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Doctoral theses (biochemistry, biology, biophysics, ecology and environmental, zoology) defended in Lithuania in 2024

Compiled by Justina DAGYTĖ and Indrė LIPATOVA

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STRUCTURAL AND BIOCHEMICAL STUDIES OF AN ARGONAUTE AND ITS ASSOCIATED PROTEIN FROM *ARCHAEOGLOBUS FULGIDUS* (Biochemistry)

Edvardas Golovinas

Scientific supervisors:
Dr Elena Manakova, Dr Mindaugas Zaremba
Vilnius University

Dissertation defended:
29 February 2024

This doctoral thesis investigates a truncated long-B pAgo from *Archaeoglobus fulgidus* (AfAgo) and its associated protein, AfAgo-N, aiming to elucidate their structural and biochemical characteristics. The research explores the oligomeric state of AfAgo *in vitro*, revealing its ability to form homodimeric complexes and looped dsDNA structures, a feature not previously observed among pAgos. Furthermore, AfAgo displays specificity for the 5′-terminal AUU sequence of the guide RNA and demonstrates RNA-guided ssDNA targeting *in vitro*, characteristics not previously described for this protein, expanding our understanding of pAgo functionalities. Moreover, a novel protein, AfAgo-N, is identified within the same operon as AfAgo in *A. fulgidus*. Structural analysis reveals the equivalence of AfAgo-N to the N-L1-L2 domains of long pAgos. It forms a heterodimeric complex with AfAgo, termed fAfAgo, via the same dimerisation surface used by AfAgo to form homodimers. fAfAgo exhibits RNA-guided DNA targeting activity and displays enhanced affinity for DNA targets compared to stand-alone AfAgo. These findings significantly broaden our understanding of pAgo functionalities, unveiling previously unexplored features such as homodimerisation and sequence specificity beyond the first 5′-nucleotide. This comprehensive characterisation of AfAgo and AfAgo-N lays the groundwork for future research into their possible roles in defense mechanisms or other functions and paves the way to investigate other possible split pAgo systems.

THE ANALYSIS OF THE MOLECULAR MECHANISMS OF RESISTANCE TO 5-FLUOROURACIL AND OXALIPLATIN IN HUMAN COLORECTAL CARCINOMA CELLS (Biochemistry)

Vilmantė Žitkutė

Scientific supervisor:
Dr Aušra Sasnauskienė
Vilnius University

Dissertation defended:
24 April 2024

Colorectal cancer (CRC) is the second leading cause of death worldwide. In this work, we evaluated molecular changes in resistant CRC cell sublines induced by 5-FU or OxaPt. We detected changes in the levels of cytokines, serpins, and autophagy proteins in chemotherapeutic drug-resistant CRC cells and evaluated the role of autophagy in chemoresistance. We showed that cells with 5-FU-induced resistance have a higher cytokine expression compared to cells with OxaPt-induced resistance, while 5-FU or OxaPt increases cytokine expression in cells that have not adapted to the drug treatment. We also found that serpin B level is elevated in resistant sublines, whereas drug exposure increases it in parental cells and sublines with resistance induced by 5-FU. In this study, we found that the level of the autophagy protein ATG12 is elevated in the HCT116 cells with OxaPt-induced resistance and that the drugs decrease the level of ATG12 in the HCT116 line and its sublines but increase it in the SW620 line and its sublines. We showed that ATG12 is important for the survival of CRC cells and for OxaPt resistance. We also found that the level of autophagy receptor p62 is increased in HCT116/FU cells with 5-FU-induced resistance and that the decrease in p62 protein level after drug treatment correlates with cells' drug sensitivity. We demonstrated that p62 promotes survival and drug resistance of the HCT116 line and its sublines. Our results suggest that p62 impacts chemoresistance by stimulating prosurvival signalling.

IDENTIFICATION OF OVARIAN FOLLICULAR FLUID CELLS AND MOLECULAR FACTORS AND INVESTIGATION OF THEIR POSSIBLE USE IN REPRODUCTIVE MEDICINE (Biochemistry)

Brigita Vaigauskaitė-Mažeikienė

Scientific supervisors:
Prof. Dr Rūta Navakauskienė,
Assoc. Prof. Dr Edita Kazėnaitė
Vilnius University

Dissertation defended:
21 November 2024

Infertility is a reproductive system disorder characterised by the inability to conceive after 12 months or more of regular, unprotected sexual intercourse. According to the latest data, the prevalence of infertility is increasing and currently affects as many as one in six couples of reproductive age. Each year, more research is conducted not only to identify the causes of infertility but also to explore factors that could improve the chances of conception for these couples. One of the main research areas focuses on ovarian tissue and follicular fluid. The aim of the dissertation was to investigate the regenerative and therapeutic properties of ovarian follicular fluid stromal cells and to assess their clinical applicability in treating unexplained infertility. This study involved molecular analyses of follicular fluid and endometrial stromal cells from infertile women. It was found that the expression of genes involved in DNA demethylation processes, apoptosis, and immune response modulation was higher in the endometrium of non-conceiving patients compared to follicular fluid, potentially contributing to implantation disorders in cases of infertility. Preclinical *in vivo* studies revealed that both primary and cultured stromal cells from follicular fluid can partially restore chemotherapy-induced infertility, suggesting their potential application in clinical practice for oncology patients of reproductive-age.

INVESTIGATING THE ROLE OF EPIGENETIC FACTORS AND THEIR ALTERATIONS IN THE FUNCTIONING OF THE FEMALE REPRODUCTIVE SYSTEM (Biochemistry)

Deimantė Žukauskaitė

Scientific supervisor:
Prof. Dr Rūta Navakauskienė
Vilnius University

Dissertation defended:
6 December 2024

Infertility affects approximately 10–15% of reproductive-age couples worldwide. Successful embryo implantation depends not only on embryo quality but also on the readiness of the endometrium, the uterine lining. During the preparation of this dissertation, we analysed changes in epigenetic and other molecular factors in endometrial stromal cells (ESC) during decidualisation and in response to the embryo-secreted factor, human chorionic gonadotropin (hCG). The study revealed that the level of H4hyperAc specifically increases in the promoter regions of genes associated with decidualisation (WNT4, HAND2, STAT5A), while the DNA methylation level in these regions remains unchanged. We also confirmed that the primary properties of mesenchymal stromal cells in ESC isolated from cryopreserved endometrial tissue are unchanged. However, in these cells, changes in the DNA methylation levels and expression of factors regulating this modification (DNA methyltransferases) were detected. Our study found that hCG enhances the functionality of endometrium, increasing H3K27Ac levels in the regions of genes associated with decidualisation (FOXO1), and implantation (HOXA10, HAND2), as well as inducing changes in ESC secreted extracellular vesicles miRNAs. Evaluating molecular factors changes in ESC during decidualisation enhances understanding of their role in physiological and pathological processes in the endometrium, and aids in identifying strategies for infertility diagnosis and treatment.

MOLECULAR PROFILING OF SYNAPSES DURING BRAIN CIRCUIT REFINEMENT (Biochemistry)

Daina Bujanauskienė

Scientific supervisor:
Dr Urtė Neniškytė
Vilnius University

Dissertation defended:
12 December 2024

The brain development depends on critical periods marked by heightened synaptic plasticity. Once these periods close, plasticity declines. Recent investigations underscore the potential role of local synaptic protein synthesis in regulating this process. During critical periods of plasticity, the dynamic formation and elimination of synapses rely on molecular signals denoted as 'don't eat-me' and 'eat-me', among which externalised phosphatidylserine (PS) is a known signal. However, the biochemical processes initiating synapse elimination remain unclear. To study local transcriptome changes during these periods, synaptosomes – isolated synaptic terminals – were analysed using fluorescence-activated synaptosome sorting (FASS) to obtain excitatory synapse-enriched samples. These samples, however, have reduced ribosomal RNA (rRNA) content, complicating traditional RNA integrity assessments. A PGK1-based 5′:3′ assay was developed for reliable mRNA integrity evaluation in rRNA-depleted samples. mRNA sequencing revealed that synaptic transcripts primarily regulate cellular transport, synaptic transmission, and local protein synthesis, with dynamic changes during visual cortex development. A PS-targeting probe based on the lactadherin (MFG-E8) C2 domain was also designed to visualise and quantify exposed PS on synapses, aiding the study of synaptic pruning. This research enhances our understanding of synaptic plasticity and provides tools to study brain development and remodeling processes.

INVESTIGATION OF PLASMA MEMBRANE REPAIR AND CELL DEATH MECHANISMS AFTER ELECTROPORATION AND APPLICATION IN ANTICANCER THERAPY (Biology)

Dovilė Uždavinytė

Scientific supervisor:
Assoc. Prof. Dr Ingrida Šatkauskienė
Vytautas Magnus University

Dissertation defended:
19 January 2024

The aim of this study was to investigate the impact of electroporation on the damage and restoration of cancer cell membranes and to examine the effects of these methods on cell viability and the effectiveness of cancer treatment. The context of the study was based on the goal of understanding mechanisms that could optimise modern therapeutic strategies for cancer treatment. During the study, it was observed that intracellular compounds (e.g., ATF, proteins) released during electroporation negatively affected cell viability. However, a medium termed electroporation supernatant (EP SN) demonstrated the ability to suppress or delay cell death. Furthermore, ANXA4 aggregation on the membrane after electroporation was demonstrated, highlighting its importance in membrane restoration after damage. The combined treatment method with BLM (Ca^{2+} electroporation and chemotherapy) activated a systemic immune response against the tumour and effectively suppressed its growth. The results provided valuable information about the response of cancer cells to treatment, allowing an understanding of the impact of different therapeutic methods. The different cell death modes observed after electroporation and the role of ANXA4 in membrane restoration open new possibilities for future cancer therapy. The synergistic effect of the combined treatment method also demonstrates the potential to develop effective strategies for cancer treatment and activate the body's immune system against the tumour.

MICROORGANISMS OF THE GENUS *DIAPORTHE* AND *XANTHOMONAS* IN FABACEAE PLANTS (Biology)

Dovilė Čepukoit

Scientific supervisor:
Dr Daiva Burokienė
Nature Research Centre

Dissertation defended:
28 March 2024

Plants belonging to the Fabaceae family are among the most important in the food and medicine industry due to their diverse uses. These plants are frequently attacked by various fungal and bacterial pathogens. They are characterised by a variety of lifestyles, such as endophytes on intact leaves and stems, saprotrophs on decaying wood and leaves, and pathogens. Bacteria of the genus *Xanthomonas* Dowson are also found in Fabaceae plants and many of these bacterial species are under investigation because of the significant economic damage they can cause. In Lithuania, however, there is **a** lack of information on the damage these pathogens cause to the country's plants. Therefore, plants from the Fabaceae family were chosen as the object of study, including both native Lithuanian plants species and alien plants growing in natural ecosystems, as well as the microorganisms of the genera *Diaporthe* and *Xanthomonas* that colonise them. The diversity of the genus *Diaporthe* in *Caragana arborescens*, *Caragana frutex*, *Cytisus scoparius*, *Robinia ambigua*, and *Robinia pseudoacacia* plants and the diversity of *Xanthomonas* in roots and nodules of *Cytisus scoparius*, *Trifolium* sp. and *Coronilla varia* was determined by different molecular methods. The pathogenicity of the investigated microorganisms for their host plants and/or indicator plants was evaluated.

SHORT-TERM AND LONG-TERM DIETARY NITRATE SUPPLEMENTATION INFLUENCE ON THE FUNCTIONAL STATE AND PERFORMANCE OF THE ORGANISM (Biology)

Tomas Liubertas

Scientific supervisor
Prof. Dr Jonas Liudas Poderys
Lithuanian Sports University

Dissertation defended:
12 April 2024

The thesis combines findings from five scientific articles employing a generalised exploratory approach to explore the impact of dietary nitrate supplementation on organism functionality in general. The primary goal of the study was to evaluate the effects on functional states and performance in living organisms, predominantly focusing on humans, while simultaneously seeking a deeper comprehension of the mechanisms underlying nitrate supplementation. Besides human trials, the study used *Drosophila melanogaster* and mice as analytical models due to their genetic similarities to humans, ease of breeding, and shorter lifespan. This made them ideal candidates for studying the effects of dietary interventions like nitrate supplementation on lifespan and health outcomes within a reasonable experimental timeframe. Longevity emerged as a natural outcome of the study, serving as a proxy for improved organism functionality. Results indicated a substantial influence on the functional state and performance of the organisms under study. This effect was manifested in various aspects, notably in enhanced performance markers and increased longevity, which served as indicators of improved organism functionality. In summary, the thesis presents a comprehensive exploration of the influence of dietary nitrate supplementation on organism functionality. By employing an exploratory approach, the study did not limit itself to predefined assumptions, unraveling potential relationships between variables instead.

GENETIC DIVERSITY OF VECTOR-BORNE PATHOGEN STRAINS IN DOMESTIC CATS AND THEIR ECTOPARASITES (Biology)

Miglė Razgūnaitė

Scientific supervisor:
Prof. Dr Jana Radzijevskaja
Vytautas Magnus University

Dissertation defended:
24 April 2024

The dissertation aims to evaluate infection of domestic cats (*Felis catus*) and their ectoparasites (fleas and ticks) with vector-borne pathogens, to identify pathogen strains and their genetic diversity using molecular research methods. There is a critical need for research on feline vector-borne pathogens, as domestic cats are seldom screened for these pathogens due to their often asymptomatic nature. Diagnosis typically relies on presumptive assessments based on the history of animals' exposure to ectoparasites. Monitoring the prevalence of these pathogens in animals can serve as an early warning mechanism for potential outbreaks, facilitating prompt intervention to curb disease transmission. This study marks the first identification of two causative agents of cat scratch disease, *Bartonella henselae* and *Bartonella clarridgeiae*, in domestic cats and cat fleas in Lithuania. Additionally, it presents the first molecular detection and characterisation of the genetic diversity of *Mycoplasma* bacteria in domestic cats and their ectoparasites in Lithuania. Furthermore, the study reports, for the first time, the presence of diverse *Rickettsia* pathogens in domestic cats and their ectoparasites in Lithuania, including the first detection of *Rickettsia felis* in cats in the Baltic countries.

INVESTIGATION OF SUB-MICROSECOND PULSE RANGE ELECTROPORATION AND ELECTROCHEMOTHERAPY FOR ERADICATION OF TUMORS AND INDUCTION OF AN IMMUNE RESPONSE (Biology)

Eivina Radzevičiūtė-Valčiukė

Scientific supervisor:
Dr Irutė Girkontaitė, Dr Vytautas Kašėta
Centre for Innovative Medicine

Dissertation defended:
31 May 2024

The aim of this thesis was to characterise sub-microsecond high-frequency (MHz range) electroporation in the context of drug and gene electrotransfer and to determine the anticancer immune response. The results showed that the delivery of nsPEF using MHz pulse repetition frequencies improves the efficacy of electroporation several-fold compared to the equivalent low-frequency protocols due to transmembrane potential (TMP) accumulation throughout the high frequency burst. The MHz repetition frequency sub-microsecond PEF protocols ensure equivalent or superior efficacy of short-term and long-term transfection of mammalian cells when compared to the μsPEF protocols (100 μs × 8). The evaluation of ATP depletion using D-luciferin oxidation reaction for detecting the extent of electroporation ensures equivalent sensitivity when compared to conventional methods of detecting electroporation-induced membrane permeabilization (e.g., YO-PRO-1). Bleomycin and Ca^{2+} ions nsECT modulate cellular immunity and promote humoral antitumour immune responses. Moreover, sub-microsecond treatments reduce tumor progression and extend the lifespan of mice.

EFFECTS OF LOCAL HEATING AND WHOLE-BODY HYPERTHERMIA ON NEUROMUSCULAR AND COGNITIVE FUNCTION IN YOUNG AND OLD HEALTHY ADULTS (Biology)

Junli Wang

Scientific supervisor:
Prof. Dr Marius Brazaitis
Lithuanian Sports University

Dissertation defended:
21 June 2024

The purpose of the thesis was to investigate age-related differences in motor and cognitive functioning under local and whole-body hyperthermia conditions in healthy young and older men. The results showed that the spinal and supraspinal reflex excitability of older adults increased during local knee-heating application. The improved motor drive transmission observed in older adults was accompanied by increases in voluntarily induced isometric peak torque production, the rate of torque development, and isokinetic plantar flexion/dorsiflexion peak torque production. In conclusion, our study reveals that neuromuscular performance during fatigue-provoking sustained isometric exercise under severe whole-body hyperthermia conditions declined in both age groups, but severely hyperthermic older males were less susceptible to neuromuscular fatigue. This is likely due to the lower heat stress experienced by older males, which can be attributed to a suppressed sympathetic autonomic response, impaired dopamine response, and lower circulating prolactin levels. Our main finding was that healthy elderly individuals improved cognitive flexibility with an increase in Tre of ≈2.5°C, but their memory was maintained. This improvement paralleled the lower physiological strain and greater cortisol response to WBH compared with young adults.

ROLE OF isomiRNA IN PATHOGENESIS OF COLORECTAL CANCER (Biology)

Rokas Lukoševičius

Scientific supervisor:
Dr Simonas Juzėnas
Lithuanian University of Health Sciences

Dissertation defended:
26 June 2024

MicroRNAs (miRNAs) are essential molecules, which are involved in post-transcriptional gene regulation, contributing to various diseases, including cancer. It has been shown that not only known canonical miRNAs have importance in normal physiological or pathological processes, but multiple miRNA isoforms (isomiRs) as well. Despite the increased availability of fecal occult blood tests and colonoscopy-based CRC screening programs, colorectal cancer (CRC) still remains the third most common cancer and the leading cause of cancer-related deaths worldwide, which means that more precise methods for early, non-invasive disease detection are urgently needed. There are studies implying association of isomiRs with different cancers by simply analysing small RNA sequencing data, however, there is no knowledge of functional relevance of isomiR and their targetome in CRC done *in vitro*. In this study, isomiR profile of CRC tissue and commercial cell lines was analysed using small-RNA-seq data. The most deregulated miRNAs (miR-125b-2-3p; miR-183-5p; miR-1246) and their 5' isoforms were selected for further investigation by implementing loss-of-function cell experiments. Most prominent tumour-promoting result in loss-of-function experiments demonstrated miR-1246 and its two 5' isoforms were selected to explore its candidate target genes. The targetome of canonical miR-1246 and its two 5' isoforms were determined using RNA-seq and *in silico* target prediction analysis. The pathway enrichment analysis of targets deregulated by miR-1246 and its two 5' isoforms indicated that the three studied miR-1246 variants partially shared signaling pathways, but in some cases isomiRs demonstrated stronger effect than canonical miRNA.

ALTERATIONS OF CIRCULATING AND TISSUE-ASSOCIATED MICROBIOME PROFILES IN THE CASE OF GASTRIC AND COLORECTAL CANCERS (Biology)

Darja Nikitina

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

Dissertation defended:
27 June 2024

As awareness of the impact of microbiota on human health increases, the importance of exploring the influencing factors on the final microbiome profile is also growing. Gastric and colorectal cancers, both of which are highly prevalent and often asymptomatic in their early stages, demand a deeper exploration of their risk factors including microbiota. This study provides new insights into: (1) bacterial profiles of stomach and colon tissues in cases of tumourous, tumour-adjacent, pre-cancerous, and healthy mucosa, (2) bacterial DNA signatures profile in blood plasma in the cases of gastric cancer, colorectal cancer, and colon adenoma, (3) the relationship between the clinical data of gastric and colon cancer patients and the DNA profiles of their tissues and circulating bacterial DNA profile, (4) the influence of blood plasma sampling and preparation on bacterial microbiome analysis, and (5) the influence of the selected microbiota technique on the analysis of the bacterial microbiome. Overall, this study contributes to a deeper understanding of the complex bacterial community in both health and disease. Further, the study offers valuable insights into the standardisation of microbiome study approaches. Finally, the results of this work may be useful for future scientific research in the fields of biomarkers and microbiome-based therapy development for gastric and colorectal cancers.

POTENTIAL ANTIMICROBIAL MECHANISM OF SILVER NANOPARTICLES SYNTHESIZED BY THERMOPHILIC *GEOBACILLUS* GENUS BACTERIA (Biology)

Kotryna Čekuolytė

Scientific supervisor:
Prof. Dr Eglė Lastauskienė
Vilnius University

Dissertation defended:
4 July 2024

The increasing resistance of microorganisms to currently used antimicrobial agents requires the search for alternative treatments. Silver nanoparticles (AgNPs) could be one alternative to antibiotics and antifungal drugs. AgNPs have a complex antimicrobial effect on cells, simultaneously directly affecting various parts of the cell and its functions. Currently, most of the commercially available AgNPs are obtained by physical and chemical methods, but these methods are expensive and environmentally unfriendly. As an environmentally friendly alternative, biological methods of AgNP synthesis are used. Although much is known about AgNPs obtained using mesophilic organisms, there is still a lack of research on AgNPs obtained using thermophilic organisms. AgNPs obtained using thermophilic bacteria of the genus *Geobacillus* are investigated in this thesis. The mechanism of antimicrobial action of AgNPs against bacteria and yeasts associated with human skin diseases was evaluated. The effect of these AgNPs on cell membranes was determined, whether they cause oxidative stress, whether caspases are activated in yeast cells after exposure to AgNPs, whether programmed death of yeast cells occurs. In addition, exposure to AgNPs was combined with electroporation to reduce the toxic effects of AgNP for the first time. The cytotoxic effect of these AgNPs on human keratinocyte cells was also investigated to determine whether AgNPs obtained using thermophilic bacteria are safe for practical use.

THE INFLUENCE OF KNEE JOINT ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTIVE SURGERY METHODS ON THIGH MUSCLE STRENGTH AND PHYSICAL ACTIVITY RECOVERY (Biology)

Vytenis Trumpickas

Scientific supervisor:
Prof. Dr Algimantas Paulauskas
Vytautas Magnus University

Dissertation defended:
5 July 2024

Conducted from 2008 to 2017, this study included 60 subjects with an ACL tear, with a mean age of 31.4 years (range 25 to 39 years). Subjects were randomised into two groups (30 per group): GT group – ACL reconstruction performed using a patellar tendon graft; SMGT group – ACL reconstruction performed using a semitendinosus and gracilis muscle graft. All subjects followed the same rehabilitation protocol and were assessed using the Tegner physical activity scale preoperatively, at three months and at 12 months postoperatively. The results showed that in patients who underwent ACL reconstruction, knee extensor strength was restored within eight months, regardless of graft type. Flexor strength recovered faster with the GT graft (five months) than with the SMGT graft (eight months). At all study stages, extensor strength remained similar, while flexor strength was significantly higher with the GT graft at an angular velocity of 30°/s. It was found that patients who received the GT graft had greater leg function symmetry when performing single-leg hop tests for time compared to those who received the SMGT graft, six months after surgery. Evaluating the return to the previous level of physical activity, it was determined that patients who underwent ACL reconstruction using either GT or SMGT grafts returned to their previous level of physical activity 12 months after surgery. The type of graft did not influence the return to previous physical activity levels.

GEOBACILLUS LIPOLYTIC ENZYMES: STRUCTURE-FUNCTION RESEARCH AND APPLICATION IN POLYCAPROLACTONE RECYCLING (Biology)

Vilius Malūnavičius

Scientific supervisor:
Assoc. Prof. Dr Renata Gudiukaitė
Vilnius University

Dissertation defended:
27 August 2024

Due to their capability to function and remain stable at higher temperatures, catalyse hydrolysis, ester synthesis and transesterification reactions, the lipolytic enzymes (lipases and carboxylesterases) of *Geobacillus* origin have intriguing fundamental reseach and industrial application potential. Due to a lack of knowledge, the aim of this work is to improve the understanding of lesser known 55 kDa *Geobacillus* carboxylesterases as well as evaluate the application of *Geobacillus* lipolytic enzymes in polyester recycling. The results showed that site-specific mutagenesis of amino acids Gly108 and Ala410 located near the active centre, and Leu226 substitutions to arginine had a negative, while Leu411 substitution to alanine had a positive effect on GDEst-95 activity. Using error-prone PCR, three GDEst-95 variants: GDEst-RM1, GDEstRM2, GDEst-RM3, with improved properties were generated. The mutated variants possessed improved Vmax and had a higher optimal working temperature when compared to unmodified GDEst-95. GD-95RM and GDEst-lip can perform PCL film hydrolysis: 1 mg of GD95RM could degrade more than 264.0 mg of PCL weight; GDEst-lip 1 mg could degrade more than 134.0 mg of PCL weight. The addition of organic solvents could significantly improve PCL film hydrolysis by using GD-95RM or GDEst-lip enzymes. After adding 25% ethanol to the PCL degradation mixture, GD-95RM enzyme (1 mg) could degrade 2.4 times more of PCL (646.0 mg), after adding 50% methanol – GDEst-lip (1 mg) could degrade three times more than of PCL (442.7 mg). Adding organic solvents to the reaction mixture, results in synthesis of ethyl-hydroxyhexanoate or methyl-hydroxyhexanoate by both GD-95RM and GDEst-lip.

BLOOD CIRCULATION OF EPICARDIAL NERVES AND GANGLIA IN PIGS (Biology)

Dmitrij Kvitka

Scientific supervisor:
Prof. Dr Dainius Haroldas Pauža
Lithuanian University of Health Sciences

Dissertation defended:
28 August 2024

The research described in this thesis aimed to investigate in detail the blood vessels of the epicardial nerve plexus of the pig, their distribution, variety, and structure and to compare the data of this study with analogous studies of the nerve blood vessels of the human heart in order to evaluate the pig, as a suitable animal model for its use in further studies to improve the treatment of cardiac arrhythmias by the method of local trans-coronary ethanol neuroablation. It was determined that pig epicardial nerves and nerve ganglia are supplied with blood by both coronary and non-coronary (extra-cardiac) blood vessels. The blood vessels branching off from the coronary arteries and feeding the epicardial nerves and ganglia are their fifth-sixth order branches – arterioles, which branch into a network of capillaries in these nerves and ganglia. The results of this study provide new fundamental knowledge about the structural organization of the intrinsic nervous system and are significant for thoracic and cardiac surgery, as they allow successful invasive cardiac operations with less trauma to the epicardial plexus and its blood vessels, both in animal experiments (pigs) and in clinical practice, using trans-coronary ethanol ablations for the treatment of cardiac arrhythmias.

MOLECULAR AND CLINICAL PROGNOSTIC INDICATORS OF KIDNEY CANCER (Biology)

Algirdas Žalimas

Scientific supervisor:
Prof. Dr Sonata Jarmalaitė
Vilnius University

Dissertation defended:
4 October 2024

The study provides scientifically based information about the oncological safety of active surveillance of small kidney tumours. It identifies the prognostic factors of aggressive kidney cancer that can help the urologist choose the right treatment tactics. The work evaluated the predictive value of epigenetic markers in determining aggressive forms of kidney cancer and predicting rapid tumour growth.To our knowledge, this study is the first to analyse DNA methylation in urine samples from patients with small renal tumours and demonstrates its potential as a non-invasive tool for the detection of small (up to 4 cm in diameter) malignancies. According to our available data, studies that evaluated the influence of clinical data such as obesity, smoking, elevated arterial blood pressure, metabolic syndrome, dyslipidemia, or the psychoemotional state of the patient on the progression of small, observed kidney tumours, i.e., for rapid tumour growth, have not been performed so far. Based on the fact that Lithuania has one of the highest rates of incidence and mortality of kidney cancer in Europe, it is necessary to evaluate the effectiveness of systemic treatment in patients with advanced kidney cancer.

ASSESSMENT OF NANOPARTICLE GENOTOXICITY AND ITS CELLULAR UPTAKE BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (Biology)

Milda Babonaitė

Scientific supervisor:
Prof. Dr habil. Juozas Rimantas Lazutka
Vilnius University

Dissertation defended:
17 December 2024

According to European Commission Recommendation 2022/C 229/01, nanomaterials are described as materials with one or more external dimensions in the size range of 1 nm–100 nm. Due to their small size, composition, shape, and surface functionalities, nanoparticles (NPs) possess unique physicochemical properties. As a result, the number of anthropogenic NPs being synthesised and applied in various fields, such as medicine, food science, cosmetics, pharmaceuticals, and electronics, continues to rise. This exponential growth raises concerns about the potential negative effects of NPs on living organisms. This study explored the genotoxicity of 11 different types of NPs. Using human peripheral blood mononuclear cells and established genotoxicity assays. Based on the results, we identified three distinct mechanisms of NP-induced genotoxicity. NPs that were efficiently internalised and induced ROS formation – such as Co_3O_4, Al_2O_3, and polystyrene (PS) – caused not only primary DNA damage but also significant chromosomal aberrations. In contrast, NPs that did not induce ROS (e.g., PVP-Ag, SiO_2) resulted solely in primary DNA strand breaks. The study also showed that NP uptake and ROS generation might be limited (Au-NPs); however, they still caused a statistically significant increase in primary DNA damage. The results of this study provide theoretical insights into NP-induced genotoxic mechanisms and underscore the importance of comprehensive testing in the field of nanogenotoxicology. They may also contribute to developing new recommendations for nanoparticle research and safety assessment.

THE RELATIONSHIP BETWEEN HEARING AND SPEECH PERCEPTION IN PATIENTS WITH AUTOIMMUNE RHEUMATIC DISEASES (Biology)

Vija Vainutienė

Scientific supervisor:
Dr Justinas Ivaška
Centre for Innovative Medicine

Dissertation defended:
18 December 2024

The aim of this dissertation was to analyse and evaluate the relationship between hearing and speech perception in patients with autoimmune rheumatic diseases – systemic vasculitis. During the study, a new Lithuanian speech audiometry methodology was developed, including lists of bisyllabic spondaic and phonetically balanced words for assessing speech thresholds and speech perception. The results showed that half of the patients with systemic vasculitis exhibited hearing impairment, most commonly presenting as sensorineural hearing loss. It was found that hearing disorders were more frequent in systemic vasculitis patients with ANCA antibodies detected in their blood. To ensure early detection of hearing impairment, it is recommended that all patients with autoimmune systemic vasculitis be referred for an otolaryngologist consultation and hearing assessment. For speech audiometry in Lithuania, the Lithuanian speech audiometry methodology developed in this dissertation is recommended.

THE PHARMACOLOGICAL EFFECTS OF DICHLOROACETATE, VALPROIC ACID AND THEIR COMBINATION ON RAT THYMOCYTES AND THE DEVELOPMENT OF ADULT HUMAN GLIOBLASTOMA (Biology)

Milda Juknevičienė

Scientific supervisor:
Prof. Dr habil. Donatas Stakišaitis
Lithuanian University of Health Sciences

Dissertation defended:
18 December 2024

Dissertation aimed to investigate the effects of valproic acid (VPA), dichloroacetate (DCA), and their combination (VPA-DCA) on rats, their thymocytes, as well as adult GBM cell lines, and GBM primary cells *in vitro* and their tumours using an experimental model *in vivo*; to confirm the hypothesis that VPA–DCA has a synergistic effect that allows to reduce drug dosage. The objectives were: (1) To determine the effects of single-dose and long-term treatment with VPA–NaDCA on rats, the excretion of Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+} in 24-hour urine, concentration of glucose, testosterone in blood, the thymus and number of Hassall's corpuscle in it, kidneys, thymocytes cell cycle, the expression of Slc5a8, Slc12a2, and Slc12a5 in the thymocyte, and gender-related differences. (2) To determine the effects of VPA on the expression of Slc5a8, Slc12a2, and Slc12a5 in rat thymocytes and the expression of SLC5A8, SLC12A2, SLC12A5, CDH1, and CDH_2 in U87 and T98G cells *in vitro*. (3) To determine the effects of MgDCA, VPA–MgDCA, and TMZ on tumour progression in U87 and T98G glioblastoma cell lines on the CAM, expression of EZH2, PCNA, p53 *in vivo*, and expression of the SLC5A8, SLC12A2, SLC12A5, CDH1, and CDH_2 in tumour cells *in vitro*. (4) To investigate the effect of VPA–MgDCA on tumour development from patient's GBM primary cells on the CAM, expression of PCNA, EZH_2, p53, and expression of SLC5A8, SLC12A2, SLC12A5, CDH1, and CDH_2 as well as pro-apoptotic genes.

INVESTIGATING RECOVERY STRATEGIES TO OPTIMIZE PERFORMANCE AND WELL-BEING IN BASKETBALL (Biology)

Marco Pernigoni

Scientific supervisor:
Dr Daniele Conte
Lithuanian Sports University

Dissertation defended:
20 December 2024

The aim of this dissertation was to investigate the effectiveness of recovery strategies which have received little to no attention across basketball-specific research, despite being frequently employed by practitioners in real-world contexts. The novel findings presented in this dissertation aim to offer basketball practitioners valuable insights into the application of recovery strategies used in real-world settings. By incorporating evidence-based recovery methods within training programmes, practitioners will be able to make informed decisions enhancing performance and recovery both in the short- and long-term. Findings from this study suggest that neither active recovery nor static stretching appear to be a superior strategy for improving recovery beyond ~10–20 min post-intervention, following a training session in elite youth male basketball players. Findings indicate that foam rolling was ineffective for improving recovery of CMJ height, cardiac parasympathetic activity, muscle soreness, and perceived fatigue in female basketball players following a simulated basketball match. Results showed that a single WBC exposure (4 min, −75 to −85°C) was ineffective for improving recovery of performance, cardiac autonomic activity, and muscle soreness in high-level youth male basketball players following a simulated match.

MODELING AND ELECTROPHYSIOLOGICAL EXAMINATION OF CHEMICAL GATING OF GAP JUNCTIONS COMPOSED OF CARDIAC AND NEURAL SYSTEM-SPECIFIC CONNEXINS (Biophysics)

Rokas Mickus

Scientific supervisor:
Prof. Dr Vytenis Arvydas Skeberdis
Lithuanian University of Health Sciences

Dissertation defended:
1 March 2024

Direct intercellular communication between cells is formed by gap junctions (GJs). GJs are specialised intracellular channels composed of connexin (Cx) proteins coordinating and regulating many cell physiological processes. A GJ is composed of two hemichannels, also called connexons. Each hemichannel has voltage-sensitive fast and slow gates, but only the slow gate, also called a chemical gate, is regulated by intracellular pH, intracellular Ca^{2+} and Mg^{2+}, phosphorylation, and chemical substances. The effect of nutmeg essential oil and its components on the biophysical properties of Cx43 gap junction channels was investigated in the dissertation. The results showed that nutmeg essential oil and its three constituents (sabinene, α-pinene, and α-copaene) rapidly and reversibly inhibited endogenous Cx43 GJ channel conductance by acting through the GJ slow gates. To search for new GJ modulators, we developed QSAR and 3D-QSAR models (quantitative structure-activity relationship models), with the help of which we determined and experimentally confirmed that d-limonene acts as a Cx43 GJ inhibitor. Also, for the first time, we demonstrated that the potency of α-pinene and sevoflurane depends on Cx43 phosphorylation, which acts through positive allosteric cooperation. Our results show that CaMKII, aPKC, CDK and partly Pyk2 protein kinases modulate the potency of the Cx43 GJ blocker α-pinene.

CELLULAR UPTAKE AND ACCUMULATION STUDIES OF FUNCTIONALIZED UPCONVERTING NANOPARTICLES (Biophysics)

Evelina Kazlauskė

Scientific supervisor:
Prof. Dr habil. Ričardas Rotomskis,
Dr Vitalijus Karabanovas
Vilnius University

Dissertation defended:
14 May 2024

Upconverting nanoparticles are an excellent tool for biomedical applications that can be used in both cancer diagnosis and therapy. To increase the biocompatibility and accumulation of these nanoparticles in cancer cells, it is necessary to modify the surface of the nanoparticles with appropriate surface coatings. Additionally, surface coating is important for the formation of the necessary protein corona around the particle, which affects the endocytosis mechanisms and intercellular nanoparticles distribution in cancer cells. The aim of this work was to investigate the interaction of differently coated upconverting nanoparticles with cancer cells and to investigate the mechanisms of accumulation of nanoparticles in cells. Research has shown that rare earth metal differently coated upconverting nanoparticles are biocompatible, the protein corona improves the colloidal stability of nanoparticles, and surface coatings affect the dynamics of accumulation in cells. Cellular accumulation is also influenced by both the cell surface proteome and the composition of the protein corona formed around the nanoparticles. Moreover, it was observed that the amount of polyethylene glycol in the surface modifications of the nanoforms is an important factor regarding their accumulation in cancer cells. A higher amount of polyethylene glycol in the nanoparticle coating directly correlates with higher accumulation dynamics in the cells.

THE INVESTIGATION OF THE FACTORS MODULATING MACROPHAGE ENDOCYTOSIS: MACROPHAGE TYPE, AGE, PLASMA MEMBRANE TENSION (Biophysics)

Dovydas Gabrielaitis

Scientific supervisor:
Dr Urtė Neniškytė
Vilnius University

Dissertation defended:
13 June 2024

Macrophages are pivotal in the immune response: they engulf and digest cellular debris, pathogens, and foreign substances to maintain tissue homeostasis and protect against infections. The efficacy of macrophage phagocytosis is target type-dependant and influenced by various factors including age, macrophage type, and inflammatory state. Understanding what influences the phagocytic efficacy towards a specific target is crucial for the development and application of novel drug carriers. Consequently, in this work, factors modulating novel proteinaceous nanoparticle nanocarrier clearance by macrophages were evaluated in BV2 microglia cell line, primary microglia, and peritoneal macrophages. Macrophages are also affected by their mechanical environment that is transduced by membrane tension; however, its evaluation requires complicated single-cell techniques. Fluorescent genetically encoded membrane tension sensors can serve as an alternative. Hence, novel protein constructs were developed and tested, yielding promising genetically encoded plasma membrane tension sensors. The effect of mechanical stress and plasma membrane tension variations towards phagocytosis are being widely investigated; however, few studies investigate how plasma membrane tension changes when the macrophage is engulfing a phagocytic target. In this work, a small molecule plasma membrane tension indicator was applied to evaluate local plasma membrane tension of BV2 cells engulfing a phagocytic target.

ELECTROPHYSIOLOGICAL INVESTIGATION OF MACROALGAE *NITELLOPSIS OBTUSA* MEMBRANE CA^{2+} TRANSPORT SYSTEMS (Biophysics)

Vilmantas Pupkis

Scientific supervisor:
Prof. Dr Vilma Kisnierienė
Vilnius University

Dissertation defended:
28 August 2024

To survive in changing environmental conditions, plants must respond to external stressors by rapidly transmitting information from the locally affected area of the plant to other parts of their body and initiating a wide range of responses. This signal transduction function is carried out by electrical signals, i.e. membrane polarisation waves. The mechanism of action potential generation in plants is functionally understood: the initial depolarisation is triggered by an increase in the cytoplasmic Ca^{2+} concentration which activates Cl^- channels; the activity of K^+ channels and H+-ATPases realises the repolarisation. However, there is a lack of knowledge on the electrophysiological properties of Ca^{2+} channels and their potential control by second messengers. It has been hypothesised that, in analogy with animal systems, Ca^{2+} channels may be activated by inositol phosphates, but no receptor homologues have been found in plants. In this thesis, the effects of Ca^{2+} channel modulators on the Characean alga *Nitellopsis obtusa* were investigated using electrophysiological methods: the potential blockers verapamil, tetrandrine, NED-19, La^{3+} were used to block Ca^{2+} channels, while the potential activators inositol 1,4,5-trisphosphate (IP3) and inositol hexakisphosphate (IP6) were used to activate them. Most of the blockers were found to have non-specific effects and IP6 was found to be more consistent with the function of a hypothetical secondmessenger than IP3.

HALLMARKS OF AMINO ACID METABOLISM IN BREAST CANCER CELLS (Biophysics)

Monika Pankevičiūtė-Bukauskienė

Scientific supervisor:
Dr Sergio Bordel Velasco
Lithuanian University of Health Sciences

Dissertation defended:
11 October 2024

Altered metabolism is a hallmark of cancer. Amino acid metabolism is often disrupted in tumours to meet increased demands for building blocks and energy. This dissertation explores the role of amino acids in breast cancer cell metabolism using various -omics techniques and bioinformatics to identify potential chemotherapeutic targets. Objectives included examining the role of branched-chain amino acids (BCAAs) in cellular energetics and mevalonate biosynthesis using isotopically labelled amino acids in breast cancer cells, comparing BCAA metabolism in the blood plasma of breast cancer patients and healthy controls, and identifying alterations in amino acid metabolism by integrating metabolomic and transcriptomic data. Methods used were UPLC-ESI-MS/MS, isotope labelling, RNA sequencing, wound healing assay, and integrated genome-metabolome analysis. Results show that 34% and 14% of energy in MCF-7 and BCC breast cancer cells, respectively, come from BCAA degradation. Additionally, 67% and 22% of carbon in leucine is converted to mevalonate in MCF-7 and BCC cells, respectively. Inhibition of BCAA metabolism reduces breast cancer cell invasiveness. Three BCAA metabolites – 3-hydroxyisovalerate, 3-hydroxyisobutyrate, and mevalonate – are potential biomarkers for early breast cancer detection. Genes AHCY, PHGDH, PSPH, and CHAC1 in methionine and serine metabolism are potential drug targets for breast cancer.

DYNAMICS OF GROUND COVER IN SCOTS PINE STANDS AFTER CLEAR CUTTINGS (Ecology and environmental)

Dovilė Gustienė

Scientific supervisor:
Dr Iveta Varnagirytė-Kabašinskienė
Lithuanian Research Centre
for Agriculture and Forestry

Dissertation defended:
9 February 2024

The aim of this dissertation was to assess changes in the non-live and live ground cover in infertile Scots pine forests (*Pinetum vaccinio-myrtillosum*) with an increased stand age following clearcutting. During the research, a threshold stand age was identified, beyond which the ecological change induced by clear-cutting decreases, i.e., less intensive processes of nutrient leaching and forest floor mineralisation, and typical species composition and coverage of the ground vegetation is observed. Scots pine forests of *Pinetum vaccinio-myrtillosum* type show signs of recovery of non-live and live ground cover parameters 21–30 years after clear-cutting, i.e., in the third tree age class. The research also revealed statistically significant correlations among ground cover parameters. It was demonstrated that forest structure (canopy density) influences both non-live and live ground cover parameters. The forest floor and mineral topsoil layer showed the strongest relationships with the live ground cover components – mosses and vascular plants – and had a significant impact on ground vegetation recovery after clear-cutting. The evaluation of non-live and live ground cover components and diversity parameters does not allow the influence of clearcutting to be identified as exceptionally negative for Scots pine forests of *Pinetum vaccinio-myrtillosum* type. The higher abundance and diversity of ground vegetation species were observed in certain stages of forest development after clear-cutting, especially in younger Scots pine stands compared to the mature stands.

CONTROL OF SOSNOWSKY'S HOGWEED (*HERACLEUM SOSNOWSKYI*) SEED FORMATION USING BIOLOGICALLY ACTIVE SUBSTANCES (Ecology and environmental)

Tautvydas Žalnierius

Scientific supervisor:
Dr Sigita Jurkonienė
Nature Research Centre

Dissertation defended:
29 May 2024

The spread of alien species and their destructive impact on native ecosystems is one of the most pressing global issues today. Among the alien species registered in Lithuania, Sosnowsky's hogweed (*Heracleum sosnowskyi* Manden.) stands out for its environmental and human health hazards. Various measures, including herbicides, are used to curb the spread of Sosnowsky's hogweed, but their toxicity limits their use. Sosnowsky's hogweed produces seeds once in lifetime and then dies. Therefore, by preventing seed germination, inducing seedlessness, embryo abortion, and halting embryogenesis, its spread could be restricted. This led to the idea of using the phytohormones gibberellins and auxins to control seed formation and development in Sosnowsky's hogweed. This dissertation aimed to elucidate the effect of exogenous GA3 and other bioactive substances on fruit development in the central and lateral parts of the terminal and satellite umbels in relation to changes in the hormonal system. The research found that GA3 can contribute to the development of environmentally friendly measures for controlling the spread of invasive monocarpic hogweed species due to its ability to reduce seed germination, induce seedlessness, embryo abortion, halt embryogenesis, and reduce seed size. The results presented in this thesis provide **a** fundamental basis for an environmentally friendly technology to halt the spread of Sosnowsky's hogweed and mitigate the negative impact of this invasive plant.

ECOSYSTEM SERVICE ASSESSMENTS OF THE COASTAL ZONE: CASE STUDIES AND MANAGEMENT IMPLICATIONS (Ecology and environmental)

Esther Robbe

Scientific supervisor:
Dr Jūratė Lesutienė, Prof. Dr Gerald Schernewski
Klaipėda University

Dissertation defended:
7 June 2024

Coastal ecosystems, which are vital for biodiversity and human well-being, are increasingly affected by anthropogenic pressures. This dissertation, based on the ecosystem services assessment concept, provides insights and proposes new methods to address coastal management challenges. The study examines the ecological functions and socio-economic value of various Baltic Sea coastal habitats, with a particular focus on estuaries, sandy beaches, coastal lagoons, and macrophyte meadows. The main objective is to improve ecosystem service assessment methods that support environmental protection policies. The results confirmed that these assessment methods are valid and effective in evaluating coastal ecosystem services and proposing management solutions. A set of tools for assessing ecosystem services was developed, which can be applied in various coastal management areas, including urban coastal planning, beach management, coastal protection, and habitat restoration. The applicability of these methods was tested internationally, demonstrating their effectiveness in addressing coastal lagoon management challenges not only in the Baltic Sea region but also in other regions such as the Mediterranean Sea. Additionally, a SWOT analysis identified the strengths (interdisciplinary and holistic approach), weaknesses (limited reliability, excessive simplification), opportunities (policy integration, international harmonisation), and threats (loss of scientific interest, competing perspectives and beliefs) in applying the ecosystem services concept to coastal management. Directions for further research and potential applications of the concept were also outlined.

FUNGAL DESTRUCTIVE ACTIVITY IN THE DEGRADATION OF LINSEED OIL-BASED POLYMER COMPOSITES FILLED WITH ORGANIC WASTE MATERIALS AND ITS DEPENDENCE ON ENVIRONMENTAL FACTORS (Ecology and environmental)

Eglė Malachovskienė

Scientific supervisor:
Dr Danguolė Bridžiuvienė,
Prof. Dr Jolita Ostrauskaitė
Nature Research Centre

Dissertation defended:
12 June 2024

The increasing demand for plastics, environmental pollution, and depletion of crude oil reserves encourage the development of biodegradable polymers or their composites. When developing biodegradable materials, it is important to evaluate the role of microorganisms in their biodegradation and offer sustainable waste management solutions. The objective of this study was to evaluate the detrimental capacity of fungi on linseed oil-based polymer composites filled with organic waste materials (pine needles, pine bark, grain mill waste, rapeseed cake) under various environmental conditions. The results of enzymatic activity allowed us to select fungal strains that were used in the destructive activity studies under different pH and temperature conditions. Significant weight loss and chemical and surface structure changes in the studied materials proved the destructive potential of *Alternaria multiformis* 0065, *Cladosporium* sp. 0679, and *Fusarium* sp. 0926. The optimal pH and temperature conditions for the destructive activity of the tested fungi were also clarified. During biodegradation studies in three different soils (coniferous and deciduous forest soil and grassland soil), a significantly higher weight loss was determined in coniferous forest soil. The ATR-IR results showed that the cross-linked polymer matrix made of linseed oil broke down faster than the fillers. The obtained results can serve in the development of biopreparation or in the development of materials with desirable biodegradability.

ADVANCEMENTS IN ECOLOGICAL MONITORING THROUGH UNMANNED AERIAL VEHICLE AND SATELLITE DATA: STUDIES IN WATER CLARITY, COASTAL MANAGEMENT, AND VEGETATION DYNAMICS (Ecology and environmental)

Edvinas Tiškus

Scientific supervisor:
Dr Diana Vaičiūtė
Klaipėda University

Dissertation defended:
13 June 2024

This dissertation advances the use of Unmanned Aerial Vehicles (UAVs) and satellite imagery for monitoring eutrophication indicators and bathing water quality in aquatic and coastal ecosystems. By integrating UAV remote sensing, deep learning techniques, and a quasi-analytical algorithm, innovative approaches for water quality assessments and vegetation mapping were developed and validated. The research establishes a comprehensive UAV survey framework, demonstrating the effectiveness of sensor fusion and analytical methods for detailed vegetation analysis. This framework aids in biodiversity conservation, ecosystem structure analysis, and monitoring vegetation changes, guiding scientists and resource managers in adopting UAV technology. In aquatic vegetation assessments, the study highlights the normalised difference water index from Sentinel-2/MSI data and Yen binary thresholding, validated with UAV imagery, as effective in detecting mowing-induced changes in reed beds, particularly in areas larger than 0.1 ha. The quasi-analytical algorithm for Secchi depth estimation from UAV multispectral imagery was tested, identifying Hedley's sun glint correction method as the most precise. The study emphasises the adaptability of the algorithm and the influence of CDOM concentration and solar zenith angle on measurement accuracy. Additionally, the performance of the U-Net model in segmenting beach wrack from UAV imagery was assessed. Results indicate that RGB data combinations provide the most accurate detections, while adding multispectral and elevation data did not significantly improve segmentation accuracy, highlighting the need for site-specific data selection. This research demonstrates the potential of UAV technology for scalable and efficient environmental monitoring.

FISH AS A MODEL FOR STUDYING NANOTOXICITY: NANOPARTICLES ACCUMULATION IN FISH AND EFFECTS ON THE INTESTINAL MICROBIOTA (Ecology and environmental)

Renata Butrimienė

Scientific supervisor:
Dr Nijolė Kazlauskienė
Nature Research Centre

Dissertation defended:
12 September 2024

Products developed using nanotechnology are widely used in everyday activities, making nanoparticles an integral part of our lives. However, with the rapid development of nanotechnology, nanoparticles may become a new group of environmental pollutants and have a negative impact on ecosystems. The aim of this thesis was to investigate the accumulation of nanoparticles with different chemical compositions in salmonids at different life stages and to assess their effects on the gut microbiota and functional status of the fish. The study examined nanoparticle accumulation, stability, and effects on salmonid physiology. The antibacterial effects of QDs and Cd^{2+} on salmonid gut bacteria were examined. Illumina® MiSeq™ next-generation sequencing was used to study the gut microbiota of brown trout (*Salmo trutta*) *in vivo* under QDs treatment. The results showed that graphene oxide reduced heavy metal accumulation in rainbow trout embryos and larvae, while Co and Fe accumulation in rainbow trout depended on the size of $CoFe_2O_4$ nanoparticles and the developmental stage of the fish. Dietary CdSe/ZnS-COOH QDs accumulate Cd^{2+} in the intestine, liver and gills of juvenile brown trout. Studies have shown that nanoparticles have an effect on morphophysiological parameters (respiratory rate, heart rate) in salmonids. QDs had no significant antibacterial effect, but Cd^{2+} was temperature-dependent.

INTERACTIONS OF NEWLY ENGINEERED AND NATURAL NANO- AND MICRO- SCALE MATERIALS WITH MODEL ORGANISMS: EFFECTS, MECHANISMS AND ENVIRONMENTAL CONSEQUENCES (Ecology and environmental)

Mindaugas Kazlauskas

Scientific supervisor:
Dr Danguolė Montvydienė
Nature Research Centre

Dissertation defended:
12 September 2024

The aim of this study was to investigate the interaction of nano- and micro-sized materials with model organisms, to find out its possible mechanisms and to assess the consequences for the environment. Our data show that MIX, GO, and MIX + GO concentrations did not affect seed germination, root growth, and root and shoot biomass in most cases, but they change photosynthetic processes, enhance the production of carotenoids and H_2O_2, also activate lipid peroxidation. The effect of GO on the accumulation of the investigated metals (Cu, Cr, Ni and Zn) in the roots and shoots of *L. sativum* exposed to MIX was determined, and this is related to the ability of GO to adsorb metals from the medium, so GO at low concentrations may be a useful tool for water decontamination. Summarising the results of the investigated effects of different types, different concentrations, stability, and different sizes of NPs on aquatic and terrestrial plants, it was found that, depending on the environmental factors and media, the investigated NPs cause morpho-physiological (growth, biomass, structure) and biochemical (pigments, carotenoids, MDA, H_2O_2 amounts) changes in plants, which clearly show the phytotoxic effect of NPs. Traditional ecotoxicity assessment methods were found to be not suitable for assessing NP toxicity, because environmentally significant concentrations of NPs do not cause death of model organisms but affect metabolic processes and cause oxidative damage.

THE IMPACT OF LAND-USE CHANGE AND BIOMASS USE TO CLIMATE CHANGE MITIGATION POLICY IMPLEMENTATION (Ecology and environmental)

Vaiva Kazanavičiūtė

Scientific supervisor:
Assoc. Prof. Dr Renata Dagiliūtė
Vytautas Magnus University

Dissertation defended:
20 September 2024

In pursuit of global and EU goals, in 2021 Lithuania adopted the National Climate Change Management Agenda, which set ambitious GHG reduction goals. The changes in GHG emissions/removals in the LULUCF and other sectors and opportunities to implement the accepted climate change mitigation targets in Lithuania were assessed in this study, taking into account land use changes, the impact of biomass use, and other possible factors, influencing the GHG balance of the LULUCF sector. It has been established that the LULUCF sector will enable the achievement of climate change mitigation goals in Lithuania only in the short term (2030), while the development of bioeconomy and the increased demand for biomass will reduce the potential of this sector, i.e., the increased demand for biomass and its removal from forest ecosystem will reduce the potential of GHG removals in the long term, and the sector may become a source of GHG emissions. Meanwhile, the material and energy substitution effect could cover the GHG emissions generated in the sector due to the increased demand for biomass. During the research, additional measures to increase GHG absorption in non-forest land categories are found to be effective in Lithuania.

THE IMPACT OF PHYSICAL ACTIVITY AND ENVIRONMENTAL FACTORS ON PUBLIC HEALTH: INTEGRATED HEALTH RISK ASSESSMENT (Ecology and environmental)

Yevheniia Chebotarova

Scientific supervisor:
Prof. Dr Audrius Dėdelė
Vytautas Magnus University

Dissertation defended:
27 September 2024

This dissertation explores the associations between physical activity (PA), environmental factors, and public health outcomes in Kyiv, Ukraine, and compares these findings with European Union (EU) practices. Physical activity and environmental factors deeply impact public health, which is critical to economic stability and societal well-being. As the capital of Ukraine and a central figure in the nation's path toward EU integration, Kyiv presents a distinct opportunity to explore the associations between these factors. Given the absence of comprehensive PA guidelines in Ukraine, this research is crucial for informing policy and improving public health, especially in Ukraine's ongoing EU accession. The study identifies vulnerable groups in Kyiv based on health and lifestyle indicators, analyses the perception of environmental factors, and assesses PA levels across demographic groups. By examining best practices from the EU, this research provides recommendations for enhancing Ukraine's national health policies, focusing on increasing PA and improving overall well-being of the population. The findings highlight the importance of accessible green spaces, quality recreational infrastructure, and tailored public health interventions, particularly in the post-war period.

THE EFFECT OF METEOROLOGICAL, HELIOPHYSICAL FACTORS AND GREENNESS EXPOSURE IN THE RESIDENTIAL ENVIRONMENT ON THE PSYCHOLOGICAL STATUS AND PHYSIOLOGICAL INDICATORS WITH HEART DISEASE (Ecology and environmental)

Sonata Čerkauskaitė

Scientific supervisor:
Prof. Dr Jonė Venclovienė
Vytautas Magnus University

Dissertation defended:
18 October 2024

A long recovery process is typical of the patients after open heart surgery characteris; they are observed to have a reduced heart rate variability (HRV), and the postoperative recovery is influenced by their psychological state. The aim of the study is to evaluate the impact of meteorological/heliophysical factors on the psychological state and heart failure (HF) in patients after open heart surgery and the effect of greenery in the living environment on the health of patients with HF. The results showed a complex effect of meteorological factors on the psychological state and HRV indicators of patients after open heart surgery. Higher somatisation, depression, and anxiety scores were observed at lower relative humidity, and high depression scores were associated with atmospheric pressure fluctuations. A lower HRV was observed with a decrease in air temperature during the cold period, a higher wind speed two days before the operation, and a higher NAO index. A lower HRV is associated with a low level of geomagnetic activity two days after surgery and a low solar wind speed two days before. Lower left ventricular remodeling associated with greater greenness within a 1 km radius (by NDVI) was found, as well as a positive effect of residential greenness (by NDVI) on changes in cortisol levels after six months in patients with symptomatic HF.

ENERGETIC VALUE OF COASTAL BENTHIC ENVIRONMENT FOR BENTHIVOROUS WINTERING SEA DUCKS BASED ON THEIR FEEDING ECOLOGY (Ecology and environmental)

Paola Forni

Scientific supervisor:
Prof. Dr Darius Daunys
Klaipėda University

Dissertation defended:
25 October 2024

The aim of this work was to identify important feeding areas for wintering sea ducks, the velvet scoter (*Melanitta fusca*) and the long-tailed duck (*Clangula hyemalis*), based on the energetic value of the seabed and their energy requirements. The results demonstrated that long-tailed ducks and velvet scoters were feeding predominantly on four sessile prey species in soft-bottom areas: the bivalves *Macoma balthica*, *Cerastoderma glaucum*, *Mya arenaria* and the polychaete *Hediste diversicolor*. The analysis of the behaviour through visual observation indicated that the principal activity during the day was diving (61%), while resting, comfort, and locomotion were similar (11.1–14.0%). The transmitter data analysis showed similar results. The diving depth distribution indicated that velvet scoters preferred depths below 12 m, but they can reach three times deeper waters down to 36 m. Converting dietary and behavioural data into daily energy expenditure (DEE) yielded values of 1854 kJ/day for the long-tailed duck and 2751 kJ/day for the velvet scoter, while modeled prey biomass distribution indicated 58–632 kJ/m^2 in the environment. The areas of high prey energy availability were recorded in the southern part of the Lithuanian Curonian Spit in waters deeper than 15 m. The energy balance, derived from the ratio between prey energy available on the seabed and sea duck energetic costs indicated a relative importance of a seabed to support DEE of a single sea duck individual. The results showed that the areas where benthic prey contributes relatively more to DEE are sufficient to sustain the wintering populations of the long-tailed duck and the velvet scoter during the whole winter.

THE RESOURCE-SAVING AND WASTE-SORTING BEHAVIOUR IN LITHUANIA IN THE CONTEXT OF THE COVID-19 PANDEMIC AND WAR IN UKRAINE (Ecology and environmental)

Yuliia Matiiuk

Scientific supervisor:
Assoc. Prof. Dr Genovaitė Liobikienė
Vytautas Magnus University

Dissertation defended:
13 December 2024

The significance of resource-saving and waste-sorting behaviours has increased due to global challenges such as climate change, resource depletion, and waste management. These behaviours are crucial for achieving energy efficiency and transitioning to a circular economy. Only about 14% of global waste is recycled, while 37% ends up in landfills, highlighting the urgent need for change. In Lithuania, despite significant efforts, 6% of the population does not practice waste sorting. Crises such as the COVID-19 pandemic and the war in Ukraine have exacerbated resource-saving challenges by increasing plastic waste, household energy consumption, and energy prices, particularly in Lithuania. This study analyses the role of informational, social, financial, and convenience tools in promoting sustainable behaviours in Lithuania. It also evaluates the impact of global crises such as COVID-19 and the war in Ukraine on individual actions. The findings of this study highlight tools that are important for promoting waste-sorting and energy-saving in Lithuania. Furthermore, this research offers insights into how major global events influence resource-saving and waste-sorting behaviours, providing valuable guidance for policymakers.

SUCCESSIONS OF SOIL MICROBIAL COMMUNITIES IN HAPLIC LUVISOL AFTER FERTILIZING WITH ORGANIC FERTILIZERS (Ecology and environmental)

Diana Sivojienė

Scientific supervisor:
Assoc. Prof. Dr Audrius Kačergius
Lithuanian Research Centre
for Agriculture and Forestry

Dissertation defended:
17 December 2024

For the first time, the effects of new granulated poultry and cattle manure fertiliser rates and their combinations with biological inputs on the soil microbiome in infertile loamy soils were compared with the effectiveness of litter cattle and poultry manure and mineral fertilisers. To improve the quality of the soil, biological inputs were used in the experiment together with organic fertilisers, the composition of which included fungi *Trichoderma* spp. and nitrogenfixing bacteria *Azotobacter* spp. Metagenomic analysis of soil microbial populations made it possible to comprehensively assess microbial communities. The analysis showed **a** different taxonomic structure of bacterial and fungal communities in different fertilisation options of the agricultural field. Quantitative analysis of functional groups of soil bacteria (organotrophic, mineral nitrogen-assimilating, and nonsymbiotic diazotrophic bacteria) and fungi active representatives was performed using a cultivation-based method. Fertilisation with organic fertilisers proved to be effective in terms of the abundance of functional groups of the tested microorganisms. Overall, the obtained scientific data provides knowledge on the direct effect of organic fertilisers and their combinations with biological inputs on soil microorganisms, which play an important role in the processes of organic matter destruction in the soils fertilised with organic fertilisers. The obtained data can be applied to protect the soil from degradation, maintain the ecological functionality, balance, and sustainability of the soil.

BIBIONOMORPHA AND TIPULOMORPHA (DIPTERA: NEMATOCERA) FLIES IN THE MOST COMMON DECIDUOUS DEADWOOD (Zoology)

Ina Gorban

Scientific supervisor:
Prof. Dr Virginija Podėnienė
Vilnius University

Dissertation defended:
22 March 2024

The aim of the dissertation was to investigate the abundance, richness, and diversity of infra-orders Bibionomorpha and Tipulomorpha flies in dead wood of common deciduous trees. During the research, the species diversity of Bibionomorpha and Tipulomorpha flies in the second decay stage of dead wood was studied, and the dominance and occurrence of fly species were assessed. For the first time in Lithuania, 76 Bibionomorpha flies were recorded, of which 47 were collected from wood and 29 from soil. It was found that the composition of fly species in dead wood varies among different tree species. The highest diversity and abundance of flies were found in the dead wood of the small-leaved lime. The majority of collected flies belonged to the mycetophagous feeding group and had **a** wide zoogeographic distribution. Representatives of one species were less abundant when the dead wood was more covered with moss; individuals of another species were more abundant when the surface area of the bark was larger. It was determined that the abundance, richness, and diversity of Bibionomorpha and Tipulomorpha flies increase with higher relative humidity and precipitation. It was found that flies collected from wood are most abundant in July and August. The species diversity of flies collected from wood is not very similar to that of flies developing in the soil.

BEETLES (INSECTA, COLEOPTERA) IN THE SECOND DECAY STAGE OF THE MOST COMMON LITHUANIAN DECIDUOUS TREE SPECIES (Zoology)

Aistė Lekoveckaitė

Scientific supervisor:
Prof. Dr Virginija Podėnienė
Vilnius University

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In Lithuania, forests cover about one-third of the country's territory, and dead wood is an integral part of these forests. Species dependent on decaying or dead wood at any stage of their life cycle are called saproxylic. Insects are one of the main groups of saproxylic organisms, with beetles (order Coleoptera) being the most diverse. By using dead wood and the micro-habitats it creates as a food source, as well as for feeding, development, or overwintering, beetles contribute to the decomposition of dead wood and the cycling of nutrients in forest ecosystems. Habitat heterogeneity is a key factor in determining the diversity of beetles associated with dead wood. It can be increased indirectly by increasing the amount of deadwood or directly by using deadwood with different characteristics. Deforestation, tree loss, and insufficient quantities of large dead wood (e.g., windfallen trees, logs) in forests threaten saproxylic beetle populations across Europe, including Lithuania. Therefore, research on these organisms and the development of information on their biology and ecology are of great importance. This work represents the first comprehensive examination of beetle communities associated with dead wood of deciduous tree species in the second stage of decay (weakly decayed wood covered with bark) in Lithuania.