VILNIUS UNIVERSITY
NATURE RESEARCH CENTRE

Tomas
MAKARAS

The effects of multicomponent chemical mixtures on behavioural, physiological and biochemical parameters of different fish species

DOCTORAL DISSERTATION

Natural sciences,
Ecology and environmental sciences N 012

VILNIUS 2019

This dissertation was written between 2015 and 2019 at Nature research centre. The research was supported by Research Council of Lithuania (project „NanoTox" MIP-108/2015, and "ACTIS" MIP-S-17-10).

Academic supervisors:
Dr. Gintaras Svecevičius (2015-2016) (Nature research centre, environmental sciences, ecology and environmental sciences– N 012);
Dr. Nijolė Kazlauskienė (2016-2019) (Nature research centre, environmental sciences, ecology and environmental sciences – N 012).
This doctoral dissertation will be defended in a public meeting of the Dissertation Defence Panel:

Chairman: Dr. Kęstutis Arbačiauskas (Nature research centre, natural sciences, ecology and environmental sciences – N 012);
Members:
Dr. Irina Blinova (National institute of chemical physics and biophysics, Tallinn Estonia, natural sciences, ecology and environmental research – N 012).
Dr. Dalius Butkauskas (Nature research centre, natural sciences, ecology and environmental sciences – N 012);
Prof. Habil. Dr. Vincas Būda (Nature research centre, natural sciences, ecology and environmental sciences – N 012);
Prof. Dr. Saulius Vasarevičius (Vilnius Gediminas technical university, technological sciences, environmental engineering – T004);

Defense of the dissertation will be held at the public meeting of the Council on 17th of October 2019 at 11.00 a.m. in the meeting Room (101 a) of Nature Research Centre. Address: Akademijos 2, LT–08412, Vilnius, Lithuania, Tel. +370 5 2729257; E-mail: sekretoriatas@gamtostyrimai.lt

The text of this dissertation can be accessed at the libraries of Nature Research Centre and Vilnius University, as well as on the website of Vilnius University: *www.vu.lt/lt/naujienos/ivykiu-kalendorius*

VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRAS

Tomas
MAKARAS

Daugianarių cheminių mišinių poveikis skirtingų žuvų rūšių elgsenos, fiziologiniams ir biocheminiams rodikliams

DAKTARO DISERTACIJA

Gamtos mokslai,
ekologija ir aplinkotyra N 012

VILNIUS 2019

Disertacija rengta 2015-2019 metais Gamtos tyrimų centre.
Mokslinius tyrimus rėmė Lietuvos mokslo taryba (projektas „NanoTox” MIP-108/2015 ir ACTIS” MIP-S-17-10).

Moksliniai vadovai:
dr. Gintaras Svecevičius (2015-2016) (Gamtos tyrimų centras, gamtos mokslai, ekologija ir aplinkotyra – N 012);
dr. Nijolė Kazlauskienė (2016-2019) (Gamtos tyrimų centras, gamtos mokslai, ekologija ir aplinkotyra – N 012).

Gynimo taryba:
Pirmininkas: dr. Kęstutis Arbačiauskas (Gamtos tyrimų centras, gamtos mokslai, ekologija ir aplinkotyra – N 012).
Nariai:
dr. Irina Blinova (Nacionalinis cheminės fizikos ir biofizikos institutas, Talinas, Estija, gamtos mokslai, ekologija ir aplinkotyra N 012).
dr. Dalius Butkauskas (Gamtos tyrimų centras, gamtos mokslai, ekologija ir aplinkotyra – N 012);
prof. habil. dr. Vincas Būda (Gamtos tyrimų centras, gamtos mokslai, ekologija ir aplinkotyra – N 012);
prof. dr. Saulius Vasarevičius (Vilniaus Gedimino technikos universitetas, technologijos mokslai, aplinkos inžinerija – T004);

Disertacija ginama viešame Gynimo tarybos posėdyje 2019 m. gruodžio mėn. 17 d. 11 val. Gamtos tyrimų centro 101 auditorijoje. Adresas: Akademijos g. 2, LT-08412 Vilnius, Lietuva, tel. (8~5) 272 92 57; el. paštas sekretoriatas@gamtostyrimai.lt

Disertaciją galima peržiūrėti Gamtos tyrimų centro ir Vilniaus universiteto bibliotekose ir VU interneto svetainėje adresu:
https://www.vu.lt/naujienos/ivykiu-kalendorius

CONTENTS

ABBREVIATIONS

ASTM – the American Society for Testing Materials
CR – coughing rate
Cr↓ – 10-fold reduced concentration of chromium (Cr^{6+}) in the complex metal mixture (MIX)
Cu↓ – 10-fold reduced concentration of copper (Cu^{2+}) in the complex metal mixture (MIX)
EU – European Union
EWSs – early warning signals
GES – good ecological status
GVF – gill ventilation frequency
ISO – International Organization for Standardization
LC_{50} – concentration of the compound that is lethal for 50% of the exposed (fish) population
LL – landfill leachate (from Kairiai landfill)
MIX – complex metal (Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{6+}) mixture prepared according to the Maximum-Permissible-Concentration (MPC) for each metal in EU inland waters (Directive 2008/105/EC)
MIX↑ – increased concentration of each metal in the mixture
MIX↓ – reduced concentration of each metal in the mixture
MPC – maximum-permissible-concentration
OECD – Organization for Economic Co-operation and Development
REACH – regulation concerning the Registration, Evaluation, Authorization and Restriction of Chemicals

LIST OF PUBLICATIONS AND AUTHOR'S CONTRIBUTION

Results of the present study were presented in 9 scientific [1]publications (**Papers I-IX**) and at one national and 9 international scientific conferences. Within the text, the publications are referred to using Roman numerals.

Publications with an impact factor on the Clarivate Analytics Web of Science (WoS) database:

I. Svecevičius G, Kazlauskienė N, Slučkaitė A, **Makaras T** (**2014**) Toxicological assessment of the effects of closed landfill on neighbouring hydroecosystem. *Fresenius Environmental Bulletin* 23(11a): 2926–2932.

II. **Makaras T**, Svecevičius G, Kazlauskienė N, Montvydienė D (**2018**) Rapid detection of sublethal toxicity using locomotor activity of rainbow trout juveniles. *Bulletin of Environmental Contamination and Toxicology* 100: 221–227.

III. Stankevičiūtė M**,** Sauliutė G, **Makaras T,** Markuckas A, Virbickas T, Baršienė J (**2018**) Responses of biomarkers in Atlantic salmon (*Salmo salar*) following exposure to environmentally relevant concentrations of complex metal mixture (Zn, Cu, Ni, Cr, Pb, Cd). Part II. *Ecotoxicology* 27(8): 1069–1086.

IV. **Makaras T**, Montvydienė D, Kazlauskienė N, Stankevičiūtė M (**2019**) Comparison of behavioral and respiratory responses of European perch and rainbow trout to metal mixture in terms of rapidness and sensitivity. *Bulletin of Environmental Contamination and Toxicology*. 103(3): 391–399.

V. **Makaras T**, Razumienė J, Gurevičienė V, Šakinytė I, Stankevičiūtė M, Kazlauskienė N (**2019**) A new approach to stress evaluation in fish

[1] Declaration of contribution
Tomas Makaras designed the doctoral thesis with Gintaras Svecevičius and Nijolė Kazlauskienė. Tomas Makaras initiated and planned experiments described in Papers II, IV-VII, IX, performed all the experiments, was responsible for the all data analysis and for preparation of manuscripts. Tomas Makaras was responsible for all parts related to behavioural, physiological and biochemical testing in Papers II, IV-VI and IX. Tomas Makaras was responsible for the part of behavioural and biochemical data analysis or preparation of certain parts in Papers I, V and VIII.

using β-D-Glucose measurement in fish holding water. *Ecological Indicators* available online: doi.org/10.1016/j.ecolind.2019.105829

VI. **Makaras T**, Montvydienė D, Kazlauskienė N, Stankevičiūtė M, Raudonytė-Svirbutavičienė E (**2019**) Juvenile fish responses to sublethal leachate concentrations: comparison of sensitivity of different behavioral endpoints. *Environmental Science and Pollution Research* (DOI: 10.1007/s11356-019-07211-6).

Other peer-reviewed publications:

VII. **Makaras T**, Montvydienė D, Kazlauskienė N (**2019**) Behavioural responses of European perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) to the complex metal mixture (Pb, Zn, Cu, Cd, Ni and Cr) exposure. *Proceedings of the 7^h International Conference on Environmental Management, Engineering, Planning and Economics*. Mykonos Island, Greece, 90-97. ISBN: 978-618-5271-73-2.

VIII. Montvydienė D, **Makaras T**, Kazlauskienė N, Cibulskaitė Ž, Šulčius S (**2017**) Ecotoxicity assessment of multicomponent mixtures of different origin (landfill leachate and biomass of harmful algae bloom) using three aquatic organisms. *CEMEPE proceedings of the 6^th International Conference on Environmental Management, Engineering, Planning & Economics*, Thessaloniki, Greece, 114–123. ISBN: 978-618-5271-15-2.

IX. **Makaras T,** Svecevičius G (**2016**) The use of locomotor activity of rainbow trout juveniles for the identification of sublehtal concentrations of landfill leachate. *International Journal of Computer and Systems Engineering* 10(1): 15–21.

Presentations at national and international conferences:

1. Stasiūnaitė E, Čapukoitienė B, Eglinskaitė R, Stankevičiūtė M, **Makaras T**, Butrimavičienė L (**2019**) Haematological and biochemical indices in rainbow trout (*Oncorhynchus mykiss*) after 4-, 7- and 14-day exposure to metal mixtures. The *62^th International Conference for Students of Physics and Natural Sciences* "Open Readings 2019", 19-22^th March, Vilnius, Lithuania.
2. Sauliutė G, Stankevičiūtė M, **Makaras T** (**2019**) Biomarker responses in *Salmo salar* exposed to multicomponent metal mixtures. The *16^th*

International Conference on Environmental Science and Technology, 4-7 September, Rhodes Island, Greece.

3. **Makaras T**, Montvydienė D, Kazlauskienė N **(2019**) Behavioral responses of European perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) to the complex metal mixture (Pb, Zn, Cu, Cd, Ni and Cr) exposure. *Proceedings of the 7^{h} International Conference on Environmental Management, Engineering, Planning and Economics,* 19th- 24th May, 2019, Mykonos Island, Greece.
4. **Makaras T,** Montvydienė D, Razumienė J, Gurevičienė V, Šakinytė I, Stankevičiūtė M, Kazlauskienė N (**2019**) Behavioural and biochemical responses of rainbow trout (*Oncorhynchus mykiss*) and European perch (*Perca fluviatilis*) to complex (Pb, Cu, Cd, Ni, Zn, Cr) metal mixture exposure. The *8th SETAC Young Environmental Scientists' Meeting*" 5-10, February 2019, Ghent, Belgium.
5. Montvydienė D, **Makaras T**, Kazlauskienė N, Cibulskaitė Ž, Šulčius S, (**2017**) Ecotoxicity assessment of multicomponent mixtures of different origin (landfill leachate and biomass of harmful algae bloom) using three aquatic organisms. The *6th International Conference on Environmental Management, Engineering and Economics (CEMEPE 2017) and SECOTOX Conference*, June 25-30, 2017, Thesaloniki, Greece.
6. **Makaras T,** Svecevičius G (**2017)** Rapid detection of sublethal toxicity using fish locomotor behavior. The **12th** *International Conference of Natural and Life Sciences, The Coins,* 28th February – 2nd March, 2017, Vilnius, Lithuania.
7. **Makaras T**, Svecevičius G (**2016**) The Use of Locomotor Activity of Rainbow Trout Juveniles for the Identification of Sublethal Concentrations of Landfill Leachate. *World Academy of Science, International Conference on Environment, Energy and Biotechnology ICEEB,* 16-19th January 2016, London, the United Kingdom.
8. **Makaras T,** Svecevičius G (**2016**) Behavioural response patterns of fish exposed to polluting substances. The *11th International Conference of Natural and Life Sciences,* ***The Coins,*** **29th February – 3rd March**, 2016, Vilnius, Lithuania.
9. **Makaras T**, Svecevičius G (**2016**) Toxicity evaluation of permanently polluting hydroecosystem on fish behavior responses: comparative analysis with heavy metal toxicity (in Lithuanian). *Procedings of the 19th Conference for Junior Researchers „Science – Future of Lithuania“* 6th April, 2016, Vilnius, Lithuania.

10. Kazlauskienė N, Cibulskaitė Ž, Svecevičius G, Sauliutė G, **Makaras T,** Rotomskis R, Kulvietis V, Stankevičius M, Markuckas A, Stankevičiūtė M, Baršienė J (2016) Nanoparticles and heavy metal toxicity mechanisms in fish during ontogenesis: An interdisciplinary project. *International Conference of Natural and Life Sciences, The Coins,* 29th February – 2nd March, Vilnius, Lithuania.

ACADEMIC ACHIEVEMENTS

1. Research Council of Lithuania. Scholarship for academic achievement (2019).

INTRODUCTION

One of the key objectives of the Water Framework Directive (WFD; European Union) is to attain "Good Ecological Status (GES)" and "Good Chemical Status" of EU surface water bodies (Directive 2000/60/EC; Directive 76/464/EEC). In general, the well-being of aquatic ecosystems is a key parameter that reflects ecosystem's wealth (Summers et al. 2012). Protection of aquatic life and human health as well as maintenance of freshwater for future generations is becoming a major challenge to our society, which can be achieved by maintaining and restoring the well-being of aquatic ecosystems. However, as a result of anthropogenic activity, the aquatic environment is usually polluted with complex effluents containing various chemicals and is almost never exposed to single contaminants (Saaristo et al. 2018; Inyinbor et al. 2018). As reported by the European Environment Agency (EEA 2014), various chemicals, such as metals, pesticides and other pollutants, are responsible for the poor chemical status of surface water bodies across Europe. Mixtures are composed of different chemical substances, which, because of the synergistic action, can produce cumulative effects on organisms even though their components are below their regulatory effect concentration (SCHER et al. 2012; Bopp et al. 2018; EEA 2014).

The first stage in sustainable ecosystem management is detection of polluting compounds in the aquatic ecosystem (Bae and Park 2014). Spot sampling and analysis of water quality based on physicochemical factors are conducted to assess water quality (Bae and Park 2014; Dusabe et al. 2019). However, not all contaminants or their synergistic and antagonistic toxic effects can be determined by analyzing solely physicochemical factors. Therefore, in order to assess water toxicity, it is necessary to carry out bioassays using the most sensitive aquatic species and their test-responses that are indicative not only of acute toxicity levels, but also of post-exposure field-realistic toxicity levels in the environment (Connon et al. 2012; **Paper V**).

An increasingly popular approach to the impact assessment of toxicants and their mixtures involves examining changes in behaviour of test animals over a short period of time so as to provide a rapid evaluation and detection of toxic substances in ambient water (Legradi et al. 2018). Swimming and respiratory behaviour of certain fish species is a highly sensitive biomarker of environmental water contamination (Sárria et al. 2011; Wang et al. 2013). Alongside with behavioural analysis, measurement of other stress indicators (e.g. cortisol and glucose) within a toxicity field also showed its usefulness for stress evaluation in short-term toxicity tests (Gesto et al. 2015; Guest et al. 2016; Jiang et al. 2017; Nageswara et al. 2017). Glucose level change in fish is one of the key indicators of acute stress caused by different environmental conditions, including different amounts of chemicals (Gagnon et al. 2006; Miller et al. 2007; Nageswara et al. 2017: **Paper V**). Since behaviour integrates biochemical, physiological and ecological processes, it may be ideal for studying environmental pollution effects (Scott and Sloman, 2004).

Different fish species were used in behavioural toxicity testing to determine the impact of various chemical compounds and their mixtures (Shinn et al. 2015; Mora-Zamorano et al. 2017; Norton et al. 2019; Saaristo et al. 2019). In this thesis, the fish species used in experimental studies belong to different taxonomic and ecological groups and represent various functional positions, therefore their responses to water contamination may vary. The salmonid species, i.e. the rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*), are considered to be commercially relevant. Additionally, *O. mykiss* is referred to as a standard test species widely used in aquatic toxicology (EPA 2002a, 2002b; ASTM 2012a, 2012b). *Perca fluviatilis* and *Gasterosteus aculeatus* are non-model test species. Altough these species are relevant due to their wide distribution throughout respective continents, they are rarely used in behavioural toxicology (Sergy and Scroggins, 1990; Wibe et al. 2001; Saaristo et al. 2019). Therefore, to improve predictive models of environmental risk assessment, it is important to have a better understanding of chemicals’ and their mixtures’ toxicity not only to standard test organisms, but also to widely distributed fish species (Connon et al. 2012; Legradi et al. 2018). Future ecotoxicological research should focus on behaviour of specific pollutants-exposed fish over time as well as on different biological response endpoints and relations among them.

1. ECOTOXICOLOGICAL ASSESSMENT OF CHEMICAL MIXTURES

1.1 Environmental pollutants

Landfill leachate. Landfill leachate (LL) contains various persistent compounds (e.g. trace metals, organic and inorganic materials) that are slowly degradable or even non-degradable, and, therefore, remain in the natural environment for a long-time (Kjeldsen et al. 2002; Derco et al. 2010; **Paper VI**). Because of that and because of increasing municipal waste amounts as well as re-occurring accidental spills and outflows of effluents from improperly isolated landfills into ambient waters, leachate has emerged as a matter of worldwide concern (Przydatek and Kanownik, 2019). It is a common problem not only in the developing countries, where environmental care standards are low (Zafirakou et al. 2013; Abd El-Salam and Abu-Zuid, 2014; Raihana et al. 2014; Salem et al. 2014; Svecevičius et al. 2014; Zohra et al. 2014), but also may be an important issue in other countries where landfill sites are still operating. Although there have been some studies conducted into leachate effects on fish health (Emenike et al. 2012; Klauck et al. 2013; Salem et al. 2014; Budi et al. 2016, **Paper I**), effects of low leachate concentrations on behaviour of certain fish species are still insufficiently understood (Alkassasbeh et al. 2009; Emenike et al. 2012).

Trace metals. Trace metals pollution along with the development of human production activities, industrial and agricultural wastewater emissions, is a cause of serious concern (Tchounwou et al. 2012; AMEC et al. 2014). Metals can enter surface water through a variety of pathways including pollution point sources such as discharges from industries and municipal wastewater treatment plants, and non-point sources such as storm-water runoff, agricultural runoff, and mineral weathering (Jaishankar et al. 2014; Wuana and Okieimen, 2011). In the aquatic environment, metals exist in trace concentrations, therefore, when evaluating mixture effects at realistic exposure levels, the potential impact of background exposure should be considered (**Paper V**). However, metals are regulated on an individual basis, rather than mixtures, which is partly because the current understanding does not allow accurate prediction of toxicity of metal mixtures to aquatic organisms (Michalec et al. 2013; Meyer et al. 2015). The evaluation of realistic metal-mixture toxicity to widely distributed fish species can help develop and improve predictive models of water contamination, and thus, ensure efficient water quality monitoring and management (**Paper II** and **IV**).

Understanding and predicting effects of multicomponent mixtures is one of the great challenges facing ecotoxicology and environmental risk assessment (Jager et al. 2010; Anyanwu et al. 2018). Toxicants that are found in mixtures may differ in their modes of action as well as in species-specific toxic effects and the biological responses induced (Kienzler et al. 2016). Therefore, studies that are undertaken with a view not only to assess the impact of toxicants on organisms, but also to identify the origin of pollutants and the possible interaction of substances in a mixture, focus on different biological responses of fish rather than on separate indicators. Studying the effects of mixtures seems to be suitable, because it reflects the simultaneous exposure that fish species face in the contaminated aquatic environment (Heys et al. 2016).

This thesis, has investigated sublethal effects of LL and complex metal (Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Cr^{6+}) mixture (MIX) (as a whole mixture approach) at realistic environmental concentrations on different fish species by testing various biological endpoints.

1.2 Fish models in behavioural toxicology

Fish as bioindicators are widely used to describe natural characteristics of aquatic ecosystems and to assess habitat alterations (Chovanec et al. 2003). The impact of aquatic contaminants and their mixtures on fish could be evaluated at different levels ranging from molecular to that of population (Chovanec et al. 2003). Predictive assessment models that do not involve widely distributed and ecologically relevant non-model fish species cannot properly reflect effects of chemical mixtures on aquatic organisms.

Oncorhynchus mykiss is referred to as a standard test species commonly used in aquatic toxicology (EPA 2002a, 2002b; ASTM 2012a, 2012b, OECD, 2019) and, therefore, characterized as very sensitive to different aquatic pollutants, especially in early stages of its development (Svecevičius 2009, 2012; Le Bihanic et al. 2014; Sovova et al. 2014; Shinn et al. 2015; Santos et al. 2019). Sensitivity of a test species is usually compared with that of a standard (*O. mykiss*, *Danio rerio, etc.*) species.

Salmo salar is included in annexes II and V of the European Union (EU) Habitats Directive as a species of European significance. The species is an economically (subsistence, recreational and commercial fisheries) and ecologically important throughout the European region (Kulmala et al.

2012). Although, *S. salar* is a non-model fish, there is a growing interest in using *S. salar* behavioural analysis for toxicity assessment.

Perca fluviatilis and *G. aculeatus* are relevant due to their wide distribution in Europe, however, these species are rarely used in behavioural toxicity studies. Most of the behavioural toxicology studies on perch species were conducted using Yellow perch (*Perca flavescens*), which is native to inland waters of North America (Lacroix and Hontela, 2004; Azizishirazi et al. 2014; Mora-Zamorano et al. 2017). However, combined effects of aquatic pollutants in mixtures on *P. fluviatilis* through fish behavioural alterations have received less attention from researchers. Only very few studies investigated behavioural effects of *P. fluviatilis* exposed to specific organic pollutants (Brodin et al. 2013; Saaristo et al. 2019) and metal mixtures (Svecevičius, 2007).

Changes in *G. aculeatus* behaviour under the impact of water contaminants were scarcely investigated (Bell, 2001) although this species has been used in such studies as aggression-boldness (Tinbergen, 1953) and schooling behaviour (Wark et al. 2011). *G. aculeatus* has the potential to be used alongside with other model fishes for gaining insights into the mechanisms of chemical agents' toxicity affecting behaviour. Moreover, this fish species is easy to breed, maintain in the laboratory and can be also used in behavioural testing as an alternative test species to the standard ones such as *O. mykiss* and zebrafish (*Danio rerio*) due to very similar innate behaviours (Norton et al. 2019).

1.3 Behavioural assays

Behavioural testing is a relatively common test procedure, because it integrates biochemical-physiological and ecological processes (Scott and Sloman, 2004). The growing focus of applied behavioural analysis on adverse environmental effects on fish seems to correspond with the wider use of improved computational software tools in more detailed quantitative analysis of fish behaviour (Bae and Park 2014; Delcourt et al. 2013; Calfee et al. 2016; Xia et al. 2018). Therefore, behavioural endpoints of certain fish species are considered to be one of the earliest-warning signals that have proven their sensitivity, effectiveness and usefulness in ecological risk assessment (Scott and Sloman, 2004; Hellou, 2011, Bae and Park, 2014).

The most straightforward application of specialized behavioural analysis software involves measuring of such basic behavioural characteristics as swimming velocity, the distance travelled, frequency and duration of fish movements (Tierney, 2011; Benhaim et al. 2012; Melvin et al. 2017). Swimming behaviour (locomotor activity) of fish is considered to be highly relevant for establishing fish survival in natural environment, including food search, response to predators or successful reproduction (Brodin et al. 2014; Gui et al. 2014). Meanwhile, fish respiratory parameters, such as gill ventilation frequency, depth or amplitude of ventilation and coughing (gill purge reflex) rate, are known to be relatively sensitive to water contamination (Wang et al. 2013; **Paper IV**).

An increasingly popular approach to the impact assessment of aquatic pollutants involves examining changes in behaviour of test fish over a short period of time (Melvin, 2017). However, there is a lack of studies into the basic behavioural patterns of test species in the course of time and the relevance and efficacy of different endpoints commonly used in early toxicity assessment of ambient surface waters, effluent discharge or chemical mixtures (Robinson, 2009; Brandão et al. 2015; Kochhan et al. 2015; Xie et al. 2015; Ren et al. 2016). More consideration is required to gain a better understanding of baseline behaviour and sources of variability so as to validate experimental design choices and ensure quality of behavioural data (Melvin, 2017; O'Neill et al. 2018; Deakin et al. 2019).

In some cases, behavioural responses or response patterns of the fish exposed to contaminants or chemical mixtures may be overlooked due to the inappropriate selection of behavioural endpoints for short-term (e.g. few hours) testing, and that may influence the outcomes and interpretations in behavioural toxicology (Sárria et al. 2011; Melvin, 2017). Comparative analysis of a wide range of endpoints and their dynamic changes in combination with reasonable exposure duration and observation interval is required to provide a more accurate representation of the results and to reveal the possible mechanisms and pathways associated with specific behavioural responses of certain fishes to different aquatic pollutants (Calfee et al. 2016; **Paper IV**).

1.4 Glucose level as a stress indicator

The measurement of stress responses in aquatic organisms may provide early warning signals of the adverse effects that chemical contaminants produce on the aquatic environment (Hook et al. 2014. Fish response to stress is characterized by a series of biochemical and physiological changes, causing release of stress hormones into the bloodstream (Zahangir et al. 2015). Stress hormones (e.g. cortisol and catecholamines) released by the endocrine system (the primary response) in stressful conditions activate glucose production through gluconeogenesis (the secondary response) leading to the elevation of glucose level in fish blood (Nakano et al. 2014).

Blood-glucose measurements along with the behavioural analysis performed within a toxicity field also showed their usefulness for stress evaluation in short-term toxicity tests (Jiang et al. 2017; **Paper III**). Blood-glucose measuring is simple, fast and cost-effective compared to other biochemical endpoints, e.g. cortisol measurement. Blood-glucose elevation is described as a non-specific physiological-biochemical stress response to chemical stimuli (Jiang et al. 2017). Glucose as an important indicator of stress is continuously being monitored using *in vivo* blood analysis following acute or chronic exposure to different stress stimuli, e.g. physical stress (Lowe and Davison, 2005), handling (Welker et al. 2007) and chemical substances (Gagnon et al. 2006; Miller et al. 2007; Nageswara et al. 2017). However, blood-glucose measurement for acute stress evaluation was indicated as an inaccurate indicator (Iversen et al. 2003; Perez-Casanova et al. 2008; Fast et al. 2008; **Paper II** and **V**). Meanwhile, *ex vivo* [e.g. fecals (Felix et al. 2013), mucus (Guardiola et al. 2016), and water (Fanouraki et al. 2008)] measurements of biochemical (glucose, cortisol) stress indicators were indicated as more promising techniques.

This thesis has investigated blood glucose level changes over time in the fish species exposed to different chemical mixtures and has evaluated the new approach to the non-invasive (in fish holding water) measurement of glucose as a reliable indicator of stress in the fish exposed to chemical stimuli.

1.5 Effects of pollutants on fish behaviour

Effects of aquatic pollutants can be evaluated at various levels of biological organization, including physiological and biochemical levels, cells level, the level of individual organisms and the population level. Observations of behaviour give a unique ecological perspective, in which physiological, biochemical and ecological consequences of environmental pollution are interrelated (Steinberg, 2003; Weis et al. 2001). Behavioural and physiological alterations can be viewed as early warning signals (EWS) and indicators of exposure to toxic chemicals and their complex mixtures (Weis et al. 2001).

High concentration of metals adversely affect various organs/systems in fish and can alter synthesis and release of stress hormones (e.g. cortisol and catecholamines) or other metabolites (e.g. glucose), which may be associated with behavioural changes (Kuz'mina and Garina, 2001; Øverli et al. 2002; Scott and Sloman, 2004; Martínez-Porchas et al. 2009; Mishra and Mohanty, 2009; Archard et al. 2012). Metals also may affect fish olfactory neurons and, as a result, cause olfactory-evoked avoidance or attraction response (Tierney et al. 2010; Sovová et al. 2014). Witeska et al. (2005) revealed that affected olfactory neurons in fish can inhibit gill ventilation responses. Couture and Kumar (2003) demonstrated that metals have a significant impact on the metabolic enzyme activity in fish associated with glycolysis (e.g., lactate dehydrogenase (LDH)), trichloroacetic acid (TCA) cycle (e.g., citrate synthase), and the electron transport chain (e.g., cytochrome C oxidase). Additionally, the impaired ATP production could lead to the observed hypoactivity in certain fish species. It is known that trace metal ions usually cause hyperactivity (Sandheinrich 2014), while organic matter mainly exerts a narcotic effect (hypoactivity or lethargy) in fish (Gonçalves et al. 2008; Clarke et al. 2015). Behavioural studies reviewed by Robinson (2009) indicated that various organic compounds at specific sublethal concentration may exert different fish responses such as hypo- or hyper-activity-related avoidance or attraction response, changes in intra- and inter-specific social behaviour (Robinson, 2009).

A few studies have shown correlations between behavioural and physiological indicators of toxicity and have therefore succeeded in eliminating the complicating effects faced when comparing different behavioural and physiological studies. Beauvais et al. (2001) found that exposure of *O. mykiss* to pesticide carbaryl decreased brain acetylcholinesterase (AChE) activity and this was correlated with decreased swimming behaviour. Belanger et al. (2006) revealed a causal relationship between olfactory epithelial activity and hyperventilation under exposure to chemical substances.

Toxicants may differ in their modes of action as well as in species-specific toxic effects and the behavioural responses induced (Kienzler et al. 2016). Michalec et al. (2013) argued that avoidance behaviour of organisms is not dependent on toxic properties of chemical contaminants. Some video-tracking approaches using fish locomotion are able to differentiate among fish behavioural responses induced by different pollutants (Ašmonaitė et al. 2016). The authors stated that body movements of the fish exposed to single Ag^{+} ions differ from those of the fish exposed to Ag-based nanoparticles. However, Gonçalves et al. (2008) established that behavioural patterns in locomotor activity of the fish exposed to different polycyclic aromatic hydrocarbons (PAHs) and to their mixtures are similar. Effects of contaminants on target cells, and induced behavioural responses are variable and do not show a clear pattern or consistency among species (Vanderberg et al. 2012; Sandheinrich, 2014). Such differences in fish behavioural patterns may be predetermined by physiological, ecological and behavioural traits, possibly representing different behavioural strategies of fish species towards adapting to the contaminated environment (Mittelbach, 2014). However, there is little information concerning the possibility of differentiating among patterns of fish behavioural response to the stress caused by various chemicals and their mixtures with different toxic characteristics, especially during a short period of time (Michalec et al. 2013; Wang et al. 2013; Meyer et al. 2015; Ašmonaitė et al. 2016).

To evaluate the real impacts of aquatic pollution not only on the organism but on whole ecosystems, an integrative and multidisciplinary studies is required to increase the significance and usefulness of behavioural testing as wide range biomarker for aquatic toxicology. Furthermore, to provide a better understanding of mechanistic aspects and the interaction between behavioural effects and physiological-biochemical processes associated with the regulation and production of different stress hormones and metabolites in the fish exposed to various stressors is clearly needed in future studies.

SCIENTIFIC NOVELTY OF THE THESIS

1. For the first time, the appropriate acclimation duration before actual locomotor activity testing was established for *Oncorhynchus mykiss*, *Salmo salar*, *Perca. fluviatilis* and *Gasterosteus aculeatus*.
2. For the first time, acclimation-induced differences in the locomotor activity of rheophilic (*O. mykiss* and *S. salar*) and eurytopic (*P. fluviatilis* and *G. aculeatus*) fish species were evaluated.
3. For the first time, rapidness and sensitivity of locomotor activity and respiratory responses of model (*O. mykiss*) and non-model (*S. salar* and *P. fluviatilis*) fish species to landfill leachate (LL) and the complex metal (Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} and Cr^{6+}) mixture (MIX) prepared at maximum-permissible-concentration (MPC) level were evaluated.
4. The relationship among the investigated locomotor activity endpoints of *O. mykiss* exposed to different multicomponent chemical mixtures was determined.
5. For the first time, it was established that locomotor activity and respiratory responses of the non-model species *P. fluviatilis* to MIX exposure were insensitive compared to those of the model species *O. mykiss.*
6. For the first time, significant differences in the locomotor activity and blood-glucose level of *S. salar* were established on exposure to MIX containing 10-fold reduced concentrations of essential metals Cr^{6+} or Cu^{2+}.
7. For the first time, significant glucose level changes in *O. mykiss* holding-water induced by fish exposure to MIX were found.
8. A new approach to non-invasive glucose measurement procedure for studying short-term stress responses induced by chemical stimuli in fish was developed and applied.

THEORETICAL AND PRACTICAL SIGNIFICANCE

1. The effect of acclimation duration on behavioural characteristics of fish species from different taxonomic and functional guilds was revealed, which is an important step in identifying appropriate acclimation duration before actual behavioural testing of fish.
2. The obtained results extend the knowledge of locomotor and respiratory behaviour patterns of different fish species exposed to multicomponent chemical mixtures.
3. MIX at MPC level and MIX with reduced concentrations of essential metals (Cr^{6+} or Cu^{2+}) significantly affect the locomotor activity of fish. These findings can be used for explaining the possible behavioural mechanisms underlying the effect of chemicals at environmentally relevant concentrations.
4. Specific locomotor activity and respiratory endpoints of Salmonids can be used as early-warning signs in water toxicity assessment.
5. Comparative data on MIX-exposed fish glucose levels in blood and in fish-holding water as well as the determined interrelation between them are useful for gaining a better understanding of the possible physiological mechanisms induced by chemical stimuli.
6. The proposed non-invasive glucose measurement procedure could be a valuable tool for stress evaluation in aquaculture, fish welfare and toxicological research.
7. The behavioural data obtained in this study will be useful for the regulation and standartization of fish behavioural testing.

THE AIM AND OBJECTIVES OF THE THESIS

The aim of the thesis was to experimentally investigate the effects of acclimation duration and the impact of exposure to multicomponent chemical mixtures on different fish species' behavioural, respiratory characteristics as well as on the glucose content in fish blood and fish holding-water and to evaluate rapidness and sensitivity of the tested biological responses to mixtures.

Thesis objectives were as follows:

1. To evaluate the locomotor activity of fish during acclimation and to determine appropriate acclimation duration for different fish species.
2. To determine acute toxicity of landfill leachate (LL) and complex metal mixture (MIX) to different fish species.
3. To evaluate patterns of locomotor activity and respiratory responses of different fish species depending on LL and MIX concentrations, exposure duration and fish age/size.
4. To assess the importance of changes in concentrations of essential metal (Cr^{6+} or Cu^{2+}) in MIX for the observed locomotor activity in *S. salar*.
5. To evaluate changes in fish locomotor activity and respiration in response to LL- and MIX- exposure in terms of rapidness and sensitivity.
6. To evaluate glucose level in *O. mykiss* blood and holding-water as an indicator of LL- and MIX-caused stress.

STATEMENTS TO DEFEND

1. The time needed for different fish species to acclimate before actual toxicity testing is variable.
2. Fish behavioural and respiratory responses to exposure depend on LL and MIX concentrations, exposure duration, fish age/size.
3. 10-fold reduction of essential metals' (Cr^{6+} and Cu^{2+}) concentrations in MIX induce significant differences in *S. salar* behaviour and blood-glucose level.
4. Angular velocity is the most sensitive locomotor activity endpoint in *O. mykiss* exposed to LL and MIX.
5. Gill ventilation frequency (GVF) is the most sensitive and rapid endpoint among the locomotor and respiratory responses examined in LL- and MIX-exposed *O. mykiss*.
6. *Perca fluviatilis* is behaviourally insensitive to MIX exposure.
7. Changes in *O. mykiss* blood-glucose level in response to exposure to LL and MIX are insignificant.
8. Changes in glucose level in *O. mykiss* holding-water in response to MIX exposure show that water-glucose as an endpoint is more sensitive than blood-glucose level, and more sensitive than the tested locomotor activity and respiratory endpoints.

2. MATERIALS AND METHODS

The simplified scheme of this thesis experimental design (including the fish species used, tested endpoints and test chemicals) is presented in Table 1.

The fish species used for experimental testing and their maintenance are described in each paper (**Papers I-IX**).

The effects of multicomponent mixtures such as landfill leachate (LL) and complex metal mixture (MIX) on mortality, behavioural and biochemical endpoints were investigated (**Papers I-IX**).

Hexavalent chromium (Cr^{6+}) as a reference toxicant is recommended for use in standard toxicity tests (ISO 7346-1:1996a; ISO 7346-2:1996b; ISO 7346-3:1996c; EPA 2002a, 2002b) and was used in behavioural testing. Cr^{6+} as a pollutant is important because it is referred to as a selected water quality indicator (SCORECARD 2005) (**Paper II**).

LL samples were collected in October 2016 from the drainage collection reservoir at the Kairiai landfill (WGS: 118.55°55′46.74"N, 23°23′28.4"E). The sampling procedure was conducted in accordance with the Operating Procedure for Wastewater Sampling (EPA, 2013) (**Paper I, II, VI, VIII** and **IX**).

Six sampling sites (No. 0, 1, 2, 3, 4 and 5) in the neighbouring hydro-ecosystem around the Kairiai landfill were selected at increasing distances from the leachate-holding reservoirs in the drainage channel, the Ginkūnai pond and the creek, the distances being about 10, 800, 1300, 2200, 2900 and 3200 meters along the water flow from the reservoirs, respectively (**Paper I**).

MIX was prepared in accordance with the Maximum-Permisible-Concentrations (MPC) of metals ions (Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{6+} mg/L, respectively) accepted for inland waters in EU (Directive 2008/105/EC). Each metal concentration in the MIX (Pb^{2+}, – 0.014, Cu^{2+}– 0.01, Cd^{2+}– 0.0015, Ni^{2+}– 0.034, Zn^{2+} – 0.1, Cr^{6+}– 0.01 mg/L, respectively) solution was considered as the primary test concentration of the metal mixture. Arrows (↑) indicate an increase in the concentration of each metal in a mixture solution, i.e. it was increased compared to the primary concentration of MIX solution (**Paper III-V** and **VII**).

The methods employed for measuring concentrations of elements in LL and MIX, in experimental water and deep-well (control) water are described in respective **Papers I-IX**.

Acute toxicity assays for 96 h LC_{50} determination in fish were performed following the procedure (ISO 7346-2:1996b) (with some modifications) described in **Papers IV, VIII** and Montvydienė et al. (2019) (submitted).

The methods used for locomotor activity testing are described in **Papers II-IV**, **VI**, and **IX;** and those used for respiratory testing in **Papers I and IV**. Locomotor activity and respiratory endpoints were assessed using video tracking software: non-comercial *Fish tank monitor*, which was originally created and developed at the Nature Research Center (Lithuania), and the commercially available *Ethovision XT* (Noldus Information Technology, the Netherlands) (Fig. 1 and 2).

The experimental procedure used for the behavioural testing of fish during acclimation was described in **Papers II** and **III.** The experimental procedure for collecting behavioural data (unpublished) on fish acclimation is described in detail in Makaras et al. (2019) (submitted).

The method for blood-glucose (β-D-glucose) level evaluation in fish blood is described in **Papers III**, **V** and **VI,** and the method employed for glucose evaluation in fish holding-water is described in detail in **Paper V**.

Water toxicity classification on fish used by comparing behavioural endpoints with standard (reference) toxicity test results through regression analysis was described in **Paper I**.

The statistical methods employed are presented in detail in each **Paper** (**I-IX**).

The Ethical Commission of the Baltic Laboratory Animal Science Association, Lithuania, approved experimental procedures; Lithuanian State Food and Veterinary Office (permit no. G2-69; 2017.07.25). All experiments on fish were conducted in accordance with local and EU regulations (Directive 2010/63/EU).

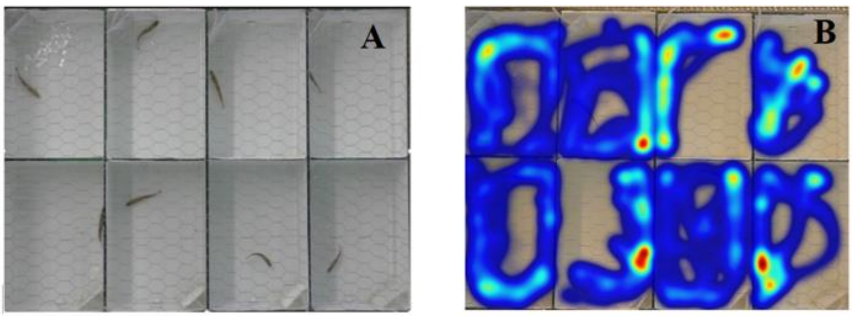
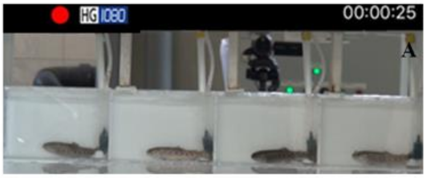


Fig 1. Video recording (A) and analysis (B) of *O. mykiss* locomotor activity using Ethovision XT software (Noldus Information Technology, the Netherlands).

1 pav. *Vaivorykštinio upėtakio lokomotorinio aktyvumo registravimas (A) ir analizė (B) naudojant Ethovision kompiuterinę programą (Noldus Informacinės sistemos, Nyderlandai).*



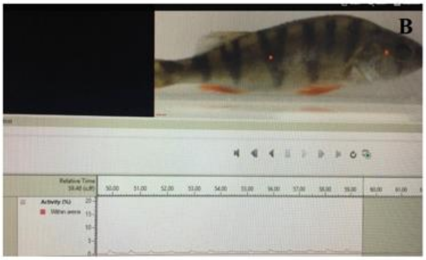


Fig. 2 Video tracking of *O. mykiss* respiratory responses (A) and respiratory analysis of *P. fluviatilis* (B) using Ethovision XT software (Noldus Information Technology, the Netherlands).

2 pav. *O. mykiss kvėpavimo rodiklių registravimas (A) ir P. fluviatilis kvėpavimo rodiklių analizė (B) naudojant Ethovision kompiuterinę programą (Noldus Informacinės technologijos, Nyderlandai).*

Table 1. Scheme of the thesis experimental design (including fish species, test chemicals, and biological endpoints) used for fish behavioural and biochemical testing.

1 Lentelė. *Eksperimentinių bandymų dizainas (žuvų rūšys, cheminės medžiagos ir biologiniai rodikliai) taikytas žuvų elgsenos ir biocheminiuose tyrimuose.*

Fish species	**Test chemicals**	**Endpoints**	**Exposure duration**	**Observational timeframes**	**Paper**
Oncorhynchus mykiss *Salmo salar* *Perca fluviatilis* *Gasterosteus aculeatus*	Control (deep-well) water	Average velocity (cm/s) Maximum velocity (cm/s) Angular velocity (deg/s)	4 h	10 min	**II**, **III** and unpublished data
Oncorhynchus mykiss	Sampling sites*	Coughing rate (part of 96 h LC_{50})	1 h	1 min	**I**
	LL, Cr^{6+}	Locomotor activity (Response index (RI) value from 0 to 100)	30 min	After 5, 10 and 30 min	**II, IX**
		LC_{50} (%)	96 h	24 h	**VII** and unpublished

					data
	LL	Average (cm/s) Maximum (cm/s) Angular (deg/s) velocity Movement duration (%)	2 h	10 min	**Paper VI**
		Blood-glucose (mmol/L)	2 h	2 h	
	MIX	Blood-glucose (mmol/L) β-D-glucose in water (μmol/L)	3 h	1 h	**V**
Oncorhynchus mykiss *Perca fluviatilis*	MIX	LC_{50} (%)	96 h	24 h	**IV**, **VII**
		Average velocity (cm/s) Maximum velocity (cm/s) Angular velocity (deg/s) Movement duration (%) Gill ventilation frequency (GVF) (count/min) Coughing rate (CR) (count/min)	2 h	10 min	
Salmo salar	MIX, Cr↓, Cu↓	Average velocity (cm/s) Movement duration (%)	2 h	10 min	**III**
		Blood-glucose (mmol/L)	2 h	2 h	

*The hydro-ecosystem incorporated in the landfill area consisted of six sampling sites (No. 0, 1, 2, 3, 4, 5), which were chosen at increasing distances from the leachate-holding reservoirs in the drainage channel, the pond and the creek, the distances being about 10, 800, 1300, 2200, 2900 and 3200 meters along the water flow from the reservoirs, respectively.

3. RESULTS AND DISCUSSION

The main results are presented in this section. The detailed results are given in each publication (**Papers I–IX**).

3.1 Behavioural differences among different fish species during acclimation

3.1.1 Effect of acclimation duration on locomotor activity of different fish species

The effect of acclimation duration (4 h) on behavioural characteristics of fish species from different taxonomic and functional guilds: rheophilic salmonids rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), and eurytopic European perch (*Perca fluviatilis*) and three-spined stickleback (*Gasterosteus aculeatus*) was investigated. Salmonids were obtained from recirculating aquaculture systems, and *P. fluviatilis* and *G. aculeatus* from ponds. Suitable acclimation duration for the test fish species and fish behavioural patterns (locomotor activity) during acclimation were determined based on specific endpoints (average, maximum and angular velocity). This study demonstrated that fish change their swimming behaviour over time fundamentally, and that acclimation duration may influence behavioural data analysis and interpretation before toxicity testing.

Results of the experiment on *O. mykiss* locomotor activity revealed significant differences in such tested endpoints as average, maximum and angular velocity, although maximum velocity showed lower variability over time than the other endpoints. According to average and angular velocity endpoints, the locomotor activity of *O. mykiss* significantly decreased during 3–4 h of acclimation (unpublished data). Additionally, the analysis of *O. mykiss* locomotor activity endpoints showed a significant decrease in fish average velocity after 2 h of acclimation, the same change in movement duration endpoint being observed after 1 h of acclimation. However, the average velocity was found to be stable in relation to time compared to movement duration (**Paper II**: Figs. 1A and B).

The locomotor activity of *S. salar* showed a similar behavioural tendency to that of *O. mykiss* (unpublished data). A significant decrease in locomotor activity was determined after 3–4 h of acclimation, while changes in angular velocity data were established a bit earlier, i.e. within 2 h of acclimation. Additionally, the most stable variation in average velocity and movement duration endpoints between data time points (every 10 min) was recorded after 2 h of acclimation (**Paper III**: Figs. 7 A and B).

However, *P. fluviatilis* showed the opposite behavioural tendency to that of salmonids. The locomotor activity of the fish transferred into new experimental conditions initially decreased, after which it started increasing during approximately the first hour of acclimation (unpublished data).

Meanwhile, behavioural endpoints of *G. aculeatus* did not change significantly over time, although slight behavioural changes were observed during the first 30 min of acclimation (unpublished data). The differences observed in behavioural data of *P. fluviatilis* and *G. aculeatus* show that their locomotor activity was more stable (less variable) over time than that of the tested salmonid species.

3.1.2 Behavioural differences among fish species during acclimation

The behavioural data, which were collected within 1 h observational timeframes, were analyzed to compare behavioural differences between unacclimated and acclimated fish species during the 4 h acclimation period (unpublished data). Fish were accepted as acclimated when locomotor activity reached the baseline activity level.

Based on the selected endpoints, the data obtained demonstrated clear differences between behavioural characteristics of rheophilic and eurytopic fish species during the 4 h acclimation period (unpublished data). These differences mainly lay in locomotor activity changes during acclimation, which in salmonids decreased, in *P. fluviatilis* increased and in *G. aculeatus*, initially decreased and afterwards increased. Significant behavioural differences among the test species were found only in average velocity and angular velocity, while maximum velocity showed very high behavioural data variance indicating no significant behavioural changes among the test species during the acclimation testing.

The average velocity of unacclimated *O. mykiss* and *S. salar* was found to be significantly different from that of *P. fluviatilis* and *G. aculeatus.* The locomotor activity of acclimated *O. mykiss* and *S. salar* significantly decreased compared to that of the unacclimated fish at the beginning of the experiment (unpublished data). The locomotor activity of unacclimated and acclimated *O. mykiss* and *S. salar* were similar, no significant differences between these species being observed in all the tested endpoints (unpublished data). No significant changes in behaviour of unacclimated and acclimated *P. fluviatilis* and *G. aculeatus* fish were found (unpublished data). The values determined for unacclimated *P. fluviatilis* significantly differ from those of other test species indicating the lowest locomotor activity of the fish *(P. fluviatilis),* while after normalization to the baseline activity level, they were within a close range of those of salmonid species (unpublished data). The locomotor activity of acclimated *G. aculeatus* was found to be significantly higher than that of *O. mykiss, S. salar* and *P. fluviatilis.*

Overall, the results of this study revealed that the effect of acclimation period on rheophilic species such as *O. mykiss* and *S. salar* was greater than on eurytopic species *P. fluviatilis* and *G. aculeatus*, indicating that acclimation period is important in managing fish stress before the onset of behavioural observations.

3.1.3 Appropriate acclimation period for different fish species

The obtained results revealed that in order to adjust fish locomotor activity to more stable baseline levels (fish accepted as acclimated), acclimation of *O. mykiss* and *S. salar* should last at least for several hours (2 h), and that of *P. fluviatilis* for at least 1 h (**Paper II, III** and unpublished data). Although no significant behavioural changes were established for *G. aculeatus*, a short period (e.g. 30 min) of acclimation could be applied to this fish due to the slight changes in locomotor activity observed during a 30 min acclimation period (**Paper VII**).

The effect of acclimation on behaviour of rheophilic fish (*O. mykiss* and *S. salar*) species was strong compared to that of eurytopic fish (*P. fluviatilis* and *G. aculeatus*). The obtained results indicated that acclimation duration is important in managing fish stress before starting behavioural observation.

For the first time, differences in behaviour of rheophilic (*O. mykiss* and *S. salar*) and eurytopic (*P. fluviatilis* and *G. aculeatus*) fish species, which are distributed in the temperate climate zone, during the acclimation period were evaluated. Only very few studies have investigated the effect of acclimation on such eurytopic fish species as adult mosquitofish (*G. holbrooki*) and guppy (*P. reticulate*), which are distributed in the tropic climate zone (Melvin et al. 2017; O’Neill et al. 2018). Moreover, these species are widely used in behavioural toxicity testing, while in our case, such test species as *S. salar*, *P. fluviatilis* and *G. aculeatus* have been rarely used in such a type of research (Sergy and Scroggins, 1990; Wibe et al. 2001; Saaristo et al. 2019).

According to Melvin et al. (2017), most of the studies assessing locomotor activity, learning/memory and anxiety, acclimate different fish species for less than 1 h. In cases of inappropriate duration of fish acclimation (e.g. duration is too short), the fish behaviour observed may not reflect the actual impact of toxicants due to the interference of effects caused by both stressors (new environment and toxicants) leading to high variability of fish behaviour. On the other hand, longer acclimation period may be required for the detailed characterization of fish behavioural patterns.

Since behavioural testing is not clearly standardized and experimental setup may depend on the aim of the study, acclimation period setup in toxicity testing remains an important issue across studies. Overall, the acclimation period may vary among fish species from different ecological groups, thus it is necessary to establish appropriate acclimation period before starting actual behavioural toxicity experiments.

3.2 Acute toxicity of chemical mixtures to fish species

The ecotoxicological effect of LL and MIX on mortality of different development stages of fish was investigated (**Paper IV, VII** and **VIII**).

LL exposure. The determined 96 h LC_{50} (SD values) of LL to different development stages (fry, juveniles (0+) as well as to elder juveniles (1+) of *O. mykiss* varied from 3.0 % (0.6) to 3.6 % (0.3). However, no significant differences in sensitivity to LL were found among different development stages of *O. mykiss* indicating fish tolerance to LL irrespective of the tested fish development stage. The calculated toxic units (TU) for LL showed that this mixture is highly toxic to all the tested development stages of *O. mykiss* and therefore, toxicity of the tested LL can be assigned to Ecotoxicity Class IV (highly toxic) (**Paper X**; unpublished data).

MIX exposure. Acute (96 h LC_{50}) toxicity of MIX was tested to evaluate sensitivity differences between MIX-exposed rheophilic (model) and eurytopic (non-model) fish species, represented by *O. mykiss* and *P. fluviatilis*, respectively. The observed 96 h LC_{50} (95 % CI) values for MIX-exposed *O. mykiss* and *P. fluviatilis* were 50.1 % (45.5-55.8 %) and 26.8 % (22.5-31.8 %), respectively, compared to the primary MIX solution (MIX concentration equal to 1 %) prepared in accordance with maximum-permissible-concentrations (MPC) accepted for inland waters in EU (Directive 2008/105/EC) (**Paper IV**: Table 3). Acute toxicity results showed that *P. fluviatilis* individuals were approximately 1.9-fold less tolerant to MIX exposure than those of *O. mykiss* (**Paper IV**: Table 3; **Paper IX**).

The effect of LL on all development stages of *O. mykiss*, except for the embryo stage, was uniform suggesting much broader negative implications for this species dynamics and food web structure as well as a lack of development stage-related resistance to harmful compounds. The sensitivity of early development stages of fish to the impact of LL was also noted in some studies by other authors (Alkassasbeh et al. 2009; Emenike et al. 2012; Qiu et al. 2016).

Some acute toxicity studies using perch and trout species got the opposite results. Svecevičius (2006) determined that the 96-h LC_{50} values of Cr^{6+} for *P. fluviatilis* and for *O. mykiss* were within a close range. According to the findings reported by Lacroix and Hontela (2004) and Couture et al. (2008), yellow perch (*Perca flavescens*) was notably less resistant to metals exposure than *O. mykiss*. Taylor with co-authors (2003) established that according to 96- h LC_{50}, *P. flavescens* was more resistant to Cu than *O. mykiss*. The reason why the 96 h LC_{50} value for *P. fluviatilis* was found to be lower than that for *O. mykiss* is probably that perch was farmed in ponds, whereas *O. mykiss* was hatchery-reared.

Turschwell and White (2016) demonstrated that an organism's housing environment can cause significant plastic changes in fitness-related traits including brain size and resting metabolic rate (RMR). Hatchery-reared and laboratory-housed fish were described as having a significantly higher RMR than wild-caught fish. The data obtained in this thesis allow hypothesizing that hatchery rearing and housing may lead to the evolution of adaptive self-regulation mechanisms in fish, explaining *O. mykiss* susceptibility. Reduced sensitivity may be a physiological response to the chronic stress of being housed, to commercial feed and its additives and other stress stimuli during hatchery rearing. Therefore, decreased sensitivity of hatchery-reared fish species to pollutants can be suspected. Moreover, Turschwell and White (2016) concluded that due to the current standard laboratory housing conditions, captive animals may be non-representative of their wild populations.

The contrasting acute toxicity results could generate a discussion of the hypothesis that over time, the animals lab-reared for breeding and keeping and fed with artificial leash can adapt and become more resistant to experimental conditions (**Paper IV**).

3.3 The impact of LL and MIX on complex behaviour of different fish species

3.3.1 Locomotor activity and respiratory alterations in LL-exposed *O. mykiss*

The impact of water of the potentially-affected hydro-ecosystem, neighbouring the closed Kairiai landfill, on the respiratory behaviour of *O.*

mykiss and the extent of toxicity attenuation recorded with increasing distance from the landfill in the direction of water were evaluated (**Paper I**).

The data reported in **Paper I** showed that water samples from the drainage channel (sites No. 0) increased the coughing rate (CR) of *O. mykiss* and were found to be extremely toxic (equal to 0.86 part of 96 h LC_{50}) to the test fish. Water samples from the drainage channel (No. 1) and Ginkūnai pond (No. 2) were „moderately toxic“ (equal to 0.28 and 0.26 part of 96 h LC_{50}, respectively (**Paper I**: Table 1). Toxicity of water samples from the Ginkūnai pond (No. 3 and 4) and Švedė creek (No. 5) to *O. mykiss* was found to be low (**Paper I**). The relationship between the integral toxicity level of test water and the distance from leachate-holding reservoirs was established.

These results suggest that landfill leachate, which flows from holding reservoirs to the drainage channel and further into the Ginkūnai pond and Švedė Creek, is still extremely toxic. Sampling site water toxicity is significantly related to the distance from the pollution source. Water toxicity decreases with increasing distance from the pollution source (**Paper I**).

Alterations in locomotor activity of *O. mykiss* (3-month old) exposed to sublethal concentrations of LL and Cr^{6+} were investigated at selected test-intervals (after 5, 10 and 30 min of exposure), making it possible to differentiate and evaluate behavioural fish response patterns to multicomponent mixtures and single metal ions.

At all test intervals, the locomotor activity of the fish exposed to leachate concentrations of 0.05 %, 0.1 % and 0.2 % relevantly increased (**Paper II**: Fig. 2). Significant differences in fish behaviour were established after 5 min of exposure reaching a peak within 10 min (1.7-fold higher than no-response level) (**Paper II**: Fig. 2b; **Paper IX**). The increased locomotor activity of the fish remained significantly higher than the no-response level until the end of the testing session.

The highest tested Cr^{6+} concentration (2 mg/L) was found to induce a significant increase (from 1.5 to 1.6-fold higher than no-response level) in the locomotor activity of the fish at all test-intervals (**Paper II**: Fig. 2). At 1 mg/L concentration of Cr^{6+}, a similar locomotor activity response was observed in fish after 5 min (1.5-fold higher than no-response level) of exposure, reaching a peak within 10 min (1.6-fold higher than no-response level).

It was found that LL-exposed fish were more responsive than those exposed to Cr^{6+} (**Paper II**: Fig. 2). The data showed that the locomotor activity of fish depends on a toxicant and its concentrations, but not on the selected exposure duration.

Fish response to sublethal concentrations of LL and Cr^{6+} manifested itself in the following locomotor activity changes: (1) increased locomotion (2) peak locomotor activity (3) decreased locomotion.

Michalec et al. (2013) argued that avoidance behaviour of organisms does not depend on toxic properties of chemical contaminants. However, it is known that in a short period of time, most organic compounds exert a narcotic effect (hypoactivity or lethargy) or cause a hyperactivity syndrome in fish, while trace metal ions usually cause hyperactivity (Sandheinrich 2014). Additionally, chemicals found in mixtures can synergistically interact producing a cumulative effect on fish behaviour, which is stronger than that of single components (Svecevičius 2012). Some approaches of fish locomotion video-tracking are able to differentiate between different pollutants-induced fish behavioural responses (Ašmonaitė et al. 2016). The authors stated that body movements of fish exposed to single Ag^{+} ions differ from those of the fish exposed to Ag-based nanoparticles. Gonçalves et al. (2008) established that behavioural patterns of the fish exposed to different PAHs and to their mixtures are similar. The data reported in **Paper II** show that the behavioural response patterns of *O. mykiss* elicited by different pollutants (mixture of organic and inorganic compounds (LL) and single metal ions (Cr^{6+})) cannot be differentiated.

The behavioural results obtained proved effectiveness of the test in providing valuable information about the time required for the test species to elicit behavioural responses to pollutants reflecting species sensitivity to the latter (**Paper II**). Moreover, results of this thesis suggest that to elicit significant responses of the fish (in this case *O. mykiss*) to the impact of single metal ions (Cr^{6+}) or sublethal concentrations of complex mixtures (LL), it is sufficient to track fish locomotor activity for as little as 1 h (usually within 30-min).

To enhance the understanding of time-related changes in *O. mykiss* behavioural characteristics, and relationships among behavioural endpoints of the fish subjected to short-term (2 h) exposure to leachate, dynamics of different behavioural endpoints were investigated, their rapidness and efficacy being compared (**Paper VI**).

Our results revealed that the locomotor activity of *O. mykiss* exposed to LL solutions varied significantly during the first 2 h of exposure depending on the endpoints analyzed (**Paper VI**: Fig. 1; Table 4). Overall, after a 2-h exposure, almost in all treatments groups, the locomotor activity of fish showed a tendency to decrease with increasing leachate concentration.

The average velocity of the fish exposed to 0.5 % leachate solution (0.138 part of 96 h LC_{50}) significantly decreased compared to that of the control group fish , while a significant increase in angular velocity of the fish was observed under exposure to 0.25 % (0.069 part of 96 h LC_{50}) LL concentration (**Paper VI**: Fig. 1). The endpoints of maximum velocity, movement duration and body mobility did not show significant changes under exposure to different LL concentrations (**Paper VI**: Fig. 1). Only the endpoints of average velocity, maximum velocity, and angular velocity were found to be significantly affected by the interaction of LL concentration and exposure duration. (**Paper VI**: Fig. 2, Table 4). Rapid changes in fish locomotor activity (average and angular velocities) were recorded after 1 min of exposure to the highest leachate concentrations (0.25% and 0.5%) (**Paper VI**: Fig. 2). The analysis of behavioural endpoints revealed that the locomotor activity of leachate-exposed fish was mainly characterized by rotational movements associated with increased angular velocity rather than by more or less straight swimming patterns (**Paper VI**: Fig. 2).

The decreased locomotor activity of the leachate-exposed *O. mykiss* may indicate a lethargic effect (narcosis) caused by organic contaminants that are usually found in landfill leachate in high concentrations (Gonçalves et al. 2008; Clarke et al. 2015). On the other hand, the decreased locomotor activity (hypoactivity) of fish could be interpreted as a behavioural fish strategy in the presence of toxic compounds, when fish have to cope with stressful conditions (Lushchak 2011; Hamilton et al. 2017; **Paper VI**).

Several studies have investigated effects of organic compounds on certain fish species using multiple endpoints and their interrelationships (Brandão et al. 2015; Kochhan et al. 2015; Xie et al. 2015; Ren et al. 2016). However, in such studies, fish were exposed to relatively high concentrations of single organic pollutants, and not to their mixtures, which could have represented a more realistic scenario of chemicals exposure in an aquatic environment. Furthermore, the effects of organic contaminants on fish were assessed after several days of exposure. Thus, the lack of data on behavioural responses as well as the suitability and efficacy of multiple endpoints of different fish species exposed not to single contaminants but to their mixtures at environmentally relevant concentrations, especially in rapid toxicity assessment tests, calls for additional investigations.

Some studies have indicated that in fish, leachate exposure causes behavioural, cellular, physiological and biochemical alterations of varying degree (Emenike et al. 2012; Klauck et al. 2013; Salem et al. 2014; Budi et al. 2016). Investigations into LL toxicity and its effects on fish (*Cyprinus carpio*) behaviour showed that leachate causes a decline in general activity, loss of balance, breathing difficulties, excessive mucosal secretion and fish gathering at the surface for breathing (Alkassasbeh et al. 2009). However, controversial findings indicating that not all organic compounds have a lethargic effect manifesting itself in decreased locomotor activity of fish (hypoactivity) have been also reported(Brandão et al. 2015; Xie et al. 2015).

The current investigation (**Paper VI**) into behavioural patterns of *O. mykiss* showed that fish activity did not vary greatly over time, and that significant changes in fish activity were recorded during the first minutes of exposure indicating rapidness of response. Similarly, changes in the locomotor activity and respiratory behaviour of MIX-exposed *O. mykiss* were observed after 1 min of exposure (**Paper IV**). Significant changes in the swimming activity of *S. salar* under exposure to MIX were recorded after 10 min, while significant behavioural response peaks at different time points (**Paper III**). The data reported for different fish species in other studies show that significant changes in fish behaviour were observed during 1 h of exposure. Thus, the selected 2-h observational duration is the most suitable time frame for water quality monitoring based on fish behavioural changes (Svecevičius 2007, 2009; Huang et al. 2014).

The study results presented in **Paper VI** suggest that some of the endpoints used for the assessment of pollution effects on fish behaviour might be more appropriate or indicative than others. Some studies established that different behavioural endpoints may reflect different response sensitivity of fish (Chen et al. 2011; Steele et al. 2018) and therefore, in order to better evaluate pollution-induced alterations in fish behaviour, it is necessary to use a combination of different behavioural endpoints, which can reflect different toxicity mechanisms of a multicomponent mixture (**Paper IV** and **VI**).

3.3.2 Locomotor activity and respiratory alterations in MIX- exposed fish

Behavioural and respiratory responses of *P. fluviatilis* and *O. mykiss* exposed for a short period of time (2-h) to the metal (Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{6+}) mixture (MIX), which was prepared taking into consideration the maximum-permissible-concentrations (MPC) of these metals set for inland waters in EU (Directive 67 2008/105/EC) (Table 2), were investigated. The hypothesis that behavioural and respiratory responses of *P. fluviatilis* are sensitive early-warning signs of pollution and can detect metal mixture exposure effects at much lower concentrations than traditional ecotoxicological endpoints, was tested.

Results of the analyzed behavioural and respiratory responses of *P. fluviatilis* and *O. mykiss* to the tested MIX solutions during 2-h observation periods showed a decrease in fish locomotor activity with increasing concentration of MIX (**Paper IV**: Table 4; **Paper VII**). A significant decrease in the average velocity (2.23-fold) and in maximum velocity (3.4-fold) was observed in *P. fluviatilis* exposed to the highest tested concentrations of MIX20↑ as compared to the control level. Changes in the endpoints of angular velocity and movement duration in response to different treatments were found to be insignificant (**Paper IV**; Table 4; **Paper VII**).

In the case of *O. mykiss*, exposure to MIX5↑ and MIX10↑ solutions caused a significant increase (1.6-fold) only in angular velocity (**Paper IV**: Table 4). Moreover, angular velocity data demonstrated a significant effect of the interaction between exposure duration and treatment (**Paper IV**: Fig. 2). A significant increase (1.5-fold) in fish angular velocity was recorded after 1- and 10-min exposure to the highest concentration of MIX10↑ solution. Although, the locomotor activity of both MIX-exposed species showed a decreasing tendency, their behavioural activity patterns were rather different. The swimming pattern of *P. fluviatilis* was mainly characterized by more or less straight movements rather than rotational movements, whereas that of *O. mykiss* mainly by rotational movements.

The observed hypoactivity may have occurred as a result of metal-induced metabolic system impairment (Couture and Kumar, 2003; Mishra and Mohanty, 2009). These results are in agreement with the findings about *O. mykiss* exposure to LL (**Paper II**), in which case the locomotor activity significantly decreased at the beginning of exposure, remaining low until the end of the experiment. The observed hypoactivity could be explained by the behavioural adaptation strategy of fish to the polluted environment. The locomotor activity of fish is usually considered to be a non-specific response to contaminants rather than a stimulus-specific one (Svecevičius 2009). Thus, adaptive behavioural responses may enable fish to cope with stressful conditions (e.g. chemical stress).

Analysis of respiratory responses of the MIX-exposed fish (**Paper IV**: Table 4) showed that CR of *P. fluviatilis* exhibited no significant differences among treatments. Meanwhile, analysis of GVF dynamics was not feasible because the amplitude of gill movements in the tested fish was too small to be properly identified and observed through the recorded video data. Such gill movements were also recorded in non-exposed perch individuals and were considered to be normal in laboratory conditions. The analysis of changes in respiratory responses of MIX-exposed *O. mykiss* yielded more interesting results. No significant differences in fish GVF were found among treatments. However, analysis of the effect produced by the interaction of exposure duration and treatment revealed that it was significant for GVF (**Paper IV**: Fig. 2). The increased (1.25-fold) GVF was observed after 1 min of exposure to MIX2.5↑, MIX5↑ and MIX10↑, while after 10-min exposure, GVF decreased and no significant changes compared to the control level were recorded throughout the 2-h observation (**Paper IV**: Fig. 2).

The respiratory responses of *O. mykiss* and *P. fluviatilis* recorded in our study were rather different from those reported by Svecevičius (2005, 2009). In his studies, GVF and CR of MIX-exposed *O. mykiss* were found to increase with increasing concentrations of Cr^{6+} and metal mixture. Wang et al. (2013) found that single metal (Hg^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}) ions stimulated different types of respiratory reactions in different cyprinid fish species. The increased GVF observed in *O. mykiss* after 1-min of exposure to MIX is likely to be related to the interaction of metal ions with fish olfactory sensory neurons, which evoke the initial fish response to toxic substances in water **(Paper IV**). Such an explanation of the results obtained is in agreement with the findings reported by Belanger et al. (2006), who revealed a causal relationship between olfactory epithelial activity and hyperventilation under exposure to chemical substances. Meanwhile, after 10-min of exposure, GVF decreased and no significant changes compared to the control level were recorded throughout the 2-h observation period **(Paper IV**), which could be related to the chemical lesion of olfactory sensory neurons inhibiting gill ventilation responses (Witewska et al. 2005). Azizishirazi et al. (2014) also recorded impairment of olfaction in wild yellow perch *P. flavescens* chronically exposed to metals.

Some studies showed that locomotor acitivity of *O. mykiss* was a more sensitive endpoint than GVF and CR (Svecevičius 2005, 2009). Tierney et al. (2010) concluded that locomotor activity can be initiated through chemosensory irritation because fish oflcatory system is involved in formation of avoidance response. A similar study (Svecevičius 2012) on the effects caused by binary metal (Cu^{2+} and Zn^{2+}) mixture on adult *Rutilus rutilus* identified CR as the most sensitive and informative behavioural response. The controversial findings about chemicals-induced changes in fish locomotor and respiratory activity may suggest species-specificity that could be predetermined by chemosensory mechanisms and physiological compensatory-adaptive mechanisms on cellular and molecular levels, as well as contaminant-specificity including the interaction between chemicals in complex mixtures that may pose a challenge to predicting mixture toxicity, especially in short-term toxicity tests **(Paper IV**).

Overall, CR of *O. mykiss* was found to be insensitive to MIX exposure and, in the course of fish exposure to different concentrations of MIX solutions, it fluctuated. No significant differences were found in CR between control and treatment groups, although, coughing, as we observed in the control group, could be regarded as a normal trait of fish respiratory activity.

Alterations, induced by short-term (2 h) exposure to MIX and to MIX with reduced (10-fold) concentrations of essential metals Cr↓ and Cu↓, in behavioural patterns of *S. salar* were studied (**Paper III**). *S. salar* responded to MIX and Cu↓ (10-fold reduced Cr^{6+} concentration in MIX) exposure by different peaks of the average velocity endpoint, no peaks being observed in behavioural patterns of Cr↓-exposed fish. (**Paper III**: Fig. 7). Mean values of the response to Cr↓ exposure was apparently much lower than MIX and Cu↓ (10-fold reduced Cu^{2+} concentration in MIX) and did not significantly differ from control. However, the results of movement duration endpoint showed no significant difference between control and Cu↓ treatment. Only MIX treatment was found to be a significant compared to control for both tested endpoints (average velocity and movement duration) (**Paper III**: Fig. 7). The meaningful results (increase) in locomotor activity of *S. salar* to mixtures treatment were observed after 10 min of exposure, while at different time points indicating the significant peaks (the highest observed values) of the behavioural response. In addition, the response patterns of fish based on average velocity data (to MIX and Cu↓ exposure) could be characterized in the following order: (1) increased velocity, (2) peak of velocity, (3) decreased velocity.

The obtained results for *S. salar* exposed to MIX showed that interactions at low metal exposure concentrations are likely to occur and elicit toxicologically significant effects in fish behaviour (**Paper III**). However, little concern was given to understand the interaction and the effects caused by combined metals and its concentrations in complex mixture and how it may affect complex fish behaviour over the short period. Different activity patterns in swimming behaviour of fish may suggest about possible synergistic or antagonistic actions of contaminants. Analysed behavioural data reported in **Paper III** suggest that Cr^{6+} presence in the mixture at different concentrations may have a role in fish behavioural response. According to average velocity data, it was found that Cr↓ mixture (10 times reduced Cr^{6+} concentration) exhibited a weaker effect on fish behaviour compared to the Cu↓ mixture (10 times reduced Cu^{2+} concentration) exposure. Data based on recorded movement duration of the same fish showed a different significance of the response to mixtures (**Paper III**: Fig. 7).

More studies are required to ascertain what endpoints are the most appropriate for elucidating response behaviours specific to different classes of contaminants. Thus, we suppose that fish behavioural studies should cover not only contaminants-induced changes in different endpoints but also dynamic changes in fish behavioural patterns over time. In agreement with other studies (Eissa et al. 2009; Wang et al. 2013; Ašmonaitė et al. 2016), this thesis suggests that investigation of behavioural patterns in toxicity testing would be useful for the identification of possible mechanisms and pathways associated with other biological processes in an organism, and potentially for the identification of adverse effects of chemicals. Thus, it might be necessary to carry out a complex behaviour analysis using a combination of multiple parameters.

3.4 Invasive and non-invasive glucose measurement for stress evaluation in different fish species exposed to LL and MIX

3.4.1 Blood-glucose level changes in LL- and MIX-exposed fish

Blood-glucose level changes as stress indicators in different fish species (*O. mykiss* and *S. salar*) under exposure to multicomponent mixtures (LL and MIX) were investigated (**Paper III, V** and **VI**). Blood-glucose levels in LL-exposed *O. mykiss* showed a tendency to increase with increasing LL concentration after 2 h of exposure (**Paper VI**: Fig. 3). However, high variance in blood-glucose concentration was observed at each leachate concentration, no statistically significant difference being found among treatments (**Paper V:** Fig. 3).

Blood-glucose levels measured in *O. mykiss* individuals exposed to different concentration MIX solutions (**Paper V**: Fig. 3) were similar. The blood-glucose level in the fish exposed to deep-well water (control) and to different concentration MIX solutions (MIX5↓ (5-fold decreased concentration of each metal compared to primary MIX) and MIX) used to vary throughout the test irrespective of the concentration tested. Although blood-glucose levels in *O. mykiss* showed a tendency to decrease with increasing treatment concentration after 3 h exposure (**Paper V**: Fig. 3), no statistically significant differences were found among treatments (**Paper V**: Table 3).

The blood-glucose level in *S. salar* individuals exposed to different MIX solutions (MIX, Cu↓ (10-fold reduced Cu^{2+} concentration in MIX) and Cr↓ (10-fold reduced Cr^{6+} concentration in MIX)) significantly increased compared to the control level after 2 h of exposure (**Paper III**: Fig. 6). However, the amount of blood-glucose in fish exposed to different MIX solutions did not differ.

A number of studies have shown that in short-term tests the release of stress hormones and glucose into fish blood could be observed relatively fast, during the first 1-2 h of exposure to different stressors (Jentoff et al. 2005; Zahangir et al. 2014; Wu et al. 2015, **Paper V**). In some cases, changes in blood-glucose levels were recorded some days (Gagnon et al. 2006; Langiano and Martínez, 2008; Miller et al. 2007) after exposure or even were not recorded at all. Most studies reported significant elevations in levels of fish blood-glucose some days after exposure to different chemicals (Pucher et al. 2014; Hoq et al. 2015; Nageswara et al. 2017). Moreoever, blood glucose as an indicator of acute stress was evaluated as inaccurate (Iversen et al. 2003; Perez-Casanova et al. 2008; Fast et al. 2008). Although most fish follow a generalized pattern of stress response, the obtained contrasting study results reveal that induction of stress response in fish and fish tolerance to different types of stressors are species-specific.

Overall, the strongly contrasting study results suggest that blood-glucose seems to be an unreliable stress indicator and should be used only in combination with other endpoints to complement toxicity data (**Paper V** and **VI**).

3.4.2 Glucose level changes in holding-water of MIX-exposed *O. mykiss*

The analysis of changes in levels of water-glucose released by *O. mykiss* exposed to deep-well (control) water and to MIX yielded the opposite results compared to those of blood-glucose measurement (**Paper V**). Glucose levels in fish-holding water used to steadily increase with increasing test duration (**Paper V**: Fig. 3). The levels of water-glucose released by MIX- exposed *O. mykiss* significantly increased (1.9-fold) after 3 h of exposure compared to the control level (**Paper V**: Fig. 3). The content of water-glucose released by *O. mykiss* under exposure to MIX5↓ solution (5-fold reduced concentration of each metal in MIX) showed a similar tendency towards increase, no statistically significant differences being found between treated fish groups and control groups at the same time points.

Analysis of the data, collected within 3 h (every 1 h) observational time frames, on contents of water-glucose released by MIX-exposed fish revealed significant differences among different fish treatment groups (**Paper V**: Fig. 3). The estimated glucose levels released into water by MIX5↓-exposed *O. mykiss* were higher than those released by the fish exposed to deep-well (control) water. The levels of glucose released into water by the fish treated with MIX5↓ and MIX were found to differ significantly from those released by fish of the control group (**Paper V**: Fig. 3; Table 4).

These results suggest that the elevation of water- β-D-glucose level like that of cortisol may be a relatively rapid stress response. Many studies have investigated the release of corticosteroids into water by the fish exposed to different types of stressors (Scott and Ellis, 2007; Fanouraki et al. 2008; Lower et al. (2015). Measurements of biochemical (corticosteroids) stress indicators in fish using feces (Felix et al. 2013), mucus (Guardiola et al. 2016) or water (Fanouraki et al. 2008) were characterized as more promising.

Most of the above-mentioned studies examined the impact of handling or crowding stress on fish. However, to our knowledge, no studies have been conducted employing the technique of glucose measurement in fish holding-water, especially in that of the fish exposed to chemical mixtures at environmentally relevant concentrations. Thus, to confirm the reliability of glucose as an indicator of stress in fish under chemical stimuli, a new approach to non-invasive glucose measurement is necessary. Glucose measurement in water using an appropriate biosensor could be a useful tool for assessing environmental risk (ERA) and could complement the existing test batteries used for assessing contaminant exposure and effects (**Paper V).**

3.5 Comparison of different endpoints of LL- and MIX-exposed fish by rapidness and sensitivity

Dynamics of the selected rainbow trout behavioural endpoints over time and interrelations among them were studied. These behavioural endpoints were compared by response rapidness and efficacy (**Paper II-VI**). Results of this study revealed clear differences in sensitivity of the tested behavioural endpoints of different fish species (Table 4).

The locomotor activity of *O. mykiss* juveniles (3-month old, 0+) was found to be very sensitive to LL exposure, subject to significant changes under exposure to 0.05 % LL solution equivalent to 0.01 part of 96 h LC_{50}, which is 100-fold lower than the established 96 h LC_{50} for this species

(**Paper I**). The relative sensitivity of the selected endpoints was evaluated for elder juveniles (yearlings, 1+) of *O. mykiss* by comparing effective LL concentration values (**Paper VI**; Table 5). The ascertained effective LL concentration (lowest tested) values for the studied behavioural endpoints of average and angular velocity were 0.5 % (0.138 part of 96 h LC_{50}) and 0.25% (0.069 part of 96 h LC_{50}), respectively. The data showed that significant changes in the average velocity of test fish were recorded under exposure to the LL concentration that was 7.25-fold lower than the 96-h LC_{50} value for *O. mykiss* (yearlings) (data not shown). Meanwhile, significant changes in angular velocity were observed at the LL concentration that was 14.5-fold lower than the above mentioned 96-h LC_{50} value for *O. mykiss* (**Paper VI**: Table 5). These results prove that angular velocity is the most sensitive of all the endpoints used for assessing *O. mykiss* locomotor activity response to LL exposure (Table 2).

The examined differences among locomotor, respiratory and biochemical endpoints in response rapidness and sensitivity to short-term MIX exposure revealed that gill ventilation frequency (GVF) was the most rapid response of *O. mykiss* to MIX exposure (**Paper IV**: Table 5). Significant changes in GVF were recorded after 1 min of exposure to the concentration of MIX5↑ (5-fold increased concentration of each metal in MIX compared to MPC concentrations) equivalent to 0.05 part of 96-h LC_{50}, which was approximately 20-fold lower than the 96-h LC_{50} value determined for *O. mykiss*. Meanwhile, only angular velocity of all the locomotor activity endpoints tested showed significant changes (after 1 min) in response to MIX10↑ exposure (10-fold increased concentration of each metal in the mixture) equivalent to 0.1 part of 96-h LC_{50}, which was approximately 10-fold lower than the 96-h LC_{50} value for this fish (**Paper IV)**: Table 5). The level of water-glucose released by *O. mykiss* was described as the most sensitive of all the endpoints tested (**Paper V**). Significant changes in water-glucose levels were observed under exposure to the concentration of MIX5↓ equivalent to 0.004 part of 96 h LC_{50} or 250-fold lower than 96-h LC_{50} (**Paper IV**: Fig. 3)

Both behavioural endpoints (average velocity (cm/s) and movement duration (%)) investigated in *S. salar* proved to be sensitive to MIX exposure. In comparison with the control at the same time points, the average velocity of the exposed fish significantly increased after 30 min of exposure to MIX, significant changes in fish movement duration being observed after 10 min of exposure (**Paper III**: Fig. 7). *S. salar* exposed to Cu↓ showed significant changes in average velocity and movement duration after 10 min of exposure, while fish exposed to Cr↓ showed changes in these endpoints after 20 and 30 min of exposure, respectively (**Paper III**: Fig. 7). Significant changes in fish behavioural response peaks were established after 2 h of exposure to MIX and Cu↓. In addition, the level of blood-glucose in *S. salar* as an indicator of chemical stimuli-induced stress was also found to be a sensitive endpoint of the fish. In *S. salar* individuals, significant changes (increase) in blood-glucose level were established after 2 h of exposure (**Paper III:** Fig. 6).

The average and maximum velocities of *P. fluviatilis* also showed significant changes under exposure to MIX solution, the concentration of which was equivalent to 0.746 part of 96-h LC_{50}. This concentration is 1.34-fold lower than the observed 96-h LC_{50} value for *P.fluviatilis* (**Paper IV**: Table 3). As to respiratory responses of this fish, CR showed no significant changes in the course of exposure to MIX, while detection and observation of GVF was not feasible even in pre-exposed fish.

Specific endpoints of locomotor and respiratory responses of the tested salmonids were found to be highly sensitive to LL and MIX exposure, however, the studied behavioural responses of *P. fluviatilis* proved to be quite insensitive to the impact of MIX. In *O. mykiss*, the most indicative and sensitive locomotor activity endpoint was angular velocity (Table 2).

Table 2. Comparison of locomotor activity, respiratory and biochemical endpoints in different fish species under exposure to LL and different MIX solutions.

Fish species	**Test chemical**	**Response**	**Endpoints**	**Effective concentration (part of 96-h LC_{50})**	**Response time (response rapidness)**
O. mykiss (3 month old)[a]	LL	Locomotor activity	General activity (RI values)	0.05 % (0.01)	5 min
O. mykiss (yearlings)[b]	LL	Locomotor activity	Average velocity (cm/s)	0.5 % (0.138)	1 min
			Maximum velocity (cm/s)	–	–
			Angular velocity (deg/s)	0.25 % (0.069)	1 min
			Movement duration (%)	–	–
			Mobility (%)	–	–
		Biochemical	β-D-glucose in blood (mmol/L)	–	–
	Cr^{6+} (reference substance)	Locomotor activity	General activity (RI values)	0.5 mg/L 1 mg/L	10 min 1 min
	MIX	Locomotor activity	Average velocity (cm/s)	–	–
			Maximum velocity (cm/s)	–	–
			Angular velocity (deg/s)	MIX5↑ (0.1) MIX10↑ (0.2)	1 min 1 min

			Movement duration (%)	–	–
		Respiration	Gill ventilation frequency (GVF) (count/min)	MIX2.5↑ (0.05)	1 min
			Coughing rate (CR) (count/min)	–	–
		Biochemical	β-D-glucose in blood (mmol/L)	–	–
			β-D-glucose in water (μmol/L)	MIX5↓ (0.004)	3 h
S. salar	MIX	Locomotor activity	Average velocity (cm/s)	MIX	30 min (1-2 h)
			Movement duration (%)	MIX	10 min (1-2 h)
	Cu↓		Average velocity (cm/s)	Cu↓	10 min (1-2 h)
			Movement duration (%)	Cu↓	10 min
	Cr↓		Average velocity (cm/s)	Cr↓	30 min
			Movement duration (%)	Cr↓	20 min
	MIX, Cu↓, Cr↓	Biochemical	β-D-glucose in blood (mmol/L)	MIX, Cu↓, Cr↓	2 h
P. fluviatilis	MIX	Locomotor activity	Average velocity (cm/s)	MIX20↑ (0.746)	2 h
			Maximum velocity (cm/s)	MIX20↑ (0.746)	2 h
			Angular velocity (deg/s)	–	–
			Movement duration (%)	–	–
		Respiration	Gill ventilation frequency (GVF) (count/min)	Could not be observed	–
			Coughing rate (CR) (count/min)	–	–

[a] the size of *O. mykiss* individuals (0+) (length and weight, mean ± SEM) exposed to LL and Cr^{6+} was 6.35 ± 0.61 cm and 3.80 ± 0.59 g,respectively.
[b] the size of *O. mykiss* individuals (1+) (length and weight, mean ± SEM) exposed to LL was 10.2 ± 0.15 cm and 10.86 ± 0.45 g, respectively.
"–" no significant differences in fish response were established throughout exposure to landfill leachate (LL) or complex metal mixture (MIX).
MIX was prepared in accordance with the maximum-permissible-concentrations (MPC) of (Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{6+}) set for inland waters in EU (Directive 2008/105/EC). Arrows (↑) indicate how much the concentration of each metal in a mixture solution was increased compared to the primary concentration of MIX solution (equal to 1 % of MIX solution).
Cu↓ – 10-fold reduced concentration of copper in MIX.
Cr↓ – 10-fold reduced concentration of hexavalent chromium in MIX.
[a] the size of tested *O. mykiss* (0+) (length and weight, mean ± SEM) to LL and Cr^{6+} was 6.35 ± 0.61 cm and 3.80 ± 0.59 g
[b] the size of tested *O. mykiss* (1+) (length and weight, mean ± SEM) to LL exposure was 10.2 ± 0.15 cm and 10.86 ± 0.45 g
"–" no significant differences in fish response were established during landfill leachate (LL) or complex metal mixture (MIX) exposure over time.
MIX was prepared in accordance with the maximum-permissible-concentrations (MPC) of (Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{6+}) set for inland waters in EU (Directive 2008/105/EC). Arrows (↑) indicate how much the concentration of each metal in a mixture solution was increased compared to the primary concentration of MIX solution (equal to 1 % of MIX solution).
Cu↓ – 10-fold reduced concentration of copper in MIX.
Cr↓ – 10-fold reduced concentration of hexavalent chromium in MIX.

[a] *tirtų žuvų O. mykiss (ilgis ir svoris, vidurkis ± SEM) veikiant sąvartyno filtratu (LL) ir* Cr^{6+} *buvo 6.35 ± 0.61 cm and 3.80 ± 0.59 g*
[b] *tirtų žuvų O. mykiss (ilgis ir svoris, vidurkis ± SEM) veikiant sąvartyno filtratu (LL) buvo 10.2 ± 0.15 cm and 10.86 ± 0.45 g*
"–" nenustatyti reikšmingi skirtumai tarp žuvų elgsenos atsako į sąvartyno filtrato (LL) arba MIX poveikį ir kontrolės visa bandymo laikotarpį.
Metalų (Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} ir Cr^{6+}*) koncentracijos MIX mišinyje buvo paruoštos pagal nustatytas didžiausias leidžiamas koncentracijas (DLK) Europos vidaus vandenyse (Direktyva 2008/105/EC). Rodyklės (↑) rodo kiek kartų kiekviena metalo koncentracija mišinyje buvo padidinta lyginant su pradine MIX koncentracija.*
Cu↓ – 10 kartų sumažinta vario koncentracija mišinyje MIX.
Cr↓ – 10 kartų sumažinta šešiavalenčio chromo koncentracija mišinyje MIX.

This thesis has emphasized the importance of investigating and documenting the relevance and efficacy of different endpoints used for toxicity evaluation in ambient surface waters, effluent discharge and chemical mixtures. Finally, to provide truly meaningful behavioural results that are relevant to biological exposure consequences for an organism, it is essential that research be directed onto understanding linkages between specific behavioural and biochemical-physiological processes (Brandão et al. 2015; Kochhan et al. 2015; Xie et al. 2015; Ren et al. 2016). However, there is still a shortage of information on the identification of toxic effects and their dependence on the endpoints used in rapid toxicity assessment or toxic effect tests.

The summarized data on effects of environmental pollutants (multicomponent mixtures) on fish behavioural and physiological-biochemical responses in relation to exposure duration are presented in the empirical model (Fig. 3). The performance of normal behaviour by individual fish follows specific physiological sequences, which are triggered by external (e.g. chemical) stimuli acting via neural networks. Disruption of these sequences before completion is likely to result in detrimental behavioural alterations. Initiation of these sequences is also affected by numerous physiological and environmental influences. In terms of response time, behavioural responses include the first fish response aimed to reduce effects of exposure to water contamination, since behavioural changes can occur within seconds after a chemical stimulus has been encountered. Physiological-biochemical responses may also ensue, and together with central and peripheral changes in physiology determine behavioural responses to stimuli (Scott and Sloman, 2004).

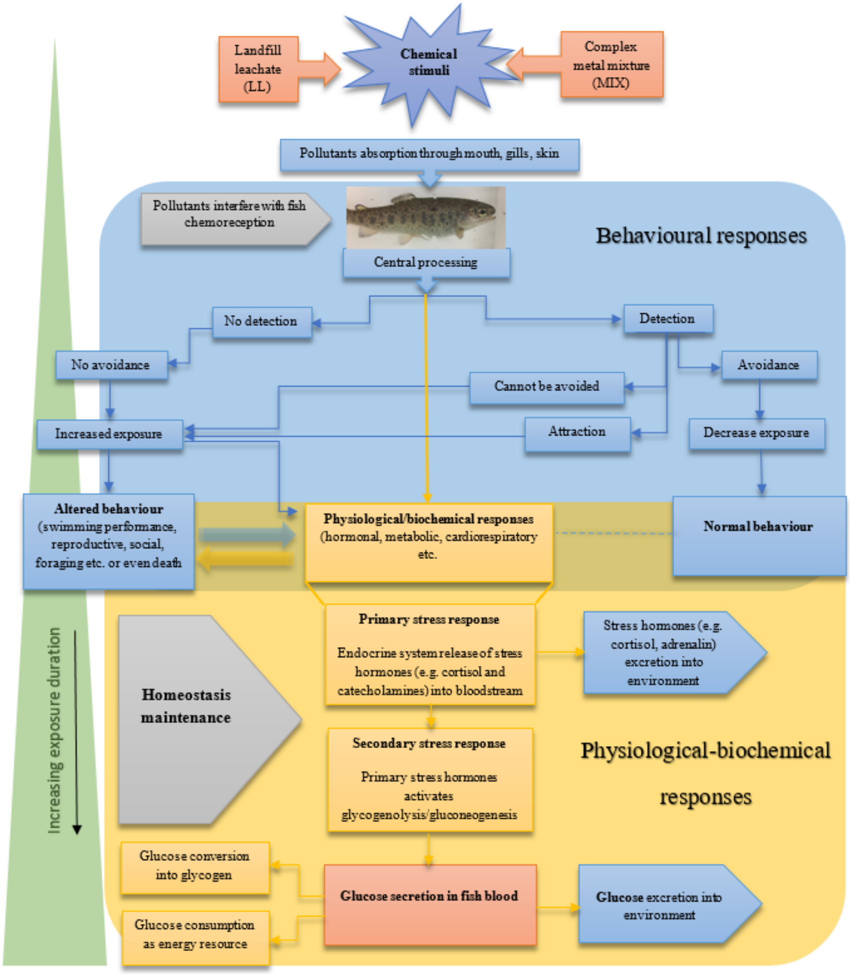


Fig. 3 The simplified scheme (empiric model) of the effects exerted by environmental pollutants on fish behavioural and physiological-biochemical responses (Modified scheme of Beitinger (1990) and Scott and Sloman (2004)).

3 pav. *Supaprastinta schema (empirinis modelis) aplinkos teršalų poveikio žuvų elgsenai ir fiziologiniams/biocheminiams procesams (papildyta schema pagal Beitinger (1990) ir Scott ir Sloman (2004)).*

Overall, findings of this thesis have revealed that chemical exposure-induced behavioural changes in certain fish species can be detected very fast, i.e. within a few minutes of exposure, which is most likely due to the well-developed olfactory system, while exposure-induced significant glucose level changes in fish bloodstream resulting in glucose release into water can occur a few hours later under exposure to much lower concentrations of chemicals than those that elicit behavioural changes. Integrative behavioural and physiological-biochemical studies could provide a better understanding of the inter-relationships and mechanistic aspects of the biological processes taking place in different fish species exposed to aquatic pollutants.

FOR MORE DETAILED RESULTS AND DISCUSSION SEE PAPERS I-IX

CONCLUSIONS

1. The duration needed for the rheophilic fish species (*O. mykiss* and *S. salar*) to acclimate before actual behavioural testing is 2 h and that for the eurytopic species *P. fluviatilis* is 1 h. The acclimation duration recommended for *G. aculeatus* is at least 30 min.
2. According to the 96 h LC_{50} value, tolerance of different *O. mykiss* development stages (fry, juveniles (0+) as well as that of elder juveniles (1+) to LL did not differ significantly. *P. fluviatilis* was found to be 1.9-fold less tolerant to MIX exposure (96 h LC_{50} value – 26.8 %) than *O. mykiss* (96 h LC_{50} value – 50.1 %).
3. The locomotor activity of LL-exposed *O. mykiss* juveniles (age 0+) significantly increased with an increasing LL concentration, in contrast to *O. mykiss* juveniles (age 1+), whose locomotor activity decreased. When compared by effective LL concentrations (0.05 % equal to 0.01 part of 96 h LC_{50}), *O. mykiss* juveniles (age 0+) were found to be 5-fold more sensitive to LL exposure than *O. mykiss* juveniles (age 1+)) (0.25 % equal to 0.069 part of 96 h LC_{50}).
4. Differences were found between locomotor activity response patterns of MIX-exposed *O. mykiss* individuals, whose locomotor activity decreased under exposure, and *S. salar* individuals, whose locomotor activity, on the contrary, increased. The locomotor activity of *O. mykiss* and *P. fluviatilis* significantly decreased with increasing MIX concentrations. The locomotor activity response of *O. mykiss* to MIX exposure was found to be 4-fold more sensitive than that of *P. fluviatilis*.
5. A significant increase in the average velocity and movement duration of *S. salar* exposed to MIX and MIX with 10-fold reduced Cu^{2+} concentration was observed during 1-2 h of exposure. Differences were found between locomotor activity peaks of the fish treated with MIX and those exposed to MIX with reduced 10-fold Cu^{2+} concentration.
6. Angular velocity was found to be the most sensitive locomotor activity endpoint of *O. mykiss* exposed to LL and MIX at the concentrations equal to 0.069 and 0.1 part of 96 h LC_{50}, respectively. GVF was found to be the most rapid and sensitive endpoint of all the tested behavioural and respiratory endpoints of the fish exposed to MIX (0.05 part of 96 LC_{50}). A significant decrease in angular velocity and an increase in GVF of *O. mykiss* were established after 1 min exposure to LL and MIX, respectively.

7. The average velocity and maximum velocity were the most sensitive endpoints of *P. fluviatilis* exposed to MIX (0.746 part of 96 h LC_{50}). A significant decrease in the average and maximum velocity was established after 2 h of exposure. According to 96 h LC_{50} values, the investigated behavioural and respiratory endpoints of *P. fluviatilis* were insensitive to MIX exposure.
8. Significant changes in the level of glucose (β-D-glucose) released into water by *O. mykiss* were established after 3 h exposure to MIX, while blood-glucose level in LL- and MIX-exposed fish did not change significantly over time. A significant increase in β-D-glucose in water released by *O. mykiss* was observed at the MIX concentration equal to 0.004 part of 96 h LC_{50}.
9. The β-D-glucose level in water (0.004 part of 96 h LC_{50}) was found to be a more sensitive indicator of MIX exposure-induced stress than the tested locomotor activity (for angular velocity – 0.1 part of LC_{50}) and respiratory (for GVF – 0.05 96 h part of LC_{50}) endpoints of *O. mykiss*.

SANTRAUKA

Vienas iš Vandens pagrindų direktyvos (Vandens pagrindų direktyva, Europos Sąjunga) tikslų yra pasiekti Europos Sąjungos paviršinių vandens telkinių „gerą ekologinę būklę“ (angl. GES) ir „gerą cheminę būklę“ Europos Sąjungos paviršinių vandens telkiniuose (Direktyva 2000/60/EC; Direktyva 76/464/EEC). Gera vandens ekosistemų būklė yra pagrindinis parametras, užtikrinantis ekosistemų teikiamas paslaugas (Summers ir kt. 2012). Vandens ekosistemų tvarumo palaikymas ateities kartoms yra laikomas dideliu iššūkiu mūsų visuomenei. Tai galima pasiekti tik palaikant ir atkuriant vandens ekosistemų gerą ekologinę būklę. Tačiau vandens aplinka dėl antropogeninės žmonių veiklos yra nuolat teršiama daugianariais cheminiais mišiniais, pavyzdžiui, nuotekomis ir kitomis cheminėmis medžiagomis, tačiau niekada pavienėmis medžiagomis (Saaristo ir kt. 2018; Inyinbor ir kt. 2018). Pagal Europos aplinkos agentūros duomenis (EAA, 2012), įvairios cheminės medžiagos, tokios kaip metalai, pesticidai ir kiti teršalai, lemia blogą paviršinių vandens telkinių cheminę būklę visoje Europoje. Mišiniai, sudaryti iš skirtingų cheminių medžiagų, dėl sinergetinio poveikio tarp elementų gali sukelti stipresnį toksinį poveikį vandens organizmams nei pavienės cheminės medžiagos, kurios esant žemiau leidžiamų koncentracijų aplinkoje dažnai nesukelia jokio neigiamo poveikio organizmams (SCHER ir kt. 2012; Bopp ir kt. 2018; EEA 2014).

Vienas iš pirmųjų tvaraus ekosistemos valdymo etapų yra toksinių medžiagų nustatymas vandens ekosistemoje (Bae ir Park 2014). Vandens kokybė vertinama pagal fizikocheminius parametrus ir vandens kokybės ilgalaikį monitoringą (Bae ir Park 2014; Dusabe ir kt. 2019). Tačiau, tik analizuojant fizikocheminius parametrus, sudėtinga tiksliai įvertinti tiriamų mišinių sinergetinį ar antagonistinį poveikį vandens organizmams. Todėl, norint tinkamai įvertinti vandens toksiškumą, būtina atlikti biologinius tyrimus, naudojant jautriausias vandens organizmų rūšis ir jų atsako rodiklius, kurie yra informatyvūs ne tik ūmaus toksiškumo sukeliančių koncentracijų ribose, bet ir realių gamtinėje aplinkoje esamų medžiagų koncentracijų ribose (Connon ir kt. 2012; **V publikacija)**.

Siekiant greitai aptikti toksines medžiagas vandenyje ir įvertinti jų koncentracijos pavojingumą vandens biotai, vis dažniau toksinių medžiagų ir jų mišinių poveikio vertinimui naudojama tiriamų gyvūnų elgesenos pokyčių per trumpą laiką analizė (Legradi ir kt. 2018). Tam tikrų žuvų rūšių plaukiojimo ir kvėpavimo pokyčių vertinimas yra labai jautrus aplinkos vandens užterštumo rodiklis (Sárria ir kt. 2011; Wang ir kt. 2013). Kartu su elgsenos tyrimais, kiti streso vertinimo rodikliai (pavyzdžiui, kortizolis ir gliukozė) taip pat parodė savo tinkamumą trumpalaikiuose toksiškumo bandymuose vertinant streso poveikį organizmams (Gesto ir kt. 2015; Guest ir kt. 2016; Jiang ir kt. 2017; Nageswara ir kt. 2017). Gliukozės koncentracijos pokyčiai (pavyzdžiui, žuvų kraujyje) yra vienas pagrindinių žuvų ūmaus streso rodiklių, kuris jautriai ir pakankamai greitai reaguoja į kintančias aplinkos sąlygas ir vandens cheminę sudėtį (Gagnon ir kt. 2006; Miller ir kt. 2007; Nageswara ir kt. 2017; **V publikacija**). Kadangi individo elgesena integruoja biocheminius, fiziologinius ir ekologinius procesus, tokie tyrimai gali būti tinkami tiriant aplinkos taršos poveikį organizmams (Scott ir Sloman 2004).

Tiriant įvairių cheminių junginių ir jų mišinių poveikį žuvims, jų elgesenai naudojamos įvairios žuvų rūšys (Shinn ir kt. 2015; Mora-Zamorano ir kt. 2017; Norton ir kt. 2019; Saaristo ir kt. 2019). Kadangi eksperimentiniuose tyrimuose buvo naudotos žuvų rūšys, priklausančios skirtingoms taksonominėms, ekologinėms ir funkcinėms grupėms, vandens toksinis poveikis žuvims gali skirtis ir priklausyti nuo rūšies. Vaivorykštinis upėtakis (*Oncorhynchus mykiss*) ir atlantinė lašiša (*Salmo salar*) yra laikomi komerciškai reikšmingomis žuvų rūšimis. Be to, *O. mykiss* yra laikoma modeline rūšimi, plačiai naudojama vandens toksikologijoje (EPA 2002; ASTM 2012a, 2012b, OECD, 2019), o *P. fluviatilis* ir *G. aculeatus* yra nemodelinės rūšys, tačiau jos yra reikšmingos dėl savo plataus paplitimo Europoje, bet, deja, su jomis atlikta mažai tyrimų vertinant vandens toksiškumą (Sergy ir Scroggins 1990; Wibe ir kt. 2001; Saaristo ir kt. 2019). Todėl, norint tiksliau įvertinti ar prognozuoti cheminių medžiagų ir jų mišinių poveikį vandens organizmams, būtina vertinti jų toksiškumą ne tik modeliniams eksperimentiniams objektams, bet ir plačiai paplitusioms žuvų rūšims. Gauti nauji duomenys papildys jau esamus cheminių medžiagų ir jų mišinių toksiškumo vertinimo modelius, skirtus cheminių medžiagų poveikio aplinkai rizikos vertinimui (Connon ir kt. 2012; Legradi ir kt. 2018). Tyrimai, vertinantys daugianarių mišinių sukeliamą toksinį poveikį žuvims, aiškinantys toksinio poveikio mechanizmus, nustatantys skirtingų tiriamų biologinių rodiklių tarpusavio ryšius ir jų jautrumą, yra labai svarbūs ekotoksikologiniu požiūriu ir bus aktualūs ateityje.

DARBO NAUJUMAS

1. Pirmą kartą nustatyta aklimacijos trukmė *Oncorhynchus mykiss, Salmo salar, Perca fluviatilis Gasterosteus aculeatus* žuvų rūšims yra reikalinga prieš vykdant žuvų lokomotorinio aktyvumo tyrimus.
2. Pirmą kartą buvo nustatyti lokomotorinio aktyvumo skirtumai tarp reofilinių *O. mykiss ir S. salar* ir euritopinių *P. fluviatilis* ir *G. aculeatus* žuvų rūšių aklimacijos metu.
3. Pirmą kartą įvertintas modelinių (*O. mykiss*) ir nemodelinių (*S. salar* ir *P. fluviatilis*) žuvų rūšių lokomotorinio aktyvumo ir kvėpavimo reakcijų greitis ir jautrumas veikiant jas sąvartyno filtratu (SF) ir modeliniu metalų (Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} ir Cr^{6+}) mišiniu (MIX), paruoštu pagal didžiausias į gamtinę aplinką leidžiamas metalų koncentracijas (DLK).
4. Įvertintas ryšys tarp tirtų *O. mykiss* lokomotorinio aktyvumo rodiklių veikiant žuvis skirtingais daugianariais cheminiais mišiniais.
5. Pirmą kartą nustatyta, kad tirti *P. fluviatilis* lokomotorinio aktyvumo ir kvėpavimo rodikliai yra nejautrūs mišinio MIX poveikiui, palyginti su modelinės žuvų rūšies *O. mykiss* tirtais rodikliais.
6. Pirmą kartą buvo nustatyti reikšmingi *S. salar* lokomotorinio aktyvumo ir gliukozės koncentracijos kraujyje pokyčiai mišinyje MIX 10 kartų sumažinus būtinųjų metalų Cr^{6+} ir Cu^{2+} koncentracijas.
7. Pirmą kartą buvo nustatyti reikšmingi gliukozės koncentracijos pokyčiai vandenyje veikiant *O. mykiss* mišiniu MIX.
8. Sukurta ir pritaikyta nauja neinvazinė gliukozės koncentracijos matavimo procedūra tiriant cheminių dirgiklių sukeltas trumpalaikes streso reakcijas žuvyse.

MOKSLINĖ IR PRAKTINĖ DARBO REIKŠMĖ

1. Skirtingos taksonomijos ir funkcinės grupės žuvų elgsenos ypatumų nustatymas aklimacijos laikotarpiu yra svarbus žingsnis pasirenkant tinkamą aklimacijos trukmę prieš vykdant žuvų elgsenos tyrimus.
2. Gauti duomenys suteikė naujų žinių apie skirtingų žuvų rūšių lokomotorinio aktyvumo ir kvėpavimo atsakų dėsningumus veikiant žuvis daugianariais cheminiais mišiniais.
3. Mišinys MIX, paruoštas pagal DLK, ir būtinųjų metalų (Cr^{6+} arba Cu^{2+}) sumažinimas mišinyje MIX sukelia reikšmingus žuvų lokomotorinio aktyvumo pokyčius. Šie duomenys gali būti panaudoti aiškinant galimus

žuvų elgsenos mechanizmus veikiant žuvis gamtoje nustatomomis realiomis cheminių medžiagų koncentracijomis.
4. Nustatyti specifiniai lašišinių žuvų lokomotorinio aktyvumo ir kvėpavimo rodikliai gali būti naudojami kaip ankstyvojo įspėjimo rodikliai vertinant vandens toksiškumą.
5. Įvertintas ryšys tarp gliukozės koncentracijų žuvų kraujyje ir vandenyje veikiant žuvis mišiniu MIX padės geriau suprasti poveikio, sukelto cheminio streso, fiziologinius mechanizmus žuvyse.
6. Sukurta nauja neinvazinė gliukozės matavimo procedūra, kuri gali būti pritaikyta akvakultūros, žuvų gerovės ir toksikologinių tyrimų metu vertinant žuvų patirtą stresą.
7. Žuvų elgsenos tyrimų rezultatai bus naudingi žuvų elgsenos tyrimams reglamentuoti ir standartizuoti.

DARBO TIKSLAS IR UŽDAVINIAI

Darbo tikslas: eksperimentiškai ištirti aklimacijos ir daugianarių cheminių mišinių poveikį skirtingų žuvų rūšių elgsenai, kvėpavimui ir gliukozės koncentracijai kraujyje ir vandenyje bei nustatyti tirtų biologinių reakcijų greitį ir jautrumą mišiniams.

Darbo uždaviniai:

1. Įvertinti ir palyginti skirtingų žuvų rūšių lokomotorinį aktyvumą aklimacijos laikotarpiu ir nustatyti optimalią jos trukmę.
2. Nustatyti ir palyginti sąvartyno filtrato (SF) ir daugianarių metalų mišinių (MIX) ūminį poveikį skirtingoms žuvų rūšims.
3. Nustatyti skirtingų žuvų rūšių lokomotorinio aktyvumo ir kvėpavimo reakcijų ypatumus, priklausomai nuo SF ir mišinio MIX koncentracijų, poveikio trukmės ir žuvies amžiaus / dydžio.
4. Nustatyti *S. salar* lokomotorinio aktyvumo ypatumus, 10 kartų sumažinus būtinųjų metalų (Cr ir Cu) koncentracijas mišinyje MIX.
5. Įvertinti skirtingų žuvų rūšių atsakų greitį ir jautrumą SF ir mišinio MIX poveikiui pagal lokomotorinio aktyvumo ir kvėpavimo rodiklius.

Nustatyti ir palyginti gliukozės kaip streso rodiklio koncentracijos pokyčius *O. mykiss* kraujyje ir vandenyje veikiant SF ir mišiniui MIX.GINAMIEJI TEIGINIAI

1. Aklimacijos trukmė, reikalinga skirtingoms žuvų rūšims iki poveikio daugianariais mišiniais, yra nevienoda.
2. Žuvų elgsenos ir kvėpavimo reakcijos priklauso nuo SF ir mišinio MIX koncentracijų, poveikio trukmės, žuvų rūšies ir jų amžiaus.
3. Būtinųjų metalų Cr^{6+} ir Cu^{2+} 10 kartų koncentracijos sumažinimas mišinyje MIX yra reikšmingas *S. salar* elgsenai ir gliukozės koncentracijos pokyčiams žuvų kraujyje.
4. Kampinis greitis yra jautriausias ir greičiausias *O. mykiss* lokomotorinio aktyvumo rodiklis veikiant juos skirtingais SF ir MIX mišiniais.
5. *Oncorhynchus mykiss* žiaunų ventiliacijos dažnis (ŽVD) jautriausia ir greičiausia reakcija į tiriamų mišinių SF ir MIX poveikį.
6. *Perca fluviatilis* elgsenos reakcijos yra nejautrios mišinio MIX poveikiui.
7. *Oncorhynchus mykiss* gliukozės koncentracijos pokyčiai kraujyje esant mišinių SF ir MIX poveikiui yra nereikšmingi.
8. Gliukozės koncentracijos pokyčiai vandenyje, paveikus *O. mykiss* mišiniu MIX, yra jautresnis rodiklis nei gliukozės koncentracijos pokyčiai kraujyje ir jautresnis nei tirti elgsenos ir kvėpavimo rodikliai.

TYRIMŲ MEDŽIAGA IR METODIKA

Svarbiausi šioje disertacijoje taikyti metodai (naudotos žuvų rūšys; tirti rodikliai ir cheminės medžiagos) yra pateikti 1 lentelėje. Išsami informacija apie naudotus metodus pateikta kiekvienoje **publikacijoje** (**I–XI publikacijos**).

Žuvų rūšys, naudotos eksperimentiniuose tyrimuose, ir jų laikymo sąlygos yra aprašytos kiekvienoje **publikacijoje** (**I–IX publikacijos**).

Ištirtas daugianarių mišinių – sąvartynų filtrato (SF) (angl. LL) ir metalų mišinio (angl. MIX) – poveikis žuvų mirtingumui, elgsenos ir biocheminiams rodikliams (**I–XI publikacijos**).

Šešivalentis chromas (Cr^{6+}) žuvų elgsenos tyrimuose buvo naudojamas kaip referentinė toksinė medžiaga. Jis rekomenduojamas naudoti, vykdant standartinius toksiškumo bandymus (ISO 7346-1: 1996a; ISO 7346-2: 1996b; ISO 7346-3: 1996c; EPA 2002a, 2002b). Cr^{6+} yra svarbus, kaip vienas iš vandens kokybės rodiklių (SCORECARD 2005) (**II publikacija**).

SF mėginiai buvo surinkti 2016 m. spalio mėn. iš drenažo surinkimo rezervuaro Kairių sąvartyne (WGS: 118,55° 55′46,74" šiaurės platuma, 23° 23′28,4" rytų ilguma). Mėginiai tyrimui buvo imami vadovaujantis nuotekų mėginių ėmimo procedūra (EPA 2013) (**I, II, VI**, **VIII** ir **IX publikacijos)**.

Šešios vandens mėginių ėmimo vietos (Nr. 0 ir 1 (drenažo kanalas), 2, 3 ir 4 (Ginkūnų tvenkinys) ir 5 (Švedės upelis)), priklausomai nuo atstumo, buvo pasirinktos vandens ekosistemoje aplink Kairių sąvartyną, t. y. atitinkamai maždaug 10, 800, 1300, 2200, 2900 ir 3200 m nuo Kairių sąvartyno filtrato (SF) surinkimo rezervuaro (**I publikacija**).

MIX buvo paruoštas pagal didžiausias leistinas metalų (Pb, Cu, Cd, Ni, Zn ir Cr mg/L) koncentracijas (angl. MPC), kurios yra taikomos Europos paviršiniams vandenims (Direktyva 2008/105/EB). Kiekvieno metalo koncentracija mišinio MIX (Pb^{2+}, – 0,014, Cu^{2+} – 0,01, Cd^{2+} – 0,0015, Ni^{2+} – 0,014, Zn^{2+} – 0,1, Cr^{6+} – 0,01 mg/L) tirpale buvo laikoma pradine (angl. *primary*). Rodyklės (↑) rodo, kiek kartų buvo padidinta metalo koncentracija mišinio tirpale, palyginti su pradine mišinio MIX koncentracija (**III**, **V** ir **VII publikacijos**)

Cheminių medžiagų koncentracijų (gręžinio) vandenyje bei mišiniuose SF ir MIX nustatymo metodai yra aprašyti kiekvienoje **publikacijoje** (**I–IX publikacijos**).

Ūmaus toksiškumo bandymai, nustatant 96 val. letalią koncentraciją, sukeliančią 50 proc. individų imties žūtį (96 val. LC_{50}), žuvims buvo atlikti pagal standartizuotą ISO procedūrą su kai kuriomis modifikacijomis (ISO 7346-2: 1996b) ir išsamiau aprašyti **IV, VIII publikacijose** ir Montvydienė ir kt. (2019) rankraštyje (įteiktas spaudai).

Žuvų elgsenos (lokomotorinio aktyvumo) tyrimų metodai yra išsamiau aprašyti **II–IV, VI** ir **IX publikacijose**, respiracinės (kvėpavimo) fiziologinių tyrimų metodai aprašyti **I ir V publikacijose**. Lokomotorinio aktyvumo ir kvėpavimo rodikliai buvo registruojami ir analizuojami naudojant žuvų elgsenos registracijos ir analizės kompiuterines programas: Gamtos tyrimų centre (Lietuva) „Fish Tank Monitor“, ir „Ethovision XT“ („Noldus“ informacinės technologijos, Nyderlandai) (1 ir 2 pav.).

Eksperimento dizainas, naudotas žuvų aklimacijos tyrimuose, aprašytas **II** ir **III publikacijose**. Neskelbti žuvų aklimacijos tyrimų rezultatai išsamiai aprašyti Makaras ir kt. (2019) rankraštyje (įteiktas spaudai).

Gliukozės (β-D-gliukozės) koncentracijos nustatymo metodas žuvų kraujyje aprašytas **III**, **V** ir **VI publikacijose**, o β-D-gliukozės įvertinimo vandenyje metodas išsamiai aprašytas **V publikacijoje**.

Vandens toksiškumo klasifikacija žuvims pritaikyta remiantis lyginamosios analizės pagrindu: toksiškumas elgesenos parametrams palygintas su standartiniais (referentinės medžiagos) toksiškumo bandymo rezultatais pagal regresinę analizę, kuri yra išsamiai aprašyta **I publikacijoje**.

Statistiniai metodai, taikyti gautų duomenų analizei, išsamiai aprašyti kiekvienoje **publikacijoje** (**I–IX publikacijos**).

Lietuvos valstybinė maisto ir veterinarijos tarnyba išdavė Leidimą atlikti bandymo su gyvūnais projektą (Nr. G2-69; 2017.07.25). Visi bandymai su žuvimis buvo atlikti laikantis ES reglamento (Direktyva 2010/63/ES).

REZULTATAI IR JŲ APTARIMAS

Šiame skyriuje pateikti svarbiausi rezultatai. Išsamūs rezultatai pateikti atitinkamose **publikacijose** (**I–IX**).

Aklimacijos trukmės nustatymas skirtingoms žuvų rūšims pagal elgsenos rodiklius

Buvo nustatytas aklimacijos (4 val.) poveikis skirtingų taksonominių ir funkcinių grupių žuvų rūšių elgsenos ypatumams: buvo tirtos migruojančios reofilinės lašišinės žuvys – vaivorykštinis upėtakis (*Oncorhynchus mykiss*) ir atlantinė lašiša (*Salmo salar*) ir nemigruojančios euritopinės žuvys – paprastasis europinis ešerys (*Perca fluviatilis*) ir trispyglė dyglė (*Gasterosteus aculeatus*). Aklimacijos laikotarpiu buvo įvertinti žuvų elgsenos (lokomotorinio aktyvumo) dėsningumai, remiantis specifiniais rodikliais (vidutinis, maksimalus ir kampinis greičiai), ir nustatytas tinkamas aklimacijos laikas skirtingoms žuvų rūšims. Reikšmingi *O. mykiss* lokomotorinio aktyvumo pokyčiai buvo nustatyti visuose tirtuose rodikliuose. Šis tyrimas parodė, kad laikui bėgant žuvys iš esmės keičia savo plaukimo elgesį ir kad aklimacijos trukmė gali turėti įtakos tolimesnei gautų elgsenos duomenų analizei ir interpretacijai prieš atliekant toksiškumo tyrimus.

Priklausomai nuo vidutinio ir kampinio greičio rodiklių, *O. mykiss* lokomotorinis aktyvumas aklimacijos laikotarpiu reikšmingai sumažėjo 3-4 val. (nepublikuoti duomenys). Be to, ištirti *O. mykiss* lokomotorinio aktyvumo rodikliai parodė reikšmingą žuvų vidutinio greičio sumažėjimą po 2 val. aklimacijos, o judėjimo trukmę – po 1 val. aklimacijos. Tačiau *O. mykiss* vidutinis greitis, palyginus su judėjimo trukme, buvo stabilesnis laiko atžvilgiu (**II publikacija**: 1A ir B pav.). Panaši *O. mykiss* lokomotorinio aktyvumo tendencija buvo nustatyta ir *S. salar* lokomotoriniam aktyvumui (nepublikuoti duomenys). Reikšmingas *S. salar* lokomotorinio aktyvumo sumažėjimas buvo nustatytas 3–4 val., o kampinio greičio – 2 aklimacijos valandą. Mažiausia *S. salar* vidutinio greičio ir judėjimo trukmės rodiklių variacija laike (kas 10 min.) buvo nustatyta po 2 valandų aklimacijos (**III publikacija**: 7 pav. A ir B).

Perca fluviatilis atveju, palyginus su lašišinėmis žuvimis, pastebėta priešinga elgsenos pokyčių tendencija. *P. fluviatilis* lokomotorinis aktyvumas, po perkėlimo į eksperimentines sąlygas, sumažėjo ir ėmė didėti per pirmąją aklimacijos valandą (nepublikuoti duomenys). Tuo tarpu reikšmingų *G. aculeatus* elgsenos skirtumų aklimacijos laikotarpiu (4 val.) nebuvo nustatyta (nepublikuoti duomenys). Nedideli elgsenos pokyčiai buvo stebimi pirmąsias 30 aklimacijos minučių (nepublikuoti duomenys). Remiantis nustatytais elgsenos skirtumais, *P. fluviatilis* ir *G. aculeatus* lokomotorinis aktyvumas laike buvo stabilesnis nei tirtų lašišinių žuvų.

Žuvys buvo laikomos aklimuotomis, kai jų lokomotorinis aktyvumas pasiekė stabilų aktyvumo lygį laike (po aklimacijos). Gauti duomenys parodė reikšmingus lokomotorinio aktyvumo skirtumus tarp reofilinių ir euritopinių žuvų rūšių 4 val. aklimacijos laikotarpiu (nepublikuoti duomenys). Nustatyta, kad prieš aklimaciją (neaklimuotų) *O. mykiss* ir *S. salar* vidutinis greitis reikšmingai skiriasi nuo neaklimuotų *P. fluviatilis* ir *G. aculeatus* vidutinio greičio. Po aklimacijos (aklimuotų) *O. mykiss* ir *S. salar* lokomotorinis aktyvumas eksperimento pradžioje reikšmingai sumažėjo, palyginus su žuvimis prieš aklimaciją (su neaklimuotomis) (nepublikuoti duomenys). Neaklimuotų ir aklimuotų *O. mykiss* ir *S. salar* lokomotorinis aktyvumas buvo panašus, reikšmingų skirtumų tarp šių rūšių nenustatyta (nepublikuoti duomenys). Taip pat reikšmingų elgsenos skirtumų tarp neaklimuotų ir aklimuotų *P. fluviatilis* ir *G. aculeatus* žuvų rūšių nenustatyta (nepublikuoti duomenys). Neaklimuotų *P. fluviatilis* lokomotorinis aktyvumas buvo mažiausias, palyginus su kitų tirtų žuvų rūšių, tačiau aktyvumo lygiui nusistovėjus, jis buvo artimas lašišinių žuvų aktyvumui (nepublikuoti duomenys). Aklimuotų *G. aculeatus* lokomotorinis aktyvumas buvo reikšmingai didesnis, palyginti su *O. mykiss*, *S. salar* ir *P. fluviatilis*.

Gauti rezultatai parodė, kad aklimacijos trukmė O *mykiss* ir *S. salar* yra 2 val. o *P. fluviatilis* – 1 val. Nors reikšmingų *G. aculeatus* elgsenos pokyčių nenustatyta, tačiau, atsižvelgiant į nežymius lokomotorinio aktyvumo svyravimus, registruotus per pirmąsias 30 aklimacijos minutes, šiai rūšiai rekomenduojama taikyti trumpą aklimaciją (30 min.) (**II, III publikacijos** ir nepublikuoti duomenys).

Pirmą kartą įvertintas aklimacijos poveikis vidutinio klimato zonoje paplitusių reofilinių (*O. mykiss* ir *S. salar*) ir euritopinių (*P. fluviatilis* ir *G. aculeatus*) žuvų rūšių elgsenai. Buvo atlikta tik keletas eksperimentų, tiriančių aklimacijos poveikį nemigruojančioms (euritopinėms) žuvų rūšims kaip *G. holbrooki* ir gupijai (P. *reticulate*), kurios paplitusios tropinio klimato juostoje (Melvin ir kt. 2017; O'Neill ir kt. 2018). Be to, šios rūšys yra plačiai naudojamos elgsenos tyrimuose, o *S. salar*, *P. fluviatilis* ir *G. aculeatus* retai naudojamos tokiuose tyrimuose (Sergy ir Scroggins 1990; Wibe ir kt. 2001; Saaristo ir kt. 2019).

Dauguma mokslininkų ekotoksikologiniuose tyrimuose, vertinant žuvų lokomotorinį aktyvumą, aklimuoja skirtingas žuvų rūšis mažiau nei 1 val. (Melvin *et al.* 2017). Netinkamos žuvų aklimacijos trukmės atvejais (pavyzdžiui, trukmė per trumpa) stebimas žuvų elgesys gali neatspindėti tikrojo medžiagų toksinio poveikio dėl patirto streso, kurį sukėlė nauja aplinka ir toksinės medžiagos, o tai gali lemti elgsenos kintamumą. Deja, elgsenos tyrimai nėra standartizuoti, todėl tinkamo aklimacijos laikotarpio nustatymas išlieka aktualia problema ekotoksiškumo tyrimuose. Įvairių ekologinių grupių žuvų aklimacijos trukmė gali skirtis, todėl prieš pradedant elgsenos tyrimus, būtina nustatyti tinkamą aklimacijos laikotarpį.

SF ir MIX ūminis toksiškumas skirtingoms žuvų rūšims

Sąvartyno filtrato (SF) (daugiausia sudaro organiniai junginiai) ir daugianario metalų mišinio (MIX) ekotoksikologinis poveikis (mirtingumas) nustatytas skirtingo vystymosi stadijų žuvims (**IV, VII** ir **VIII publikacijos**).

Nustatytos SF 96 val. LC_{50} (letali koncentracija, sukelianti 50 proc. imties invididų žūtį (±SD)) vertės *O. mykiss* esant skirtingoms vystymosi stadijoms (mailius, jaunikliai (0+) ir jaunikliai (1+)) svyravo nuo 3,0 proc. (0,6) iki 3,6 proc. (0,3). Reikšmingų skirtumų SF poveikiui tarp *O. mykiss* skirtingų vystymosi stadijų nebuvo nustatyta (nepublikuoti duomenys).

Apskaičiuoti SF toksiškumo vienetai (TV) parodė, kad mišinys yra labai toksiškas *O. mykiss* visose tirtose jo vystymosi stadijose. Atsižvelgiant į sukeliamą toksinį poveikį, SF galima priskirti IV ekotoksiškumo klasei (labai toksiškas) (**VIII publikacija**: 5 lentelė, 2 pav.). SF poveikio tyrimai žuvims ankstyvose jų vystymosi stadijose buvo tirti Alkassasbeh ir kt. 2009; Emenike ir kt. 2012; Qiu ir kt. 2016. Alkassasbeh ir kt. (2009) nustatė, kad SF (mėginiai paimti iš skirtingų sąvartynų) 96 val. LC_{50} *Cyprinus carpio* jaunikliams (vidutinė kūno masė – 0,92 ± 0,24 g, o vidutinis ilgis – 3,83 ± 0,19 cm) atitinkamai buvo 1,132, 2,0 ir 3,822 proc. Eimenike ir kt. (2012) parodė, kad veikiant SF *Pangasius sutchi* ir

Clarius batrachus 96 val. LC_{50} atitinkamai buvo 3,2 proc. ir 5,9 proc. Panašūs rezultatai gauti veikiant *O. mykiss* jauniklius Kairių SF.

Ištirtas ir nustatytas mišinio MIX ūminis poveikis *O. mykiss* (modelinė) ir *P. fluviatilis* (nemodelinė) žuvų rūšims. Nustatytos MIX 96 val. LC_{50} (95 proc. PI) vertės *O. mykiss* ir *P. fluviatilis* atitinkamai buvo 50,1 proc. (45,5–55,8) ir 26,8 proc. (22,5–31,8), palyginti su pradiniu mišiniu MIX (pradinė MIX koncentracija prilyginta 1 proc.), paruoštu pagal ES paviršiniuose vidaus vandenyse didžiausias leistinas koncentracijas (DLK) (Direktyva 2008/105/EB) (**IV publikacija**: 3 lentelė). Ūmaus toksiškumo rezultatai parodė, kad *P. fluviatilis* yra 1,9 karto jautresni MIX poveikiui nei *O. mykiss* (**IV publikacija**: 3 lentelė; **IX publikacija**).

Žinomi priešingi cheminių medžiagų ūmaus toksiškumo *O. mykiss* ir *P. fluviatilis* tyrimų rezultatai. Svecevičius (2006) nustatė, kad Cr^{6+} 96 val. LC_{50} vertės *P. fluviatilis* ir *O. mykiss* buvo panašios. Lacroix ir Hontela (2004) ir Couture ir kt. (2008) tyrimai parodė, kad *P. flavescens* yra labiau atsparesnis metalų poveikiui nei *O. mykiss*. Taylor su bendraautoriais (2003) nustatė, kad remiantis 96 val. LC_{50} verte, *P. flavescens* buvo atsparesnis Cu poveikiui nei *O. mykiss*. Mažesnę 96 val. LC_{50} vertę *P. fluviatilis* nei *O. mykiss* galėjo lemti tai, kad tirti ešeriai buvo auginami atviruose tvenkiniuose, o *O. mykiss* – veislyne.

Turschwell ir White (2016) tyrimas atskleidė, kad organizmo laikymo aplinka gali sukelti reikšmingus smegenų dydžio ir medžiagų apykaitos greičio (angl. *resting metabolic rate* (RMR)) pokyčius. Žuvų veislynuose išaugintos ir laboratorijose laikomos žuvys turi reikšmingai didesnį RMR nei natūralioje aplinkoje sugaunamos žuvys. Remiantis disertacijoje pateiktais duomenimis, galima teigti, kad žuvų veisimas ir laikymas nenatūralioje aplinkoje gali paskatinti adaptacinių savireguliacijos mechanizmų, turinčių įtakos *O. mykiss* jautrumui, evoliuciją. Sumažėjęs veislyne auginamų žuvų jautrumas gali būti fiziologinis atsakas į lėtinį stresą, patiriamą dėl laikymo nelaisvėje, šėrimo komerciniais pašarais su priedais ir kitų dirgiklių, esančių žuvų veislynuose. Dėl to gali sumažėti ir šių žuvų jautrumas teršalams. Turschwell ir White (2016) pateikė išvadą, kad atsižvelgiant į esamas standartines laikymo laboratorijose sąlygas, nelaisvėje laikomi gyvūnai gali būti nereprezentatyvūs, palyginti su laukinėmis populiacijomis.

Šioje disertacijoje pateiti kontrastingi ūmaus toksiškumo rezultatai leidžia iškelti hipotezę, kad veislynuose auginamos ir dirbtiniais pašarais maitinamos žuvys su laiku gali prisitaikyti ir tapti atsparesnės cheminių medžiagų poveikiui (**IV publikacija**).

SF ir MIX poveikis skirtingų žuvų rūšių elgsenai ir kvėpavimo rodikliams

Tyrimo metu buvo įvertintas vandens mėginių, paimtų iš hidroekosistemos, esančios šalia uždaryto Kairių sąvartyno, toksinis poveikis *O. mykiss* kvėpavimui. Didėjant atstumui nuo sąvartyno, nustatytas toksiškumo mažėjimas (**I publikacija**).

Vandens mėginiai, paimti iš drenažo kanalo (stotis Nr. 0), sukėlė reikšmingą *O. mykiss* kosėjimo dažnio (reversinio kvėpavimo) (KD) padidėjimą ir buvo labai toksiški (koncentracija, sukėlusi KD pokyčius, sudarė 0,86 nuo 96 val. LC_{50} dalį) žuvims (**I publikacija**). Vandens mėginiai iš drenažo kanalo (Nr. 1) ir Ginkūnų tvenkinio (Nr. 2) buvo „vidutiniškai toksiški" (atitinkantys 0,28 ir 0,26 dalis nuo 96 val. LC_{50} (**I publikacija**: 1 lentelė)). Nustatytas Ginkūnų tvenkinio (Nr. 3 ir 4) ir Švedės upelio (Nr. 5) vandens mėginių mažas toksiškumas *O. mykiss* (**I publikacija**). Regresinės analizės rezultatai parodė, kad tiriamų vandens mėginių toksiškumas priklauso nuo Kairių SF rezervuarų atstumo.

Remiantis gautais rezultatais, galima teigti, kad SF vis dar yra labai toksiškos nuotekos, kurios iš rezervuarų patenka į kanalizacijos kanalą, o toliau į Ginkūnų tvenkinį ir Švedės upelį. Vandens toksiškumas mažėja, didėjant atstumui nuo taršos šaltinio (**I publikacija**).

Nustatyti *O. mykiss* (3 mėn. amžiaus (0+)) reikšmingi lokomotorinio aktyvumo pokyčiai, veikiant žuvis subletaliomis SF ir Cr^{6+} koncentracijomis po 5, 10 ir 30 min. poveikio, ir atsako dėsningumai (**II publikacija**). *O. mykiss,* paveiktų 0,05 proc., 0,1 proc. ir 0,2 proc. SF koncentracijomis, lokomotorinis aktyvumas padidėjo po 5, 10 ir 30 min. (**II publikacija**: 2 pav.). Reikšmingi žuvų lokomotorinio aktyvumo skirtumai nustatyti po 5 min. poveikio, o jo pikas – po 10 min. (1,7 karto didesnis, palyginus su kontrole) (**II publikacija**: 2b pav.; **XI publikacija**). Reikšmingas *O. mykiss* (3 mėn. amžiaus (0+)) lokomotorinio aktyvumo padidėjimas, veikiant 1 mg/L Cr^{6+} koncentracija, nustatytas po 5 min. (1,5 karto didesnis nei kontrolė), o aktyvumo pikas nustatytas po 10 min. (1,6 karto didesnis nei kontrolė) poveikio. Rezultatai parodė, kad *O. mykiss* yra jautresnis SF subletalių koncentracijų poveikiui nei Cr^{6+} (**II publikacija**: 2 pav.). Duomenys parodė, kad *O. mykiss* lokomotorinis aktyvumas priklauso nuo cheminės medžiagos ir jos koncentracijos, tačiau nepriklauso nuo poveikio trukmės. *O. mykiss* atsakas į subletalias SF ir Cr^{6+} koncentracijas pasireiškia šiais lokomotorinio aktyvumo pokyčiais: (1) lokomotorinio aktyvumo padidėjimas, (2) lokomotorinio aktyvumo pikas, (3) lokomotorinio aktyvumo mažėjimas (**II publikacija**: 2 pav.).

Tyrimo rezultatai parodė, kad SF ir Cr^{6+} poveikis *O. mykiss* lokomotoriniam aktyvumui buvo panašūs, t. y. aktyvumas didėjo ir vėliau mažėjo (**II publikacija**).

Michalec ir kt. (2013), Gonçalves ir kt. (2008) tyrimai parodė, kad žuvų elgsenos atsakas į chemines medžiagas nepriklauso nuo tirtų medžiagų tipo, tačiau Ašmonaitė ir kt. (2016) nustatė, kad zebražuvės (*Danio rerio*), paveiktos Ag^{+}, kūno judesiai skiriasi nuo žuvų, paveiktų Ag nanodalelėmis. Sandheinrich (2014) teigia, kad organiniai junginiai gali sukelti žuvims tiek hipoaktyvumą (arba letargiją), tiek hiperaktyvumą, o mažos metalų jonų koncentracijos dažniausiai sukelia hiperaktyvumą. Kiti tyrimai atskleidė, kad ne visi organiniai junginiai pasireiškia letarginiu poveikiu (Brandão ir kt. 2015; Xie ir kt. 2015). Be to, cheminės medžiagos mišiniuose gali veikti sinergetiškai ir turėti didesnį poveikį žuvų elgsenai nei atskiri komponentai (Svecevičius 2012).

Siekiant nustatyti *O. mykiss* jauniklių (metinukų (1+)) lokomotorinio aktyvumo ypatumus laike ir ryšius tarp skirtingų rodiklių, buvo tirti skirtingi elgsenos rodikliai, veikiant žuvis SF (**VI publikcija**). Palygintas *O. mykiss* metinukų (1+) lokomotorinio aktyvumo skirtingų rodiklių atsako greitis ir jautrumas, veikiant žuvis SF (**VI publikcija**). Nustatyta, kad po 2 val. poveikio žuvų lokomotorinis aktyvumas mažėjo, didėjant SF koncentracijai (**VI publikacija**. 1 pav.; 4 lentelė). Reikšmingas *O. mykiss* vidutinio greičio sumažėjimas, palyginus su kontroline grupe, buvo nustatytas esant 0,5 proc. SF koncentracijai (kuri sudarė 0,138 dalį nuo 96 val. LC_{50}), o reikšmingas kampinio greičio padidėjimas – esant 0,25 proc. koncentracijai (0,069 dalis nuo 96 val. LC_{50}) (**VI publikacija**: 1 pav.). SF poveikis nesukėlė reikšmingų tirtų žuvų didžiausio greičio, judėjimo trukmės ir kūno mobilumo pakitimų (**VI publikacija**: 1 pav.). *O. mykiss* metinukų lokomotorinio aktyvumo (vidutinio ir kampinio greičio) pokyčiai buvo nustatyti po 1 min, veikiant juos didžiausioms SF koncentracijoms (0,25 proc. ir 0,5 proc.) (**VI publikacija**: 2 pav.). Pagrindinių lokomotorinio aktyvumo komponenčių analizė parodė, kad *O. mykiss* visi tirti lokomotorinio aktyvumo rodikliai tarpusavyje koreliuoja, nors šių rodiklių jautrumas SF poveikiui skiriasi (**VI publikacija**).

Užsienio mokslininkų tyrimai parodė, kad SF poveikis sukelia skirtingus žuvų elgsenos, fiziologinius ir biocheminius pakitimus (Emenike ir kt. 2012; Klauck ir kt. 2013; Salem ir kt. 2014; Budi ir kt. 2016). Alkassasbeh ir kt. (2009) parodė, kad sąvartynuose susidaręs filtratas sukelia *Cyprinus carpio* elgsenos ir fiziologinius pokyčius: bendrą lokomotorinio aktyvumo sumažėjimą, pusiausvyros praradimą, sutrikdytą kvėpavimą ir žuvų sutelkimą paviršiuje, suaktyvintą gleivių sekreciją. Sumažėjęs žuvų lokomotorinis aktyvumas (hipoaktyvumas) gali būti aiškinamas ir pakitusia žuvų elgsenos strategija esant stresinėms sąlygoms (Lushchak 2011; Hamilton ir kt. 2017; **VI publikacija**).

Chen ir kt. (2011) ir Steele ir kt. (2018) tyrimų metu nustatė, kad skirtingi elgsenos rodikliai gali atspindėti skirtingą žuvų atsako jautrumą į cheminių medžiagų poveikį. Tyrimų metu gauti rezultatai leidžia manyti, kad vieni elgsenos rodikliai, vertinant teršalų poveikį žuvų elgsenai, gali būti tinkamesni ir jautresni nei kiti (**VI publikacija**). Todėl, siekiant tinkamai įvertinti teršalų sukeltus žuvų elgsenos pokyčius ir išaiškinti daugianarių cheminių mišinių toksiškumo mechanizmus, būtina vertinti kelis ar daugiau skirtingus elgsenos rodiklius (**IV** ir **VI publikacijos**).

Atliktų elgsenos tyrimų duomenys su skirtingomis žuvų rūšimis atskleidė, kad reikšmingi žuvų elgsenos pokyčiai stebimi per pirmąją poveikio valandą (Svecevičius 2007, 2009; Huang ir kt. 2014; **IV publikacija**). Šioje disertacijoje gauti rezultatai parodė, kad nustatyta žuvų elgsenos pokyčių svyravimų trukmė 2 val. ir yra tinkama vandens kokybės stebėsenai atlikti.

Ištirti *P. fluviatilis* ir *O. mykiss* elgsenos ir kvėpavimo pokyčiai, veikiant žuvis mišiniu MIX, paruoštu pagal ES paviršiniuose vidaus vandenyse didžiausias leistinas metalų koncentracijas (DLK) (Direktyva 67 2008/105/EC). Nustatytas *P. fluviatilis* ir *O. mykiss* lokomotorinio aktyvumo mažėjimas, didėjant MIX koncentracijai (**IV publikacija**: 4 lentelė; **IX publikacija**). Gauti rezultatai parodė, kad veikiant žuvis mišiniu MIX *P. fluviatilis* lokomotoriniam aktyvumui labiau būdingi tiesiniai, o *O. mykiss* – sukamieji judesiai (plaukiojimas ratu).

Couture ir Kumar (2003), Mishra ir Mohanty (2009) nustatė, kad hipoaktyvumas gali būti stebimas dėl metalų sukelto metabolizmo sutrikimo. Tai patvirtina ir mūsų gauti rezultatai, tiriant SF poveikį *O. mykiss* (**II publikacija**). Taip pat hipoaktyvumas gali būti siejamas su žuvų elgsenos strategija, prisitaikant prie užterštos aplinkos (**II publikacija**).

Tyrimai parodė, kad mišinys MIX reikšmingai nepaveikė *P. fluviatilis* tirtų kvėpavimo rodiklių (žiaunų ventiliacijos (ŽVD) ir kosėjimo dažniai (KD)) (**IV publikacija**: 4 lentelė), tačiau jau po 1 min. poveikio mišiniu MIX reikšmingai pakito *O. mykiss* ŽVD (**IV publikacija**: 2 pav.). Gauti tyrimo rezultatai nesutampa su Svecevičiaus (2005, 2009) atliktais tyrimais, kurių metu ŽVD ir KD didėjo didėjant Cr^{6+} ir metalų mišinio koncentracijoms. Wang ir kt. (2013) tyrimai parodė, kad pavieniai metalai (Hg^{2+}, Cu^{2+}, Cd^{2+} ir Zn^{2+}) paveikia skirtingus karpinių žuvų kvėpavimo rodiklius. Padidėjęs *O. mykiss* ŽVD (po 1 min. MIX poveikio) gali būti stebimas dėl metalo jonų ir žuvų uoslės neuronų sąveikos, kuri lemia pirminį žuvų atsaką į vandenyje esančias chemines medžiagas (**IV publikacija**). Tai patvirtina ir kiti moksliniai tyrimai (Belanger ir kt. 2006). Tuo tarpu po 10 min. poveikio *O. mykiss* ŽVD sumažėjo, tačiau esant ilgesnei poveikio trukmei reikšmingų pokyčių, palyginti su kontrole, nenustatyta (**IV publikacija**). Tai gali būti susiję su uoslės receptorių cheminiu pažeidimu, kas lemia žiaunų ventiliacijos sumažėjimą ir slopinamą (Witewska ir kt. 2005). Azizishirazi ir kt. (2014) taip pat nustatė, kad metalų poveikis sutrikdo normalią laukinių *P. flavescens* jutimines funkcijas.

O. mykiss lokomotorinis aktyvumas nurodomas kaip jautresnis rodiklis, palyginus su ŽVD ir KD (Svecevičius 2005, 2009). Tierney ir kt. (2010) tyrimai atskleidė, kad žuvų lokomotorinio aktyvumo padidėjimas gali būti siejamas su vengimo reakcija, kurią sukelia uoslės receptorių dirginimas. Svecevičius (2012), tirdamas dvinarių (Cu ir Zn) mišinių poveikį, nustatė, kad *Rutilus rutilus* KD buvo jautriausias ir informatyviausias elgsenos rodiklis. Prieštaringi rezultatai, gauti tiriant žuvų lokomotorinį aktyvumą ir kvėpavimo reakcijas, leidžia teigti, kad egzistuoja rūšies specifiškumas, kurį lemia fiziologiniai kompensaciniai-adaptaciniai mechanizmai ląsteliniu ir molekuliniu lygmenimis (**IV publikacija**). Mūsų tyrimų metu nustatyta, kad *O. mykiss* KD nėra jautrus rodiklis mišinio MIX poveikiui.

Ištirti *S. salar* elgsenos ypatumai po 2 val. poveikio MIX ir MIX su 10 kartų sumažintomis būtinųjų metalų Cr↓ ir Cu↓ koncentracijomis (**III publikacija**). Nustatyti *S. salar* vidutinio greičio padidėjimo pikai MIX ir Cu↓ 1–2 val. poveikio laike, o veikiant Cr↓ – reikšmingo poveikio laike nenustatyta (**III publikacija**: 7 pav.). MIX poveikis sukėlė reikšmingus vidutinio greičio ir judėjimo trukmės padidėjimus, palyginti su kontrole. Dėl mišinio MIX ir Cu↓ poveikio nustatyti *S. salar* lokomotorinio aktyvumo dėsningumai: (1) padidėjęs lokomotorinis aktyvumas, (2) lokomotorinio aktyvumo pikas, (3) sumažėjęs lokomotorinis aktyvumas.

Gauti *S. salar* elgsenos rezultatai dėl MIX poveikio, parodė, kad mažos metalų koncentracijos mišinyje gali sąveikauti ir sukelti toksikologiškai reikšmingą poveikį žuvų elgsenai (**III publikacija**). Iki šiol mažai atlikta tyrimų, siekiant įvertinti galimą metalų sąveiką daugianariuose mišiniuose ir atsirandančių sąveikų poveikį žuvų elgsenai.

Skirtingi žuvų elgsenos ypatumai gali atskleisti teršalų sinergetinį ar antagonistinį poveikį. Išanalizuoti *S. salar* elgsenos duomenys, pateikti **III**

publikacijoje, rodo, kad Cr^{6+} buvimas skirtingomis koncentracijomis mišinyje gali turėti įtakos žuvų elgsenai. Remiantis *S. salar* vidutinio greičio rezultatais, nustatyta, kad Cr↓ mišinys (10 kartų sumažinta Cr^{6+} koncentracija) turėjo silpnesnį poveikį žuvų elgsenai, palyginus su Cu↓ mišiniu (10 kartų sumažinta Cu^{2+} koncentracija) (**III publikacija**: 7 pav.).

Ateityje reikėtų atlikti daugiau tyrimų, kad būtų galima spręsti, kurie elgsenos rodikliai yra tinkamiausi ir jautriausi vertinant žuvų elgsenos atsaką į skirtingų teršalų poveikį. Taip pat žuvų elgsenos tyrimai turėtų apimti ne tik teršalų sukeltą skirtingų rodiklių pokyčių vertinimą, bet ir dinaminius žuvų elgsenos pokyčius poveikio laike.

Gliukozės koncentracijos pokyčiai žuvų kraujyje ir vandenyje veikiant SF ir MIX mišiniais

Gliukozės koncentracijos pokyčiai žuvų (*O. mykiss* ir *S. salar*) kraujyje kaip streso rodiklis buvo vertinamas veikiant žuvis SF ir MIX (**III, V** ir **VI publikacijos**). Rezultatai parodė, kad veikiant *O. mykiss* SF ir MIX nesukelia reikšmingų gliukozės koncentracijos pokyčių žuvų kraujyje (**V publikacija**: 3 pav; **VI publikacija**: 3 pav.). Veikiant *S. salar* (2 val.) skirtingais MIX (MIX, Cu↓ (10 kartų sumažinta Cu^{2+} koncentracija mišinyje MIX) ir Cr↓ (10 kartų sumažinta Cr^{6+} koncentracija mišinyje MIX)) mišiniais reikšmingai padidino gliukozės koncentraciją šių žuvų kraujyje, tačiau skirtumų tarp poveikio grupių skirtingais MIX mišiniais nenustatyta (**III publikacija**: 6 pav.).

Užsienio mokslininkų nuomone, gliukozės koncentracijos padidėjimas žuvų kraujyje gali būti nustatomas jau po 1–2 val. poveikio, vieną valandą veikiant žuvis skirtingo tipo stresoriais (cheminėmis medžiagomis, žuvų transportavu ir pan.) (Jentoff ir kt. 2005; Zahangir ir kt. 2014; Wu ir kt. 2015, **V publikacija**). Kitu atveju, sukeltas stresas žuvims gali ir nesukelti gliukozės koncentracijos pokyčių kraujyje arba šie pokyčiai gali pasireikšti tik po kelių poveikio dienų (Gagnon ir kt. 2006; Langiano ir Martínez 2008; Miller ir kt. 2007; Pucher ir kt. 2014; Hoq ir kt. 2015; Nageswara ir kt. 2017). Ūmaus toksiškumo tyrimuose gliukozės koncentracijos pokytis žuvų kraujyje buvo įvertintas kaip netikslus rodiklis (Iversen ir kt. 2003; Perez-Casanova ir kt. 2008; Fast ir kt. 2008). Atsižvelgiant į prieštaringus tyrimų rezultatus galima teigti, kad gliukozės koncentracijos pokyčiai kraujyje yra nepatikimas streso rodiklis, todėl turėtų būti naudojamas tik kartu su kitais toksiškumo vertinimo rodikliais (**V** ir **VI publikacijos**).

Atlikus gliukozės (β-D-gliukozės) koncentracijos vandenyje matavimus, paveikus *O. mykiss* MIX, buvo gauti priešingi rezultatai, palyginti su gliukozės koncentracijos kraujyje matavimais (**V publikacija**). Gliukozės koncentracija žuvų laikymo vandenyje didėjo, ilgėjant poveikio laikui (**V publikacija**: 3 pav.). MIX ir MIX5↓ poveikis sukėlė reikšmingus gliukozės koncentracijos pokyčius vandenyje, palyginus su kontroline grupe (**V publikacija**: 3 pav., 4 lentelė). Remiantis rezultatais, galima teigti, kad β-D-gliukozės koncentracijos padidėjimas vandenyje yra greitas atsakas į stresą, sukeltą MIX.

Atlikta daug tyrimų, kurių metu vertinta žuvų išskiriamų kortikosteroidų koncentracija vandenyje, esant skirtingų stresorių poveikiui (Scott and Ellis 2007; Fanouraki ir kt. 2008; Lower ir kt. (2015). Biocheminis streso indikatorius (kortizolis) buvo vertintas žuvų išmatose (Felix ir kt. 2013), gleivėse (Guardiola ir kt. 2016) ar vandenyje (Fanouraki ir kt. 2008) ir pasiūlytas kaip tinkami streso vertinimo rodikliai. Daugelio eksperimentų metu buvo tiriamas laikymo nelaisvėje sukelto streso poveikis žuvims. Iki šiol nebuvo atlikta tyrimų, taikant gliukozės koncentracijos matavimus žuvų laikymo vandenyje, paveikus žuvis cheminiais mišiniais, paruoštais pagal aplinkoje esančias realias metalų koncentracijas. Norint patvirtinti gliukozės vandenyje koncentraciją, kaip cheminio streso rodiklį, būtina atlikti daugiau tokių tyrimų siekiant tobulinti neinvazinio gliukozės matavimo procedūrą. Gliukozės koncentracijos pokyčių vertinimas vandenyje, naudojant specifinį biojutiklį, galėtų būti naudinga priemonė, vertinant cheminių medžiagų riziką aplinkai ir galėtų papildyti esamus biožymenis, naudojamus vertinant teršalų poveikį gyviems organizmams (**V publikacija**).

Skirtingų žuvų rūšių elgsenos, fiziologinių ir biocheminių atsako rodiklių greitumo ir jautrumo palyginimas

Žuvų rūšių elgsenos, fiziologinių ir biocheminių atsako rodiklių greitumo ir jautrumas palyginimas parodė, kad *O. mykiss* jaunikliai (3 mėn. amžiaus) pagal lokomotorinio aktyvumo pokyčius yra 5 kartus jautresni nei *O. mykiss* metinukai veikiant juos SF mišiniu.

Kampinio greitis nustatytas kaip jautriausias *O. mykiss* lokomotorinio aktyvumo rodiklis į SF ir mišinio MIX, kurių koncentracijos atitinkamai sudarė 0,069 ir 0,1 dalį nuo 96 val. LC_{50}, poveikį. ŽVD nustatytas kaip greičiausias ir jautriausias tirtas elgsenos ir kvėpavimo rodiklis į MIX, kurio koncentracija sudarė 0,05 dalį nuo 96 val. LC_{50}, poveikį. Reikšmingi kampinio greičio ir ŽVD pokyčiai nustatyti po 1 min. poveikio SF ir MIX mišiniais.

Vidutinis ir maksimalus greičiai buvo nustatyti kaip jautriausi *P. fluviatilis* lokomotorinio aktyvumo rodikliai į mišinio MIX, kurio koncentracija sudarė 0,746 dalį nuo 96 val. LC_{50}, poveikį. Reikšmingas vidutinio ir maksimalaus greičių sumažėjimas buvo nustatytas po 2 val. poveikio mišiniu MIX. Ištirti *P. fluviatilis* lokomotorinio aktyvumo ir kvėpavimo rodikliai parodė šios rūšies mažą jautrumą poveikiui MIX, palyginti su nustatyta 96 val. LC_{50} verte.

β-D-gliukozės koncentracijos pokyčiai *O. mykiss* kraujyje laiko atžvilgiu buvo nereikšmingi veikiant žuvis daugianariais mišiniais, tačiau nustatytas reikšmingas β-D-gliukozės koncentracijos vandenyje padidėjimas po 3 val., paveikus *O. mykiss* mišiniu MIX, kurio koncentracija sudarė 0,004 dalį nuo 96 val. LC_{50}. β-D-gliukozės koncentracija vandenyje, paveikus *O. mykiss* (metinukus) mišiniu MIX, yra jautresnis rodiklis (sudarė 0,004 dalį nuo 96 val. LC_{50}) nei gliukozės koncentracija kraujyje, 25 kartus jautresnis nei kampinis greitis (sudarė 0,1 dalį nuo 96 val. LC_{50}) ir 12,5 karto nei ŽVD (sudarė 0,05 dalį nuo 96 val. LC_{50}).

IŠVADOS

1. Aklimacijos trukmė *Oncorhynchus mykiss* ir *Salmo salar* prieš elgesenos reakcijų registravimą yra 2 val., o *Perca fluviatilis* – 1 val. *Gasterosteus aculeatus* rekomenduojama aklimacijos trukmė – 30 min.
2. *O. mykiss* jautrumas skirtingais vystymosi etapais (mailius, jaunikliai (0+) ir jaunikliai (1+)) SF poveikiui pagal 96 val. LC_{50} vertes reikšmingai nesiskyrė. *P. fluviatilis* buvo 1,9 karto mažiau jautrus mišiniui MIX (96 val. LC_{50} – 26,8 proc.) nei *O. mykiss* (96 val. LC_{50} – 50,1 proc.).
3. *O. mykiss* jauniklių (3 mėn. amžiaus (0+)) lokomotorinis aktyvumas reikšmingai didėjo, o metinukų (1+) sumažėjo, didėjant SF koncentracijai. *O. mykiss (*3 mėn. amžiaus (0+)) pagal lokomotorinio aktyvumo pokyčius yra 5 kartus jautresnis SF poveikiui (0,05 proc. SF koncentracija, sudarė 0,01 dalį nuo 96 val. LC_{50}) nei *O. mykiss* metinukai (1+) (0,25 proc. SF koncentracija sudarė 0,069 dalį nuo 96 val. LC_{50}).
4. *Oncorhynchus mykiss* lokomotorinis aktyvumas reikšmingai mažėjo, o *S. salar* – didėjo didėjant MIX koncentracijai. Lokomotorinis aktyvumas tiek *O. Mykiss,* tiek *P. fluviatilis* reikšmingai mažėjo didėjant MIX koncentracijai. *O. mykiss* MIX poveikiui yra 4 kartus jautresnis nei *P. fluviatilis* pagal lokomotorinio aktyvumo pokyčius.

5. *S. salar* vidutinis greitis ir judėjimo trukmė reikšmingai padidėjo 1–2 val. laikotarpiu veikiant žuvis MIX ir MIX su 10 kartų sumažinta Cu^{2+} koncentracija. Nustatyti reikšmingi *S. salar* lokomotorinio aktyvumo dėsningumai (lokomotorinio aktyvumo pikas veikiant žuvis MIX ir MIX su sumažinta 10 kartų Cu^{2+} koncentracija.
6. *Oncorhynchus mykiss* kampinis greitis yra jautriausias lokomotorinio aktyvumo rodiklis į SF ir mišinio MIX poveikį, kurių koncentracijos atitinkamai sudarė 0,069 ir 0,1 dalis nuo 96 val. LC_{50}. Žiaunų ventiliacijos dažnis (ŽVD) nustatytas kaip greičiausias ir jautriausias tirtas elgsenos ir kvėpavimo rodiklis į MIX poveikį, kurio koncentracija sudarė 0,05 dalį nuo 96 val. LC_{50}. Reikšmingas kampinio greičio sumažėjimas ir ŽVD padidėjimas nustatyti po 1 min. poveikio MIX.
7. Vidutinis ir maksimalus greičiai buvo nustatyti kaip jautriausi *P. fluviatilis* tirti lokomotorinio aktyvumo rodikliai į mišinio MIX poveikį, kurio koncentracija sudarė 0,746 dalį nuo 96 val. LC_{50}. Reikšmingas vidutinio ir maksimalaus greičių sumažėjimas buvo nustatytas po 2 val. poveikio mišiniu MIX. Ištirti *P. fluviatilis* lokomotorinio ir kvėpavimo elgsenos rodikliai parodė *P. fluviatilis* tirtų elgsenos rodiklių nejautrumą poveikiui MIX, palyginti su 96 val. LC_{50} verte.
8. Nustatyta, kad β-D-gliukozės koncentracijos pokyčiai *O. mykiss* kraujyje laiko atžvilgiu buvo nereikšmingi veikiant žuvis daugianariais mišiniais. Tuo tarpu reikšmingas β-D-gliukozės koncentracijos vandenyje padidėjimas, paveikus *O. mykiss* mišiniu MIX, buvo nustatytas po 3 val. poveikio MIX, kurio koncentracija sudarė 0,004 dalį nuo 96 val. LC_{50}.
9. Nustatyta, kad β-D-gliukozės koncentracijos pokyčiai vandenyje, paveikus *O. mykiss* mišiniu MIX, yra jautresnis rodiklis (sudarė 0,004 dalį nuo 96 h LC_{50}) nei gliukozės koncentracijos pokyčiai kraujyje ir jautresnis nei tirti lokomotorinio aktyvumo (kampinio greičio pokyčiai prie MIX koncentracijos, kuri sudarė 0,1 dalį nuo 96 val. LC_{50}) ir kvėpavimo (žiaunų ventiliacijos dažnio pokyčiai prie MIX koncentracijos, kuri sudarė 0,05 dalį nuo 96 val. LC_{50}) rodikliai.

REFERENCES

Abd El-Salam MM, Abu-Zuid GI (2014) Impact of landfill leachate on the groundwater quality: A case study in Egypt. Journal of Advanced Research 6(4): 579–586

Alkassasbeh MYJ, Heng LY, Surif S (2009) Toxicity testing and the effect of landfill leachate in Malaysia on behavior of common carp (Cyprinus Carpio L., 1758; Pisces, Cyprinidae). American Journal of Environmental Sciences 5: 209–217.

AMEC, IEEP and REC (2014) Study on: Contribution of industry to pollutant emissions to air and water. Final report. AMEC Environment and Infrastructure UK Limited

Anyanwu BO, Ezejiofor AN, Igweze ZN, Orisakwe OE (2018) Heavy Metal Mixture Exposure and Effects in Developing Nations: An Update. Toxics 6(4):65

Archard GA, Earley RL, Hanninen AF, Braithwaite VA (2012) Correlated behaviour and stress physiology in fishexposed to different levels of predation pressure. Functional Ecology 26: 637–645

ASTM E1604-12 (2012b) Standard Guide for Behavioral Testing in Aquatic Toxicology, ASTM International, West Conshohocken, PA, pp. 1–17

ASTM E1711-12 (2012a) Standard Guide for Measurement of Behavior During Fish Toxicity Tests, ASTM International, West Conshohocken, PA, pp. 1–15

Ašmonaitė G, Boyer S, Souza KB, Wassmur B, Sturve J (2016) Behaviuoral toxicity assessment of silver ions and nanoparticles on zebrafish using a locomotion profiling approach. Aquatic Toxicology 173: 143–153

Azizishirazi A, Dew WA, Bougas B, Dashtban M, Bernatchez L, Pyle GG (2014) Chemosensory mediated behaviors and gene transcription profiles in wild yellow perch (Perca flavescens) from metal contaminated lakes. Ecotoxicology and Environmental Safety106: 239–245

Bae MJ, Park YS (2014) Biological early warning system based on the responses of aquatic organisms to disturbances: a review. Science of the Total Environment 466–467: 635–649

Beauvais SL, Jones SB, Parris JT, Brewer SK, Little EE (2001) Cholinergic and behavioural neurotoxicity of carbaryl and cadmium to larval rainbow trout (*Oncorhynchus mykiss*). Ecotoxicology and Environmental Safety 49: 84–90

Beitinger TL (1990) Behavioural reactions for the assessment of stress in fishes. Journal of Great Lakes Research 16(4): 495–528

Belanger RM, Corkum LD, Li W, Zielenski BS (2006) Olfactory sensory input increases gill ventilation in male round gobies (Neogobius melanostomus) during exposure to steroids. Comparative Biochemistry and Physiology - Comp Biochem Physiol A Mol Integr Physiol 144(2): 196–202

Bell AM (2001) Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback, *Gasterosteus aculeatus*. Animal Behaviour 62: 775–780

Benhaim D, Péan S, Lucas G, Blanc N, Chatain B, Bégout ML (2012) Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*). Applied Animal Behaviour Science 141(1–2): 79–90

Bopp SK, Barouki R, Brack W, Dalla Costa S, Dorne JCM, Drakvik PE, Faust M, Karjalainen TK, Kephalopoulos S, van Klaveren J, et al. 2018 Current EU research activities on combined exposure to multiple chemicals. Environmental International 120, 544–562

Brandão FP, Rodrigues S, Castro BB, Goncalves F, Antunes SC, Nunes B (2015) Short-term effects of neuroactive pharmaceutical drugs on a fish species: Biochemical and behavioural effects. Aquatic Toxicology 144-145: 218–229

Brodin T, Fick J, Jonnson M, Klaminder J (2013) Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations. Science 339: 814–815

Brodin T, Poviano S, Fick J, Klaminder J, Heynen M, Jonsson M (2014) Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 369(1656): 20130580

Budi S, Suliasih BA, Othman MS, Heng LY, Surif S (2016) Toxicity identification evaluation of landfill leachate using fish, prawn and seed plan. Waste Management 55: 231–237

Calfee RD, Puglis HJ, Little EE, Brumbaugh WG, Mebane CA (2016) Quantifying Fish Swimming Behaviour in Response to Acute Exposure of Aqueous Copper Using Computer Assisted Video and Digital Image Analysis. Journal of Visualized Experiments 108: 53477

Chen TH, Wang YH, Wu YH (2011) Developmental exposures to ethanol or dimethylsulfoxide at low concentrations alter locomotor activity in larval zebrafish: Implications for behavioral toxicity bioassays. Aquatic Toxicology 102: 162–166

Chovanec A, Hofer R, Schiemer F (2003) Fish as bioindicators. In: Markert BA, Breure AM, Zechmeister HG (eds) Bioindicators and biomonitors: principles, concepts and applications. Elsevier, Amsterdam, 6: 639–675

Clarke BO, Anumol T, Barlaz M, Snyder SS (2015) Investigating landfill leachate as a source of trace organic pollutants. Chemosphere 127: 269–275

Connon RE, Geist J, Werner I. Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. Sensors (Basel). 2012;12(9):12741–12771. doi:10.3390/s120912741Couture P, Busby P, Gauthier C, Rajotte JW (2008) Seasonal and regional variations of metal contamination and condition indicators in yellow perch (Perca flavescens) along two polymetallic gradients. I. factors influencing tissue metal concentrations. Human and Ecological Risk Assessmen 14: 97–125

Couture P, Kumar PR (2003) Impairment of metabolic capacities in copper and cadmium contaminated wild yellow perch (Perca flavescens). Aquatic Toxicology 64(1): 107–120

Deakin AG, Buckley J, Alzu'bi HS, Cossins AR, Spencer JW, Al'Nuaimy W, Young IS, Thomson JS, Sneddon LU (2019) Automated monitoring of behaviour in zebrafish after invasive procedures. Scientific Reports 9: 9042

Delcourt J, Denoel M, Ylieff M, Poncin P (2013) Video multitracking of fish behaviour: a synthesis and future perspectives. Fish and Fisheries 14: 186–204

Derco J, Gotvajan AŽ, Zagorc-Končan J, Almásiová B, Kassai A (2010) Pretreatment of landfill leachate by chemical oxidation processes. Chemical papers 64: 237–245

Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy.

Directive 2008/105/EC of the European Parliament and of the Council of 496 16 December 2008 on environmental quality standards in the field of water 497 policy, amending and subsequently repealing Council Directives 82/176/EEC, 498 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 499 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Communities L 500 348, p 0084–0097

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes

Directive 76/464/EEC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community http://data.europa.eu/eli/dir/1976/464/oj.

Dusabe MC, Wronski T, Gomes-Silva G, Plath M, Albrecht C, Apio A (2019) Biological water quality assessment in the degraded Mutara rangelands, northeastern Rwanda. Environmental Monitoring and Assessment 191: 139

EEA (European Environment Agency), Waterbase – Rivers. https://www.eea.europa.eu/data-and-maps/data/waterbase-rivers-10

Eissa BL, Ossana NA, Ferrari L, Salibian A (2009) Quantitative Behavioral Parameters as Toxicity Biomarkers: Fish Responses to Waterborne Cadmium. Archives of Environmental Contamination and Toxicology 58(4):1032–1039

Emenike CU, Fauziah SH, Agamuthu P (2012) Characterization and toxicological evaluation of leachate from closed sanitary landfill. Waste Management & Research 30: 888–897

EPA (2002a) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012, 5th edn. Office of Water, Washington

EPA (2002b) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA-821-R-02-013, 4th edn. Office of Water, Washington

EPA (2013) Wastewater Sampling. U.S. Environment Protection Agency Science and Ecosystem Support Division Athens, Georgia, SESD Operating Procedure 1–24

Fanouraki P, Papandroulakis N, Ellis T, Mylonas CC, Scott AP, Pavlidis M (2008) Water cortisol is a reliable indicator of stress in European sea bass, *Dicentrarchus labrax*. Behaviour 145(10): 1267–1281

Fast MD, Hosoya S, Johnson SC, Alfonso LOB (2008) Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linneaus) subjected to short- and long- term stress. Fish and Shellfish Immunology 24: 194–204

Félix AS, Faustino AI, Cabral EM, Oliveira RF (2013) Non-invasive measurement of steroid hormones in zebrafish holding-water. Zebrafish 10(1), 110–115.

Gagnon A, Jumarie C, Hontela A (2006) Effects of Cu on plasma cortisol and cortisol secretion byadrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 78(1): 59–65

Gesto M, Hernández J, López-Patiño MA, Soengas JL, Míguez JM (2015) Is gill cortisol concentration a good acute stress indicator in fish? A study in rainbow trout and zebrafish. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 188: 65–69.

Gonçalves R, Scholze M, Ferreira AM, Martins M, Correia AD (2008) The joint effect of polycyclic aromatic hydrocarbons on fish behavior. Environmental Research 108: 205–213

Guardiola FA, Cuesta A, Esteban MA (2016) Using skin mucus to evaluate stress in gilthead seabream (*Sparus aurata* L.). Fish and Shellfish Immunology 59: 323–330

Guest TW, Blaylock RB, Evans AN (2016) Development of a modified cortisol extraction procedure for intermediately sized fish not amenable to whole-body or plasma extraction methods. Fish Physiology and Biochemistry 42(1): 1–6

Gui F, Wang P, Wu C (2014) Evaluation approaches of fish swimming performance. Agricultural Sciences 5: 106–113

Hamilton PB, Rolshausen G, Webster TUM, Tyler CR (2017) Adaptive capabilities and fitness consequences associated with pollution exposure in fish. Philos Trans R Soc Lond B Biol Sci 372(1712): 20160042

Heys KA, Shore RF, Pereira MG, Jones KC, Martin FL (2016) Risk assessment of environmental mixture effects. RSC Advances 6: 47844–47857

Hellou J (2011) Behavioural ecotoxicology, an “early warning” signal to assess environment quality. Environmental Science and Pollution Research 18: 1–11

Hook SE, Gallagher EP, Batley GE (2012) The role of biomarkers in the assessment of aquatic ecosystem health. Integrated Environmental Assessment and Management 10(3): 327–341

Hoq T, Hasan MR, Haque N (2015) The Effect of Chromium on Glucose Content of Freshwater Fish, *Heteropneustes fossilis*. American Journal of Zoological Research 3(1): 1–3

Huang Y, Zhang J, Xiaobo H, Huang T (2014) The use of zebrafish (Danio rerio) behavioral responses in identifying sublethal exposures of deltamethrin. International Journal of Environmental Research and Public Health 11:3650–3660

Inyinbor AA, Adebesin BO, Oluyori AP, Adelani-Akande TA, Dada AO, Oreofe TA (2018) Water pollution: effects, prevention, and climatic impact, in Water Challenges of an Urbanizing World, IntechOpen Limited, London, UK.

ISO 7346-1 (1996a) Water quality—determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] Part 1: Static method. ISO, Switzerland

ISO 7346-2 (1996b) Water quality—Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] Part 2: Semi-static method. ISO, Switzerland

ISO 7346-3 (1996c) Water quality—Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] Part 1: Flowthrough method. ISO, Switzerland

Iversen M, Finstad B, McKinley RS, Eliassen RA (2003) The efficacy of metomidate, clove oil, Aqui-S™ and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-educing capacity. Aquaculture 221:549–566

Jager T, Vandenbrouck T, Baas J, De Coen WM, Kooijman SA (2010) A biology-based approach for mixture toxicity of multiple endpoints over the life cycle. Ecotoxicology 19(2): 351–361

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology 7(2): 60–72.

Jentoff S, Aastveit AH, Torjesen PA, Andersen O (2005) Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 141(3): 353–358

Jiang D, Wu Y, Huang D, Ren X, Wang Y (2017) Effects of nutritional history on stress response in gibel carp (*Carassius auratus gibelio*) and largemouth bass (*Micropterus salmoides*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 210: 9–17

Kienzler A, Bopp SK, Linden SVD, Berggen E, Worth A (2016) Regulatory assessment of chemical mixtures: Requirements current approaches and future perspectives. Regulatory Toxicology and Pharmacology 80: 321–334

Kjeldsen P, Barlaz MA, Rooker AP, Baun A, Ledin A, Christensen TH (2002) Present and Long-Term Composition of MSW Landfill Leachate: A Review. Critical Reviews in Environmental Science and Technology 32(4): 297–336

Klauck CR, Rodrigues MAS, da Silva LB (2013) Toxicological evaluation of landfill leachate using plant (*Allium cepa*) and fish (*Leporinus obtusidens*) bioassays. Waste Management & Research 31:48–53

Kochhan D, Meyersieck JM, Valdez DFX, Val AL (2015) Biochemical and behavioural responses of the Amazonian fish *Colossoma macropomum* to crude oil: the effect of oil layer on water surface. Ecotoxicology and Environmental Safty 111: 32–42

Kulmala S, Haapasaari P, Karjalainen TP, Kuikka S, Pakarinenm T, Parkkila K, Romakkaniemi A et al. (2012) Ecosystem services provided by the Baltic salmon—a regional perspective to the socio-economic benefits associated with a keystone species, In Socio-economic importance of ecosystem services in the Nordic Countries—synthesis in the context of The Economics of Ecosystems and Biodiversity (TEEB), Copenhagen Nordic Council of Ministers, p 266–276

Kuz'mina V, Garina D (2001) Glucose, Insulin, and Adrenaline Effects on Some Aspects of Fish Feeding Behaviour. Journal of Evolutionary Biochemistry and Physiology 37: 154–160

Lacroix A, Hontela A (2004) A comparative assessment of the adrenotoxic effects of cadmiumin two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens*. Aquatic Toxicology 67:13–21

Langiano VC, Martínez CBR (2008) Toxicity and effects of a glyphosate-based herbicide on the neotropical fish *Prochilodus lineatus*. Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology 147(2): 222–231

Le Bihanic F, Morin B, Cousin X, Menach KL, Budzinksi H, Cachot J (2014) Developmental toxicity of PAH mixtures in fish early life stages. Part I: adverse effects in rainbow trout. Environmental Science and Pollution Research 21(24): 13720–13731

Legradi JB, Di Paolo C, Kraak MHS, et al. 2018. An ecotoxicological view on neurotoxicity assessment. Environmental Science Europe. 30(1):46.

Lowe CJ, Davison W (2005) Plasma osmolarity, glucose concentration and erythrocyte responses of two Antarctic nototheniid fishes to acute and chronic thermal change. Journal of Fish Biology 67: 752–766

Lower N, Moore A, Scott AP, Ellis T, James JD, Russell IC (2005) A non-invasive method to assess the impact of electronic tag insertion on stress levels in fishes. Journal of Fish Biology 67(5): 1202–1212

Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology 101: 13–30

Makaras T, Montvydienė D, Stankevičiūtė M, Kazlauskienė N, Virbickas T (2019) The effect of acclimation on behavioural characteristics: determine an appropriate duration of acclimation for different fish species. Journal of Fish Biology (submitted).

Martínez-Porchas M, Martinez-Cordova LR, Ramos-Enriquez R, (2009) Cortisol and Glucose: Reliable indicators of fish stress? Panamian-American Journal of Aquatic Science 4(2): 158–178

Meyer JS, Farley KJ, Garman ER (2015) Metal mixtures modelling evaluation Project: 1. Background. Environmental Toxicology and Chemistry 34: 726–740

Melvin SD (2017) Effect of antidepressants on circadian rhythms in fish: insights and implications regarding the design of behavioural toxicity tests. Aquatic Toxicology 182: 20–30

Melvin SD, Petit MA, Duvignacq MC, Sumpter JP (2017) Towards improved behavioural testing in aquatic toxicology: Acclimation and observation times are important factors when designing behavioural tests with fish. Chemosphere 180: 430–436

Michalec FG, Holzner M, Menu D, Hwang JS, Souissi S (2013) Behavioral responses of the estuarine calanoid copepod Eurytemora affinis to sub-lethal concentrations of waterborne pollutants. Aquatic Toxicology 138-139: 129–138

Miller LL, Wang F, Palace VP, Hontela A (2007) Effects of acute and sub chronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout. Aquatic Toxicology 83: 263–271

Mishra AK, Mohanty B (2009) Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, Channa punctatus (Bloch). Science of the Total Environment 407: 5031–5038

Mittlebach GG, Ballew NG, Kjelvik MK (2014) Fish behavioral types and their ecological consequences. Canadian Journal of Fisheries Aquatic Sciences 71: 927–944

Montvydienė D, Šulčius S, Jurgelėnė Ž, Makaras T, Kalcienė V, Taraškevičius R, Kazlauskas M, Kazlauskienė N (2019) Contrasting ecotoxic effects of landfill leachate and cyanobacterial biomass on aquatic organisms. Water, Air and Soil Pollution (submitted).

Mora-Zamorano FX, Klingler R, Basu N, Head J, Murphy CA, Binkowski FP, Larson JK, Carvan MJ (2017) Developmental Methylmercury Exposure Affects Swimming Behavior and Foraging Efficiency of Yellow Perch (*Perca flavescens*) Larvae. ACS Omega 2: 4870–4877

Nageswara RNR, Srinivasa NB, Jagadish NM (2017) Thiram a fungicide induced toxicity on glycogen and blood glucose level of freshwater fish *Cyprinus carpio* (Hamilton). International Journal of Fisheries and Aquatic Studies 5: 93–96

Nakano T, Kameda M, Shoji Y, Hayashi S, Yamaguchi T, Sato M (2014) Effect of severe environmental thermal stress on redox state in salmon. Redox Biology 2: 772–776

Norton WHJ, Gutiérrez HC (2019) The three-spined stickleback as a model for behavioural neuroscience. PLoS One 14(3): e0213320

O'Neill SJ, Williamson JE, Tosseto L, Brown C (2018) Effects of acclimatisation on behavioural repeatability in two behaviour assays of the guppy *Poecilia reticulate*. Behavioral Ecology and Sociobiology 72: 166

Øverli Ø, Kotzian S, Winberg S (2002) Effects of cortisol on aggression and locomotor activity in rainbow trout. Hormones and Behaviour 42(1): 53–61

Pérez-Casanova JC, Afonso LOB, Johnson SC, Currie S, Gamperl AK (2008) The stress and metabolic responses of juvenile Atlantic con *Gadus morhua* L. to an acute thermal challenge. Journal of Fish Biology 72: 899–916.

Przydatek G, Kanownik W (2019) Impact of small municipal solid waste landfill on groundwater quality. Environmental Monitoring and Assessment 191: 169

Pucher J, Gut T, Mayrhofer R, El-Matbouli M, Viet PH, Ngoc NT, Lamers M, Streck T, Focken U (2014) Pesticide-contaminated feeds in integrated grass carp aquaculture: toxicology and bioaccumulation. Diseases of Aquatic Organisms 108(2): 137–147

Qiu A, Cai, Q, Zhao Y, Guo Y, Zhao L (2016) Evaluation of the treatment process of landfill leachate using the toxicity assessment method. International Journal of Environmental Research and Public Health. 13:1262.

Raihana U, Ismail SNS, Abidin EZ, Praveena SM (2014) Landfill Leachate Toxicity Analysis with *Orechromis mossambicus* (Mozambique Tilapia): A Review. International Journal of Sciences: Basic and Applied Research 18(2): 198–216

Ren Q, Zhang T, Li S, Ren Z, Yang M, Pan H, Xu S, Qi L, Choon TS (2016) Integrative Characterization of Toxic Response of Zebra Fish (Danio rerio) to Deltamethrin Based on AChE Activity and Behavior Strength. BioMed Research International ID 7309184, 10 pages

Robinson PD (2009) Behavioural toxicity of organic chemicalcontaminants in fish: application to ecological riskassessments (ERAs) Canadian Journal of Fisheries and Aquatic Sciences 66: 1179–1188

Saaristo M, Lagesson A, Bertram MG, Fick J, Klaminder J, Johnstone CP, Wong BBM, Brodin T (2019) Behavioural effects of psychoactive pharmaceutical exposure on European perch (*Perca fluviatilis*) in a multi-stressor environment. Science of the Total Environment 655: 1311–1320

Salem ZB, Capelli N, Grisey E, Baurand PE, Ayadi H, Aleya L (2014) First evidence of fish genotoxicity induced by heavy metals from landfill leachates: the advantage of using the RAPD-PCR technique. Ecotoxicology and Environmental Safety 101: 90–96

Sandheinrich M (2014) Role of behavior in ecotoxicology, in: Newman C. M. (Eds.), Fundamentals of ecotoxicology – The Science of pollution, 4th ed. CRC Press, New York, London, pp 265–267

Santos SW, Cachot J, Gourves PY, Clérandeau C, Morin B, Gonzalez P (2019) Sub-lethal effects of waterborne copper in early developmental stages of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicology and Environmental Safety 170: 778–788

Sárria MP, Soares J, Vieira MN, Castro LF, Santos MM, Monteiro NM (2011) Rapid-behaviour responses as a reliable indicator of estrogenic chemical toxicity in zebrafish juveniles Chemosphere 85(10): 1543–1547

SCHER, SCCS, SCENIHR 2012 Opinion on the Toxicity and Assessment of Chemical Mixtures. doi:10.2772/21444

SCORECARD (2005) Water quality indicators http://www.scorecard.org

Scott AP, Ellis T (2007) Measurement of fish steroid in water – a review. General and Comparative Endocrinology 153: 392–400.

Scott GR, Sloman KA (2004) The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology 68: 369–392

Sergy GA, Scroggins RP (1990) Canada. Conservation and Protection., Canada. Environment Canada. Biological test method: acute lethality test using three spine stickleback (*Gasterosteus aculeatus*). Ottawa: Environment Canada

Shinn C, Santos MM, Lek S, Grenouillet G (2015) Behavioural response of juvenile rainbow trout exposed to an herbicide mixture. Ecotoxicology and Environmental Safety 112: 15–21

Sovova T, Boyle D, Sloman KA, Perez CV, Handy RD (2014) Impaired behavioural response to alarm substance in rainbow trout exposed to copper nanoparticles. Aquatic Toxicology 152: 195–204

Steinberg CEW (2003) Subacute toxicity: Behavioral Disturbances, in: Ecology of Humic Substances in Freshwaters, Springer-Verlag Berlin Heidelberg, New York, pp. 266–268

Summers JK, Smith LM, Case JL, Linthurst RA (2012) A review of the elements of human well-being with an emphasis on the contribution of ecosystem services. Ambio. 41(4): 327–340. Svecevičius G (2005) Use of behavioral responses of rainbow trout Oncorhynchus mykiss in identifying sublethal exposure to hexavalent chromium. Bulletin of Environmental Contamination and Toxicology 82(5): 564–568

Svecevičius G (2006) Acute toxicity of hexavalent chromium to European freshwater fish. Bulletin of Environmental Contamination and Toxicology 77(5): 741–747

Svecevičius G (2007) The use of fish avoidance response in identifying sublethal toxicity of heavy metals and their mixtures. Acta Zoologica Lituanica 17(2): 139–143

Svecevičius G (2009) Use of behavioural responses of rainbow trout *Oncorhynchus mykiss* in identifying sublethal exposure to hexavalent chromium. Bulletin of Environmental Contamination and Toxicology 82: 564–568

Svecevičius G (2012) The acute and behavioural effects of a copper-nickel mixture on roach *Rutilus rutilus.* Bulletin of Environmental Contamination and Toxicology 89:147–151

Svecevičius G, Kazlauskienė N, Slučkaitė A, Makaras T (2014) Toxicological assessment of the effects of closed landfill on neighbouring hydroecosystem. Fresenius Environmental Bulletin 23(11): 2926–2932

Taylor LN, Wood CM, Mcdonald DG (2003) An evaluation of sodium loss and gill metal binding properties in rainbow trout and yellow perch to explain species differences in copper tolerance. Environmental Toxicology and Chemistry 22: 2159–2166

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. Experimentia Supplementum 101:133–164.

Tierney KB (2011) Behavioural assessments of neurotoxic effects and neurodegeneration in zebrafish. Biochimica et Biophysica Acta 1812: 381–389

Tierney KB, Baldwin DH, Hara TJ, Ross PS, Scholz NL, Kennedy CJ (2010) Olfactory toxicity in fishes. Aquatic Toxicology 96: 2–26

Tinbergen N (1953) Social behaviour in animals: with special reference to vertebrates. Oxford, England: Wiley

Turschwell MP, White CR (2016) The effects of laboratory housing and spatial enrichment on brain size and metabolic rate in the eastern mosquitofish, *Gambusia holbrooki*. Biology Open 5(3): 205–210

Vandenberg LN, Colborn T, Hayes TB, et al. (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine Reviews 33(3): 378–455

Wang H, Liang Y, Li S, Chang J (2013) Acute toxicity, respiratory reaction, and sensitivity of three cyprinid fish species caused by exposure to four heavy metals. PloS one 8(6): e65282

Wark AR, Greenwood AK, Taylor EM, Yoshida K, Peichel CL (2011) Heritable differences in schooling behavior among threespine stickleback populations revealed by a novel assay. PLoS One 6: e18316

Weis JS, Smith G, Zhou T, Santiago-Bass C, Weis P (2001) Effects of Contaminants on Behaviour: Biochemical Mechanisms and Ecological Consequences: Killifish from a contaminated site are slow to capture prey and escape predators; altered neurotransmitters and thyroid may be responsible for this behaviour, which may produce population changes in the fish and their major prey, the grass shrimp. BioScience 51(3): 209–217

Welker TL, Lim C, Yildrim-Askoy M, Kelsius PH (2007) Effect of buffered and unbuffered tricaine methanesulfonate (MS-222) at different concentrations on the stress responses of channel catfish, Ictalurus punctatus Rafinesque. Journal of Applied Aquaculture 19: 1–18

Wibe EÅ, Nordtug T, Jenssen BM (2001) Effects of bis(tributyltin)oxide on antipredator behavior in threespine stickleback *Gasterosteus aculeatus* L. Chemosphere 44(3): 475–481

Witewska M, Jezierska B, Wolnicki J (2005) Respiratory and hematologicoloy response of tench, *Tinca tinca* (L.) to short-term cadmium exposure. Aqua Culture 14: 141–152

Wu H, Aoki A, Arimoto T, Nakano T, Ohnuki H, Murata M, Ren H, Endo H (2015) Fish stress become visible: A new attempt to use biosensor for real-time monitoring fish stress. Biosensors and Bioelectronics 67: 503–510

Wuana RA, Okieimen FE (2011) Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology, 402647

Xia C, Fu L, Liu Z, Liu H, Chen L, Liu Y (2018) Aquatic Toxic Analysis by Monitoring Fish Behavior Using Computer Vision: A Recent Progress. Journal of Toxicology 2591924: 1–11

Xie Z, Lu G, Li S, Nie Y, Ma B, Liu J (2015) Behavioural and biochemical responses in freshwater fish *Carassius auratus* exposed to sertraline. Chemosphere 135: 146–155

Zafirakou A, Gaitanelis E, Gianni M (2013) Water Quality Monitoring and Assessment of a Small SWM's Produced Leachates. World Environment 3(3): 94–101

Zahangir M, Haque F, Islam MS (2014) Effects of acute water Ph stress on the stress indicators in zebrafish (*Danio rerio*). Proceedings of 5th International Conference on Environmental Aspects of Bangladesh, 112–113

Zahangir MM, Haque F, Mostakim GM, Islam MS (2015) Secondary tress responses of zebrafish to different pH: Evaluation in a seasonal manner. Aquaculture Reports 2: 91–96

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my first scientific supervisor Dr Gintaras Svecevičius (2015-2016) for being the greatest mentor and guiding me along the right path, for supporting and sharing his experience with me. I am so thankful for everything that I have learned from him. I could not have imagined having a better advisor and mentor for my Ph.D study.

My sincere thanks also go to my second supervisor Dr Nijolė Kazlauskienė. Her guidance helped me in the course of research and preparation of this doctoral thesis.

I wish to thank my co-authors, Dr Milda Stankevičiūtė, Dr Gintaras Svecevičius, Dr Danguolė Montvydienė, Dr Nijolė Kazlauskienė, Dr Tomas Virbickas, Dr Julija Razumienė, Dr Ieva Šakinytė, Dr Gintarė Sauliutė, Vidutė Gurevičienė, Dr Janina Greiciūnaitė, and others, for collaboration and expertise.

I express my gratitude to Dr Julija Razumienė, Vidutė Gurevičienė and Dr Ieva Šakinytė from Life Science Centre of Vilnius University for their guidance and expertise in glucose measurement experiments. I sincerely thank Dr Kęstutis Arbačiauskas and Dr Eglė Šidagytė for their suggestions concerning data analysis and Laima Monkienė for the language editing.

My gratitude is also extended to colleagues and friends Janina Pažusienė, Dr Gintarė Sauliutė and Dr Živilė Jurgelėnė for stimulating thesis-related discussions, and for all the fun we have had while working together over the last four years. I thank my colleagues at the Laboratory of Genotoxicology and the Laboratory of Fish Ecology for co-operation and assistance.

And most of all, I would like to give my special thanks to Dr Milda Stankevičiūtė, who has helped me in collecting data during the experiments, in writing manuscripts and supporting and being with me at a difficult time for me.

I am very thankful to my family and my closest friends for unconditional support and patience throughout these years.

This work was partially supported by the Research Council of Lithuania under the projects NANOTOX MIP-108/2015 and ACTIS S-MIP-17-10.

This doctoral dissertation is dedicated to the memory of my first supervisor and my friend Dr Gintaras Svecevičius.

LIST OF PUBLICATIONS

I

Toxicological assessment of the effects of closed landfill on neighbouring hydroecosystem

Svecevičius G, Kazlauskienė N, Slučkaitė A, **Makaras T**

Fresenius Environmental Bulletin 2014, 23(11a): 2926–2932

II

Rapid detection of sublethal toxicity using locomotor activity of rainbow trout juveniles

Makaras T, Svecevičius G, Kazlauskienė N, Montvydienė D

Bulletin of Environmental Contamination and Toxicology 2018, 100: 221–227

III

Responses of biomarkers in Atlantic salmon (*Salmo salar*) following exposure to environmentally relevant concentrations of complex metal mixture (Zn, Cu, Ni, Cr, Pb, Cd). Part II

Stankevičiūtė M**,** Sauliutė G, **Makaras T,** Markuckas A, Virbickas T, Baršienė J

Ecotoxicology 2018, 27(8): 1069–1086.

IV

Comparison of behavioral and respiratory responses of European perch and rainbow trout to metal mixture in terms of rapidness and sensitivity **Makaras T**, Montvydienė D, Kazlauskienė N, Stankevičiūtė M

Bulletin of Environmental Contamination and Toxicology 2019, 103(3): 391–399

IV

Comparison of behavioral and respiratory responses of European perch and rainbow trout to metal mixture in terms of rapidness and sensitivity Makaras T, Montvydienė D, Kazlauskienė N, Stankevičiūtė M

Bulletin of Environmental Contamination and Toxicology 2019, 103(3): 391–399

V

A new approach to stress evaluation in fish using β-D-Glucose measurement in fish holding water

Makaras T, Razumienė J, Gurevičienė V, Šakinytė I, Stankevičiūtė M, Kazlauskienė N

Ecological Indicators 2020, volume 109, 105829 available online 2019: doi.org/10.1016/j.ecolind.2019.105829

VI

Juvenile fish responses to sublethal leachate concentrations: comparison of sensitivity of different behavioral endpoints

Makaras T, Montvydienė D, Kazlauskienė N, Stankevičiūtė M, Raudonytė-Svirbutavičienė E

Environmental Science and Pollution Research 2019, available online (DOI: 10.1007/s11356-019-07211-6)

VII

Behavioural responses of European perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) to exposure of complex (Pb, Zn, Cu, Cd, Ni and Cr)

Makaras T, Montvydienė D, Kazlauskienė N

Proceedings of the 7^{h} International Conference on Environmental Management, Engineering, Planning and Economics. Mykonos Island, Greece, 90-97. ISBN: 978-618-5271-73-2.

VIII

Ecotoxicity assessment of multicomponent mixtures of different origin (landfill leachate and biomass of harmful algae bloom) using three aquatic organisms

Montvydienė D, **Makaras T**, Kazlauskienė N, Cibulskaitė Ž, Šulčius S

CEMEPE proceedings of 6th International Conference on Environmental Management, Engineering, Planning & Economics, 2017 Thessaloniki, Greece, 114–123. ISBN: 978-618-5271-15-2.

IX

Use of locomotor activity of rainbow trout juveniles identyfing sublehtal concentrations of landfill leachate

Makaras T, Svecevičius G

International Journal of Computer and Systems Engineering 2016, 10(1): 15–21.

NOTES

Vilniaus universiteto leidykla
Saulėtekio al. 9, LT-10221 Vilnius
El. p. info@leidykla.vu.lt,
www.leidykla.vu.lt
Tiražas 12 egz.