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THE BLUE CITY: ZOOBENTHOS COMMUNITIES AS AN INDICATOR OF URBANISATION IN THE NEMUNAS RIVER, KAUNAS CITY

Darbo vadovas (-ė): prof. dr. Algirdas Kaupinis

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ABSTRACT

Urban rivers are increasingly exposed to multiple stressors associated with land use change, yet effective tools for assessing their ecological condition remain essential for sustainable management. This study evaluated the potential of zoobenthic communities as bioindicators of water quality along an urbanisation gradient in the Nemunas River within the city of Kaunas, Lithuania. Sampling sites were selected across natural, urban centre, and peripheral areas to examine spatial variation in benthic macroinvertebrate communities in relation to physico-chemical parameters. Ecological status was assessed using the River Macrofauna Index (UMI), alongside measurements of pH, water temperature, conductivity, nutrients, chlorophyll, chloride, as well as flow velocity. UMI values were highest in natural and semi-natural areas and lowest in peripheral sections, supporting the hypothesis that urbanisation negatively influences zoobenthic community structure. Urban centre sites exhibited relatively consistent, intermediate UMI values. A significant negative relationship between UMI and both chloride concentrations was revealed, indicating the importance of ionic pollution as a driver of ecological degradation in urban rivers. Other physico-chemical parameters showed weak or non-significant relationships with UMI. Significant differences were found between seasons in metahabitat analysis, which showed homogenisation in autumn, and higher diversity in spring. Zoobenthic assemblages were dominated by invasive species, particularly *Pontogammarus robustoides* (Sars, 1894), which are not accounted for in UMI calculations and may influence interpretations of ecological status. Overall, the results demonstrate that zoobenthic communities can effectively reflect spatial patterns in water quality along urbanisation gradients, while also highlighting the need to refine bioassessment approaches to account for invasive species and urban-specific stressors.

Keywords: *Nemunas river, zoobenthic communities, River Macrofauna Index, urbanisation gradient, bioindicators, Kaunas*

1. INTRODUCTION

1.1. Urban Blue Spaces

Urban blue spaces are defined as all the natural and anthropogenic surface waters located in an urban area (Smith et al., 2021). Urban blue spaces are well known for their salutogenic qualities and the many ecosystem services they provide (Mishra et al., 2020). Water quality is considered rather important in terms of assessing overall environmental quality and a good indicator of alpha diversity (or species richness) of a particular habitat. Urban areas in particular are more prone to water quality degradation, primarily due to anthropogenic land use and its consequences. For instance, high nutrient content, acidification, increased urban runoff, and pollution have a significant impact on the hydrological, physical, and chemical structure of urban blue spaces (Calderón et al., 2019). The focal point of this study is specifically urban rivers. Historically, many cities have formed as settlements around riverbanks, as rivers are great resources, providing humans with food, water, power, as well as navigation routes for commerce, and recreation (Francis, 2012). However, anthropogenic activity has taken its toll on rivers via pollution and geomorphological modifications with severe consequences for aquatic life, causing the so-called ”Urban Stream Syndrome”. This syndrome is characterised by a complex array of symptoms including flashier hydrographs, elevated concentrations of nutrients and contaminants, and altered channel morphology. It is known to cause a decline in alpha diversity, as well as offering suitable conditions for pollution tolerant species and invasive species (Everard & Moggridge, 2011). Francis (2013) has described urban rivers as a novel ecosystem, encountering very specific challenges. For instance, due to the great amount of alterations caused by human activity, such as concrete embankments that attract only specific fauna, or offer conditions for very specific vegetation to grow, urban rivers cannot be considered fully natural ecosystems. These artificial riparian zones often lack the microhabitat complexity, such as woody debris and diverse substrate sizes, required to support specialised benthic communities.

1.2. The Urban Stream Syndrome

As mentioned above, the global phenomenon of urbanisation represents one of the most transformative forms of anthropogenic landscape degradation. As natural, permeable catchment vegetation is systematically stripped away and replaced by impervious surface areas such as asphalt, concrete, and roofing, the fundamental hydrology of the surrounding watershed is permanently altered. In stream ecology, the cumulative physical, chemical, and biological degradation resulting from these landscape alterations is formally classified as the "Urban Stream Syndrome" (Walsh et al., 2005; Kominkova, 2012).

The primary vector of this syndrome is the decoupling of the stream channel from its natural infiltration pathways. In an unimpacted catchment, precipitation is largely absorbed by soils and intercepted by vegetation canopy, feeding the fluvial system through slow, filtered groundwater flows. Conversely, urban catchments tend to direct surface runoff directly into receiving waters through engineered stormwater drainage networks. This structural connectivity, termed "effective imperviousness," acts as a hydraulic conveyor belt that delivers high volumes of surface runoff to the river channel within a highly compressed timeframe (Paul & Meyer, 2001).

The immediate consequence of the aforementioned effective imperviousness is a radically altered hydrograph. Urban rivers experience precipitation surges in peak discharge during storm events, followed by unnaturally low baseflows during dry periods. These intense hydraulic pulses generate severe erosive forces, causing channel incision, bank destabilisation, and the deterioration of benthic substrates. For macroinvertebrate communities, this represents a severe, chronic physical disturbance. The sheer force of storm-driven flows physically removes benthic organisms from the riverbed, accelerating downstream behavioral and catastrophic drift, while simultaneously destroying the stable micro-niches required for larval development (Kominkova, 2012).

1.3. The ecological significance of benthic macroinvertebrates

The ecological assembly of macroinvertebrate communities along an urbanisation gradient can be conceptualised as a series of hierarchical environmental filters. Landscape-scale stressors, such as regional land-use patterns dictate catchments-wide chemical baselines, which are subsequently modified by local riparian buffers, and ultimately expressed as specific microhabitat matrices at the benthic level. When an organism is exposed to this hierarchy within an urban matrix, the natural habitat-driven filters are frequently overwhelmed by acute and chronic chemical degradation (Alberti et al., 2007).

Industrial and highly urbanised zones act as highly restrictive chemical filters. Surface runoff washing over industrial zones, transportation corridors, and urban centres carries a complex mix of diffuse pollutants, including heavy metals (e.g., copper, zinc, lead), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, and fine suspended sediments (Nakajima & Aryal, 2018). These contaminants accumulate within the organic matter and fine fractions of the benthic sediment profile, exposing deposit-feeding and burrowing macroinvertebrates to chronic toxicity. Fine sediments pose a dual threat: they mechanically clog the spaces between gravel and cobble, suffocating hidden organisms, and they coat the respiratory surfaces of gills, impairing oxygen uptake in sensitive larvae (Schoonover et al., 2006).

Conversely, the preservation of natural riparian vegetation and permeable buffer zones can intercept and transform these stressors before they reach the instream environment. Riparian forests and wetlands act as nutrient sinks, utilising microbial denitrification to reduce nitrate loads and physically trapping sediment-bound phosphorus. Furthermore, riparian canopies provide essential microclimatic regulation by shading the water surface, which suppresses water temperatures and prevents thermal pollution driven by the Urban Heat Island (UHI) effect. Under unimpacted conditions, these vegetated zones also contribute to non local organic matter (leaf litter and woody debris), providing a complex trophic base that supports a diverse array of functional feeding groups, particularly shredders and collectors (Vannote et al., 1980).

However, in highly modified urban channels where concrete embankments and sheet piling replace natural shorelines, this riparian buffer capability is entirely lost. The loss of lateral connectivity with the floodplain means the river is transformed into an engineered canal, stripped of its self-purification capacity and microhabitat complexity (Francis, 2013).

1.4. Urban rivers as novel ecosystems

The combined physical and chemical pressures of urbanisation fundamentally restructure the evolutionary rules of the aquatic environment, forcing a transition into what ecologists define as "novel ecosystems" (Francis, 2013). Novel ecosystems are those in which human alterations have permanently changed the biophysical structures and species compositions away from historical baselines, creating entirely new environmental templates that lack natural analogs. A defining characteristic of these novel urban river channels is the pervasive onset of ecological and biotic homogenisation (Emde et al., 2012).

In pristine river systems, high macroinvertebrate diversity is largely maintained by high spatial heterogeneity. The physical environment presents an intricate mosaic of distinct microhabitats: swift flows over coarse cobbles host unique lithophilic scraper communities, slow eddies around submerged woody debris harbor specific burrowing detritivores, and dense macrophyte beds (sweep zones) provide complex structural cover and epiphytic biofilm for specialised climbers and piercers. This separation of niches allows hundreds of unique taxonomic specialists to coexist within the same river reach. However, when an urban fluvial network suffers from systemic chemical degradation, such as chronic salinisation and nutrient overload, the chemical filter operates as an overarching evolutionary bottleneck. The toxic threshold of the water column effectively nullifies the ecological benefits typically provided by physical habitat complexity. If the background water chemistry exceeds the physiological tolerances of sensitive specialists, the presence of complex macrophyte structures or clean gravel substrates becomes irrelevant.

Consequently, the localised micro-niches collapse into a single, functionally uniform environment where only a handful of highly plastic, stress-tolerant generalists can survive. This results in statistical similarity between distinct structural zones (such as benthic kick samples vs. macrophyte sweep samples), signaling a profound loss of biodiversity between habitats (beta diversity) and a systemic structural flattening of the entire aquatic food web (Emde et al., 2012).

The ecological vulnerability of novel urban ecosystems is frequently exploited by non-indigenous invasive species, which find ideal conditions for establishment within modified river channels. One of the most prolific vectors of aquatic biological invasions, especially relevant to the European context is the Ponto-Caspian corridor, an intricate network of natural rivers, artificial canals, and shipping routes linking the Caspian and Black Seas to the interior river basins of Northern and Western Europe. Urbanised, heavily regulated river channels act as highly efficient highways for these Ponto-Caspian invaders, leading to major shifts in community composition on a continental scale (Van Riel et al., 2006).

The spectacular success of Ponto-Caspian invaders within urbanised river systems is driven by a highly specialised composition of functional traits that align with the pressures of the Urban Stream Syndrome. Having evolved in the dynamic, fluctuating, and naturally brackish environments of the Caspian and Black Sea deltas, these organisms possess high natural tolerances for elevated conductivity, high salinity, and severe temperature fluctuations. When introduced into an urban river experiencing chronic salinisation, eutrophication, and high water temperatures, these non-native species are pre-adapted to tolerate the exact chemical filters that eliminate native specialists. Furthermore, many urban rivers feature extensively modified bank structures, such as artificial rock armour, concrete embankments, and sheet piling. These vertical, hard, engineered surfaces do not mimic natural riparian zones, but they closely replicate the rocky, wave-swept shorelines of lakes and estuaries to which Ponto-Caspian species are evolutionarily adapted. Native stream insects, which rely on depositional zones, leaf litter packs, and soft fine-gravel substrates, find these vertical concrete surfaces completely uninhabitable. In contrast, invasive Ponto-Caspian amphipods, dreissenid mussels, and gastropods aggressively colonise the cracks, biofilms, and micro-crevices of artificial embankments, building high population densities that crowd out any remaining native fauna (Van Riel et al., 2006). This interaction between physical habitat modification and chemical stress creates an environment which accelerates biological invasions.

1.5. Anthropogenic pollutants

While nutrient enrichment and heavy metal contamination are widely cited vectors of urban river degradation, the profound impact of anthropogenically induced salinisation, specifically driven by the accumulation of chloride (Cl^-) anions , has increasingly emerged as a main driver of biotic decline in cold-climate urban centres (Jin et al., 2011; Jamshidi et al., 2020). The primary source of this ionic stressor is the heavy application of rock salt (NaCl) and chemical brines for winter de-icing operations on roads, highways, and sidewalks. Once dissolved, chloride ions don't degrade. Instead, they remain highly mobile, entering the fluvial system through direct stormwater outfalls or infiltrating shallow groundwater aquifers, leading to persistent, chronic salinisation (Evans & Frick, 2002).

The toxicity of elevated chloride concentrations on freshwater macroinvertebrates is the root of the disruption of fundamental physiological processes, specifically osmoregulation. Freshwater organisms are hyperosmotic to their environment, meaning their internal body fluids contain a significantly higher concentration of dissolved ions than the surrounding river water. To survive and maintain cellular homeostasis, these organisms must continuously prevent the loss of internal salts while minimising excessive water influx. This is achieved through active ion transport, where specialised cells located in epithelial tissues, such as the tracheal gills of mayflies (*Ephemeroptera*) and caddisflies (*Trichoptera*), actively absorb sodium (Na^+) and chloride (Cl^-) ions from the water column against a steep concentration gradient (Fink, 2021).

Furthermore, different macroinvertebrate taxa exhibit diverse physiological sensitivities to this ionic stress. Sensitive groups, particularly the *Ephemeroptera, Plecoptera*, and *Trichoptera* (the EPT taxa), possess thin, highly permeable cuticles designed to increase oxygen absorption in pristine environments. This high permeability makes them exceptionally vulnerable to rapid fluxes in external salinity, leading to a sudden decline in EPT richness and abundance along salinisation gradients (Evans & Frick, 2002).

Contrastingly, certain highly tolerant taxa, including many *Diptera* (e.g., *Chironomidae*), *Odonata,* and specific macro-molluscs, possess specialised cuticular properties or superior hyporheic osmoregulatory mechanisms that allow them to tolerate high ionic strengths. They readily occupy the vacant niche space left behind by the collapse of sensitive native species (Jin et al., 2011).

This study will investigate several physico-chemical parameters that were linked with urbanisation. These parameters are as follows: pH, water temperature, conductivity, total nitrates, and total phosphates.

pH is deemed to be somewhat of a ”master variable” in determining water quality. In natural water bodies, most reactions are pH reliant, meaning that waters contain both acids and bases, and their opposing interactions along with other biological processes, such as photosynthesis, determine water pH (Boyd, 2019). Fluctuations in pH can significantly alter the bioavailability of heavy metals and the toxicity of ammonia, thereby exerting direct physiological stress on aquatic organisms.

Conductivity is a rather reliable way of detecting different types of anthropogenic pollutants in fresh waters that present themselves as electrically charged ions, for instance chloride, heavy metals, pharmaceuticals, and nutrients (De Sousa et al., 2014). Conductivity is used as a measure of salinity, ionic strength, and total dissolved solids in natural waters (McCleskey et al., 2012). In urban contexts, spikes in conductivity often serve as a proxy for the influx of point-source and non-point-source discharges into the fluvial system.

Increased water temperature may be linked to urbanisation via the Urban Heat Island Effect (Hathway & Sharples, 2012), and is also linked with other physicochemical indicators, such as pH and conductivity. Thermal pollution in urban rivers can depress dissolved oxygen levels and accelerate metabolic rates in ectothermic aquatic species, potentially exceeding their thermal tolerance limits.

Flow velocity can affect population densities and community compositions of many aquatic organisms including many macroinvertebrates (Nautiyal and Mishra, 2022).

Nitrogen and Phosphorus compounds are the main drivers of eutrophication, a process rather widespread as a result of anthropogenic activity, and very common in heavily urbanised areas (Côte et al., 2021). Excessive loading of these limiting nutrients stimulates primary production, leading to algal blooms and subsequent hypoxic conditions during decomposition.

Chloride anions, which are linked to salinity, tend to come in higher concentration in urban rivers than in their non-urban counterparts, as a result of road salt usage during winter, exceeding ecological quotas (USGS, 2019). The persistence of these anions in the groundwater-surface water interface can lead to chronic salinisation, permanently altering the osmotic balance required for freshwater macroinvertebrate survival.

1.6. Ecological analysis of riparian systems

To accurately diagnose the ecological health of highly modified novel ecosystems, modern bioassessment frameworks rely on multi-metric indices that consolidate complex ecological data into digestible management metrics. In Lithuania, the standardised protocol for assessing the ecological status of surface running waters is the River Macroinvertebrate Index (UMI) (Lithuanian Ministry of Environment, 2007). The structural reliability of the UMI stems from its integration of four distinct biological markers, each designed to capture a unique facet of community response to environmental degradation.

The first component, the Danish Stream Fauna Index (DSFI), is an indicator system optimised primarily for tracking organic pollution and subsequent dissolved oxygen depletion. The DSFI evaluates the presence and relative abundance of specific key indicator groups, scaling its score based on the sensitivity of the most delicate organisms detected within the sample.

This is supplemented by the Average Score Per Taxon (ASPT), derived from the foundational British Biological Monitoring Working Party (BMWP) system. The ASPT assigns a static tolerance score ranging from 1 (highly tolerant) to 10 (highly sensitive) to macroinvertebrate families. By calculating the arithmetic mean of these scores across all families present, the ASPT provides a measure of community sensitivity that is robust against variations in sample size or seasonal sampling effort.

The final two metrics of the UMI focus on community structure and composition. The DEP metric quantifies the absolute taxonomic richness of the *Diptera, Ephemeroptera,* and *Plecoptera* families, tracking the critical balance between pollution-tolerant true flies and pollution-sensitive insects. This is paired with the EHP-CrHi metric, which calculates the proportional ratio between highly sensitive insect groups (*Ephemeroptera, Heteroptera, Plecoptera*) and generally tolerant, long-lived macro-crustaceans (*Crustacea*) and leeches (*Hirudinea*). By averaging these four metrics, the UMI generates a comprehensive score reflecting the ecological integrity of the system.

However, a critical structural limitation exists within the architecture of classical bioassessment indices like the UMI. These tools were historically conceptualised and calibrated during the late 20th century, when the primary threat to freshwater rivers was organic enrichment from untreated municipal sewage and agricultural runoff. Consequently, their scoring scales are optimised to detect saprobiotic gradients and oxygen depletion. They do not possess explicit calibrations for the unique, non-saprobiotic stressors of the modern urban matrix, such as chronic chloride salinisation, synthetic chemical pollution, or intense biological contamination by non-native invasive species. This gap creates a substantial risk of diagnostic error in urban environmental management.

Aquatic macrofauna is used as a water quality bioindicator, as they are generally very sensitive to environmental conditions and respond to both natural and anthropogenic changes. Their relatively sedentary nature allows them to integrate the effects of short-term environmental stressors, providing a holistic reflection of the ecosystem's ecological integrity. Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), collectively known as EPT taxa, are among the most significant indicators in water quality assessment. These groups are among the first to react to pollution, with their abundance decreasing as water quality deteriorates. The number of EPT taxa (EPT_taxa) and their relative abundance (EPT_%) are positively correlated with water quality assessment indices (such as BMWP and DSFI) (Višinskienė & Bernotienė, 2012). However, the reliability of zoobenthic communities in assessing water quality can be complicated by the presence of invasive species. For instance, high abundances of non-indigenous molluscs, such as *Potamopyrgus antipodarum* (J.E. Gray, 1843) and *Lithoglyphus naticoides* (C. Pfeiffer, 1828), can lead to substantial biological contamination and affect conventional metrics on the ecological quality of surface waters (Butkus et al., 2014).

1.7. Local case-study: Nemunas River, Kaunas City

To properly evaluate these global ecological paradigms within a localised context, the city of Kaunas, Lithuania, serves as an exceptionally rigorous geographic case study. The Nemunas River is the fourth-largest river system in the Baltic Sea basin and stands as the hydrographic spine of Lithuania. Historically, the geographic confluence where the Neris River empties into the Nemunas dictated the location and socio-economic development of

Kaunas city, transforming it into a vital historical node for trade, navigation, and industry since the 5th century (Maciukėnaitė & Povilaitienė, 2013). Today, this historical footprint has evolved into a dense urban-industrial matrix that exerts severe, multi-faceted pressures on the fluvial corridor.

The unique value of Kaunas as a macro-ecological laboratory is driven by a profound upstream hydrological modification: the presence of the Kaunas Hydroelectric Power Plant (HPP) and its massive associated reservoir, constructed immediately upstream of the urban core in 1959. The Kaunas HPP acts as a major disruptor of the river's longitudinal connectivity, permanently altering sediment transport, trapping natural gravel fractions, and regulating downstream flow regimes through artificial hydro-peaking operations (Jakimavičius & Kriaučiūnienė, 2009). Furthermore, the reservoir functions as a massive thermal and chemical regulator. In winter, it buffers water temperatures, while in summer and autumn, it can trap nutrients and discharge plankton-rich, oxygen-stratified water directly into the downstream urban reach.

As the Nemunas flows past the HPP dam, it immediately enters the Kaunas urban space, transforming from a natural fluvial system into a highly controlled urban blue space. Over a spatial gradient of less than twenty kilometers, the river transitions through sharply contrasting land-use zones. It moves from the relatively well-vegetated parklands of Panemunė, through the dense, historic commercial centre (Senamiestis) featuring extensive vertical concrete embankments, and ultimately into the western industrial zones of Lampėdžiai. This compact spatial arrangement packs an intense urban gradient into a single, continuous river reach, allowing researchers to track the progressive onset of the Urban Stream Syndrome without the confounding variables introduced by shifting ecoregions or sweeping latitudinal changes.

The zoobenthic communities in the Nemunas River are characterised by a significant shift from native species dominance to an overwhelming presence of non-indigenous species, particularly Ponto-Caspian amphipods, molluscs, and North American crayfish. This shift often results in the homogenisation of the benthic community, where specialised native niches are replaced by generalist invaders. This has led to a reduction in native biodiversity and overall lower taxonomic richness and abundance in the main river course, indicating a degraded ecological state in many sections. The Nemunas acts as a major pathway for biological invasions, and the continued expansion of invasive species is expected to further influence its zoobenthic communities. The city of Kaunas is an interesting case study for this research, as the Kaunas Water Reservoir, constructed for a hydroelectric power plant is known to have contributed to the rapid spread of invasive species and decreasing water quality in Nemunas (Arbačiauskas et al., 2011). The reservoir acts as a thermal and hydrological regulator, creating a "stepping stone" environment that facilitates the acclimatisation and proliferation of non-native taxa.

1.8. The main invader: *Pontogammarus robustoides*

Within the novel ecosystem of the urban Nemunas, the premier biological actor is the invasive Ponto-Caspian amphipod *Pontogammarus robustoides* (Sars, 1894). Introduced into Lithuanian waters during intentional acclimatisation programs in the 1960s to increase fish food resources, *P. robustoides* has systematically dominated large, regulated rivers and reservoirs across the Baltic basin (Arbačiauskas et al., 2011). The species represents a textbook example of an invasive engineer capable of altering community assembly patterns.

Physiologically, *P. robustoides* possesses an extraordinary tolerance profile that aligns perfectly with the chemical pressures characterising the Kaunas urban reach. It can comfortably tolerate high salinity and is capable of withstanding a large range of temperatures, maintaining active metabolic and reproductive functions under temperature regimes that trigger thermal shock in native species.

Behaviorally, *P. robustoides* is an aggressive, opportunistic omnivore. It functions simultaneously as a shredder of macro-vegetation, a voracious predator of smaller native insect larvae (such as chironomids and early-instar mayflies), and a rapid consumer of biofilms. This trophic plasticity allows it to secure a stable energy base even in highly degraded urban channels stripped of natural leaf-litter inputs (Bankovska et al., 2018).

Crucially, the Kaunas Reservoir acts as a massive invasion engine and source population for this species. The stable, slow-flowing, and mineral-rich environment of the reservoir allows *P. robustoides* to build high densities. These populations are then steadily discharged downstream into the urban Nemunas.

Once in the city channel, they leverage their aggressive behaviors and high reproductive output to outcompete and physically displace native macroinvertebrate specialists (Šidagytė & Arbačiauskas, 2016). This creates a profound "Index Paradox" within the UMI framework, because *P. robustoides* belongs to the Gammaridae family, which often receives a moderate tolerance score in standard organic pollution metrics (Stark, 1998). Its overwhelming abundance can artificially inflate family-level richness metrics, masking the reality that the native ecosystem has effectively collapsed and been replaced by a monoculture of an invasive generalist.

1.9. Aim and objectives

This study aims to evaluate the potential of zoobenthic communities as bioindicators of water quality along an urbanisation gradient in the River Nemunas, in Kaunas. By sampling sites across a range of land use types, including natural areas, peripheral or industrial zones, and urban centres, the study seeks to determine how changes in land use influence the composition and structure of benthic invertebrate communities. This involves an analysis of community assembly patterns and the shifting dominance of functional feeding groups in response to anthropogenic disturbance. The ultimate goal is to assess whether these communities can reliably reflect spatial variations in water quality and environmental stress associated with urban development. By quantifying taxonomic sensitivity and tolerance levels, the research intends to validate the diagnostic reliability of biological metrics within highly modified fluvial systems. In order to achieve this goal, the study has the following objectives:

1. To investigate the relationships between zoobenthic community structure, mesohabitat types, and key physico-chemical parameters. This objective focuses on identifying the specific environmental filters, such as substrate grain size and dissolved oxygen gradients, that dictate the spatial distribution of macroinvertebrate assemblages.
2. To assess whether changes in zoobenthic communities correspond with the intensity and type of surrounding urban land use and identify key environmental and anthropogenic factors shaping zoobenthic communities in urban aquatic systems. The emphasis here is placed on disentangling the effects of diffuse surface runoff and point-source effluents on the biotic integrity of the river.
3. To evaluate the effectiveness of using zoobenthic communities as indicators of ecological status and water quality in support of sustainable river management. This involves benchmarking biological indices against chemical water quality standards to determine their utility in long-term biomonitoring frameworks and urban restoration strategies.

1. METHODOLOGY

2.1 Study area

In order to conduct this study, 7 sampling locations were selected. For further analysis, the locations were coded. The Panemunė site (PN) is located in the Eastern side of the city, in a park with relatively high vegetation cover, and somewhat abundant avifauna, such as mallard ducks (*Anas platyrhynchos,* (Linnaeus, 1758)), common coots (*Fulica atra* (Linnaeus, 1758)), common gulls *(Larus canus* (Linnaeus, 1758)), among others. The Old city centre, further on referred to as Senamiestis (SM) site, as opposed to the previous site, is known for more foot traffic, less vegetation, predominantly grass, and concrete embankments on a portion of the river. The confluence of the Nemunas and Neris site (ST) is also located in close proximity to Senamiestis. It is an interesting site to research, as it offers insight into the combined water of two rivers, thus the different particularities of Nemunas between the locations were followed.

The Lampėdžiai site (LZ) is located in the Western part of Kaunas, closer to industrial areas of the city. It is characterised by sandy substrate, low vegetation, mainly shrubs and grasses, and an old navigational dock. The 2 Neris River sites (NC-located in the city centre and ND-located in the Northern periphery of the city) offered valuable information regarding the Nemunas - Confluence - Neris water gradient.

The Jiesia River mouth site (JI), located nearby the Panemunė neighbourhood, offers valuable information, especially for determining differences between Nemunas, as well as Neris. Similarly to the site in Panemune, it is located in a semi-natural area, further away from the city centre, however, unlike the aforementioned site which is characterised by forest vegetation such as tall trees, shrubs, and grasses, this site has lower vegetation, such as grasses and plenty of reedbeds.

Figure 1. Map of the sampling locations and position within Lithuania (Google Maps, n.d.)

2.2. The physico-chemical measurements

The physico-chemical measurements were conducted separately from the zoobenthic measurements, in several iterations. First of all, 0.5L of water was collected from each location and analysed in laboratory conditions. pH and conductivity were measured using a *WTW inoLab 720* meters for pH and conductivity, which offer instantaneous readings.

Following the pH and conductivity measurements, the inorganic compounds were measured using the chromatography method. Chromatographic analysis consists of sample introduction, separation, detection of the separated analytes and processing and evaluation of the data obtained. The detectors record the composition of the mobile phase exiting the chromatograph at different times, convert it into an electrical signal and transmit it to the computer for data processing. The river water sample tainted with drowning agents was filtered under vacuum using a 0.8 μm membrane filter. Next, a 25 ml cylinder was placed into the filter flask, and the stem of the SPE disc tray was placed into the cylinder. Then, 5 ml of C_2H_5OH was spread on the sorbent. Once a few drops of solvent have escaped, vacuum was applied, and the process was finished by vacuuming the remaining solvent to waste. This process was repeated twice. After soaking 5 ml of ethanol C_2H_5OH under vacuum for 1 min, 5 ml of 10% ethanol was added with 1-3 mm of solvent remaining. The solvent was allowed to soak into the waste, then poured in the sample with 1 mm of solvent remaining above the sorbent (without allowing the sorbent to dry). A tiny sample of water remains on the sorbent's surface after around 20 ml was allowed to flow into a cylinder that was put inside the flask and then thrown into the waste. The extraction process was carried out by inserting the stem of the fixed SPE disc tray into a clean 25 ml cylinder that has been placed within the filter flask. One to three droplets per second is the filtration rate. Filtration was halted and the filtrate was moved into a sterile chemical vial after around 20 millilitres of water had been filtered. For chromatography, the water sample was further prepared. This method was to be carried out in 2 repetitions. This method was able to offer the concentrations of inorganic compounds containing nitrate and phosphate anions, which are indicators of eutrophication and urban pollution (Kerienė, 2024).

Another parameter measured was chlorophyll-*a*, using the spectrophotometric method. For this measurement, samples were shaken thoroughly to ensure homogeneity. A 0.5l of the sample was filtered through a membrane filter using vacuum. The obtained supernatant extract was transferred into a clean 50 ml centrifuge tube with 25 ml of 80% ethanol. For the extraction, the tubes with the ethanol and supernatant were heated to 75 °C for about 5 minutes. After a 15 minutes cooling period, the tubes were transferred to a centrifuge for about 10 minutes to facilitate extraction. The extract was then transferred to 50 ml amber glass in order to avoid sunlight damage to the sample. A spectrophotometer was then used to measure the absorbance of the extracts at 665 and 750 wavelengths through a cuvette of 5 cm, which indicates the path length of the optical cell. The same procedure was conducted after adding 0.01 ml of hydrochloric acid to 10 ml of extract, which was then let to rest for 5 minutes. The concentration of chlorophyll was then calculated using the following formula: $\rho_c = \frac{(A - A_a)}{K_c} \times \frac{R}{R-1} \times \frac{10^3 V_e}{V_s \cdot d}$

Where:

$A = A_{665} - A_{750}$ (absorbance of the extract before acidification)

$A_a = A_{665} - A_{750}$ (absorbance of the extract after acidification)

V_e= volume of extract

V_s= volume of sample

K_c= 82 µ/l*cm (specific operational spectral absorption coefficient for chlorophyll-a)

R= 1,7 (A/A_a ratio for a solution of pure chlorophyll-a which is transferred to phaeophytin by acidification)

d = path length of the optical cell (in cm)

10^3= the dimension factor to fit V_e (International Organization for Standardization, 1992).

Lastly, the water temperature and turbidity are measured *in situ.* The water temperature was measured using a portable thermometer.

2.3. Macrofauna sampling

The standard methodology for (river) macroinvertebrate research is colloquially known as ”the kicking method”. This method is suitable for eulittoral studies up to 1.2-1.5 m depth. Two samples should be collected: one from the bottom not overgrown with aquatic plants, preferably hard substrate, and the other from the bottom areas overgrown with aquatic plants. A submersible hand net (25x25 cm) was used for capture. The biotope of the study site was described according to standard requirements, including the composition of the bottom and overgrowth of aquatic vegetation. A sample was taken from the open bottom by turning the substrate, agitating the bottom to a depth of 5 cm, and dragging a hand net through the turbid water. The capture time was 3 minutes, excluding substrate turning. A sample was taken from a plant-covered area by vigorously sweeping through the plants with a hand net. If excessive plants accumulate, they are thoroughly washed and removed from the net. The capture time was 3 minutes. The samples are then preserved in 10% formaldehyde, until laboratory identification. If aquatic vegetation varies, the capture effort should be distributed proportionally to different types of vegetation (Šidagytė et al., 2013). Identification was conducted in laboratory conditions, under a light microscope, based on standard identification guides. Identification guides offer guidelines on identification macroinvertebrates, often to family level. Key parameters to look for are shape of the organism, legs, wings, abdomen, posterior, protrusions, filaments, etc. During this time velocity was also measured using traditional methods. This implies letting an object float on the surface of the river, for a set distance, while the time was being kept. Velocity was then calculated using the v = d/t formula, where:

- v = velocity
- d = distance

- t = time.

The fieldwork portion of the study was conducted in the May-June and September-October periods, as these are the months known to be more abundant with the most larvae. Spring and autumn seasonal comparison also offered a great deal of insight into water quality (Šporka et al. 2006). The results from both the biotic and physico-chemical measurements were then recorded using Excel.

Identification of macroinvertebrate specimens was conducted under standard laboratory conditions using a stereo microscope (Nikon SMZ1270). The identification process followed established taxonomic identification guides, which provide detailed criteria for distinguishing macroinvertebrates down to the species level. Key morphological characteristics were examined, including overall body shape, number and structure of legs, presence and form of wings, segmentation of the abdomen, and specific features of the posterior such as protrusions and filaments. These diagnostic traits were compared against the reference materials (Timm, 2015) to ensure accurate identification and classification of each specimen.

According to the Lithuanian Ministry of the Environment (2007), a standardised index for River Macrofauna was developed. The River Macroinvertebrate Index (UMI) is based on four other existing indices, and the UMI score is based on their arithmetic mean.

1. Danish Stream Fauna Index (DSFI) - shows the variation of indicator macroinvertebrate taxa in relation to organic pollution.
2. Average Score Per Taxon (ASPT) - measures the average sensitivity of the families in a community to organic pollution.
3. DEP - shows the number of species of *Diptera* family and *Ephemeroptera*, as well as *Plecoptera*.

4. EHP-CrHi - shows *Ephemeroptera, Heteroptera* and *Plecoptera* part of the number in the sample and common shellfish (*Crustacea*) and leech (*Hirudinea)* number in the sample difference in the proportion of individuals in the sample.

The aforementioned indices were calculated using the ASTERICS 4.04, a software created by the AQEM project, specialised in the assessment of ecological status of freshwater ecosystems (Hering et al. 2004).

2.4. Statistical analysis

First of all, descriptive statistics were conducted in order to get an overview of the data. A Shapiro-Wilk normality test was conducted in order to assess the normality of the data distribution (González-Estrada et al. 2022). Scatter plots of the UMI were created against each water parameter to visually assess possible relationships, as well as use Spearman's correlation coefficient to get an initial sense of linear or monotonic relationships between variables (Ali Abd Al-Hameed, 2022). Further on, the mesohabitat effect for macroinvertebrate metrics was tested using a Wilcoxon rank-sum test. In order to check the results, some other tests were conducted (Riffenburgh 2006). The Principal Component Analysis (PCA) is used to reduce dimensionality and identify major drivers of variation among water parameters (Zitko, 1994), and a Redundancy Analysis (RDA) was specifically useful for ecological datasets, as it was able to reveal how the zoobenthic index aligns with physico-chemical gradients (Capblancq and Forester, 2021). These tests were conducted using R Studio software.

3. RESULTS

3.1. Initial observations

Locations with similar land use types showed similarities between communities (e.g. *Odonata* species mostly present in LZ and ND). Environmentally sensitive species (EPT) were found mainly in the Panemunė (PN) location. LZ was dominated by *Gastropoda*. Invasive species were abundant in most locations. Notable species being *Lithoglyphus naticoides* (C. Pfeiffer, 1828), *Dreissena polymorpha* (Pallas, 1771), and *Pontogammarus robustoides* (Sars, 1894).

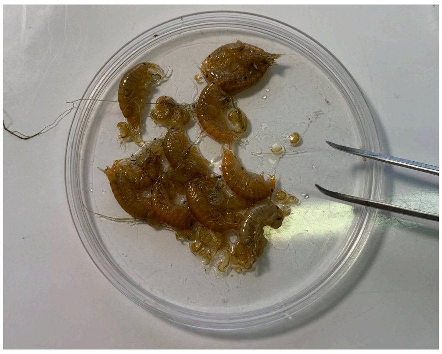

Figure 2. *Pontogammarus robustoides* (Sars, 1894)

Initial observations confirm the hypothesis that type of land use influences macrofauna communities.

Velocity determined the presence of several taxa such as *Caenis* sp. (Stephens, 1835) and *Potamanthus luteus* (Linnaeus, 1767).

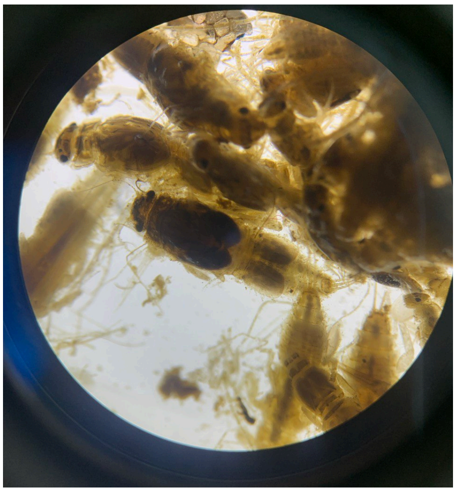

Figure 3. *Caenis sp.* under the microscope

Invasive species are present at all locations, regardless of location type, however they differ per location, which could be influenced by the origin (PN, SM – Kauno Marios (reservoir); JI- Jiesia; ST, NC, ND - Neris), as well as proximity to each other. These species are not incorporated into UMI calculations but constituted a substantial proportion of total abundance at several locations.

The map depicting the UMI scores (Figure 4) indicate lower ecological status in the locations near the city centre, based on lower biotic scores, whereas the peripheral areas show higher ecological status.

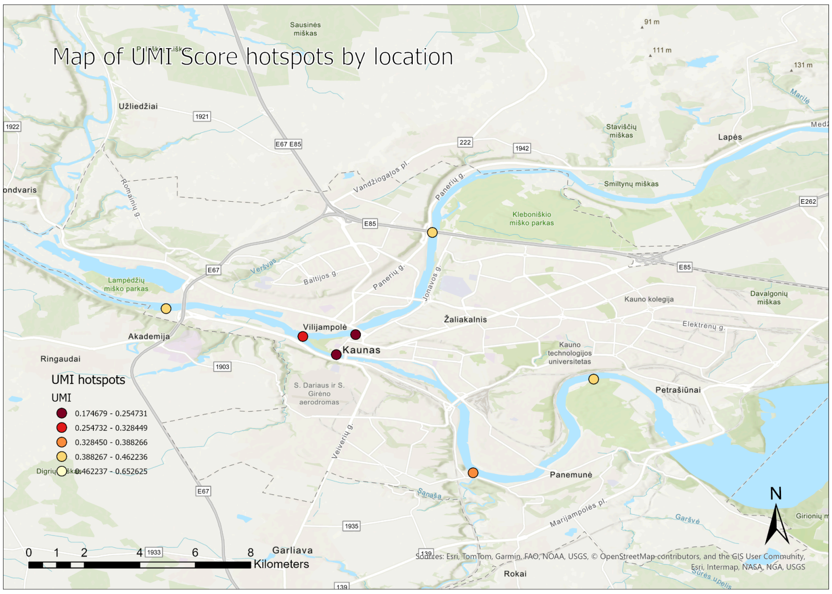

Figure 4. Map of UMI Scores

The results of the Shapiro-Wilk test suggest that the data is not normally distributed for all physico-chemical parameters, as p-values are much lower than 0.05, whereas the UMI scores are normally distributed. Based on this, the decision to conduct a Spearman correlation test was taken, as opposed to a Pearson correlation, which is used when data is normally distributed.

Table 1. **Shapiro-Wilk test results**

Parameter	**W**	**p-value**
UMI	0.9651	0.1045
Temperature	0.85099	6.138e-06
pH	0.79263	1.867e-07
Conductivity	0.83302	1.946e-06
Nitrates	0.81699	7.402e-07
Phosphates	0.94073	0.008346
Chloride	0.84996	5.734e-06
Chlorophyll-*a*	0.67901	8.717e-10
Velocity	0.8308	1.698e-06

The multivariate community assembly was visualised using an ordination biplot ($p < 0.001$), where the first two canonical axes captured 18.4% and 14.1% of the total biological variance, respectively. The ordination space revealed a profound macro-scale seasonal partition along the vertical axis (Axis 2), cleanly separating macroinvertebrate community structures between spring and autumn periods. Autumn communities were strongly directed along the positive trajectory of Axis 2, defined primarily by a high abundance of opportunistic *Chironomidae*. Conversely, spring communities clustered tightly within the negative quadrants, driven heavily by rheophilic *Simuliidae* and the freshwater gastropod *Lithoglyphus naticoides*. This directional sorting indicates that high-flow spring dynamics establish a distinct physical filter favoring flow-reliant filter feeders, whereas the autumn community realigns toward tolerant organic collectors as seasonal discharge subsides.

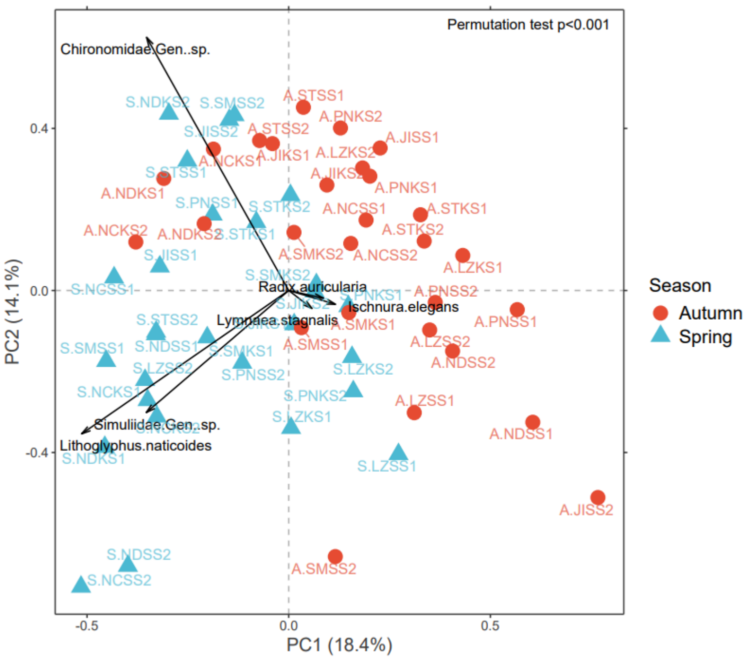

Figure 5. PCA Permutation test

3.2. Seasonal analysis

The seasonal analysis of UMI scores revealed higher variability during the Spring sampling period compared to Autumn, represented in the boxplot below (Figure 6). While median biological integrity remained relatively stable, Spring exhibited a broader range of scores, with several sites reaching UMI values >0.6. In contrast, Autumn scores were more tightly clustered at lower values, likely reflecting the cumulative impact of summer low-flow conditions and concentrated urban runoff. A general trend of lower UMI scores is observed in the autumn in Neris C, Panemunė and Jiesia, and the reverse is true for the other locations.

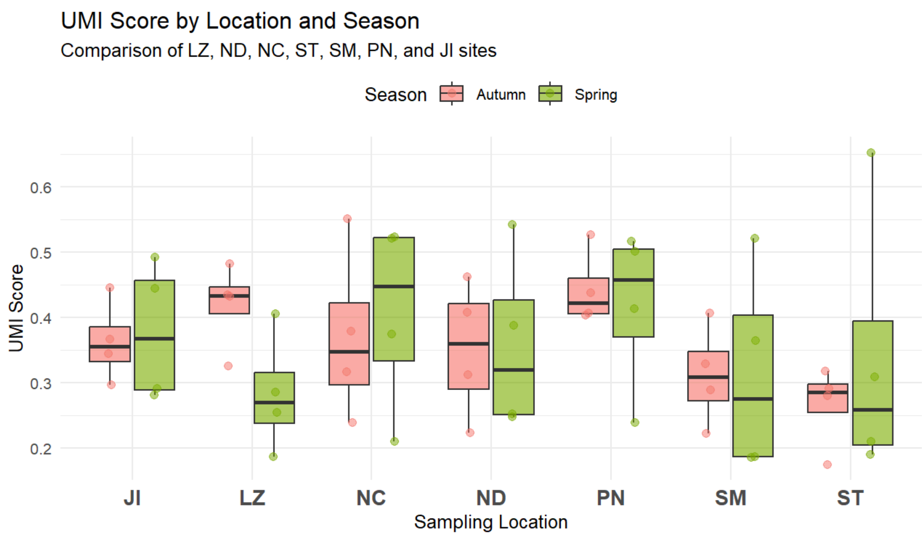

Figure 6. Seasonal comparison of UMI scores

3.3. UMI vs physico-chemical parameters

To explore the isolated, monotonic relationships between individual physico-chemical variables and the biological index, Spearman rank correlation coefficients (ρ) were calculated between UMI scores and each measured environmental parameter. The analysis revealed a uniform lack of statistically significant isolated correlations across all tested variables ($p > 0.05$). The strongest directional trend was observed between UMI scores and chloride concentrations, which exhibited a weak negative relationship ($\rho = -0.220$, $p = 0.102$), aligning with expected ecotoxicological trajectories, though failing to meet the threshold for statistical significance. Nutrient parameters, including orthophosphate ($\rho = -0.144$, $p = 0.290$) and nitrate ($\rho = 0.033$, $p = 0.809$), showed no meaningful isolated covariance with biological scores. Similarly, physical parameters such as flow velocity ($\rho = 0.032$, $p = 0.817$) and water temperature ($\rho = -0.017$, $p = 0.899$) demonstrated near-zero correlation with UMI values.

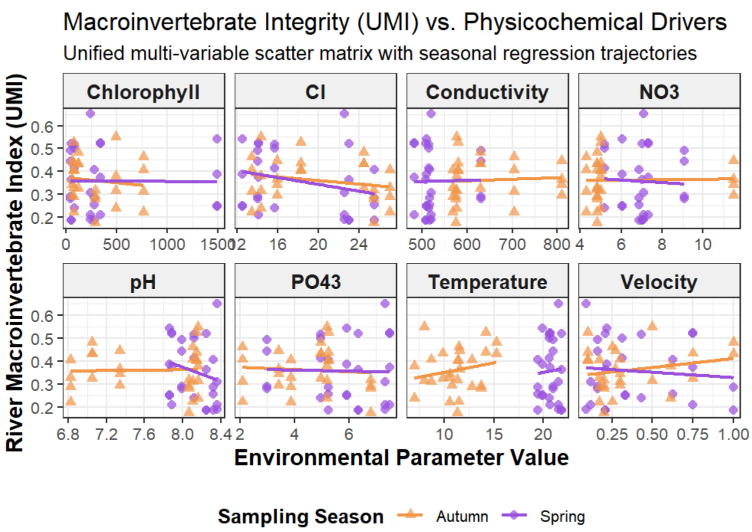

Figure 7. Plots of physico-chemical parameters vs UMI scores

To assess the macro-scale environmental and biological divergence between the two principal riparian corridors running through the urban matrix, independent Wilcoxon rank-sum tests were conducted. Physico-chemical data revealed a significant, permanent chemical asymmetry between the two systems. Overall chloride concentrations were fundamentally higher in the Nemunas River compared to the Neris River ($W = 608$, p-value < 0.001). This intense chemical split was sustained across temporal transitions, remaining highly significant during both the spring runoff period ($W = 144$, p-value $= 0.001$) and the lower-flow autumn period ($W = 160$, p-value < 0.001). However, this sharp water-chemistry divergence did not translate into a parallel divergence in macroinvertebrate community health. Biological integrity, evaluated via the River Macroinvertebrate Index (UMI), remained statistically indistinguishable between the Nemunas and Neris Rivers during both spring ($W = 62.5$, p-value $= 0.387$) and Autumn ($W = 81$, p-value $= 0.980$). This lack of biological divergence indicates that despite localised chemical variations and structural river regulation differences, both fluvial systems are subject to common urban ecological bottlenecks that restrict macroscopic community development to a structurally homogenised baseline.

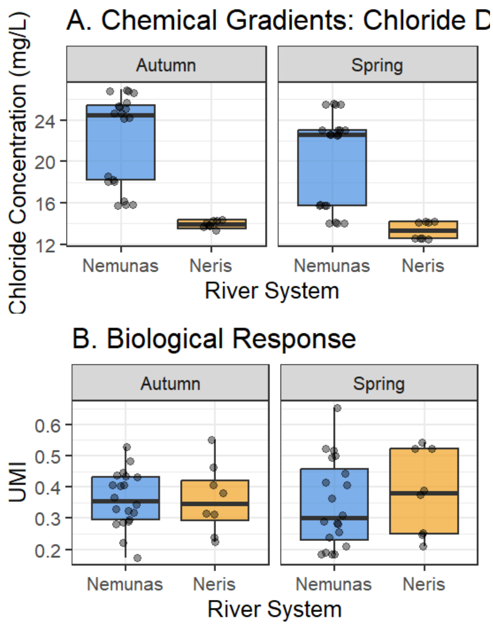

Figure 8. River comparison based on chlorides and bio index

3.4. Mesohabitat analysis

The biotic samples were collected via 2 distinct methods, kick sample (KS), which entails sampling the organisms located on the bottom of the water body, and the sweep sample (SS), where samples were collected from nearby macrophytes, in order to rule out impact of mesohabitat on biotic composition.To determine if the biological performance of instream mesohabitats varied across temporal transitions, independent Wilcoxon rank-sum tests were conducted on UMI scores for each sampling season individually. The analysis revealed that mesohabitat differentiation is highly dependent on seasonal dynamics. During the high-flow spring period, biological integrity varied significantly between mesohabitat types ($W = 151$, p-value = 0.016), indicating that macroinvertebrate community assembly is strongly partitioned by localised structural niches during early-year stress. Paradoxically, this biological distinction completely collapsed during the lower-flow autumn period ($W = 99$, p-value = 0.982). The near-identical distribution of UMI scores across autumn habitats points to a state of localised biotic homogenisation. This seasonal convergence suggests that degrading aquatic macrophytes, combined with widespread organic detrital accumulation across the river benthos, eliminates the ecological differentiation of individual mesohabitats, causing communities to merge into a single, uniform structural baseline.

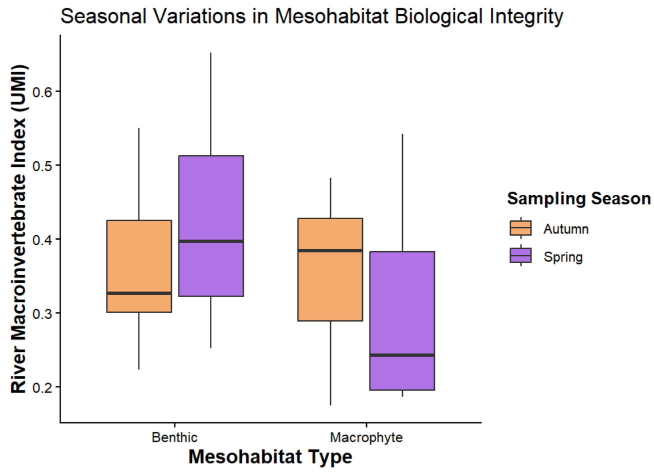

Figure 9. Mesohabitats comparison

3.5. Redundancy Analysis

To assess how local environmental parameters and micro-habitat types structure macroinvertebrate communities across seasons, separate Redundancy Analyses (RDA) were executed for autumn and spring. The Spring RDA explained a higher portion of community constraint (RDA1: 15.2%, RDA2: 9.6%), shifting toward temperature and ionic gradients. Rising water temperatures triggered a clear dominance of opportunistic *Chironomidae* along the positive axis of RDA2. High flow velocities during spring isolated heavy-shelled bivalves (*Sphaerium corneum* (Linnaeus, 1758)) within sandy navigational dock zones. Notably, the gastropod *Lithoglyphus naticoides* (C.Pfeiffer, 1828) showed a strong positive alignment with orthophosphate ($PO4^{3-}$) and chlorophyll concentrations within engineered navigation deck habitats. This confirms that the invasive species is highly effective at exploiting high-nutrient urban niches during early-year population expansions.

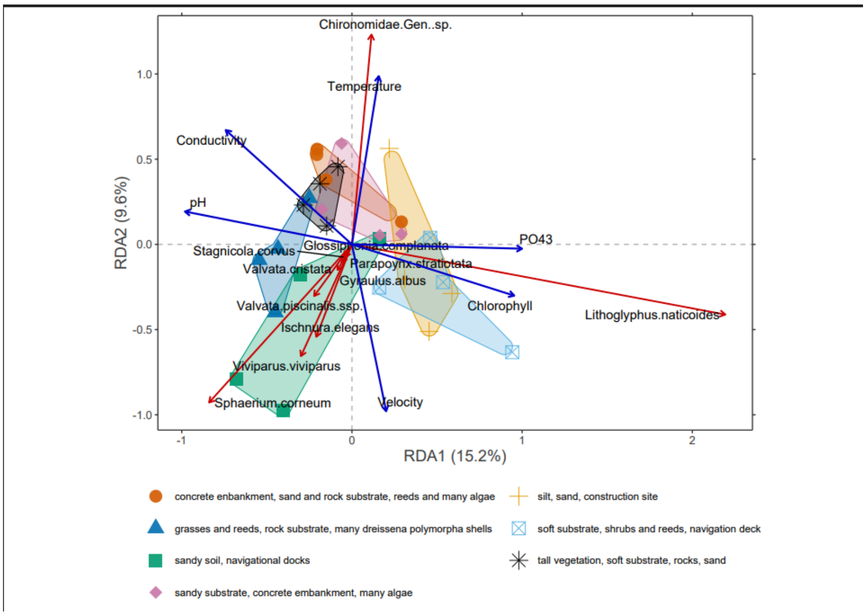

Figure 10. Spring RDA

In contrast, the autumn RDA (RDA1: 10.1%, RDA2: 8.7%) showed that community structure was driven primarily by a gradient between flow velocity and organic productivity (Chlorophyll). Flow-reliant taxa such as *Laccophilus hyalinus* (De Geer, 1774) and *Viviparus viviparus* (Linnaeus, 1758) tracked along the velocity vector within sand and dock habitats. Conversely, opportunistic *Oligochaeta* clustered along the chlorophyll vector, reflecting an accumulation of organic matter in low-flow areas.

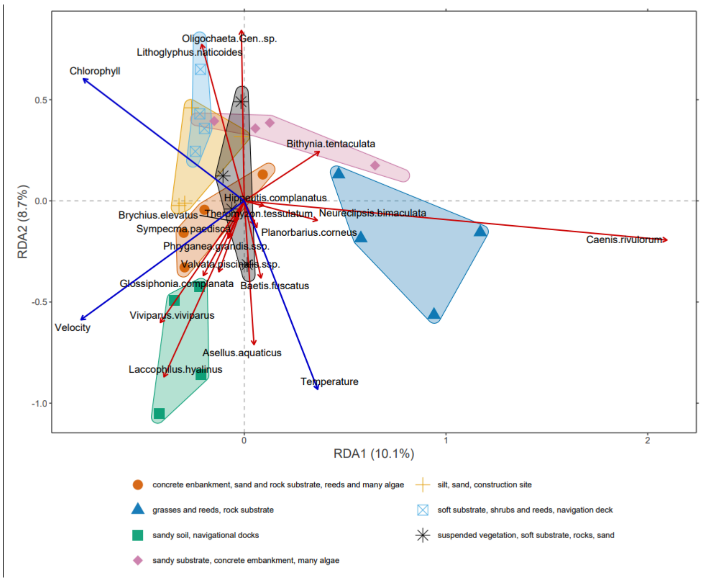

Figure 11. Autumn RDA

4. DISCUSSION

The results of this study clearly indicate a spatial gradient of ecological degradation, with the lowest UMI scores concentrated in Kaunas's urban center. This spatial pattern is likely to be linked to the concentration of chloride anions, which emerged as the primary driver of community composition in the analyses. The findings of this study demonstrate a clear, hierarchical cascading effect, where macro-scale catchment chemistry dictates micro-scale biological habitat usage across seasonal transitions. At the broadest scale, the global PCA established that seasonality acts as the overarching environmental template for the Kaunas river network, forcing a system-wide chemical shift from highly ionic, runoff-dominated conditions in the spring to productive, low-flow matrices in the autumn.

Contrary to initial expectations that nutrient loading might be the dominant stressor, urban salinisation appears to be the defining characteristic of the Nemunas in Kaunas. The elevated chloride levels observed are consistent with the "Urban Stream Syndrome," where road salt application and dense surface runoff create a toxic ionic environment (Jin et al., 2011).

Interestingly, the lower Spring UMI values likely reflect the aftermath of post-winter runoff. While the Panemunė (PN) and Jiesia (JI) sites are still within the urban boundaries, their increased distance from major arterial roads explains their higher biological integrity. It is important to note that chloride contamination is a Nemunas specific issue, as chloride levels in the 2 Neris sites are lower, regardless of proximity to roads. These sites appear to be, maintaining lower chloride levels and higher chlorophyll-a concentrations, suggesting a more impacted primary production cycle.

Despite the clear impact of chlorides observed here, the research regarding urban salinisation in Lithuania is rather scarce, with conclusive studies being conducted only in the Neries river, whose findings coincide with this one, in that Neris is less impacted by chlorides (Ratkevičius, 2014). Addressing this reality requires a transition from traditional de-icing to integrated salt-optimisation strategies. For instance, a LIFE project conducted in Trento, Italy shows promising results in changing the patterns of road salts usage based on meteorological modelling (Pretto, 2014). While alternatives like beet juice have gained popularity as organic de-icers, they are not always a suitable solution. Such organic alternatives can lead to oxygen depletion (BOD) and are significantly more expensive than traditional rock salt, presenting a conflict between infrastructure safety and biotic preservation (Gillis, 2021). Effective mitigation must therefore combine integrated policy-making (Strifling, 2017) with decentralised technical solutions like permeable pavements or specialised filtration systems (Rabaey et al., 2020).

A significant finding of this research was the lack of statistical difference between kick samples (benthic mesohabitat) and sweep samples (macrophyte mesohabitat) in autumn. This seasonal collapse of habitat distinctiveness aligns with the low-flow baseline and increased organic signature captured in the autumn data. As macrophyte beds undergo seasonal senescence and die back, they lose their structural complexity. Contrastingly, a uniform layer of organic detritus deposits across both rocky and vegetative substrates. The Autumn RDA captures the biological consequence of this localised biotic homogenisation: the community simplifies into a heavily constrained matrix driven by organic accumulation (Chlorophyll), where pollution-tolerant deposit-feeders like Oligochaeta uniformly dominate the river benthos. Collectively, these multivariate models prove that macroinvertebrate conservation in urban catchments cannot rely on static, single-season monitoring, rather, management must safeguard structural habitat refugia to buffer communities against predictable, severe seasonal environmental bottlenecks. Moreover, the chemical degradation caused by the Urban River Syndrome masks the benefits of habitat complexity. This is a vital point for urban river management, essentially restoring mesohabitats will have little to no effect if the water chemistry isn't mitigated first.

The overwhelming presence of *Pontogammarus robustoides* (Sars, 1894) signifies a shift toward a novel ecosystem in the Nemunas. This Ponto-Caspian invader thrives in high-conductivity, regulated waters, often outcompeting native taxa.

However, *P. robustoides* (Sars, 1894) presents a "monitoring paradox", because the UMI was developed based on traditional indicators of organic pollution, it does not explicitly account for the environmental impacts caused by invasive species. Consequently, the ecological status of the Nemunas may be underestimated. If an invasive species provides high abundance or richness in a sample, the index might reflect "Moderate" status, while the biotic integrity is actually lower. Future monitoring in large, regulated rivers like the Nemunas should incorporate invasion-sensitive metrics or functional trait approaches to capture the true impact of these community shifts.

Limitations and future research

While this study provides a clear picture of the urban impact, it is limited by the parameters measured. Factors such as dissolved oxygen (DO) and ammonia anions (NH_4^+), which were not sampled due to logistical constraints, are known to have significant effects on macroinvertebrate survival, particularly in eutrophic systems (Connolly, 2004); (Rius et al. 2018). Furthermore, the reliance on a single year of seasonal data means that long-term inter-annual variations in salinisation remain unknown. Future research should prioritise a broader chemical analysis and the development of an index that specifically accounts for identified urban issues, as well as further investigation into the drivers of seasonal differences.

5. CONCLUSION

1. This study aimed to assess whether zoobenthic communities can serve as reliable bioindicators of water quality along an urbanisation gradient in the Nemunas River in Kaunas. The results indicate that zoobenthic-based assessment, expressed through the River Macrofauna Index (UMI), successfully captured spatial differences in ecological status associated with proximity to urban centres.
2. The significant negative relationships between UMI and chloride concentrations highlight ionic pollution as an important stressor in urban river systems. From a management perspective, this finding emphasises the need to address salinisation sources such as road salt application and wastewater inputs when developing urban water quality management strategies. Monitoring chloride and other pollutants alongside biological indicators may therefore provide an effective early-warning system for ecological degradation in urban rivers.

3. Overall, the findings support the use of zoobenthic communities as practical and informative indicators for urban river monitoring, while also highlighting the importance of adapting assessment frameworks to account for biological invasions and site-specific urban pressures. Integrating biological, chemical, and geographic information can enhance evidence-based decision-making aimed at improving and maintaining ecological status in urban rivers.

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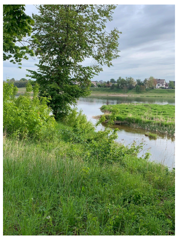
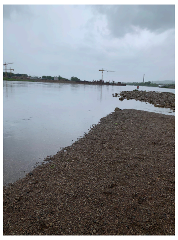
64.

8. ANNEX

Appendix 1. Location coordinates and characteristics

site nr	site name	site type	latitude	longitude	photo of the site (personal archive)
1	Panemunė	semi-natural	54.89313	23.968066	
2	Jiesia	semi-natural	54.85996	23.93148	

3	Santaka	city centre	54.89965	23.873146	
4	Senamiestis	city centre	54.89462	23.8844	
5	Lampėdžiai	periphery	54.90522	23.82832	
6	Neris D	periphery	54.9364	23.91239	
7	Neris C	city centre	54.9016	23.89014	

Appendix 2. Link to R Script

https://posit.cloud/content/12514106