigate the interspecific compatibility and productivity of legumes and grasses in agroecosystems. The study provides new insights into the properties of newer cultivars of perennial ryegrass, × festulolium, meadow fescue, white clover, red clover, sainfoin and lucerne, which are more resistant to adverse environmental conditions, and the peculiarities of their cultivation in mixtures of different species compositions and under varying nutritive conditions. Our study showed that the total sward productivity was significantly more influenced by a legume species compared to the number of species in the mixture. New results were obtained on the species composition, compatibility, and quality of swards in the first to third year of use. Significant differences in the species composition of swards were found. The most productive sward was the one with white clover, lucerne, and four species of grasses without mineral N fertiliser application, outperforming other swards fertilised with 150 kg N ha^{-1} per growing season in terms of yield and grass quality. The use of mineral N fertilisers did not improve the uniformity of herbage productivity during the growing season and the stability of sward in the individual years of use, nor the quality of sward by reducing the crude protein content.

DIVERSITY OF SMALL MAMMAL PARASITES AND FACTORS SHAPING THEIR COMMUNITIES (Ecology and environmental)

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Dissertation defended:
5 September 2022

The main objective of the dissertation was to investigate the diversity of small mammal blood protozoans and ectoparasites and to evaluate the influence of host, environment, and co-infection factors on blood protozoan, gastrointestinal helminth, and ectoparasite communities. During the research, we investigated the diversity of blood protozoans and ectoparasites, evaluated host specificity and ectoparasite dominance. Six ectoparasite and two blood protozoan species, as well as protozoans of one genus not identified to species were registered in Lithuania for the first time. The prevalence and mean intensity of parasites of thirteen orders significantly differed among

host species and among habitats. Significant differences in seasonal dynamics were recorded for parasites of seven orders. Climatic conditions influenced seasonal dynamics of parasites of six orders. Non-random co-occurrence patterns (positive and negative) were registered among parasites of all orders. A comprehensive analysis of multiple host, environment, and co-infection factors was performed for the first time. Parasites of different orders were affected by two to eight factors (from 18 possible). Host species, habitat, and air temperature influenced parasite communities the most often.

ENVIRONMENTAL NOISE EXPOSURE ASSESSMENT IN RESIDENTIAL ENVIRONMENT AND ITS IMPACT ON CHILDREN'S PSYCHOLOGICAL HEALTH (Ecology and environmental)

Jolanta Nemaniūtė-Gužienė

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Dissertation defended:
9 September 2022

All over the world the concern of professionals about the increasing level and propagation of environmental noise in the living environment is growing. Epidemiological studies show the risk of acoustic pollution to public health. The main source in agglomerations is road transport. Lithuania lacks national assessment methods, representative health impact assessments. By applying GIS, strategic noise mapping, analysing the results of Strengths and Difficulties questionnaire's SDQ Lit Sociality, Hyperactivity, Emotional, Behavioral, Communication and Total scales, the individual exposure of environmental noise to preschool children in the residential environment of Kaunas agglomeration was determined and its impact on psychological health was assessed. This is the first representative large-scale (N = 1457) noise impact study in Lithuania aimed to assess psychological health of children. A method was proposed to assess individual exposure to noise when working with large-scale data. Classification of L_{night} and L_{den} noise levels into low, medium and high noise level zones (≤40/50 dB(A); >40/50 to 49/59 dB(A); ≥50/60 dB(A), respectively) has been approved. Interim limit values for L_{night} and L_{den} noise were suggested: 50 dB(A) and 60 dB(A). In the study, the number of cases of hyperactivity increased statistically significantly up to 77% in the zone of high noise exposure and up to 63% according to Total scale. A trend of increasing social difficulties up to 33% was observed. According to Total scale, increasing L_{night} together with maternal stress statistically significantly increased the risk of problem cases

in children up to 71%. The obtained results provide new knowledge about the association between road traffic noise in the residential environment and the psychological health of preschool children.

INCREASING REPRODUCTION PARAMETERS OF THE WHITE-TAILED EAGLE, ITS PREY COMPOSITION AND INTERACTIONS WITH SOME RAPTOR SPECIES (HALIAEETUS ALBICILLA) (Ecology and environmental)

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Dissertation defended:
15 September 2022

In the twentieth century, the numbers of raptors in Europe decreased due to habitat degradation, use of the organochlorine pesticide, industrial pollution, and deliberate killing by humans. Over the last decades, many populations of raptors have been recovering as a result of the implementation of various conservation programmes and have spread in previously abandoned areas. The aim was to study the breeding performance of the recovered and increasing population of the White-tailed Eagle (*Haliaeetus albicilla*), the diet composition, and interactions with some raptor species. The data for this study were collected by the author between 1997 and 2020 from two research areas, but for each task the dataset used varied depending on the course of research. The study results indicated that breeding performance of the pairs of the White-tailed Eagle did not differ among habitats and did not decline with an increase in the density of nesting pairs. During the breeding season, fish and water birds were the most common prey and the key prey species was the Northern Pike. The species composition of the prey varied among pairs reproducing in different habitats, but the White-tailed Eagle, despite its increasing population, was not limited by the abundance of prey in the environment. The impact of the White-tailed Eagle on internationally protected bird species during the breeding season was insignificant. Adult raptors were rare White-tailed Eagle prey; however, nestlings of the Common Buzzard were more frequent as a prey. The larger-bodied White-tailed Eagle has a no effect on the smaller-bodied Lesser Spotted Eagle reproduction and its local population dynamics in a region where both species were abundant.

DIETARY ASPECTS OF HUMAN AND ANIMAL PALAEOECOLOGY: STABLE ISOTOPE DATA (Ecology and environmental)

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Dissertation defended:
30 September 2022

The bioarchaeological material collected in Lithuania has been studied fragmentary until now, but it gave the possibility to better understand the lifestyles and relationship with the environment of past inhabitants and to assess the distribution of food resources in past populations. The aim of the dissertation was to carry out a systematic analysis of carbon and nitrogen stable isotope ratios of human and animal bioarchaeological material and to evaluate their variation in the aspects of palaeodiet and palaeoecology in various historical periods, from the Iron Age to the Modern period. The stable isotope ratio method helped to understand how different dietary resources were distributed in the population, to compare individuals, and to relate the differences to the individual's age, status, region, and period of living, to assess the human influence on the diet of domestic animals. It was found that the basis of the human and animal diet consisted of C3 photosynthetic plants. In the early period (from the 2nd to the 13th century), the diets of males and females were differentiated, and the consumption of food of plant origin was higher in general, compared to the late period (from the 13th to the 19th century). There also emerge regional differences between urban, rural, coastal, and social elites.

TOOLS AND APPROACHES FOR SUPPORTING COASTAL AND MARINE POLICY IMPLEMENTATION (Ecology and environmental)

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Dissertation defended:
7 October 2022

The aim of this thesis was to assess and address current gaps between existing Decision Support Tools (DST) and their use in practice to support coastal and marine policy implementation in the Baltic Sea region. This study presents the first overview of DSTs that support the coastal and marine management of the Baltic Sea and provides recommendations to overcome existing gaps and advance future developments. For this purpose, it explores the performance of and users' satisfaction with available DSTs and analyses end-user and policy needs. Furthermore, the study addresses the lack of DSTs assessing the impacts on human welfare and linking environmental and socio-economic aspects. Instead of developing new DSTs, existing DSTs suitable to as-

sess impacts on human welfare were built upon and further developed to demonstrate their potential applications for supporting coastal and marine policy implementation. Furthermore, participatory ES assessments based on the Marine Ecosystem Service Assessment Tool (MESAT) (Inácio et al. 2018) were conducted to demonstrate and critically evaluate its practical use for supporting the implementation of the Water Framework Directive (WFD) in coastal waters. For the first time, this study provides insights into applications of participatory ES assessments at different steps of the WFD implementation cycle. Finally, with the stakeholder preference and planning tool developed in this study, a flexible and directly applicable tool to guide stakeholder discussions is provided.

BLUE MUSSEL CULTIVATION IN THE BALTIC SEA ECONOMIC AND MITIGATION POTENTIAL IN MESOHALINE COASTAL WATERS (Ecology and environmental)

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Dissertation defended:
7 October 2022

The EU Water Framework Directive (WFD) focuses on nutrient reductions to return coastal waters to the good ecological status. This study focuses on (1) the current environmental status of mesohaline inner coastal waters to illustrate their needs of internal measures to reach demanded nutrient reductions and (2) whether mussel cultivation could be a suitable strategy to improve water quality. Results showed that currently all nine water bodies did not reach the nutrient thresholds demanded by the WFD. However, coastal waters differ in nutrient pollution, indicating that some can reach the desired threshold values if internal measures are applied. The mitigation potential of mussel cultivation depends on the amount of biomass that is cultivated and harvested. However, since mussel growth is closely coupled to the salinity level, mussel cultivation in low saline environments leads to lower biomass production and inevitably to larger cultivation areas. If 50% of the case study area of Greifswald Bay was covered with mussel farms, the resulting nitrogen reduction would increase Secchi depth by 7.8 cm. However, high chlorophyll values can hamper clearance rates (<20 mg m^{-3} = 0.43 l h^{-1} dry weight g^{-1}) and therefore the mitigation potential. Also, the risk of mussel stock loss due to high summer water temperatures might affect the mitigation potential. The pilot farms had no significant effect on the total organic content of sediments beneath.

However, increased values of *Vibrio* spp. in bio deposits within the pilot farm (1.43 106 ± 1.10 106CFU 100 ml^{-1} (reference site: 1.04 106 ± 1.45 106 CFU 100 ml^{-1}) were measured with sediment traps.

COMPLEX ECOPHYSIOLOGICAL RESPONSE OF MATURE PREVAILING TREE SPECIES IN LITHUANIA TO THE CHANGES IN ENVIRONMENTAL CONDITIONS (Ecology and environmental)

Ainis Pivoras

Scientific supervisor:
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Dissertation defended:
25 November 2022

Forest ecosystems are one of the cornerstones of the biosphere actively involved in biogeochemical cycles, environmental stability, and biodiversity conservation. Also, they provide a constant timber supply and so economic stability. Most of the region's forests are human-planted, and our decisions determine their species composition and structure. By understanding the adaptive capabilities of individual species and predicting their responses to environmental change, we can make long-term sustainable decisions to maintain healthy and productive forest ecosystems and thus reduce the negative impacts of climate change. The research comprehensively assessed the impact of individual environmental factors on the annual growth, water balance and photosynthetic processes of mature, dominant tree species in the region: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) H. Karst), and silver birch (*Betula pendula* L.). The results indicate that the rise in annual temperature inhibits annual growth of birch and promotes the growth of conifers. The importance of June rainfall and temperatures on the annual growth of the species studied was highlighted. Based on sap flow and photosynthetic process measurements, exceptional sensitivity of spruce and relative insensitivity of birch to droughts and heat waves were highlighted. Based on physiological responses, the current status of mature trees and their adaptive capabilities in the context of climate change were assessed.

PHYSICAL ACTIVITY OF KAUNAS CITY RESIDENTS AND ITS ASSOCIATIONS WITH INDIVIDUAL AND ENVIRONMENTAL FACTORS (Ecology and environmental)

Žydrūnė Bartkutė

Scientific supervisor:
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Dissertation defended:
25 November 2022

The decreasing levels of physical activity (PA) are caused by the modern sedentary lifestyle, low physical activity at work, the COVID-19 pandemic restrictions. Sustainable urban mobility is becoming inseparable from physical activity, a particularly important component of a healthy lifestyle and aging. There is a lack of multifaceted studies examining active mobility and its associations in Lithuania. In this study, for the first time, in addition to subjective evaluation of PA of the Kaunas city residents (questionnaire of the residents, $N = 1111$), an objective assessment of residents' PA is introduced (smartwatch, $N = 20$), associations between active mobility that meets the physical activity recommendations (≥150 min/week), and a wide complex of factors (demographic, socioeconomic, health, lifestyle, behavioural, seasonal, environmental) are analysed. It was determined that 10.4% of Kaunas city residents fulfilled the physical activity recommendations by walking and cycling. Kaunas can be regarded as having a low prevalence of active mobility (11.4%). Sufficient physical activity among males was associated with unemployment, not using a car, a healthy diet, and higher leisure-time physical activity; among females, with higher leisure-time physical activity, lower BMI, and older age. The objective assessment revealed that those fulfilling physical activity recommendations spent less time in domestic and transportation microenvironments, their heart rate was higher in walking and other indoor microenvironments compared to those not achieving the recommendations. The obtained results revealed the need to consider a complex approach while promoting active mobility and thus contributing to sustainable urban mobility in specific social groups.

REINTRODUCTION OF THE ATLANTIC STURGEON (*ACIPENSER OXYRINCHUS* MITCHILL, 1815) IN LITHUANIAN WATERS (Ecology and environmental)

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Scientific supervisor:
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Dissertation defended:
7 December 2022

The dissertation presents the results of laboratory and field research on the Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill, 1815) and the reintroduction of this species in Lithuanian waters. Studies were carried out using various classical ichthyological, genetic, and physiological methods and by applying specific methods of hydroacoustic tagging to study the migration of sturgeon juveniles. Using mtDNA markers, the genetic structure of the sturgeons exhibited and stored in the repositories at the Kaunas Tadas Ivanauskas Museum of Zoology was investigated and the species affiliation of these specimens was clarified. The effect of transportation on the blood indices of Atlantic sturgeon was evaluated depending on the light and dark phases, and the recovery time of the blood indices was determined. Using modern monitoring and tagging methods, migration of the Atlantic sturgeon in the Nemunas, Neris, and Šventoji rivers was studied. Spatial distribution was determined, and the mortality rate was estimated in the Curonian Lagoon and coastal zone of the Baltic Sea. The obtained results of the research add to the knowledge about the species of sturgeon family (Acipenseridae) fish that used to inhabit Lithuanian waters. The research results and conclusions presented in the dissertation were important and useful in the preparation of the programme and action plan for the restoration of the Atlantic sturgeon population in Lithuania as well as for reintroducing sturgeons in the Baltic Sea in cooperation with other countries and international organizations.

THE INFLUENCE OF INTENSIVE AND ORGANIC AGRICULTURE ACTIVITY ON THE QUALITY OF GROUND AND SURFACE WATER (Ecology and environmental)

Laura Čiteikė

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Dissertation defended:
20 December 2022

In order to evaluate the influence of organic and intensive agriculture on the quality of underground and surface water, river water and groundwater taken from organic and intensive agriculture fields bordering on the studied rivers, were studied in this work and a comparative analysis of these water samples was performed. The research provided new data that the impact of organic farming on the condition of underground water (at the depth of 4–5 m) was minimal. Therefore, this data can be useful for promoting

the development of ecological agriculture and for the preparation of new environmental legislation. It was found during the research that the amount of nutrients in the underground water in the areas of intensive agricultural farms was significantly higher than in organic farms; therefore, based on this data, it is possible to commit farmers engaged in intensive agriculture to periodically conduct tests of the quality of the ground water in the soil and cultivated fields and to encourage more responsible use of mineral fertilizers. Considering the fact that almost 52 per cent of Lithuania's territory is agricultural land, the reduction of intensive agricultural farms or the promotion of organic farms would significantly contribute to the improvement of surface and underground water quality.

AVIAN HAEMOSPORIDIAN PARASITES: FACTORS INFLUENCING THE TRANSMISSION OF TROPICAL SPECIES IN TEMPERATE ZONE (Zoology)

Elena Platonova

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Dissertation defended:
20 January 2022

In this thesis, we studied factors influencing the spread haemosporidian parasites (genera *Haemoproteus* and *Plasmodium*) of tropical-origin in temperate climate zone and virulence to European birds. (1) We showed that tropical-origin *Haemoproteus nucleocondensus* completes its sporogony in *Culicoides impunctatus*, which is abundant in Europe. (2) We determined *Haemoproteus* spp. DNA in five *Culicoides* species and defined *C. kibunensis* as a natural vector for *Haemoproteus minutus* in Europe. (3) We experimentally demonstrated that inoculation of infected donor blood to a recipient bird is a valid method to study the development of *Plasmodium* parasites in the avian blood and to estimate virulence for the host. (4) We found that the tropical-origin *Plasmodium collidatum* is highly virulent for Eurasian Siskins and, although mosquitoes of *Culex pipiens* were resistant to the infection, *P. collidatum* should be considered as a potential threat for European birds. Finally, (5) it was shown that tropical-origin *P. relictum* (pGRW4) completes its sporogony in European vectors even under temporary exposure to low temperature. However, the co-infection with *P. relictum* (pSGS1) suppresses the development of pGRW4 in birds and may have a negative effect on the transmission of this tropical parasite in Europe. The obtained results reveal the complexity of factors influencing the successful transmission of haemosporidian parasites and need for additional research on vector-borne diseases.