KLAIPĖDA UNIVERSITY

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SURFACE-GROUNDWATER INTERACTIONS, HYDROLOGICAL REGIME AND LAND USE: IMPACTS ON STREAM FUNCTIONING FOR SALMONID SPAWNING AND INCUBATION PHASES

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Abstract

The Baltic Sea hosts populations of Atlantic salmon (Salmo salar L.) and sea trout (Salmo trutta L.), which have historically used many Lithuanian watercourses for spawning and juvenile recruitment. Therefore, knowledge about the anthropogenically modified factors behind the functioning of these habitats, particularly hydrological extremes and land use in the catchment, is of practical importance in implementing effective conservation and restoration measures. This thesis presents a multiscale approach to understanding the major environmental drivers and threats in three Lithuanian rivers (Blendžiava, Smiltaitė, Šventoji) hosting salmonid spawning habitats.

At the watershed level, land cover is correlated with the physicochemical parameters dictating different water quality responses during hydrological fluctuations in the three rivers. The Blendžiava Stream presents well-expressed riparian zones and agricultural areas with the consistent presence of natural features and covered soils, a trait positively correlated with high oxygen values across the entire year, including during drought periods. The Smiltaitė (syn. Smeltaitė) Stream, with its high share of urbanized areas and uncovered cultivated soils in its catchment area, displays physicochemical parameters characterizing a high trophic status. The Šventoji River (original Lithuanian name is Pajūrio Šventoji – Coastal Šventoji) is characterized by a low amount of suspended solids all year round due to the extensive coverage of forested areas in the watershed. In contrast, in the Blendžiava Stream watershed, flood periods have the potential to mobilize a high load of suspended solids, enhanced by the dominance of agricultural areas, a steeper bed, and valley slopes.

Regarding spawning areas, the spatiotemporal variations of the intragravel water parameters is investigated. Hyporheic water in the nest area has higher values in conductivity and ammonium, signalling eutrophic conditions. This is in line with the findings in surficial water quality from the Smiltaitė Stream. As the incubation period approaches the final phase, temperature and oxygen values drop down to below the level necessary for larvae survival in all three rivers. Moreover, periods of low waterflow enhance the presence of nutrients and organic matter that not only act as a further oxygen sink, but also boost the growth of ephyphiton layers both on and inside the nest, hampering water circulation and oxygen delivery. The structure of the egg pocket ensures better water circulation and, thus, more oxidative conditions. Higher values of oxygen and nitrate coming from the hyporheic water withdrawn from the proximity of the egg pocket contrast with lower values in the hyporheic water coming from the periphery of the nest.

This study also highlights the influence of groundwater in the spawning areas through the incubation period. The main benefit connected to upwelling groundwater seeps is improved intragravel circulation. This not only guarantees adequate oxygen supply and the removal of metabolic waste, but also exerts a desilting action, aiding the movement and consequent swim up of alevins. This ecosystem service appears pivotal in systems with high percentages of fine particles in the streambed. Mesocosm experiments were carried out to explore egg pocket biogeochemistry in terms of respiration and excretion rates, in parallel with the fluxes coming from eggs incubated in water alone. Oxygen consumption increases as the incubation period goes on, in parallel with the advancement of the developmental stage and the expansion of biofilm growth. Reconstructed egg pockets acted as ammonium sources, reflecting increases in the metabolic excretion of larvae at the final stages. Nevertheless, as incubation progresses, ammonium and nitrate removal increases, thanks to the formation of oxic-hypoxic patches aiding nitrification-denitrification processes.

The multiscale approach helps to understand deficiencies found in a specific stretch of watercourse in relation to peculiarities driven by land use. The consideration of physicochemical changes during hydrological fluctuations allows for the detection of the moments in which systems are pushed toward the threshold of their "good ecological status" in terms of the Water Framework Directive (WFD 2000/60/EC). This enables the identification of potential gaps in water quality otherwise hidden by standard monitoring. The use of laboratory setups allows for the fine-scale exploration of biogeochemical processes inside salmonid nest cores, which themselves drive the successful survival of the organisms that exploit this environment in nature. This can yield predictions regarding the potential of real habitats to host spawning grounds, and can also help motivate conservation measures.

Keywords

Lotic ecosystems, physicochemical water quality, land use, hydrological extremes, multiscale approach, salmonids, boreal region.

Reziumė

Baltijos jūroje yra sutinkamos vienos svarbiausių Atlanto lašišų (Salmo salar L.) ir šlakių (Salmo trutta L.) populiacijų, kurių nerštui ir jauniklių augimui didelę reikšmę turi ir Lietuvos upės. Žinios apie antropogeninius veiksnius, lemiančius upių funkcionavimą – ypač hidrologinius ekstremumus ir žemėnaudą baseine – yra labai svarbios efektyvioms šių buveinių apsaugos ir atkūrimo priemonėms įgyvendinti. Ši disertacija pristato daugiasluoksnį požiūrį, padedantį suprasti pagrindinius aplinkos veiksnius ir grėsmes trijose Lietuvos upėse (Blendžiavoje, Smiltaitėje, Šventojoje), kuriose yra lašišinių žuvų nerštavietės.

Baseino lygmeniu, žemėnaudos tipas koreliuoja su fizikocheminiais parametrais, nulemiančiais skirtingą vandens kokybės atsaką hidrologinių svyravimų metu trijose upėse. Blendžiavos upelis pasižymi gerai išreikštomis pakrančių zonomis ir žemės ūkio naudmenomis kartu su natūraliais plotais, kas pozityviai koreliuoja su aukštu ištirpusio deguonies kiekiu visus metus, įskaitant žemo vandens lygio laikotarpius. Smiltaitės upelyje, kur didelę dalį baseino užima urbanizuotos teritorijos ir dirbami laukai, fiksuojamos vandens parametrų reikšmės, būdingos aukšto trofinio statuso sistemoms. Šventosios upė pasižymi nedideliu suspenduotų dalelių kiekiu ištisus metus dėl didelio miškingų teritorijų užimamo ploto baseine. Priešingai, Blendžiavos baseine potvynių metu mobilizuojamas didelis suspenduotų dalelių kiekis, ką lemia žemės ūkio naudmenų dominavimas baseine ir reikšmingi vagos bei slėnio nuolydžiai.

Nerštaviečių lygmeniu in situ buvo nagrinėjamos vidugruntinio vandens fizikocheminės savybės sezono eigoje. Hiporėjinis vanduo lizdo zonoje pasižymi didesniu elektriniu laidumu ir amonio koncentracija, rodydamas eutrofines sąlygas, kas sutampa su paviršinio vandens kokybės tyrimų rezultatais Smiltaitėje. Lašišinių žuvų ikrų inkubacijos pabaigoje temperatūra ir deguonies koncentracija visose trijose upėse nukrenta žemiau lervų išgyvenimui būtino lygio. Be to, žemo vandens lygio periodai sustiprina maistmedžiagių ir organinių medžiagų akumuliaciją, kurios ne tik veikia kaip papildomas deguonies „siurblys“, bet ir skatina bioplėvelės augimą ant lizdo ir jo viduje, trukdydami vandens cirkuliacijai ir deguonies tiekimui. Iš kitos pusės, ikrų dėties struktūra užtikrina geresnį vandens pratekėjimą ir deguonies sąlygas. Aukštesnės deguonies ir nitrato koncentracijos hiporėjinio vandens mėginiuose, imtuose iš lizdo zonos ties ikrų dėtimis kontrastuoja su žemesnėmis koncentracijomis hiporėjiniame vandenyje, imtame iš lizdo pakraščių. Darbe nustatyta akivaizdi gruntinio vandens įtaka nerštavietėms per inkubacijos periodą. Išsikraunančio gruntinio vandens ties neršto lizdais pagrindinis privalumas – pagerėjusi vidugruntinė vandens cirkuliacija. Tai ne tik garantuoja pakankamą deguonies tiekimą ir metabolinių atliekų pašalinimą,

bet ir veikia deziltuojančiai, palengvinant lervų judėjimą ir iškilimą į lizdo paviršių. Ši ekosisteminė paslauga yra ypač svarbi tose sistemose, kur upės dugne gausu smulkių nuosėdų.

Darbe panaudota laboratorinė mezokosmų sistema leido tirti lašišinių žuvų ikrų dėties biogeochemiją, eksperimentiškai mikromastelyje įvertinant individualių ikrų ir ikrų dėties su jos substratu kvėpavimo ir ekskrecijos rodiklius. Rekonstruotos lašišinių žuvų ikrų dėtys pradžioje veikė kaip amonio šaltinis, atspindėdamos lervų metabolinę ekskreciją, tačiau inkubacijai tęsiantis amonio ir nitrato pašalinimas didėjo dėl oksicinių–hipoksinių mikrozonų formavimosi ir aktyvių nitrifikacijos–denitrifikacijos procesų. Deguonies suvartojimas didėjo kartu su inkubacijos ir lervų vystymosi pažanga bei bioplėvelės augimu.

Darbe taikytas daugiapakopis tyrimas padeda integruotai vertinti sudėtingą neršto buveinių funkcionavimą laike ir skirtingais masteliais. Makromasteliu, specifiniuose upės ruožuose aptiktos nepalankios sąlygos yra susijusios su žemės naudojimo ypatumais baseine. Fizikocheminių pokyčių stebėjimas atsižvelgiant į hidrologinius svyravimus leidžia tyrėjams geriau identifikuoti vandens kokybės dinamiką ir suprastėjusios ekologinės būklės momentus, kurie lieka nepastebėti naudojant standartinį monitoringo protokolą. Laboratoriniai tyrimai suteikia galimybę detaliai analizuoti biogeocheminius procesus lašišinių žuvų lizduose, kurių rezultatai gali padėti prognozuoti realių buveinių tinkamumą nerštavietėms ir atrinkti maksimaliai efektyvias litofilinių gyvūnų buveinių atkūrimo ir apsaugos priemones.

Reikšminiai žodžiai

Upių ekosistemos, fizikocheminė vandens kokybė, žemėnauda, hidrologiniai svyravimai, daugiapakopis vertinimas, lašišinių žuvų nerštavietės, borealinis regionas.

Table of Content

1

Introduction

Of all the teleosts, salmonids are among the most extensively studied taxa (Bjornn et al., 1991; Crisp, 2008; Louhi et al., 2008; Malcolm et al., 2003a; Nika, 2011). Many salmonid species hold significant socio-economic value (Bloomer et al., 2016), related to the many forms of recreational and professional fisheries (Geist et al., 2002; Liu et al., 2019; Malcolm et al., 2003a). Additionally, being at the top of their food webs, they play a key role in the functioning of both marine and freshwater ecosystems via the top-down regulation of the population dynamics of other fish taxa (Bottom et al., 2008; Rudstam et al., 1994; Wipfli et al., 2010). Many salmonid species are suffering from general decline due to overfishing (Williams et al., 2024) and habitat loss and degradation (Conallin, 2004; Malcolm et al., 2004). Numerous populations of Atlantic salmon (Salmo salar) and sea trout (Salmo trutta) foraging in the Baltic Sea exploit Lithuanian watercourses for spawning. However, Lithuania has faced and continues to undergo rapid and drastic changes in land use, which can also affect the health of freshwater ecosystems (Gorgoglione et al., 2020; Pulg et al., 2013; Sutherland et al., 2002). It is in this context that conservation actions which seek to combat salmonid stock depletion by targeting different critical life stages should be considered (Smialek et al., 2021; Sternecker et al., 2013). The restoration and increase of the number and extent of suitable spawning areas has been proven to reinforce local populations

(Kondolf et al., 2008; Smialek et al., 2021; Sternecker et al., 2013). Nevertheless, these actions should be framed in relation to the peculiarities of the watershed – – to deliver long-lasting and effective improvements (Malcolm et al., 2003a; Malcolm et al., 2005; Soulsby et al., 2001).

Restoration actions find common ground in the key points of the Water Framework Directive (WFD 2000/60/EC). The WFD is a pivotal law at the European level requiring Member States to perform management actions to achieve "good ecological status" (GES) for inland water bodies. GES is defined not only by physicochemical water parameter classes, which should fall into specific ranges, but also by the composition of macroinvertebrate, macrophyte, and fish communities. If one of these physicochemical classes or communities does not reflect the expected range, taxa composition, and abundance, the investigated waterbody fails to achieve GES. It is evident how a system with proper ecological functionality should hold proper ranges in the physicochemical water parameters and community structures, thus matching the GES parameters.

Salmonids belong to the stenoecian fish species, which are sensitive to pollution events (Brinkman et al., 2009; Eddy, 2005) and habitat alteration (Bjornn et al., 1991; Crisp, 2008; van Treeck et al., 2020). They can act as umbrella species (Bell, 2015; Obester et al., 2022), meaning that the protection and restoration of their community will positively affect many other fish and macroinvertebrates that share the same habitats. It is apparent how spawning area restoration will also benefit related communities, with a higher likelihood of attaining GES. In the case of the present work, not only the spawning areas, but also the entire watershed is investigated. Ecological functionality linked to watershed ecosystems relates to their capacity to metabolize nutrients, retain solids, and dampen the intensity of runoff into the watercourse (Guo et al., 2024; Huang et al., 2024; Swanson et al., 2017). This, in turn, relates to a more stable water environment that experiences fewer physicochemical fluctuations, which can represent stress for dwelling organisms. This will translate into higher watercourse viability, allowing communities to flourish. In this regard, it has been acknowledged that landscape ecology is a tool to improve the ecological functionality of an ecosystem (Turner, 2005; Turner et al., 2001). The improved ecological functionality of watersheds and watercourses becomes a key trait in dampening the stressors generated by hydrological fluctuations, which in turn are enhanced by ongoing climate change. Hydrological fluctuations have the potential to alter the physicochemical water quality in relation to watershed-specific land cover features (Adjovu et al., 2023; Calles et al., 2007; Dumas et al., 2007; Soulsby et al., 2001).

In the present climate change scenario, Lithuanian watercourses will experience spring flood decrement and the elongation of summer low flow periods (IPCC, 2014). Additionally, the water temperature will register an overall increment of more than 5 °C (Čerkasova et al., 2024; Šarauskienė et al., 2018). This means that hydrological shifts are expected to increase in duration and intensity, worsening the functionality of those watercourses already suffering from multiple stressors at the watershed level. These stressors are connected to anthropogenic pressures near the watercourse (e.g., road construction, bank and riverbed alteration) or spread around it (e.g., wastewater from industries and housing, agricultural practices, and tree-felling) (Binkley et al., 1993; Burke, 2011). These stressors not only increase the inputs of chemical substances (e.g., pesticides, heavy metals, inorganic nitrogen, N–, and phosphorous, P–, fertilizers) (Burke, 2011), but can also promote streambed erosion connected to increased water scour potential (Billi et al., 2022; Pulg et al., 2013; Sutherland et al., 2002). Prolonged periods of low water flows or intense high flood events linked to specific land use coverage have the potential to alter the ecological functionality of a watercourse (Dahm et al., 2003; Stanley et al., 1997). In other words, the interaction of climate change and human pressures can worsen the functionality and resilience of riverine systems (Brooks, 2009; Nohara et al., 2006).

On the one hand, prolonged periods of low water flow connected to point and non-point source pollution problems can trigger high trophic status in the water column (Bernal et al., 2013; Wilby et al., 2006). This is more prominent in those watersheds with higher proportions of urbanized areas due to the increased presence of wastewater treatment plants and septic tanks directly in contact with the watercourse, which release water rich in nutrients into the stream (Lange et al., 2012; Larned et al., 2010). However, the absence of dilution capacity due to the lack of rainfall will lead to the concentration of such nutrients and the associated overgrowth of primary producers (Hosen et al., 2019; Poff et al., 2010; Woelfle-Erskine et al., 2017). In turn, this leads to oxygen depletion phenomena due to organic matter accumulation (Burke, 2011; Greig et al., 2005). Such situations can be worsened by the rising temperatures typical of the spring and summer seasons (Conallin, 2004).

On the other hand, the higher intensity of precipitation and flood events has the potential to erode watershed soil and release high quantities of suspended solids into the watercourse (Adjovu et al., 2023; Soulsby et al., 2001). This will be more evident in those watersheds with higher proportions of agricultural areas, which are more prone to erosion (Pulg et al., 2013; Sternecker et al., 2013; Sutherland et al., 2002). Moreover, high runoff rates will increase non-point pollution due to higher soil leaching, which is connected to the organic matter released into the watercourse and translates into increased concentrations of N– and P– (Jarvie et al., 2005; Severini et al., 2023).

Urbanized areas are also connected to an increased area of impermeable surfaces, which increase runoff power. The massive entrance of water into the stream then leads to streambed scour phenomena (Opperman et al., 2005; Sutherland et al., 2002). On the contrary, areas with high forest coverage will better deal with runoff situations, with less suspended solids and nutrient retention rates (Billi et al., 2022; Pulg et al., 2013; Sutherland et al., 2002). Organic matter is better degraded and absorbed by these types of surfaces before entering the stream (Gorgoglione et al., 2020). Finally, roots and branches can dampen the force with which runoff enters the watercourse, leading to less streambed erosion and suspended solid intrusion (Chen et al., 2011; Maraseni et al., 2016; Van Esbroeck et al., 2016). Evidently, watershed land use triggers different responses during hydrological fluctuations. Knowledge about how these systems function during such hydrological shifts provides an understanding of physicochemical water quality and how such changes can inhibit the achievement of GES or hinder the survival of stenoecian species.

Salmonids lay their eggs in gravel nests (also called redds) (Crisp, 2008). Each nest is formed by several egg pockets made out of larger stones, and eggs are laid into egg pockets (Bjornn et al., 1991). The nests are built in the hyporheic zone (Crisp, 2008), a boundary habitat between the surface water above and the groundwater beneath the aquifer (Calles et al., 2007). Since salmonids spawn in areas with gravel and pebbles, the hyporheic environment is also called the intragravel environment. In the intragravel environment, eggs are laid at a depth of 10–15 cm (DeVries, 2000) where they hatch, becoming embryos (also known as larvae or alevins). The overall duration of the intragravel period in the area that this study concerns can last up to six months (Nika, 2011), ending with the emergence (called the swim-up phase) of young fish (called fry). During the intragravel period, eggs are dependent on hyporheic water quality.

The spawning habitat selection of salmonids usually relies on substratum composition, water depth, and velocity (Neumann et al., 2016). These are indeed acknowledged as classical parameters to estimate the potentiality of salmonid spawning habitats (Neumann et al., 2016). However, the presence of surface-groundwater (SW-GW) interactions can play a non-negligible role in driving the spawning site selection process (Mouw et al., 2014). SW-GW interactions are defined as the twist of water currents from the surface water column which locally intrudes into the streambed, mixing with the regional groundwater generated from the aquifer (Stanford et al., 2005). Different temperature and oxygen gradients can privilege the selection of surface- or groundwater-dominated sites (Stanford et al., 1993). The exploitation of zones of surface water discharge into the streambed (downwelling) or groundwater recharge into it (upwelling) is linked to the physicochemical benefits that these two water types can provide. However, the physicochemical quality of surface and groundwaters will vary according to the lithology across which such water is percolating (Briggs et al., 2018). Also, seep residence time and interaction with the surrounding habitat (e.g., the connected presence of geomorphic bed features, nursery refugia, spawning gravel, etc.) will define the importance that SW-GW interactions have in a specific spawning context (Baxter et al., 2000; Bean et al., 2015).

It is clear how the selection of zones dominated by surface- or groundwater will result from interaction with the other aforementioned factors. Groundwater at boreal latitudes can confer a stable thermal regime, thus avoiding freezing conditions for eggs (Lorenz et al., 1989). Additional groundwater into the hyporheos improves overall water circulation (Stanford et al., 1993), increasing oxygen delivery and waste removal (Lorenz et al., 1989). Finally, a stable groundwater supply can dampen nest dewatering phenomena connected to low surface water flows (Casas-Mulet et al., 2015). The nest will naturally accumulate fine materials (sand, silt, and clay) delivered by the flow throughout the incubation phase. Eventually, this fine fraction will cake onto the nest, clogging its interstices and preventing the surface water from circulating. The ageing phenomenon of the hyporheic nest matrix is worsened if the high trophic status allows consistent biofilm overgrowth around the nest (Conallin, 2004). This process is enhanced during prolonged low water flow periods, when dilution is minimized and the slow current allows optimal biofilm growth (Burke, 2011). Low flow periods will also increase fine sediment deposition due to the loss in sediment transport capacity (Guarch-Ribot et al., 2016). The sum of these phenomena will lead to the complete suffocation and armouring of the nest surface layer, making it unsuitable for the stenoecian salmonid embryos (Malcolm et al., 2010; Pulg et al., 2013). Overall, increased primary productivity and streambed suffocation during prolonged low water periods will lead to oxygen drops, which are dangerous for the developing larvae (Calles et al., 2007; Dumas et al., 2007).

On the other hand, intense flood events can deliver high quantities of fine materials which have the potential to obstruct the nest in a single spate flow event (Malcolm et al., 2004; Pulg et al., 2013; Sternecker et al., 2013; Sutherland et al., 2002). This aspect is also connected to the sagging of oxygen into the streambed due to impaired surface water intrusion. Lower intragravel water circulation is additionally connected to the accumulation of N–compounds directly generated by meiofauna and egg metabolism. Such by-products are toxic for the developing eggs (Brinkman et al., 2009; Greig et al., 2005; Rubin et al., 1996). Increased waterflow is connected to higher yields in streambed scours, and consequent nest displacement (Chapman, 1988; Rubin et al., 1996). It is clear how land cover and interaction with these hydrological shifts represent stressors for the success of salmonid incubation. However, as previously explained, this will increase with the ongoing climate change. From this dynamic picture, it is clear how the physiological rates of incubating eggs will be bound to the hydrological shifts and land use cover occurring on a watershed scale.

The present thesis aims to integrate three spatial levels of investigation – 1) macroscale, in km; 2) mesoscale, in m; and 3) microscale, in cm – to deliver a comprehensive picture of the functioning of an ecosystem in terms of the suitability of spawning areas and potential stressors for the incubation of salmonid embryos (Figure 1).

1) At the macroscale, i.e., the watershed level, the thesis seeks to explore an array of physicochemical water parameters in three lotic Lithuanian ecosystems (Blendžiava, Smiltaitė, and Šventoji) holding salmonid reproductive habitats. At this scale, wa-

ter quality is related to different land usage, and varies in response to hydrological fluctuations. The macro-scale investigation allows watershed-dependent stressors pertaining to water quality to be observed, particularly those that miss GES and/or critical thresholds for the survival of incubating embryos and juvenile rearing.

2) At the mesoscale, i.e., the spawning area level, the main findings outlined in the macroscale are further explored, assessing the hyporheic water quality directly coming from the spawning areas of the three systems mentioned above. Physicochemical hyporheic water parameters outside the ranges required for successful larvae incubation are then outlined. Using the same scale, the composition of SW-GW interactions is evaluated during spawning and emergence. Further attention is devoted to the role played by SW-GW interactions during the selection of spawning sites by the Oncorhynchus, Salmo, and Salvelinus salmonid genera.

3) At the microscale, i.e., the salmonid nest level, the reconstruction of the core parts of a salmonid nest, i.e., the egg pockets, is carried out to explore the nest's biogeochemistry regarding respiration and excretion rates. An attempt is then made to quantify the role of biofilm in the constitution of the measured respiration and excretion rates. The feasibility of using this setup to directly assess the productivity of different spawning areas by mimicking the conditions encountered in nature is further questioned.

Results describing deficiencies in the ecological status of spawning areas at the mesoscale are intersected with the peculiarities of land cover and physicochemical water quality outlined at the macroscale. Findings from the time-based flux evaluation measured in the mesocosm experiments (at the mesoscale) give insights into the functioning and evolution of the nest through the incubation period. High-resolution snapshots can capture egg-biofilm interactions, delivering information on the most critical moments that the developing eggs encounter in the natural environment. The workflow scaffold presented here aims to provide a basis for similar assessments when considering the conservation and restoration of habitats and related freshwater lithophilic fish species.

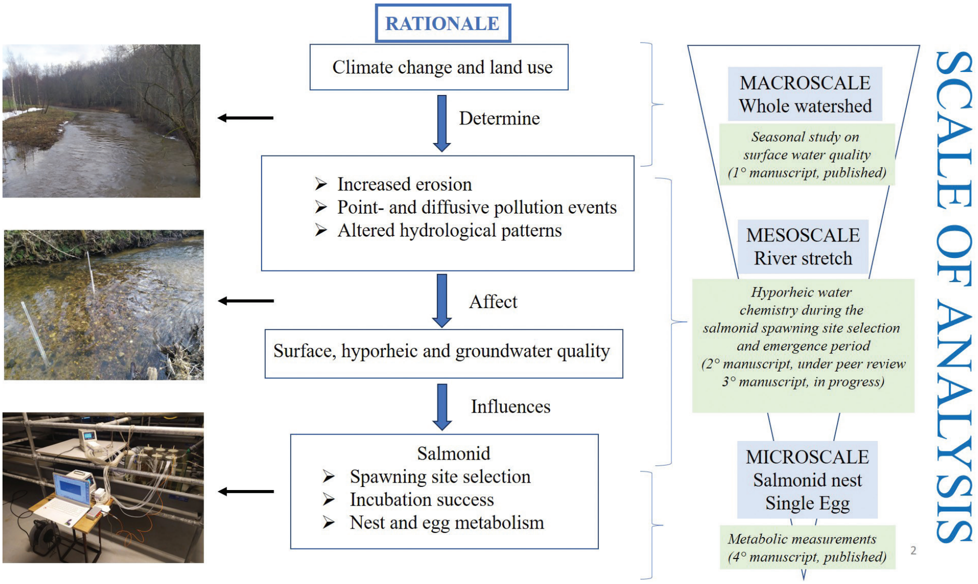

Figure 1. Conceptual framework of the main aspects discussed in the thesis and how they are nested on different scales of analysis.

1.1. Aim and objectives

The aim of this study is to investigate how hydrological fluctuations, SW-GW interactions, and land use affect stream functioning and the related physicochemical factors responsible for salmonid spawning and incubation success.

The following objectives were thus outlined:

1. to analyse the physicochemical water parameters in three Lithuanian watercourses and relate their variations to watershed land use and hydrological regimes;
2. to review the scientific literature on the salmonid genera (Salmo, Oncorhynchus and Salvelinus) in lotic ecosystems in order to evaluate the role of SW-GW interactions in shaping salmonid spawning site selection mechanisms;
3. to investigate the composition of SW-GW interactions in the spawning stretches of the study areas of point 1), and relate it to salmonid spawning and emergence times;
4. to analyse hyporheic water quality in the spawning stretches of the study areas of point 1), and relate it to salmonid incubation conditions;
5. to track the metabolic evolution of reconstructed egg pockets under laboratory conditions in terms of respiration and excretion rates in relation to egg developmental stages to understand the role played by biofilms.

1.2. Novelty

The thesis presents methodological and conceptual novelties. On a methodological level, different scales of investigation are tackled with a multidisciplinary approach, which involves various methods. Surface water sampling and GIS tools are used to characterize the macroscale, and hyporheic chemistry analyses are employed for the mesoscale in order to explore SW-GW interactions in the salmonid nests. Finally, the microscale foresees the establishment of mesocosms and the quantification of fluxes. On a conceptual level, this study seeks to understand the functioning of salmonid-spawning lotic ecosystems by interweaving different scales of investigation. The interplay of these scales should provide a comprehensive approach integrating different aspects that can ensure successful salmonid reproduction. Thus, stressors related to single spawning stretches are connected to the interactions between specific land use and hydrological regimes.

1.3. The scientific and applied significance of the results

The scientific significance of these results is related to the novel methods applied for in situ and laboratory investigations. Concerning in situ measurement, previous scientific work in this study area (Nika, 2011) has assessed hyporheic water chemistry by relating it to embryo survival. However, the present work intends to explore SW-GW interactions in spawning areas for the first time. In this study, the hyporheic water dataset is enlarged with major ions (e.g., K^+, Ca^{2+}, F^-, Cl^-, etc.) and gases of biological interest (N_2, Ar) in order to determine the most suitable traces of groundwater presence inside the hyporheic zone. At the laboratory level, flow-through systems are already employed to track biogeochemical cycles at Klaipeda University (Politi, 2022). However, in this study, this methodology is adapted to account for intragravel flow conditions, which are consistently higher than those typically used in traditional flow-through system experiments. Moreover, a systematic literature review is carried out on the impact of SW-GW interactions on the Oncorhynchus, Salmo, and Salvelinus salmonid genera during spawning.

The applied significance of these results is related to the workflow design employed to investigate the ecosystems on multiple scales. Each scale delivers practical methodologies to stakeholders to better understand ecosystem functioning and track potential stressors. At the macroscale, samples taken during hydrological shifts are paired with land cover composition analysis; at the mesoscale, techniques to withdraw and assess hyporheic water from the salmonid nests are used; finally, at the microscale, laboratory setups are used to mimic and test different hyporheic environments. Watershed-specific solutions can then follow the workflow to comprise a multi-scale assessment.

These solutions span from ecological landscape management at the watershed level to specific restoration works on the habitats of single watercourses. The sum of these actions will improve the overall ecological status of the targeted watercourse. This will aid in fulfilling the WFD guidelines, which will translate into specific river basin management plans and measurement programs – the main EU instruments required to achieve GES. Finally, these same actions will increase the resilience of these ecosystems, enabling them to combat those pressures that could hinder the reproductive potential of salmonids in Lithuanian watercourses.

1.4. Scientific approval

The results of this study were presented at the following conferences:

1. Baltic Sea Science Congress 2023, Helsinki, Finland. Benetti, R.; Nika, N.; Bartoli, M. "Responsiveness to surface-groundwater interactions by spawning salmonids: site selection patterns and control mechanisms in Oncorhynchus, Salmo and Salvelinus genera." Poster presentation.
2. Free Flowing Rivers Congress 2024, Groningen, Netherlands. Benetti, R.; Nika, N.; Bartoli, M. "Responsiveness to surface-groundwater interactions by spawning salmonids: site selection patterns and control mechanisms in Oncorhynchus, Salmo and Salvelinus genera." Poster presentation.
3. Jūros ir krantų tyrimai 2024: nacionalinė jūros mokslų ir technologijų konferencija. Benetti, R.; Politi, T.; Bartoli, M.; Nika, N. Metabolic Rates of Rainbow Trout Eggs in Reconstructed Salmonid Egg Pockets. Oral presentation.
4. AIIAD (Italian Freshwater Ichthyological Association) national symposium 2024, Parma, Italy. Benetti, R.; Severini, E.; Nika, N.; Cerkasova N.; Magri M.; Bartoli, M. 2024. Water quality in the framework of climate change- and land use- driven pressures: lessons from three boreal streams. Oral presentation.

1.5. Publications

The material in this study was presented in two original publications in peer-reviewed scientific journals. It is also based on the materials of three manuscripts, one of which has been submitted:

1. Benetti, R.; Severini, E.; Nika, N.; Cerkasova, N.; Magri, M.; Bartoli, M. Seasonal Patterns of Water Chemistry into Three Boreal Rivers: Implication for Salmonid Incubation and Rearing in the Frame of Hydrological Extremes and Land Use Contexts. Water 2024, 16, 3352. https://doi.org/10.3390/w16233352 (in the text referred to as Paper I)

2. Benetti, R.; Politi, T.; Bartoli, M.; Nika, N. Metabolic Rates of Rainbow Trout Eggs in Reconstructed Salmonid Egg Pockets. Water 2024, 16, 612. https://doi.org/10.3390/w16040612 (in the text referred to as Paper II)
3. Benetti, R.; Bartoli, M.; Nika, N. Responsiveness to surface-groundwater interactions by spawning salmonids: site selection patterns and control mechanisms in lotic ecosystems by Oncorhynchus, Salmo and Salvelinus genera. Journal of Fish Biology (manuscript resubmitted on 10 March 2025, 2nd peer-review round, in the text referred to as Manuscript I).
4. Benetti, R.; Bartoli, M.; Nika, N. Hyporheic water during the salmonid incubation period into three boreal rivers (manuscript in preparation, in the text referred to as Manuscript II).
5. Benetti, R.; Bartoli, M.; Nika, N.; Severini, E. Surface-groundwater interactions during salmonids spawning and emergence time: insights at boreal latitudes (manuscript in preparation, in the text referred to as Manuscript III).

Authors' contributions

Paper I:

Author contributions: conceptualization, R.B., M.B.; methodology, R.B., E.S., N.C., M.M., M.B.; validation, N.N., M.B.; formal analysis, R.B., E.S., N.C.; investigation, R.B., N.N., M.M.; resources, N.N., M.B.; data curation, R.B., E.S.; writing—original draft preparation, R.B., E.S., M.B.; writing—review and editing, N.N., N.C., M.M., M.B.; visualization, R.B., N.C.; supervision, E.S., N.N., M.B.; project administration, M.B.; funding acquisition, N.N., M.B..

Paper II:

Author contributions: conceptualization, N.N.; methodology, all co-authors; validation, N.N., M.B. and R.B.; investigation, R.B., T.P.; data curation, R.B.; writing—original draft preparation, R.B.; writing—review and editing, all co-authors.; funding acquisition, N.N., M.B.

Manuscript I:

Author contributions: conceptualization, R.B.; literature research, R.B.; figures, R.B.; validation, M.B. and N.N.; writing—original draft preparation, R.B.; writing—review and editing, M.B. and N.N.

Manuscript II:

Author contributions: conceptualization, all authors; methodology, R.B., E.S., M.B.; validation, E.S., N.N., M.B.; formal analysis, R.B., E.S.; investigation, R.B., E.S.; resources, N.N., M.B.; data curation, R.B., E.S.; writing—original draft preparation, R.B., E.S.; writing—review and editing, E.S., N.N. and M.B.; visualization, R.B., E.S.; supervision, E.S., M.B.; project administration, M.B.; funding acquisition, N.N., M.B.

Manuscript III:
Author contributions: conceptualization, all authors; methodology, R.B., M.B.; validation, N.N., M.B.; formal analysis, R.B.; investigation, R.B.; resources, N.N., M.B.; data curation, R.B.; writing—original draft preparation, R.B.; writing—review and editing, N.N. and M.B.; visualization, R.B.; supervision, N.N., M.B.; project administration, M.B.; funding acquisition, N.N., M.B.

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R.B.: Rudy Benetti; E.S.: Edoardo Severini; N.N.: Nerijus Nika; N.C.: Natalja Čerkasova; M.M.: Monia Magri; T.B.: Tobia Politi; M.B.: Marco Bartoli.

1.6. Thesis structure

This dissertation is comprised of 10 chapters: introduction, literature review, material and methods, results and discussion, recommendations, conclusions, acknowledgements, references, summary in Lithuanian, and supplementary materials. The material is presented in 120 pages, 27 figures, and 7 tables. The dissertation refers to 331 literature sources. The dissertation is written in English with an extended summary in Lithuanian.

1.7. Abbreviations

Abbreviation	Definition	Explanation
SW-GW	Surface-groundwater [interactions]	Zones in the watercourse where the presence of surface water flowing into the riverbed (i.e., downwelling) or groundwater issuing from the riverbed (i.e., upwelling) is registered.
EC	Electric conductivity	Physicochemical water parameter.
T	Temperature	Physicochemical water parameter.
DO	Dissolved oxygen	Available oxygen in the water column.
L	Live	Reconstructed egg pockets packed with 100 live eggs during the mesocosm experiments.

Abbreviation	Definition	Explanation
L+D	Live + dead	Reconstructed egg pockets packed with 50 live and 50 dead eggs during the mesocosm experiments.
CA	Correspondence analysis	Used to identify relationships from discrete data (e.g., percentages) coming from contingency tables.
PCA	Principal component analysis	Unconstrained (does not follow a priori data clustering) summarization technique for multivariate data matrices. It allows the visualization of similarities/differences among data (e.g., sampling stations) and their relationships with environmental variables. Euclidean distances from the multivariate data matrix are reported in the 2D space following an eigenvalue equation.
NMDS	Non-metric multidimensional scaling	Unconstrained (does not follow a priori data clustering) summarization technique for multivariate data matrices. It allows the visualization of similarities/differences among data (e.g., sampling stations) and their relationships with environmental variables. Euclidean distances from the multivariate data matrix are reported in the 2D space following an iterative algorithm. It does not make any assumption of the data.
ISA	Indicator species analysis	A technique used to determine the most influential environmental variables in a given dataset.

1.8. Glossary

Term	Definition	Explanation
Respiration/ Excretion	$\frac{\Delta C \times V}{n \times \Delta t}$	$\Delta C/\Delta t$ (mg or µg $L^{-1}h^{-1}$) is the variation of O_2 or N-NH_4^+ concentration during incubation, V (L) is the volume of the incubation chamber, and n is the number of incubated eggs.
Flux	$(C_{out} - C_{in}) \times Q$	C_{out} and C_{in} (mg or µg $L^{-1}h^{-1}$) are the concentrations of O_2 or dissolved inorganic N at the core outlet and inlet, respectively, and Q (L h^{-1}) is the water flow.
Nest Centrum	Egg pockets	The sampling site targeted the intragravel water from the egg pockets. The egg pockets are patches constituted by coarser gravel, and the female action during the construction phase winnows out the fine fraction. These conditions are representative of those encountered by eggs.

Term	Definition	Explanation
$N\text{-}NH_4^+$	Ammonium nitrogen	Reduced form of inorganic nitrogen.
$N\text{-}NO_2^-$	Nitrite nitrogen	Oxidized form of inorganic nitrogen.
$N\text{-}NO_3^-$	Nitrate nitrogen	Oxidized form of inorganic nitrogen.
Hyporheic zone	Ecotone between the actual water-course bed and the water column	This boundary habitat ranges from a few centimetres to several metres. Lithophilic fish species usually lay their eggs inside this ecotone.
Near Nest	Nest periphery	The sampling site aimed to target the intragravel water conditions faced at the nest periphery, where the female's action only minimally alters the spawning gravel.

2

Literature review

2.1. The influence of hydrological extremes and land use on running water ecosystems

(material in section 2.1 from Paper I)

In the context of river ecology, hydrological extremes are defined as exceptional, prolonged low flow periods or intense flood events (Brooks, 2009), and are regarded as one of the most crucial challenges of our century (Nohara et al., 2006). Hydrological extremes are triggered by the interplay of ongoing climate change and human pressures; they have the potential to compromise river functioning and the resilience of riverine ecosystems, leading to habitat loss and the degradation of ecological communities (Dahm et al., 2003; Stanley et al., 1997).

Across the globe watercourses are experiencing low flow events, the intensity and prolongation of which are increasing due to ongoing climate change (Brooks, 2009). Water scarcity episodes are becoming more common in temperate climates (Sutherland et al., 2008), and this leads to the hydrological intermittency phenomenon, also called the Mediterraneization process (Bernal et al., 2013; White et al., 2023; Wilby et al., 2006) as it is common in watercourses in the Mediterranean region. There, watercourses which have historically held a perennial waterflow regime have experienced moments in which their waterflows have been interrupted in recent decades, leaving portions of the streambed uncovered (Bernal et al., 2013; White et al., 2023; Wilby et al., 2006). The absence of perennial flow, i.e., droughts, has multiple consequences on the functioning of a running-water environment (Vannote et al., 1980). Low flow periods cause the alteration of biogeochemical processes, dissolved and particulate matter transport, changes in SW-GW interactions, and shifts in community structure (Lange et al., 2012; Larned et al., 2010). This is because longitudinal water transport is a pivotal element of these lotic environments (Poff et al., 2010; Woelfle-Erskine et al., 2017), and during low discharge events the limited dilution capacity results in nutrient accumulation, favouring the growth of primary producers (Hosen et al., 2019).

In low-flushed, stagnant areas, low flow discharge can lead to organic matter accumulation and oxygen depletion (Burke, 2011; Greig et al., 2005), a scenario that is worsened by temperature rise (Conallin, 2004). Additionally, hydrological intermittency can also lead to a loss in the sediment transport activity of running water. This translates into the increased deposition and accumulation of fine fractions over the riverbed (Guarch-Ribot et al., 2016). The clogging of interstices prevents water circulation beneath the streambed, leading to the suffocation of the intragravel environment. Clogged areas are then unsuitable for the most sensitive macrofauna taxa and the majority of the intragravel stages of lithophilic fish (Barlaup et al., 1998; Malcolm et al., 2010; Pulg et al., 2013). Moreover, flow reduction inevitably leads to a rise in nutrient concentration. This can become harmful to sensitive cold-water species like salmonids, particularly when combined with increased temperatures and the reduction of oxygen (Calles et al., 2007; Dumas et al., 2007).

Under the present climate change scenario (IPCC, 2014), the latest predictions for Lithuanian rivers for the end of the 21st century are a significant decrease in spring floods, an increase in summer low flow events, and an increase in water temperature of more than 5 °C (Čerkasova et al., 2024; Šarauskienė et al., 2018). Systems that already show altered patterns in water quality during hydrological extremes are likely to see further deterioration in their conditions. Events that currently represent a stressor for cold-water stenoecian fish species will seriously hamper their survival in the future. Therefore, it is important to raise awareness of the watershed-related responses occurring during hydrological extremes.

However, climate change is also connected with the increasing intensity of rainfall and flood events. The latter, if associated with artificialized stream paths and with land use that favours highly erosive phenomena, may further increase siltation and riverbed scouring potential (Adjovu et al., 2023; Soulsby et al., 2001). Mechanical clogging resulting from high and prolonged suspended solid loads exerts negative impacts on the stream system. These impacts range from the impairment of gill functioning in fish (Swietlik et al., 2003) to streambed suffocation (Malcolm et al., 2004; Pulg et al., 2013; Sternecker et al., 2013; Sutherland et al., 2002). Flood phases result in higher sediment and organic matter loads, with associated increases in nitrogen

(N) and phosphorus (P) concentrations (Jarvie et al., 2005; Severini et al., 2023). Additionally, higher infiltration rates in the streambed and the reduction of intragravel waterflow result in oxygen deficiency. This may lead to the production and accumulation of potentially toxic endogenous by-products of N–compounds (Brinkman et al., 2009; Greig et al., 2005; Rubin et al., 1996).

Land use has the potential to affect the response of a drainage system during hydrological extremes, as unvegetated soil surfaces without well-established root systems are more prone to soil erosion during rainfall events. This may lead to higher loads of suspended solids into the watercourses (Pulg et al., 2013; Sternecker et al., 2013; Sutherland et al., 2002). Additionally, anthropogenic pressures in the proximity of rivers (e.g., bank and riverbed alteration, road construction) or spread within the watershed (e.g., agricultural practices, tree-felling, and wastewater from agriculture, industries, and housing) increase chemical inputs (inorganic N and P fertilizers, pesticides, and heavy metals) (Binkley et al., 1993; Burke, 2011) to freshwater bodies. They also increase the mechanical action of running water on the streambed, leading to an increased number of scouring events and impacting the spawning areas exploited by the lithophilic fish community (Bjornn et al., 1991; Chapman, 1988; Rubin et al., 1996). It is widely accepted that forested areas and buffer zones near watercourses can intercept nutrients and solid inputs during rainfall events (Barlaup et al., 1998; Billi et al., 2022; Pulg et al., 2013; Sutherland et al., 2002). These interfaces have the potential to metabolize organic substances prior to their entering the stream environment (Gorgoglione et al., 2020). Additionally, the mechanical actions of roots and branches buffer surface water runoff, thereby preventing it from entering the system, contributing to a slower rise in water levels, and dampening scouring potential (Chen et al., 2011; Maraseni et al., 2016; Van Esbroeck et al., 2016). On the contrary, impermeable surfaces allow water to rapidly enter the stream channel, favouring scouring actions (Opperman et al., 2005; Sutherland et al., 2002). The presence of wastewater treatment plants or septic tanks directly connected to the watercourse also significantly increases nutrient availability in riverine ecosystems, triggering eutrophication phenomena (Olyaei et al., 2018; Zouboulis et al., 2015).

2.2. Salmonid spawning habitat selection patterns and the role of SW-GW interactions – insights from a systematic review

(material in section 2.2 from Manuscript I)

Water velocity, depth, and substratum size are traditionally thought to be key indicators that predict the spawning habitat preferences of salmonids in fluvial ecosystems. Indeed, classical habitat models (Gallagher et al., 1999; Leclerc et al., 1995; Raleigh et al., 1984) and suitability curves (Louhi et al., 2008) primarily rely on channel hydraulics and particle size composition as the main variables to explain redd site selection (Hanrahan et al., 2004; Moir et al., 2005). Nevertheless, flow advection induced by hydraulic gradients at the local (i.e. channel bedforms − Poole et al., 2008) and regional scales (i.e. segment systems – Cardenas, 2009; Stonedahl et al., 2010) play a significant role in the selection of spawning grounds (Quinn, 2018). Geomorphic bed features and surface water velocity are used as proxies to spatially locate and define the intensity of groundwater seeps (Bernier-Bourgault et al., 2002; Fanelli et al., 2008); nonetheless, this can miss the identification of water origin and thus discriminate against measuring the real contribution of groundwater to nest site selection.

Overlooking the contribution of SW-GW exchange can lead to the biased estimation of spawning habitats (Dauble et al., 1990; Shirvell, 1989). Patchy nest distribution in alluvial floodplains can be observed despite homogeneity in channel hydraulics, and the high utilisation of just a few areas can lead to overpredictions when it comes to the utilization of spawning grounds (Geist et al., 1998; Soulsby et al., 2012). In other cases, the presence of groundwater is solely accounted for as the main factor related to the presence of dissolved oxygen (DO) (Nika, 2011), or is seen as the best parameter to model spawning habitat preferences (Fell et al., 2017). Finally, in some scenarios it represents the main variable used to explain the failure of habitat model predictions (Geist, 2000) or the different spawning behaviours of the same species in the same channel network (Leman, 1993; Mouw et al., 2014). Hence, it is apparent that the intensity and direction, either lateral or vertical, of SW-GW exchanges act synergistically with channel hydraulics when it comes to the choice of spawning areas.

SW-GW interactions are defined as the interplay of groundwater seepage generated from the water table both beneath and at the sides of the watercourse with the presence of surface water locally infiltrated into the streambed (Mouw et al., 2014; Neumann et al., 2016). The sum of these two processes can shape the quantity and quality of the hyporheic water flowing through the spawning areas, as well as the related patches of upwelling.

2.2.1. Data extraction

The present literature review examines the degree of responsiveness to SW-GW interactions among the most commonly studied salmonid species during their spawning seasons in lotic environments. Namely, it seeks to identify which salmonid species commonly use zones of groundwater recharge (i.e., upwelling) or surface water discharge (i.e., downwelling). The main control mechanisms shaping variability in the use of different SW-GW interaction regimes are commented on. The assessment examines the spawning behaviours of 14 salmonid species in relation to SW-GW interactions, gathering data from a total of 47 studies. Many studies analyse the spawning behaviour of multiple species at the same time, while others report different spawning tactics in relation to one species. Therefore, data collected is expressed in "records", where 1 record indicates the association between one (1) species and its preference for downwelling or upwelling conditions during spawning. Of the 72 records gathered, 44 deal with Pacific salmon, Oncorhynchus spp. (pink salmon – O. gorbuscha; chum salmon – O. keta; coho salmon – O. kisutch; masu salmon – O. masou; rainbow trout – O. mikyss; sockeye salmon – O. nerka; and Chinook salmon – O. tshawytscha), 11 with Salmo spp. (Atlantic salmon – S. salar; and brown trout – S. trutta), and 17 with charr, Salvelinus spp. (Arctic charr – S. alpinus; bull trout – S. confluentus; brook trout – S. fontinalis; white-spotted charr – S. leucomaenis; and Dolly Varden – S. malma) genera. The lenok (Brachymystax), taimen (Hucho), stringfish (Parahucho), and long-finned charr (Salvethymus) genera were not included due to the inconsistency of their data. The studies gathered in this survey deal with spawning strategies in lotic environments in North America, Europe, Asia, and Oceania (Figure 2). They cover a time span from 1937 to 2023 and include both peer reviewed articles and grey literature (i.e., academic theses and technical reports).

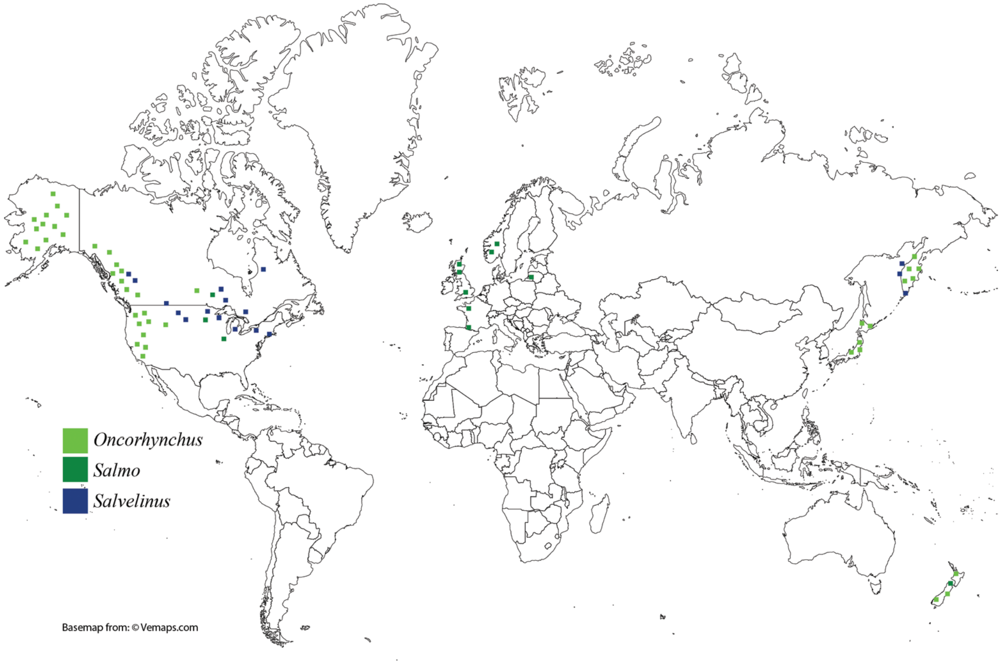

Figure 2. Map portraying the geographical distribution of the studies gathered according to the different genera (Manuscript I, in preparation).

The keywords “salmonid”, “spawning”, “hyporheic”, “upwelling”, “downwelling”, “groundwater”, “surface-groundwater interactions” and “behaviour” were used for search engine extraction using the Google Scholar, Scopus, and ISI web of knowledge databases from 2024 to 2025. Then, further material was identified from citation analysis within the selected sources (literature forward) as works that cited the investigated sources (literature backward). Studies that found a link with the selection of spawning grounds and the presence of surface- or groundwater-dominated conditions were selected. Such exchange across the hyporheos was determined by: a) tracking water movements from the water column into the spawning ground and vice-versa (upwelling-downwelling); and/or b) recognizing physicochemical differences between the water circulating inside and outside the nests – for example, in order to justify the different origin of the former. Finally, works that, although they did not explicitly measure the degree of SW-GW interactions in the spawning area, indirectly assumed the presence of downwelling (e.g., pool-riffle transitional zones) or upwelling (e.g., free-ice areas, air bubbles, sand resuspension) zones given particular environmental features were considered. This analysis considers studies that examined this interaction in stream and river systems within a local, regional, or multi-scale approach (sensu Frissell et al., 1986).

2.2.2. The reliance of the *Oncorhynchus*, *Salmo*, and *Salvelinus* genera on SW-GW interactions during spawning time

For the Oncorhynchus genus, a high usage of groundwater-fed areas is reported (Figure 3 b). According to the analysed sources, this is particularly significant for chum salmon, despite the presence of nearby zones with better water velocity, depth, and substratum size (Geist et al., 2002). Furthermore, Kobayashi (1968) noted how chum salmon disregard zones with appropriate surface water velocity and high DO saturations (97–99%) in favour of groundwater-fed areas with less favourable DO (31–74%) saturation. This tendency has also been recorded for sockeye and Chinook salmon in the context of the relationship between redd site selection and the presence of upwelling conditions. This is further confirmed, albeit to a lesser extent, for masu salmon and rainbow trout, while it has not been reported for pink and coho salmon. The spawning grounds most strongly influenced by (regional) upwelling conditions have principally been found in the alluvial floodplains of river systems, in association with mainstem habitats and side, spring, and off-channel networks, and in environments subjected to restoration works. Localized upwelling conditions are then reflected in the channel bedforms that constitute the surveyed stretch.

Mainstem habitats are usually regarded as migration routes; nonetheless, groundwater-fed patches in these areas can become spawning hotspots (Burril et al., 2010; Neumann et al., 2016) in relation to geomorphic bed features such as gravel bars and riffle heads (Aruga et al., 2023; Geist, 2000; Mesick, 2001). This can be significant in glacial-fed systems at northern latitudes, where intragravel temperatures are significantly higher than those in the surface water, thus preventing eggs from freezing and hastening their developmental process (Crisp, 1988, 1990). For example, chum salmon in the Amur River have a marked preference for patches with abundant seeps exceeding 4 °C that can help them to avoid freezing conditions (Bakkala, 1970). A similar preference was reported by Vining et al. (1985) where chum salmon preferentially spawn in the slough side channel habitats in the middle stretch of the Susitna River in Alaska, which have the potential to minimise freezing and dewatering conditions. Furthermore, as pointed out by Burril et al. (2010) and Zimmerman et al. (2012), improved vertical circulation can lead to fine removal for chum salmon, which exhibit similar spawning behaviour in the mainstem habitats of the Tanana River in Alaska, where upwelling areas are largely free from sand and silt.

Sometimes, groundwater-fed zones exploited by spawners can exhibit an opposing trend in terms of fines. This is the case in the glacial Taku River in British Columbia, where sockeye salmon intensively use groundwater-fed areas in braiding channels, which are characterized by high rates of organic silt in zones of unusually low surface water velocity (Lorenz et al., 1989). Despite the poor quality of sediment texture, these seeps improve intragravel circulation. Given the high tolerance to low oxygen levels of this species in comparison to other salmonids (Semko, 1954), the authors were confident that egg-to-fry incubation conditions would be adequate (Lorenz et al. (1989). This evidence is also supported by Garrett et al. (1998), who observed that, in the meandering sections of the Payette River in Idaho, the lacustrine ecotype of sockeye salmon (kokanee) that spawns in high densities only in discrete upwelling sections is characterized by a high proportion of fines. The characterization of hatching success with Whitlock–Vibert boxes planted in these sites revealed significantly higher survival percentages than those planted in non-upwelling zones (Garrett et al., 1998).

Even though these habitats present upwelling conditions, groundwater seeps can show a certain degree of mixing with river water, especially if intragravel circulation is affected by the presence of channel bedforms (Cherry et al., 1979). In fact, islands have the potential to generate local upwelling conditions, as highlighted by Geist (2000), where fall Chinook salmon in the mainstem section of the Columbia River massively spawned in nearby areas of river-like upwelling water, characterized by high permeability and high levels of oxygen. In the mainstem areas of the Okanagan River, most Chinook spawning occurred in phreatic-fed areas, where seep composition is a mix between real groundwater and locally intruded surface river water (Neumann et al., 2016). Redd locations were confined to stretches where the valley narrows, allowing interaction with the underlying aquifer (Neumann et al., 2016).

The presence of regional groundwater can affect local circulation inside channel bedforms – like in the Stanislaus River in California, where more than 70% of the redds were located on riffle crests with an unexpectedly positive vertical hydraulic gradient (Mesick, 2001). The presence of upwelling is commonly reported in marginal habitats exploited for spawning, as pointed out by Fell et al. (2017) in the context of chum salmon that selected groundwater-fed side channels in the mainstem reaches of the glacially fed Cheakamus River in British Columbia.

Evidence provided by Mouw et al. (2014) and Kuzishchin et al. (2010) in the wandering rivers of Kwethluk, Alaska, and Kol, Kamchatka, reports the usage of upwelling areas and the avoidance of downwelling zones in flood and spring channels. The same is reported by Aruga et al. (2023) regarding late chum salmon runs in an urbanized river system in northern Japan. The side channel in the alluvial floodplain of the Columbia River in Washington, with its warm groundwater supply, is also a favourite location for chum salmon spawners (Geist et al., 2002; Salo, 1991). However, similar studies have highlighted the local seep origin, owing to the presence of channel bedforms such as fluvial islands. It is likely that summer and fall chum salmon runs have been observed spawning in the Chena and Tanana River sloughs, receiving different types of subsurface water alimentation ranging from river water locally infiltrated into the hyporheos and seeps directly coming from the aquifer (Maclean, 2003).

Even though side environments in the floodplain can receive groundwater alimentation, the spawning site selection process can be related to the extent of seep residence time. For example, in an oxbow lake in the Cedar River in Washington state, the redd site selection of sockeye salmon was highly influenced by the presence of upwelling because of warmer seeps related to a longer residence time. However, this did not occur in a nearby man-made gravel pit with upwelling seeps characterized by a river-like signature (Hall et al., 2004).

Finally, the improvement of groundwater-fed side areas by means of widening and deepening the channel with the addition of gravel and drop-structures to create adequate spawning depth in the Squamish River in British Columbia was connected with increased usage and juvenile development by chum salmon spawners (Lister et al., 1980). To a lower extent, the literature also reports the usage of upwelling areas to spawn in groundwater-fed creeks and their spring areas, mainly due to the stable temperature regime. This life history trait has been reported for chum spawners in Hokkaido headwaters (Bakkala, 1970; Kobayashi, 1968) and sub-Arctic streams (Kogl, 1965), but also for pink salmon runs in the coastal streams of southwestern Alaska (Krueger, 1981). Rainbow trout have also been found to select groundwater-fed creeks of glacial origin underlying moraine-till deposits in Ontario, with a lack of correlation between incubation success and fine content (Sowden et al., 1985). To avoid interspecific competition for spawning grounds in the Kol basin, in the same groundwater-fed streams rainbow trout select the lower reaches (Kuzishchin et al., 2008) while masu salmon target the uppermost parts, exploiting both zones of localized downwelling at the pool-riffle interface (Kuzishchin et al., 2009).

Globally, all species analysed here rely to some extent on surface-water dominated conditions during spawning (Figure 3 b). Nevertheless, from the studies gathered here it seems that such conditions are linked with localized downwelling flows induced by geomorphic bed features (Healey, 1991; Kuzishchin et al., 2010; Savvaitova et al., 2007). This behaviour has been reported extensively: from pink salmon in Hokkaido streams (Kobayashi, 1968) to pink and Chinook spawners in the mainstem of Kol basin tributaries in Kamchatka (Kuzishchin et al., 2010; Kuzishchin et al., 2008). In the Kwethluk River in Alaska, the same life history trait is also shared by chum salmon that spawn in the mainstem areas targeting local downwelling patches in glides and pool-tailouts (Mouw et al., 2014).

When spawning occurs in sympatry, in order to avoid redd superimposition, species can be forced to select sub-optimal downwelling areas. This is the case in the Okanagan River in British Columbia, where interspecific competition with larger Chinook salmon for limited upwelling areas forces sockeye salmon to spawn in downward vertical-flow zones (Neumann et al., 2016). Chinook salmon in the Columbia River in Washington choose spawning grounds unexploited by chum spawners, where the hyporheic environment is dominated by river water (Geist et al., 2002). The usage of transitional pool-riffle zones benefiting from local downwelling circulation has also been reported for small systems, as confirmed by Hobbs (1937) and Briggs (1953) in California and New Zealand, respectively. In these studies, a major fraction of coho, Chinook salmon, and rainbow trout redds were found in pool-tailouts. The observation of this life history trait is supported by Zimmerman et al. (2012) for chum salmon summer runs that, in opposition to those in fall, spawned in a regionally surface-water-dominated creek in the Tanana River watershed.

For the Salmo genus, pool-riffle transitional zones benefitting from localized downwelling gradients are perhaps the spawning habitats most commonly exploited by Atlantic salmon and brown trout (Figure 3 a), as has already been summarized in other reviews (Fleming, 1996; Louhi et al., 2008). This tendency was observed by Hobbs (1937) and Stuart (1953) in streams in New Zealand and Scotland, respectively, in relation to the dwelling and migratory patterns of brown trout. Similarly, aerial surveys carried out by Heggberget et al. (1986) confirmed the preferential usage of downstream pool-tailouts by sympatric populations of spawning Atlantic salmon and brown trout. In watersheds in the north of France, Baglinière et al. (1990) and de Gaudemar et al. (2000) supported the aforementioned findings for Atlantic salmon, although these studies did not account for the magnitude of hydraulic gradients. In a creek in Michigan, Hansen (1975) reported that brown trout actively eschewed areas of groundwater upwelling, marked by high temperatures, that percolated through organic-rich layers. Similar behaviour was reported in a boreal stream, where Nika (2011) observed a negative relationship between nest placement and the presence of upwelling groundwater characterized by low DO content. Therefore, it is apparent

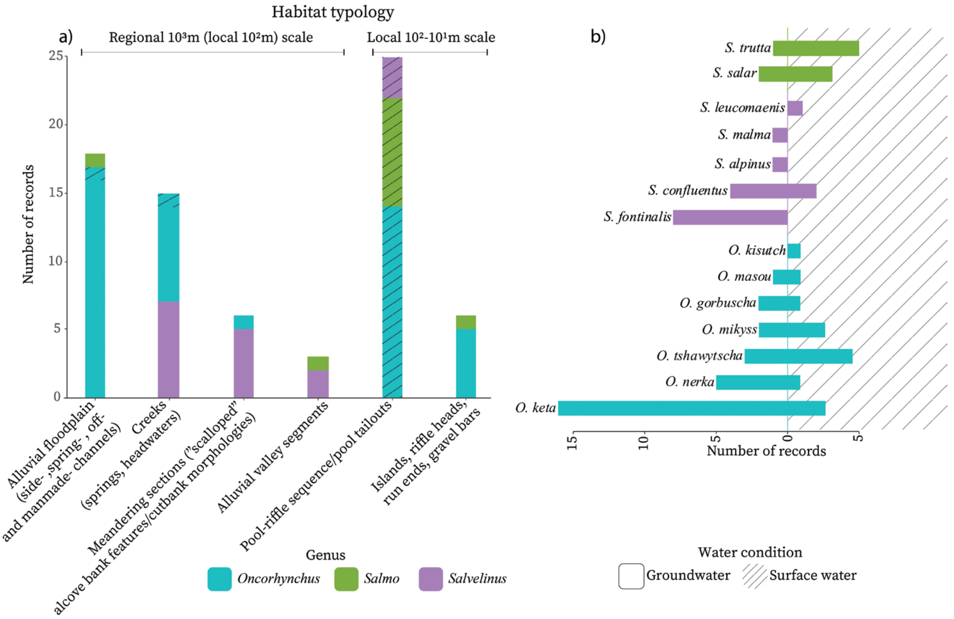

Figure 3. a) Bar charts portraying records on the occurrence of surface- (downwelling) or groundwater-dominated (upwelling) areas in relation to different habitat typologies categorised according to the scale of investigation of each reference. b) Records on the usage of surface- or groundwater-dominated areas by the different salmonid species considered in this survey. (Manuscript I, in preparation).

that, more than simply flow direction, fish will avoid upwelling groundwater in cases where the oxygen deficit can damage incubating embryos.

The use of groundwater-fed areas is well-documented for species belonging to the Salmo genus, even though the number of references is lower with respect to the Oncorhynchus and Salvelinus genera (Figure 3 b). In Scotland, valley narrowing resulting from the presence of knickpoints like bedrock outcrops and moraines can induce regional upwelling conditions and substantial gravel accumulation, which are intensively used by Atlantic salmon despite low intragravel DO content (Malcolm et al., 2005). Brown trout also opportunistically reuse brook trout redds in groundwater-fed Ontario creeks (Witzel et al., 1983). Finally, side channels experiencing generalized upwelling in alluvial floodplain systems can offer preferential spawning grounds for Atlantic salmon, mainly due to the more stable and productive environment for juveniles (Soulsby et al., 2012).

A close link exists between the use of groundwater-fed areas and specimens belonging to the Salvelinus genus (Figure 3 b), with particular regard for brook and bull trout in a variety of local and regional upwelling conditions, although the discrete usage of transitional channel bedforms with localized downwelling is also reported (Figure 3 a). For bull trout, Baxter et al. (2000) reported the preferential usage of alluvial valleys with a lateral downstream constriction (knickpoint) in the creeks of the Swan River basin in northwest Montana, which allows the emergence of groundwater and the accumulation of well-sorted spawning gravel. The redd occurrence of the same species is reported in unconfined segments without narrowing that can present a regional upwelling regime, as documented in the same region in the tributaries of the Flathead River (Bean et al., 2015). Beside the moderation of temperature, unconfinement provides an overbank flow that buffers against flood conditions, lowering bed shear-stress and thus redd scour (Buffington et al., 1999). On a local scale, in reaches presenting regional groundwater seeps, the majority of redds were spotted in pool-riffle transitional zones characterized by local downwelling that ensured high intragravel water percolation (Baxter et al., 2000; Bean et al., 2015).

In watersheds with regional upwelling characterized by deoxygenated water, brook trout nest distribution can be highly patchy, targeting discrete zones with locally rich oxygen seeps. For instance, in the Quashnet River in Massachusetts, where seep flow paths encounter organic deposits, nests were spotted in areas where the mainstem cut into the sand and gravel valley walls with highly oxygenated local seeps (Briggs et al., 2018). The aforementioned study confirms that selection is driven by DO gradients acting as a cue for spawners. This is despite the fact that upwelling spawning and non-spawning sites presented the same oxygen and temperature values in Canadian shield creeks, substantiating the hypothesis that selection would be based on other chemical gradients (Curry et al., 1995). If the striking preference of brook trout for delimited groundwater-fed areas is coupled with the scarcity of available spawning gravel, this can provoke substantial redd superimposition (Curry et al., 1995). This behaviour is further exacerbated by the fact that nests are easy to excavate with lower fine percentages because of the gravel already moved by previous spawners (Essington et al., 1998). The highly specialized spawning behaviour of brook trout is reflected in high rates of intraspecific redd superimposition, but low interspecific usage when spawning in sympatry with brown trout, where substantial redd segregation occurred because of the selection of pools with consistent upwelling in creek headwaters (Witzel et al., 1983). To avoid interspecific redd superimposition, the same life history trait is adopted by the white-spotted char in the piedmont tributaries of the Kol River in Kamchatka, where their spawning time overlaps with that of masu salmon (Kuzishchin et al., 2009).

Conversely, in the headwater tributaries of the Kol basin, Dolly Varden will select downwelling-dominated zones to avoid overlap with sympatric white-spotted charr spawners (Savvaitova et al., 2007). In many systems, the limitation of groundwater-fed zones is therefore a constraint for the recruitment of standing crops, especially if beaver damming can submerge the spawning areas, thus biasing the redd site selection mechanism (Webster, 1962). After locating groundwater-fed areas, brook trout prioritize the selection of gravel-cobble pockets offering the right substratum for egg incubation, a condition that can overcome the requirements for upwelling areas if the creek presents a shortage of unconsolidated gravel (Curry et al., 1995). Similarly, bull trout are also affected by a limitation on their spawning grounds, where they select fine gravel enclosures in upwelling sites. In this case, the intraspecific superimposition of spawning nests is also documented (Heimer, 1965).

In other contexts, the selection of upwelling sites can occur in spite of substrate composition in areas rich in sand (Briggs et al., 2018). However, due to improved water circulation, seeps can provide silt-free egg pockets that can even present a coarser substratum layer above them (Snucins et al., 1992). Less common are references that link the presence of groundwater seepage with spawning site selection in river systems. This has been ascertained for brook trout and arctic charr in the reaches of the Pigeon River in Michigan and Quebec, respectively (Benson, 1953; Cunjak et al., 1986). In the adfluvial population of the endangered coastal brook trout, Van Grinsven et al. (2012) documented the usage of a discrete proportion of the Salmon Trout River, Michigan, where spawning occurs in areas characterized by a stable temperature regime that can increase survival rates. Additionally, Benson (1953) correlated the presence of groundwater with areas without ice cover, speculating that these areas would also be free from the anchor ice on the streambed, the formation of which during freezing periods can damage incubating embryos. This issue was most recently explored by Baxter et al. (1999), who showed higher and less variable incubation rates for egg boxes placed in groundwater-fed areas chosen by bull trout spawners in the mainstem of the Chowade River in British Columbia.

2.2.3. Control mechanisms to study diversity in the use of surface-groundwater interactions

On a spatial level, the factors determining the direction and intensity of intragravel flow overlap in a scale-dependent framework (Figure 4). Water movement gradients vary from microhabitat subsystems (Tonina et al., 2009) to local exchanges generated by geomorphic bed features (Bjornn et al., 1991) and hyporheic flow path movements at the reach and segment scales (Briggs et al., 2018; Mouw et al., 2014). These three levels at which water exchanges can be interpreted add further complexity to spawning habitat selection, making it specific to each watershed system. At the microscale, it is possible to identify relatively uniform patches within a channel bedform (Frissell et al., 1986), and some features have the potential to trigger eddies and turbulence at the riverbed interface, thus generating hydraulic gradients. Many studies report the intensive use of microhabitat subsystems such as logs laying across stream sections (masu salmon, Kuzishchin et al., 2009), woody debris (brook trout, Van Grinsven et al., 2012), and large stones enclosing gravel sites (brown trout, Crisp, 2008). The structure of the redd itself, due to its bell-shaped curve, also promotes the percolation of water inside the hyporheos (Tonina et al., 2009). Moreover, the female flushes out fines during construction, allowing better water circulation inside the nest (Burke, 2011).

Therefore, all of these microhabitat subsystems enhance local flow advection in the intragravel environment, which is beneficial for egg ventilation in terms of oxygen delivery and the removal of metabolites. The local scale refers to the channel bedform units (Frissell et al., 1986), also known as geomorphic bed features, and is capable of generating localized hydraulic gradients (Stanford et al., 2005). The transitional zone of pool-riffle sections is perhaps the most relevant feature related to the selection of spawning habitat, a role that has long been recognized by fishery biologists (Bjornn et al., 1991; Heggberget et al., 1986; Hobbs, 1937; Quinn, 2018). The convex geometry of the riffle head causes the intrusion of surface water into the riverbed (downwelling); on the contrary, an opposite movement occurs in the riffle tail (upwelling) (Stuart, 1953; Vaux, 1962). These local currents across the hyporheos improve oxygen supply and metabolic waste removal, with a loosening and desilting effect for the gravel around the egg pocket (Crisp, 2008; Greig et al., 2007; Kondolf et al., 2008).

While the use of these bedform units is primarily reported for the Salmo genus, it is also consistent for Oncorhynchus and, to a lesser extent, Salvelinus, with exceptions made by those species preferring seeps originating from a wider scale. Due to their local origin, these seeps share a strong physicochemical affinity with the surface (Fanelli et al., 2008; Geist et al., 2002). Even though hydraulic gradients can be detected, this does not guarantee the area being affected by groundwater originating from beneath the water table. In this context, the majority of the studies reported spawning activity at the downstream end of the pools, although some spotted redds constructed on riffle ends experiencing a positive hydraulic head (Mesick, 2001; Neumann et al., 2016). Islands and gravel bars can also generate vertical water movement through the riverbed, so that nearby areas can be profitably selected by spawners (Geist, 2000).

The regional scale corresponds to segment systems that are portions of a watercourse with a relatively uniform lithology and channel slope composed of several reaches (Frissell et al., 1986). These are usually bound by features such as tributaries, lakes, or geomorphic knickpoints that interrupt the homogeneity of the segment. In turn, each segment is constituted by multiple reaches that range from tens to hundreds of meters long according to the watercourse type. Moraines and bedrock outcrops are examples of geomorphological and geological knickpoints, respectively (Malcolm et al., 2005), which create the abrupt restriction of the floodplain and, in steeping the river profile, became groundwater hotspots. Although regional upwelling conditions are found in these areas, fish can select zones at the pool-riffle transition with local downwelling currents (Baxter et al., 2000; Bean et al., 2015). Confined valley segments present benign incubation temperatures due to groundwater seeps (Stanford et al., 1993; Woessner, 2000), while the main trade-offs are represented by the scarcity of gravel patches for spawners and the increased frequency of scour events for redds (Buffington et al., 1999). Unconfined valley segments can also be influenced by the presence of groundwater (Cherry et al., 1979) – an advantage that is related to the abundance of spawning gravel, woody debris, and side channels increasing the overall complexity of these areas (Copp, 1989; Rich et al., 2003; Shellberg, 2002).

It is not surprising that many studies highlight alluvial floodplains in these segments as being pivotal for lithophilic spawners, especially given their vocation as nursery refugia and their low stress levels with regard to redd scours (Baxter et al., 2000; Bean et al., 2015; Copp, 1989; Montgomery et al., 1999). Expansive floodplains have high variability in terms of hydraulic gradients, which is in turn related to the great diversity in three-dimensional bedforms and channel network complexity (Stanford et al., 1993). These factors are associated with braided and wandering rivers, and present high variation in spawning habitats due to the presence of downwelling or upwelling zones. Pore-water advection, riverbed topography, and the depth of the water table dictate hydraulic gradient differences with respect to the studied scale (Gooseff, 2010; Poole et al., 2008; Stonedahl et al., 2010). Advective flowpaths mainly occur in pool-riffle sequences, glides, and runs, inducing intragravel exchange (Elliott et al., 1997; Harvey et al., 1993).

Meanwhile, islands and large gravel bars cause transverse and longitudinal water movements according to their topography in the floodplain (Stanford et al., 2005; Winter, 1999). Side channels that seasonally overflow are mainly fed by seeps during lean periods thanks to lateral intragravel circulation. Given their peripheral location and the presence of groundwater, they confer a stable environment and serve as shelter from intense freshets (Lister et al., 1980). Spring channels further away from mainstem corridors are usually less strongly influenced by scour events, and are often surrounded by riparian forest. This typology mainly concerns entombed paleochannels that are the preferential path of the alluvial aquifer, and thus benefit from consistent groundwater intake (Poole et al., 2006; Stanford et al., 1993). Thus, the mosaic of intragravel flowpaths brings situations in which segments with regional downwelling can be characterized by local upwelling reaches, and vice versa (Poole et al., 2006; Stanford et al., 2005). Diversity and limitations in the areas of groundwater-fed zones can lead to interspecific competition for spawning grounds (Geist et al., 2002; Neumann et al., 2016) alongside intraspecific variation in the life history traits of the same (Mouw et al., 2014) or different (Leman, 1993) runs. This makes it difficult to unequivocally assign preferences for upwelling or downwelling zones in expansive floodplains.

Springs and stream headwaters receiving groundwater supplies can be found in areas where the bedrock is covered by permeable layers of glacial drift materials. Therefore, high permeability allows consistent groundwater inflow, and if the seeps travel across inorganic layers then their oxygen content can be appropriate for embryo development (Sowden et al., 1985). Additionally, these environments offer patches of higher temperatures, allowing some species to spawn in harsh climates – as recorded for masu salmon at the northern edge of their distribution (Kuzishchin et al., 2009) or chum spawners for groundwater-fed headwaters in the Hokkaido region (Bakkala, 1970). Despite the presence of regional upwelling, species such as masu salmon, rainbow trout, and whitespotted charr have been found to exploit discrete zones of localized downwelling at the interface of pool-riffle sections (Kuzishchin et al., 2008; Kuzishchin et al., 2009; Savvaitova et al., 2007).

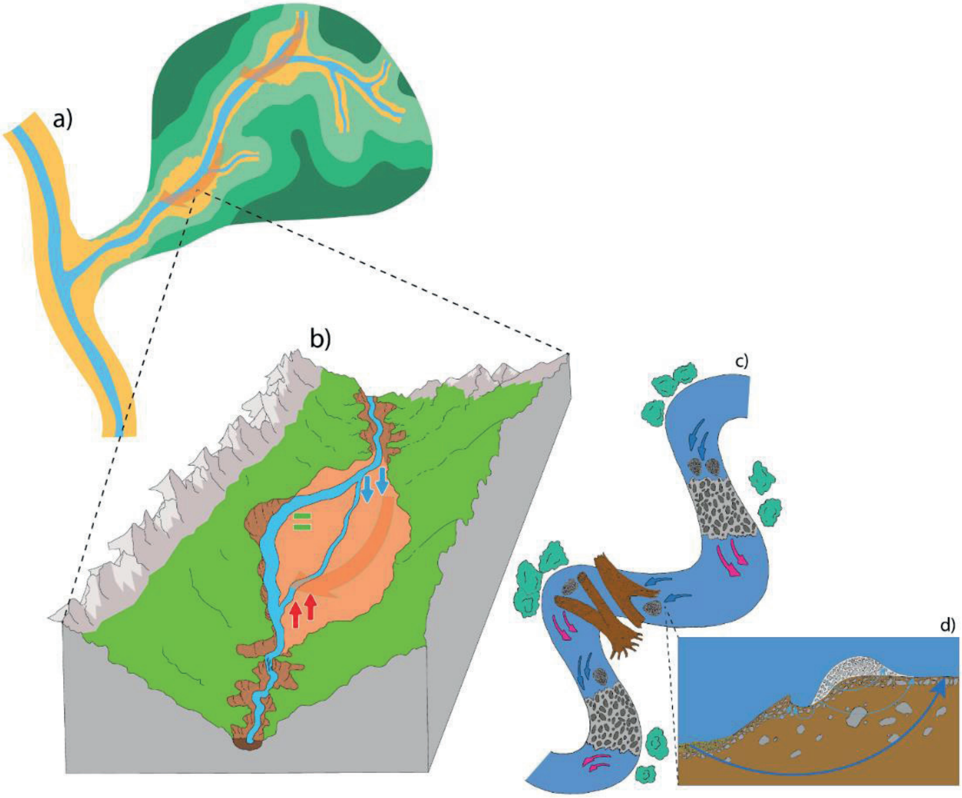

Figure 4. Direction and intensity of SW-GW interactions according to the scale of study. a) At the watershed level, regional groundwater inflow can occur in unconfined valley segments bound by a downstream knickpoint. b) At the segment level, seep intensity varies, with downwelling areas in the uppermost part, neutrality in the middle, and upwelling as the stream approaches lateral constrictions (Baxter et al., 2000). c) At the local level, pool-riffles are common geomorphic bed features that generate local downwelling currents at the riffle head, with local upwelling at the downstream pool end (Bjornn et al., 1991). d) On a microscale, redd tailspill generates its own intragravel circulation that is combined with the local seeps (Tonina et al., 2009) (Manuscript I, in preparation).

The temporal level defines the degree of groundwater exchange on a seasonal basis (Fraser et al., 1998) and in terms of short-term events caused by hydrological extremes (Malcolm et al., 2004). Periods of flood are mostly characterized by surface water from the river that recharges the underlying water table. Therefore, it is difficult to locate SW-GW interactions because of general mixing with surface runoff. On the contrary, groundwater dominates the hyporheos during low flows caused by the absence of rainfall or water trapped in the streambed due to freezing temperatures (Malcolm et al., 2003a; Malcolm et al., 2004; Soulsby et al., 2001). Indeed, upwelling conditions can be found on riffle heads if measurements are performed in a period of high groundwater discharge that covers the locally generated downwelling currents on the riffle heads (Mesick, 2001).

The physicochemical quality of the hyporheic hydrochemistry is depth-dependent, and its degree of variation reflects the presence of groundwater intrusion into the streambed (Malcolm et al., 2010). Regardless of the direction of seeps, SW-GW interactions improve intragravel circulation by adding a vertical flow dimension (Bjornn et al., 1991; Geist et al., 1998) that mitigates clogging in highly silted and sandy ground (Webster et al., 1976) and facilitates the movement and consequent swim up of larvae (Bams, 1969). Indeed, species like brook trout have been demonstrated to show a clear preference for sandy areas of groundwater seepage rather than cleaner adjacent patches without upwelling water (Witzel et al., 1983).

In turbid systems of glacial origin, groundwater zones provide clean patches for sockeye spawners in highly silted and armoured riverbeds (Lorenz et al., 1989). High oxygen content close to saturation combined with low conductivity and alkalinity are indicators of little exchange with the hyporheos, and temperatures can be highly variable (Malcolm et al., 2008, Figure 5). On the other hand, due to the longer time spent in the gravel bed, groundwater typically exhibits higher alkalinity and electrical conductivity (EC) resulting from weathering processes (Malcolm et al., 2005). Temperatures are usually stable (Silliman et al., 1993), and the favourable thermal regime ensures the faster accumulation of degree days; this constitutes the main trait by which seeps can be recognized in northern environments (Cope, 1996; Leman, 1993).

Sometimes, groundwater can have a mixed origin, including phreatic and surface sources. This condition has been found in high hydraulic conductivity contexts such as alluvial floodplains (Geist et al., 2002; Harvey et al., 1993). High permeable alluvium levels enable the constant exchange of river water across the gravel matrix, establishing upwelling zones of surface water origin (Geist, 2000; Stanford et al., 1996). A low residence time explains the affinity of these seeps with the surface water from a physicochemical point of view (Brunke et al., 1997; White, 1993). Lateral intragravel water movements in highly permeable substrata generate short-residence-time seeps in the adjacent side channels, therefore resulting in oxygen richness but colder temperatures due to the shorter intragravel flow path (Burril et al., 2010). Oxygen rates can be similar to the surface water or lower, depending on the typology of the aquifer across which seeps percolate. Indeed, pore water originating from coarse gravel in a shallow aquifer will have high oxygen content, with beneficial consequences for egg incubation during low flows (Brabrand et al., 2002; Casas-Mulet et al., 2015).

Conversely, if seeps percolate through organic-rich layers, such as peat deposits or river sediments, they can face oxygen depletion, thus becoming unsuitable for egg incubation (Mesick, 2001). Therefore, spawners will likely select downwelling areas where locally infiltrated river water maintains high oxygen rates (Cardenas et al., 2016; Tonina et al., 2009). Oxygen shortage can also occur during intense storm events, resulting in transitional phases of generalized upwelling where seeps leach intensively cultivated lands in the studied area (Malcolm et al., 2004; Soulsby et al., 2001). Moreover, oxygen sags in the hyporheos can reduce solid-phase pools of iron and manganese, the mobilization of which gives a characteristic reddish colour (Briggs et al., 2018).

Under sympatric spawning conditions, different species may compete for the same optimal groundwater-fed zones, and the overlap of the same spawning grounds can lead to biased results when assigning preferences in relation to seepage extent. Indeed, some species could be forced to select sub-optimal downwelling-dominated sites and thus worse egg incubation environments (Geist et al., 2002, Figure 5). This ecological constraint is regulated by the size of competitors (Geist et al., 2002) and their abundance in the same spawning area (Phillips et al., 2005), which are in turn watershed-specific and vary in relation to the run intensity of the surveyed year (Neumann et al., 2016). Under allopatric conditions, fall Chinook salmon used upwelling sites near fluvial islands in the Columbia River in Washington (Geist, 2000). However, during interspecific competition with chum salmon, they can select suboptimal downwelling niches in the same river. Indeed, if later chum spawners select upwelling areas, this could provoke the dislodgement of the eggs of the earlier-arriving fall Chinook salmon (Geist et al., 2002). The greater size of Chinook salmon partially forces the smaller sockeye salmon into a wider range of habitats in the Okanagan River in British Columbia. However, due to the low density of Chinook spawner runs and the high availability of groundwater sites, interspecific competition is restrained.

The plasticity of sockeye salmon in spawning among a range of surface- and hyporheic water-dominated conditions makes it difficult to properly highlight the role played by groundwater seeps (Neumann et al., 2016). In streams in southwestern Ontario, brown and brook trout populations occur in sympatry, but competition is minimal during the spawning period due to the highly specialized behaviour of brook trout, as they exclusively select seepage areas. Conversely, brown trout adapt to a wider range of conditions and, thanks to their larger body size, are able to spawn in faster currents and coarser substrata (Witzel et al., 1983). Segregation has also been recorded in the same groundwater-fed system, with masu salmon selecting local downwelling on riffle heads while the sympatric Dolly Varden target upwelling zones (Kuzishchin et al., 2009).

Life history traits result in intraspecific diversity in spawning patterns related to SW-GW interactions, and should also be accounted for (Figure 5). Among all studied species, chum salmon reported the highest degree of polymorphism. Variation in life history traits is consistent in braided systems that offer a variety of spawning niches. This is made possible if the thermal yield associated with groundwater-fed sites is consistent enough to guarantee faster growth rates with respect to mainstem areas (Mouw et al., 2014). In the Kwethluk River in Alaska, summer chum salmon select downwelling conditions in the mainstem habitats, while in side channels they prefer upwelling areas (Mouw et al., 2014). The same spatial segregation was reported by Leman (1993) in the Kamchatka River in Russia, although SW-GW interactions were not assessed.

The use of surface water discharge or ground water recharge zones is also influenced by the temporal segregation of the same species during different runs. This results in great spawning habitat differentiation within a mosaic of temperature regimes and incubation periods (Aruga et al., 2023; Lisi et al., 2013). For Pacific salmon, this translates into higher plasticity in exploiting different spawning grounds according to the run (Zolotukhin, 2019), and thus higher genetic diversity and resilience (Olsen et al., 2008). For example, in the Kol River in Kamchatka, summer chum spawners select mainstem areas, as opposed to fall spawners which target spring habitats (Kuzishchin et al., 2010). This record is consistent with chum salmon in an anthropized river in Japan, where the selection of gravel bars in the primary stream for the early run and secondary channels for the late run has been observed (Aruga et al., 2023). In Alaska, the summer ecotype of the same species selects creeks with surface-water-dominated conditions, while in the Tanana river the fall ecotype exploits upwelling patches (Zimmerman et al., 2012). Constant incubation temperatures and protection from scour events are certainly the main advantages of groundwater-fed side channels, although the main trade off could be associated with oxygen deficiency (Malcolm et al., 2005; Soulsby et al., 2012; Youngson et al., 2004).

Conversely, surface-water-dominated sites in the mainstem provide high oxygen delivery rates (Greig et al., 2007) but are more often subjected to temperature variations and streambed scouring (Mouw et al., 2014). The diversification of spawning strategies in upwelling or downwelling zones is then connected with the costs and benefits related to these areas. This results in a portfolio effect that buffers the interannual variability in temperature and water fluctuations during embryo development, thus increasing the survival chances of offspring (Schindler et al., 2010). Furthermore, this avoids the overexploitation of the same spawning stretch by conspecifics via the superimposition phenomenon (Curry et al., 1995; Essington et al., 1998).

For species that consistently spawn in groundwater-fed environments, the selection of pool-riffle transitional zones happens once the former are saturated. Additionally, late spawners will move into less extensively exploited stretches or will colonize new streams according to the density-dependent habitat model (Cope, 1996). This gradation

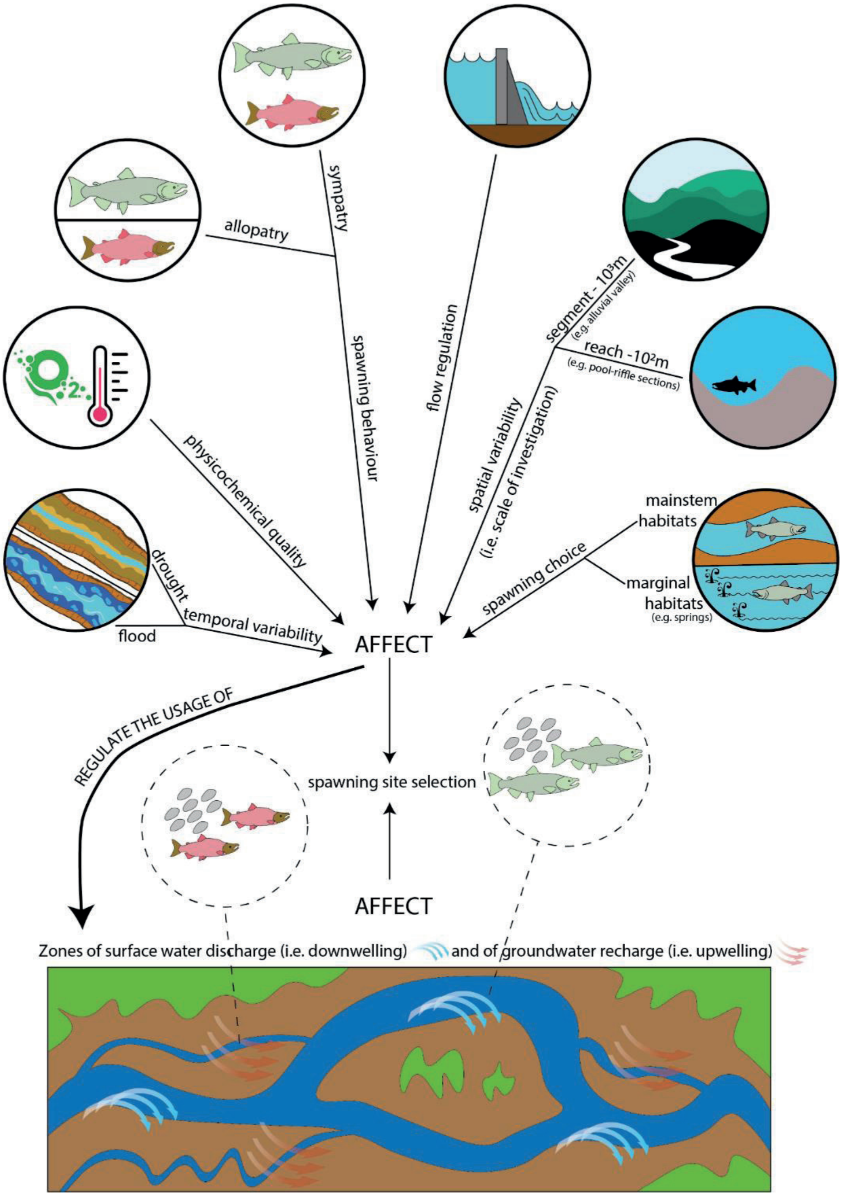

Figure 5. The role played by SW-GW interactions in spawning habitat selection and the set of factors (solid circles) leading to variations in the use of surface- or groundwater-dominated areas. From left to right: fluctuations in water discharge; DO concentration and temperature; spawning time and area shared by multiple species (sympatry) or spatiotemporal segregation (allopatry); flow regulation due to anthropogenic pressures, e.g., damming and water abstraction; scale of investigation from the different studies; and different life history traits for the same species regarding spawning site selection (Manuscript I, in preparation).

mechanism maximises egg-to-fry survival by avoiding the displacement of the eggs of early spawners (Hilborn et al., 2013). From this perspective, the presence of upwelling groundwater in marginal habitats has the potential to improve hyporheic circulation, creating new incubation niches. Thus, off-channels and side-channels partially connected to the mainstem add to the spawning environment, buffering the overexploitation of pool-riffle transitional zones and offering rearing habitats for juveniles.

In impounded watersheds, flow regulation for hydroelectric and agricultural purposes can severely affect spawning site selection and SW-GW interactions. Specifically, artificially induced high flows cool down the hyporheos, replacing the local phreatic water. Abrupt temperature drops lead to a delay in embryo development and fry emergence (Figure 5). Delay in fry swim-up may results in an overlap with the feeding windows of other juvenile species and decreased resistance to late spring floods (Fell et al., 2017). Furthermore, off-season water pulses are likely to increase scour events and consequently redd displacement while water abstraction can provoke nest dewatering (Casas-Mulet et al., 2015). Thus, hydropeaking phases undermine juvenile production and the fitness of the entire population (Geist et al., 2002; Malcolm et al., 2012; Young et al., 2011). In this scenario, given the more stable thermal regime, groundwater-fed zones can buffer against thermal fluctuations of artificially-triggered surface water pulses which can be a stressor for incubating eggs (Fell et al., 2017). Moreover, the presence of groundwater maintains the incubation environment of dewatered redds, allowing them to avoid desiccation and freezing. This provides an unquestionable advantage in those watersheds where spawning site selection is biased by water fluctuations caused by hydropeaking or hydrological intermittency (Casas-Mulet et al., 2015).

2.3. Lithuanian salmonid-spawning waters

(material in section 2.3 from Manuscript II)

Salmonidae are regarded as among the most commonly studied fish families in the world (Nika, 2011), as they hold significant economic and ecological importance (Bloomer et al., 2016; Everard, 2004). In Europe, anadromous species of this family (i.e., Salmo salar, Atlantic salmon, and Salmo trutta, sea trout) are facing a general decline (Bloomer et al., 2016; Malcolm et al., 2004) that is mainly related to their poor marine survival (Friedland et al., 2000; Ottersen et al., 2001). To counteract this marine decline, it is pivotal to maximise smolt production from freshwater ecosystems (Malcolm et al., 2003a). Thus, knowledge of the main bottlenecks related to their survival in the early freshwater stages is a prerequisite for their successful management (Malcolm et al., 2004).

Atlantic salmon (Salmo salar L.) and sea trout (Salmo trutta L.) are suffering from a general decline across Europe (Bloomer et al., 2016; Malcolm et al., 2004), with the eastern Baltic Sea region no exception. In the Baltic Sea region, Atlantic salmon and sea trout generate an important economic yield linked to the activities of both professional and recreational fisheries (Liu et al., 2019). Lithuania hosts many watercourses exploited by these two species for the completion of their life cycles (Kesminas, 2011), and the Atlantic salmon is included in the Lithuanian Red List of protected species (Witkowski et al., 2003). Baltic salmon populations mainly spawn in eastern Lithuanian rivers (e.g., the Nemunas basin), while sea trout populations occur more in the west (e.g., the Minija and Šventoji basins; (Nika, 2011). The spawning times of these two species in the study area ranges between December and January, while the intragravel phase can last until the end of April (Nika, 2011).

Lithuanian rivers fall under the Baltic Sea ecoregion and flow mainly in rather flat watersheds; indeed, 90% of Lithuanian territory lies at an altitude under 160 m. Lithuanian rivers are therefore considered lowland watercourses, with mild slopes of 0.1% on average (Jablonskis et al., 1962). Western Lithuanian rivers present a water supply mainly composed of rainwater (53%), followed by snowmelt water (29%) and groundwater (18%) (Jablonskis et al., 1962). Because of this partition, the watercourses in the study area are usually flooded in spring following the snowmelt, but are fed by groundwater during the summer and winter seasons (Jablonskis et al., 1978). Most of the watercourses in the study area rarely exceed the 20 °C year-round, making them a favourable habitat to host salmonid populations, which are regarded as stenotermich fishes (Isaak et al., 2023). The three systems investigated in this work host brown trout populations, including both residential and migratory individuals. The residential populations are supported by yearly runs of the marine-migrating ecotype (Kesminas, 2011; Nika, 2011).

Blendžiava is a third-order tributary of the River Minija, while the River Šventoji directly enters the Baltic Sea. Finally, Smiltaitė is a second-order tributary of the River Smeltalė (Fig. 1, section 3.1.1). The Blendžiava Stream falls under a protected area for salmonid spawning and juvenile rearing (Kontautas et al., 2010; Nika, 2011). The ecological status of this stream was classified as "good" by the Lithuanian Environmental Protection Agency according to the WFD classification system (Aplinkos Apsaugos Agentūra, 2024). According to salmonid monitoring data, the brown trout population density in Blendžiava is one of the highest among all Lithuanian rivers (Nika, 2011). In contrast, the Smiltaitė Stream, the ecological status of which is classified as "poor"(List of water bodies at risk (in Lithuanian), 2017; Nemunas river basin district, 2010) and displays a negative trend in its brown trout population (HELCOM, 2021).. The investigated stretch in the Šventoji River, where the majority of spawning grounds are located, has also been classified as holding "poor" ecological status (Venta river basin district, 2010). This area supports natural brown trout and Atlantic salmon populations that were recently stocked in the river, which produces self-sustaining runs (N. Nika – personal communication). Spawning time and incubation periods are thought to be one of the most critical moments during the life-cycles of salmonid (Burke, 2011; Chapman, 1988; Greig et al., 2007).

2.4. The embryonic stages of salmonids during the incubation period

(material in section 2.4 from Paper II)

Salmonids lay their eggs within the river bed, 10–30 centimetres below the interface between the water and the gravel substrate (Crisp, 2008). The nest, also called a redd, is formed by several egg pockets that represent metabolic hot-spots within an inorganic matrix with flowing hyporheic water. When preparing nests before egg deposition, salmonids displace gravel and finer grained sediments in order to remove fine material, which is then exported downstream by currents (Cope, 1996). This mechanical action serves multiple purposes: it improves water circulation within the nest while at the same time removing organic fractions that could potentially fuel microbial activity, thereby eliminating associated oxygen (O_2) consumption and the accumulation of metabolic end products.

In the context of hydrological extremes caused by climate change and to improve the efficacy of river restoration actions targeting the recovery of salmonid populations, it is important to focus research activities on the reproduction areas of salmonids, and specifically on the fine dynamics occurring in spawning sites during egg development (Greig et al., 2007; Malcolm et al., 2003b; Pulg et al., 2013). A large body of literature has thus explored these issues, seeking to understand the dynamics of egg development and the factors that regulate egg mortality (Cope, 1996; Ingendahl, 2001; Malcolm et al., 2010; Rubin et al., 1996). In this context, the role played by intragravel water circulation related to maintaining low temperatures and ensuring high O_2 solubility is pivotal (Casas-Mulet et al., 2015). These factors, in turn, are interconnected with the metabolic rates of salmonid eggs, which, like those of other organisms (Shokri et al., 2022), are expected to increase with rising temperatures as a result of climate change (Brown et al., 2004). Because the O_2 supply to these eggs is dictated by a diffusion mechanism, salmonid species that produce smaller eggs with a larger surface-to-volume ratio will be at an advantage, since their lower metabolic rates will translate into a higher thermal tolerance (Martin et al., 2020).

2.5. The biogeochemical processes occurring within the salmonid nest: the development of a mesocosm-based approach to their exploration

(material in section 2.5 from Paper II)

Within egg pockets, the respiration and excretion rates and mortality of eggs can locally modify water chemistry (Malcolm et al., 2003b). The extent of such changes depends on water renewal time and may result in positive or negative feedback for mortality (Greig et al., 2007).

The most recent studies assessing the intragravel conditions inside salmonid nests have generally been carried out in situ by means of a wide range of pore water samplers, egg incubation boxes, and substrate collectors (Greig et al., 2005; Ingendahl, 2001; Malcolm et al., 2003a). Overall, such studies try to evaluate changes in water chemistry and infer underlying processes or water circulation-related issues, such as the dominance of upwelling or downwelling conditions. Nonetheless, in situ assessments often lack spatiotemporal precision when they evaluate DO content and egg survival rates (Chapman, 1986). This is mainly because of the steep variations in DO concentrations due to variable flow regimes, uneven gravel composition in the redd, and difficulties in locating the egg pocket (Bjornn et al., 1991; Greig et al., 2005). Additionally, field studies focusing on sedimentary structure as a metric to define survival rates can overlook the influence of biochemical oxygen demand (BOD) and SW-GW interactions as additional drivers of DO availability (Greig et al., 2005; Greig et al., 2007). Thus, appraisals accounting solely for DO concentration can fail to detect DO sag zones due to stagnation linked to low intragravel flow velocities (Ingendahl, 2001; Malcolm et al., 2003b; Rubin et al., 1996).

In recent years, efforts have been made to disentangle the complex redd structure in order to assess related flowpaths and DO distribution. Tonina and Buffington (2009), for example, developed a three-dimensional hydraulic model to simulate influences on hyporheic circulation in a pool-riffle sequence after redd construction. The authors found that the fine fraction is winnowed out of the nest by the female, remarkably altering hydraulic conductivity and leading to a cascade effect on the distribution of hyporheic flowpaths and O_2 circulation. High-resolution imaging of intragravel patterns at different flow velocities and detailed observations of DO transport were conducted by Cardenas (2016), who employed a recirculating flume where an artificial salmon redd was built.

Despite the high resolution conferred by computational models (Tonina et al., 2009) and DO mapping (Cardenas et al., 2016) inside reconstructed nests, to the author's knowledge there are no studies evaluating the respiratory and excretion rates of a typical salmonid egg pocket within a gravel matrix. Therefore, a novel methodological approach is developed in this thesis in order to analyse the whole picture of the metabolic rates of egg pockets under ideal or pristine flow and water chemistry conditions. This approach then considers a wide range of simulated conditions that mimic ongoing, climate-related changes in water flow, direction, temperature, and chemistry.

3

Materials and methods

3.1. Surface water quality assessment

(material in section 3.1 from Paper I)

The three sampled watercourses are all located in western Lithuania, and are considered lowland streams. They all possess slopes with a gradient between 0.1% to 0.3% and a pool-riffle structure (Jablonskis et al., 1962) (Figure. 6).

The Blendžiava Stream is in the upper part of the Minija River catchment, between the Samogitian Highland and the Western Samogitian Plateau, in the Kretinga district. It is a third-order stream with a catchment area of 86 km^2 and an average annual discharge of 1.06 m^3 s^{-1} (Jablonskis et al., 1962). The investigated stretch falls under a protected area for salmonid spawning and juvenile rearing (Kesminas, 2011; Kontautas et al., 1994). Its ecological status was classified as “good” by the Lithuanian Environmental Protection Agency following the WFD classification system (Aplinkos Apsaugos Agentūra, 2024).

The Smiltaitė Stream has a catchment area of 32 km^2 and an average annual discharge of 0.14 m^3 s^{-1}. It flows in the Klaipėda district and is the main tributary of the Smeltalė River, which discharges its water into the Curonian Lagoon (Gailiušis et al., 2001). Smiltaitė has a self-sustaining sea trout population with a negative trend (HELCOM, 2021), perhaps due to the “poor” ecological status of this watershed according to the WFD classification (Nemunas river basin district, 2010). The river’s “poor” status is mainly caused by point source pollution from stormwater runoff discharge and household pollution (List of water bodies at risk (in Lithuanian), 2017).

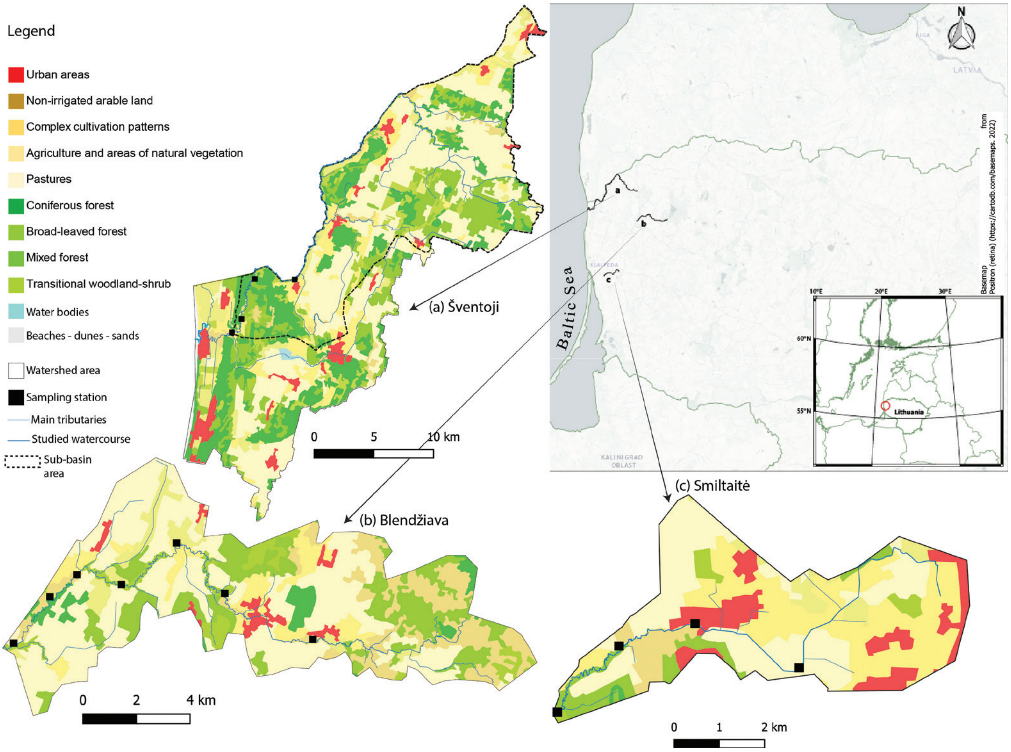

Figure 6. Map of the study area involving the three investigated watercourses with their respective watersheds, tributaries, sampling stations, and land use, with respect to the 3rd level of classification from the CORINE Land Cover project (Copernicus Land Monitoring Service). The dotted line delimits the Šventoji sub-basin area (reprinted from Paper I).

The Šventoji River is a transboundary river marking the Latvian border in the Skuodas district that flows directly into the Baltic Sea near the town of Šventoji. The Šventoji has an overall catchment area of 472 km^2 (Gailiušis et al., 2001) and an average annual discharge of 5.3 m^3 s^{-1} (Venta river basin district, 2010), and supports self-sustaining sea trout and Atlantic salmon populations (Kesminas, 2011), with the latter having been initially restocked (N. Nika – personal communication). However, only the drainage basin upstream of the sampling stations (Figure 6) was considered when calculating land use percentages in this study, which therefore refers to an area of 268 km^2. The section furthest downstream, near to the confluence with the Baltic Sea, is not suited for spawning and juvenile rearing, and was therefore not considered in this study. Although Šventoji holds a “good” ecological status, the spawning areas fall inside a section of the watercourse that flows through an area of Lithuanian territory which failed to achieve “good” ecological status, with ammonium and nitrate values falling within the “moderate” class (Venta river basin district, 2010). The area is characterized by diffuse pollution inputs from agricultural runoff, delivering inorganic nutrients and organic matter to the river (Kesminas, 2011; Venta river basin district, 2010).

The water supply of the Blendžiava is of the R-us type: 53% of the supply is from precipitation (R), 25% is from groundwater (u), and 22% is from snowmelt (s). On the contrary, the water supply for the Smiltaitė and Šventoji rivers is classified as R-s, with 67% of water input from precipitation, 26% from snowmelt, and 7% from groundwater (Jablonskis et al., 1978). Sampling stations in these three water courses are located within elective stretches for sea trout (Blendžiava and Smiltaitė) and salmon spawning (Kesminas, 2011; Kontautas et al., 1994).

Differences in land cover among the three basins were evaluated using the CORINE Land Cover (CLC) European project from 2018, at the 3rd level of accuracy (Copernicus Land Monitoring Service - https://land.copernicus.eu, accessed on 10 November 2023). The decision to attain a high resolution for land use was driven by the fact that the same land use category can have significant differences depending on the specific management typology. For example, under the same heading of “agricultural areas”, different land layouts including “pastures”, “non-irrigated arable land”, and “complex cultivation patterns” can be grouped, although their surface management will be different (Ding et al., 2016; Shi et al., 2017). Land use coverage was normalized for the respective basin areas and expressed as percentages in the contingency table.

Sampling was performed between October 2021 and August 2022 to capture physicochemical variations on a seasonal basis, following similar surveys that aimed to understand water quality in relation to watershed land usage (Gorgoglione et al., 2020; Pinardi et al., 2022). Within each season, periods of hydrological extremes such as high flow (flood) and low flow (drought) were prioritized (Figure 7). This aligns with previous works that aimed to understand changes in nutrient concentrations in relation to different hydrological conditions (Racchetti et al., 2019; Severini et al., 2023). Along with temporal variation, spatial variation was accounted for by collecting water samples throughout the entire length of the stretches investigated (Figure 7). This allowed for any upstream-downstream variations to be accounted for, as has already been proved elsewhere (Pinardi et al., 2022; Racchetti et al., 2019; Severini et al., 2024; Severini et al., 2023).

Figure 7. Water levels in the three systems investigated during drought (left) and flood (right) periods. Blendžiava (a,b), Šventoji (c,d), and Smiltaitė (e,f) (reprinted from Paper I).

In boreal ecosystems, ice cover and light limitations during the winter months have the potential to decrease primary production (Jankowski et al., 2021). This may translate into higher export rates of unprocessed nutrients through the watercourse (Thellman et al., 2021). Nevertheless, in the three investigated systems, ice cover during the sampled period was moderate, and the sampling stations were always ice-free. This reflects a global trend in which ice cover is rapidly being lost in running-water systems

(Thellman et al., 2021). Additionally, the three systems lie close to the Baltic Sea, and are therefore influenced by its climate (Čerkasova et al., 2024). Winter-thaws events systematically remove the forming ice from the watercourse surfaces – a process which left them free for the entire sampling period. Because of this, the effect of ice cover on physicochemical water quality was regarded as a minor variable in this study.

Periods of flood and drought were detected with respect to changes in the water level using real-time data from the Lithuanian Hydrometeorological Service (https://www.meteo.lt/) and validated with on-site surveys. For flood periods, an overflow of water into the dry land was observed. Drought moments were assumed when macrophytes and reference rocks, such as gravel areas that were submerged under average water levels, appeared dry and exposed to the air. Such empirical observations were validated by exceedance probability curves (Figure. 8), where flood and drought events were connected to the probability of falling in the first (Q1) and fourth (Q4) quartile, respectively.

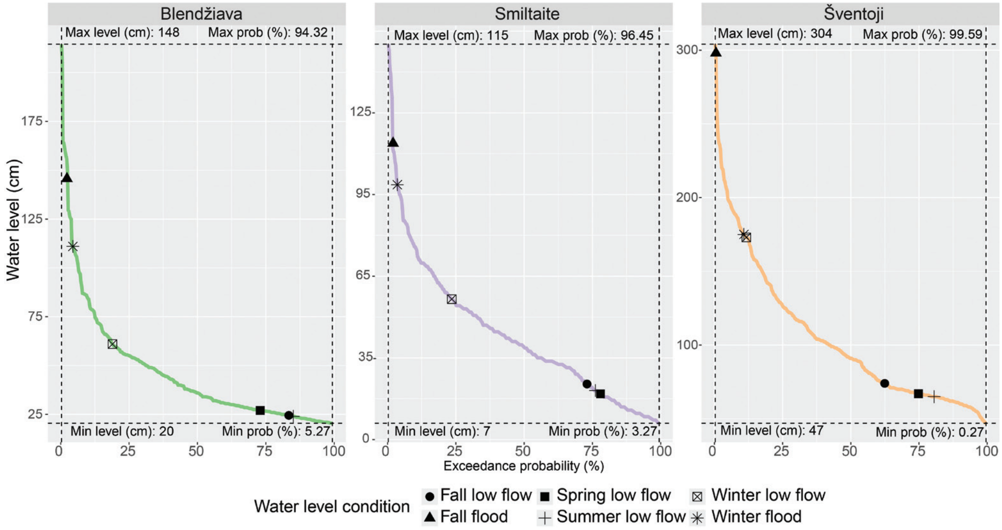

Figure 8. Exceedance probability curves for water levels in the sampled period. The different geometries in each curve indicate sampling events through all seasons, which were performed during possible periods of high or low water levels (see text for more explanation regarding quartiles) (reprinted from Paper I)

During the wettest conditions, all rivers were sampled at the Q1 point, and the fall flood sampling occurred during periods with a less-than-5% probability of occurrence, capturing peak discharge events. The driest periods during the fall, spring, and summer appeared to be positioned at the Q4 point of water levels. This was true except for the fall low flow sample from Šventoji, which was taken in conditions below Q3. These driest conditions were characterized by a probability of over 80%, representing typical low flow conditions. However, in winter, due to recurrent snowmelt episodes, it was not possible to catch a proper period of low flow. This is why, in all three systems, the winter flood and low flow events fall in the same first quartile.

To describe the physicochemical status of the water, eleven environmental variables were chosen. A YSI multiparameter probe (Pro 1030, YSI, Yellow Springs, OH, USA) was employed to measure in situ dissolved oxygen (DO, both as mg L^{-1} and saturation %), pH, electrical conductivity (EC, µS cm^{-1}), and temperature (°C, T in charts and tables). Filtered water aliquots (GF/F filters, Frisenette, 0.7 µm mesh size) were collected to detect concentrations (mg L^{-1}) of ammonium ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), and soluble reactive phosphorous ($P\text{-}PO_4^{3-}$). Nutrient concentrations were analyzed via spectrophotometric methods (A.P.H.A., 1981). Alkalinity (mmol L^{-1}) was calculated using the Gran titration method (Gran, 1952), while total suspended solids (TSS, mg L^{-1}) was observed using the gravimetric method with GF/F filters (Swietlik et al., 2003).

Correspondence analysis (CA) was used to infer the degree of similarity and difference between the three watersheds concerning the different land use configurations. The results are presented in the form of an asymmetric biplot (Bendixen, 1996), where rows indicating the different land use features are depicted on the principal coordinates (i.e. “rowprincipal”, Nenadic et al., 2007) while the watershed contribution is expressed in terms of columns (Nenadic et al., 2007).

The 11 environmental variables were summarized and visualized via Principal Component Analysis (PCA). PCAs were performed for each season and event, and although all 11 environmental variables were graphically displayed, in the description of the results only those variables that contributed most to the definition of the first two principal components (PCs) are reported. The expected average contribution was used as a cut-off point to retain statistically significant variables, represents the value if the contribution of the variables were uniform, and was calculated as follows: 1/ number of variables *100 (Kassambara, 2017).

For each watershed, variations in water chemistry between flood and drought periods were evaluated during the fall and winter seasons, when hydrological extremes occurred. A paired samples t-test was employed after checking normality and homoscedasticity assumptions. For data with unequal variances, the paired Welch t-test was employed. If data did not comply with the normality assumption, the non-parametric Wilcoxon signed-rank test for paired data was used. Among the three watersheds, variations among seasons and events were evaluated. One-way ANOVA was employed, followed by the Tukey HSD test for multiple pairwise comparisons. Prior to ANOVAs, the normality and homoscedasticity assumptions were checked. If data did not meet the assumptions, comparisons were made using the Kruskall-Wallis test, followed by Dunn’s Test for multiple comparisons. All statistical analyses were performed in the R language (R Core Team, 2023) using the rstatix and FactoMineR packages (Kassambara, 2021). Charts were built using the factoextra (Kassambara, 2016) and ggplot2 (Wickham et al., 2016) packages. All statistical tests were performed at an alpha level of 0.05, and p-values from the pairwise tests were corrected using the Bonferroni–Holm method to account for the family-wise error.

3.2. The salmonid incubation phase: hyporheic water assessment

(material in section 3.2 from Manuscript II)

The field campaign was performed during the 2021–2022 incubation season from mid-December to the beginning of May, a period defined according to previous studies (Nika, 2011). Investigations took place in the Šventoji River and the Blendžiava and Smiltaitė Stream sections, all in stretches showing an abundance of sea trout (Salmo trutta) or, in the case of Šventoji, Atlantic salmon (Salmo salar) (Figure 9; Kesminas, 2011; Kontautas et al., 1994). The Šventoji watershed holds the highest share of forests, Blendžiava holds a highly differentiated agricultural area network, while the Smiltaitė watershed presents the highest proportion of urban areas and uncovered agricultural areas. For a detailed description of these watersheds, refer to Chapter 3.1.

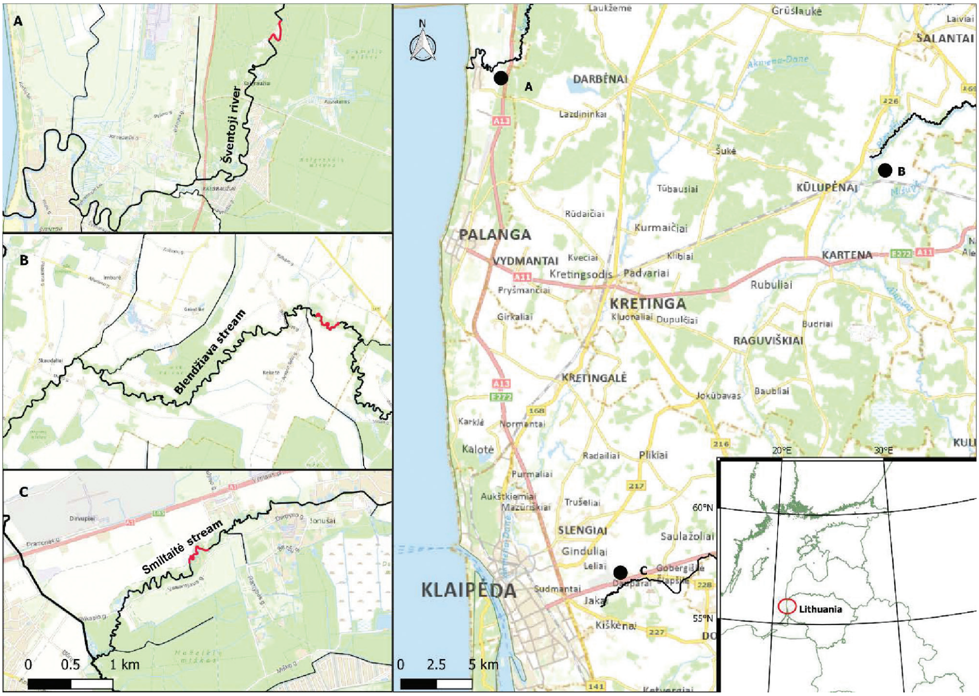

Figure 9. The study area of the three water courses. Stretches highlighted in red indicate the spawning grounds where redds were selected (Manuscript II, in preparation).

In each water system, three nests (i.e., redds) per stretch were selected to install mini-piezometers for intragravel water sampling over the incubation period. Redds were spotted in depressions (pots) in the substratum with a related downstream pile of freshly moved gravel (tailspill) in contrast to the compacted and algal-colonized streambed. To track potential differences between the nest environment and the surrounding area, the first sampling positions were located at the downstream end of the tailspill slopes (nest centrum), and the second were parallel to the tailspill but at the edge of the moved gravel area (nest periphery). The first site attempted to target the egg pockets – patches constituted by coarser gravel in which the fine fraction had been winnowed out by the female during the construction phase. These conditions are representative of those encountered by eggs, while the second sampling site represented the periphery of the redd (Figure 10 a). Sampling was performed monthly, but, due to the persistently high flows and low water visibility from December to the beginning of March, it was possible to track only the final part of the incubation season (i.e., March to the beginning of May). The final assessment was performed at the beginning of May, coinciding with peak fry emergence in these systems (Nika, 2011).

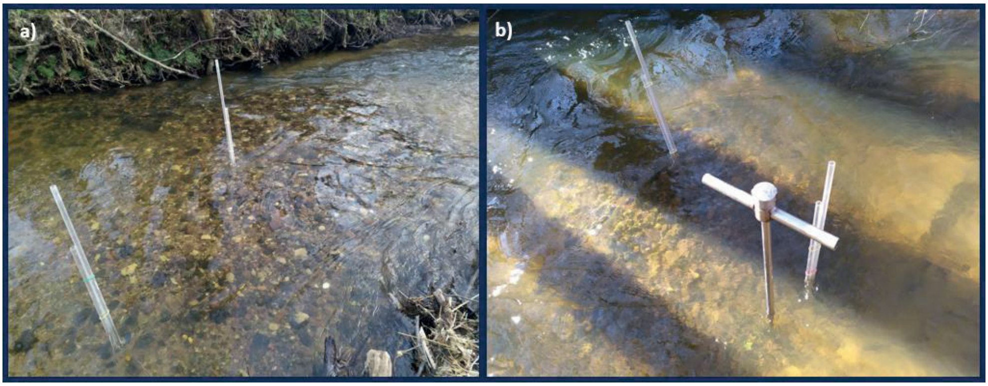

Figure 10. A typical spawning sampling site where the water from the redd was directly withdrawn. Mini-piezometer positions: (a) nest centrum and periphery; (b) with the installation apparatus (Manuscript II, in preparation).

To track hyporheic water patterns, each redd was equipped with two mini-piezometers (Figure 10 b), for a total of 18 in all systems. Each device was built according to Baxter et al. (2003), and consisted of a clear and sturdy polyvinyl chloride pipe 1.6 cm in diameter and 1.5 m in length. The bottom part was fitted with a cork and perforated in the lower 10 cm of its length, with roughly 25 holes each 0.24 cm in diameter. Mini-piezometers were embedded into the substratum with a driver apparatus (Baxter et al., 2003) at an installation depth of around 20 cm – the average depth of deposition for brown trout eggs (DeVries, 2000). Finally, the surrounding gravel was gently tamped to adhere to the sidewalls of the mini-piezometer, and thus to avoid the phenomenon of surface water leakage, otherwise known as “piping” (Kondolf et al., 2008). Mini-piezometers were inserted in the nest and left there across the entire incubation period starting from January to April. The interstitial water was sampled monthly. During each sampling operation, water was gently withdrawn to avoid air bubbling and surface water piping inside the mini-piezometer (Kondolf et al., 2008). A 150 ml syringe with a Tygon tube was employed to first purge the mini-piezometer and then to collect the sample. Beside the hyporheic water, surface water samples were also withdrawn near the mini-piezometers during sampling. Both surficial and hyporheic samples were analysed with a YSI multiprobe (Pro 1030, YSI, Yellow Springs, OH, USA) to measure intragravel and surficial DO (mg L^{-1}), EC (µS cm^{-1} at 25 °C), T (°C, in charts and tables referred as T), and pH. Additionally, the hyporheic and surficial samples were filtered (Frisenette GF/F filters) and dispensed in three test tubes to detect ammonium ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), and nitrite ($N\text{-}NO_2^-$) nitrogen via spectrophotometric methods (A.P.H.A, 2005; Rice et al., 2012; Rodier et al., 1996).

Differences in the intragravel water parameters were visualized by means of non-metric multidimensional scaling (NMDS) analyses, after a standardization process, using the vegan R-package (Oksanen et al., 2007). NMDS plots showed sampling points clustered according to the following factors: 1) stream, 2) month, 3) sampling location in the nest, and 4) difference between intragravel and surface water types. For the scope of this multivariate representation, two resemblance matrices were built using Euclidean distances (Clarke et al., 2001). The first matrix accounted only for the intragravel water records and was used to depict the clusters from point 1) to 3), while the second matrix also embedded the records from the surficial water characterization and was used for point 4). Any significant difference between the levels in each of the four factors was assessed using the one-way analysis of similarities (ANOSIM, Clarke et al., 2001) from the vegan R-package. ANOSIM relied on the same two resemblance matrices used for NMDS ordination. Indicator species analysis (ISA) was used to identify the environmental variables that best fitted ($p < 0.05$) the clusters in the NMDS plots using the indicspecies R-package (De Cáceres, 2013). The ISA results were overlaid on NMDS representations as vectors using the envift function of the vegan R-package.

The multivariate results were supported by univariate tests to highlight any significant differences in each of the 7 environmental variables (DO, T, pH, EC, $N\text{-}NO_3^-$, and $N\text{-}NO_2^-$) according to the four factors (stream, month, location in the nest, and difference between surficial and hyporheic water) considered for the NMDS plots. Differences in intragravel water parameters among the streams were evaluated for the entire incubation period using the one-way ANOVA test followed by post-hoc comparisons using the Tukey Honest test. For non-normally distributed data, the Kruskal–Wallis test was followed by Dunn’s test, as pairwise comparison was used instead. The same tests were used to detect differences in parameters between the sampling months in each stream. Differences in the physicochemical parameters of the intragravel water collected in the nest centrum and the periphery were detected with the paired sample t-test for each stream system, pooling data from the entire incubation period. The paired Wilcoxon test was used for non-normally distributed data, while the Welch t-test was used for data without equal variance. Finally, the unpaired versions of the abovementioned tests were used to check for any differences between the intragravel (coupled nest centrum and periphery) and surficial water samples.

Finally, nest centrum vs. periphery locations were contrasted to investigate the variation of intragravel DO patterns through the three different sampling occasions, combined for the three sampled basins. Since the data presented a normal distribution, the paired t-test was employed for each period. For data violating the homoschedasticity assumption, the Welch t-test was used instead. Comparatively, patterns of surficial DO variation among the three periods were assessed using the Kruskall Wallis test followed by the post hoc Dunn's test, in order to cope with the non-normality and heteroschedasticity of the data.

Univariate tests were computed with the rstatix R-package (Kassambara, 2021), plots were built with the ggplot2 R-package (Wickham et al., 2016), and both multi- and univariate tests were performed at an alpha level of $p < 0.05$.

3.3. SW-GW interactions during the spawning and emergence of salmonids in the three boreal ecosystems

(material in the section 3.3 from Manuscript III)

In situ measurements were carried out to investigate the potential contribution of groundwater in the spawning grounds of brown trout (S. trutta) and Atlantic salmon (S. salar) in the Smeltalė, Blendžiava, and Šventoji boreal watercourses. A set of physicochemical parameters was employed to uncover the presence of upwelling groundwater seeps into nests. Two critical periods were chosen for the investigation: the spawning site selection moment, in winter; and emergence time, in spring. This multidisciplinary approach was used to verify: i) if the nests are actually influenced by groundwater inputs; ii) if this input is constant from spawning to emergence; and iii) which aspects of the groundwater inputs have positive effects on the survival and metabolic performance of nests. It has previously been acknowledged that groundwater-fed spawning areas can elicit positive effects on the incubation stages of salmonids at boreal latitudes (Casas-Mulet et al., 2015; Kuzishchin et al., 2010). Moreover, a previous investigation already speculated on the presence of groundwater fed-patches overlaying a recurrent spawning area in such systems (Nika, 2011). In this study area, groundwater inputs were hypothesised to have several positive effects on eggs, including physical (i.e., temperature), chemical (i.e., oxygen supply), and hydrodynamic (i.e., intragravel water flow) benefits. The presence of groundwater is discussed in respect to similarities in ions and gases between the nest waters and proper groundwater sampled from the wells. The importance of groundwater for salmonid incubation stages in these systems is therefore discussed.

The potential influence of upwelling groundwater was investigated at the beginning and end of the incubation season in Blendžiava, Smeltalė, and Šventoji in the 2022–2023 incubation season (Figure 11). In the first field campaign in December 2022, hyporheic water samples were withdrawn from freshly built nests, in a manner representative of the conditions during spawning site selection. The second sampling campaign was performed at the end of the incubation period, in May 2023, which coincides with peak fry emergence in these systems (Nika, 2011). During both sampling events, hyporheic water samples were compared with samples from the upstream sections of the rivers and adjacent wells (representing groundwater conditions). Investigations took place in stretches located in the Šventoji River and sections of the Blendžiava and Smeltalė streams. Such sections were nested in, for Blendžiava and Šventoji, or closely downstream from, for Smeltalė, areas in which previous surficial river water monitoring which displayed an abundance of spawning grounds had been carried out (Figure 11) (Kesminas, 2011; Kontautas et al., 1994).

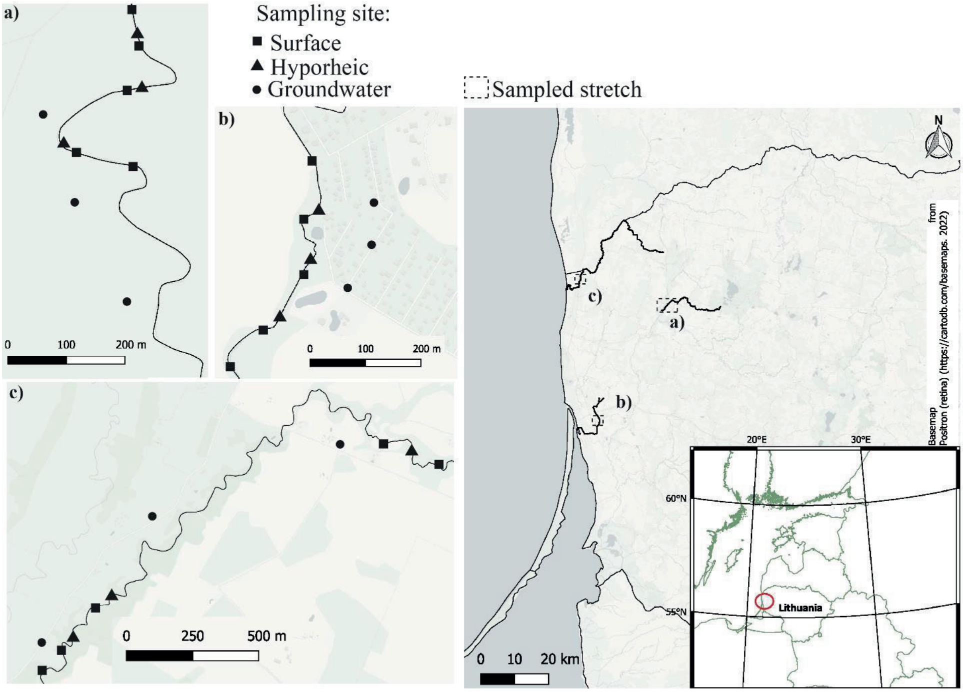

Figure 11. Study area: a) Šventoji, b) Smeltalė, and c) Blendžiava (Manuscript III, in preparation).

Hyporheic water in the nests (hereinafter called “nests”) was withdrawn from each redd by means of a hyporheic water sampler, as earlier developed by Nika (2011; Figure 12 a, c). During sampling, water was gently withdrawn to avoid air bubbling and surface water piping inside the hyporheic water sampler (Kondolf et al., 2008). A 150 ml syringe provided with a tygon tube was employed to first purge the hyporheic water sampler and then to collect the sample. At the same time, three samples from surface water (hereinafter called “river”) in the proximity of the redds were withdrawn. Finally, water from three groundwater domestic wells (hereinafter called “groundwater”, Figure 12 b) adjacent (100–300 m distance, 3–8 m depth) to each stretch was sampled. Well water should be representative of the properties of regional groundwater (Neumann et al., 2016; Severini et al., 2022; Severini et al., 2023). The groundwater heads in the wells were always higher than the rivers and as such, it was assumed that groundwater consistently feeds the investigated rivers. The general trends of groundwater level in the area, i.e., recharge and depletion periods, were derived from a nearby well located in the city of Vėžaičiai and monitored by the Lithuanian Geological Survey (LGT), with freely available data (https://www.lgt.lt/epaslaugos/elpaslauga.xhtml, accessed on 6 November 2023).

Figure 12. a) Sampling procedure for extracting hyporheic water from nests, b) typical domestic well sampled during this study, and c) hyporheic water sampler installed in the nests to withdraw the water (Manuscript III, in preparation).

Hyporheic, surficial, and groundwater aliquots were characterized in situ with a YSI multiprobe (Pro 1030, YSI, Yellow Springs, OH, USA) with regards to DO (% saturation), EC (µS cm^{-1} at 25 °C), T (°C, in charts and tables referred as T), and pH, as already used in similar studies (Fell et al., 2017; Geist et al., 2002; Malcolm et al., 2006; Malcolm et al., 2004). Water samples were filtered with a 0.2 µm syringe filter and dispensed into test tubes for the detection of major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, all in mg L^{-1}) and anions (F^-, Cl^-, N-NO_3^-, HCO_3^-, SO_4^{2-}, PO_4^{3-}, all in mg L^{-1}) by means of ion chromatography. In parallel, water aliquots were poisoned with 100 µl of 7M ZnCl to eliminate any bacterial activity and stored in glass vials for the analysis of dissolved gases such as N_2 (µM), Ar (µM), O_2 (µM), and CH_4 (nM) through the membrane introduction mass spectrometry technique (MIMS, Bay Instruments, USA). The saturation of N_2, Ar, and O_2 in water samples was calculated by contrasting the measured and theoretical concentrations derived from gas solubility at in situ conditions (Sander, 1999). Statistical analyses were performed using the R software (R Core Team, 2023). The vegan package (Oksanen et al., 2007) was used to investigate spatial relationships among the three water groups by means of nMDS plots, which were built starting from two resemblance matrices based on Euclidean distances. These two matrices were related to the two sampling events and accounted for all of the physicochemical variables and watersheds. The Ime4 package (Bates et al., 2007) was used to build linear mixed effect models for each physicochemical variable during each sampling event, gathering the data from all of the watersheds. To deal with the intrinsic variability of the watersheds derived by different land uses (Benetti et al., 2024), the term "watershed" was used as a random factor to model physicochemical variability among watersheds. The emmeans package (Lenth, 2022) was employed to perform pairwise Tukey tests on the linear mixed effect models, which reported a significant difference overall. Statistical analyses were performed at an alpha level of 0.05.

3.4. Fine dynamics in salmonid nest coupling: the mesocosm and microcosm approaches

(material in section 3.3 from Paper II)

Fluxes of dissolved O_2 and inorganic nitrogen (ammonium – N-NH_4^+ – and nitrate – N-NO_3^-) were studied in two sets of reconstructed egg pockets under controlled laboratory conditions (i.e., mesocosms). One set, in triplicate, included 100% of live "eyed stage" eggs, whereas another set, in triplicate, contained 50% live and 50% dead eggs. The egg pockets were reconstructed in transparent plexiglass cylinders packed with a mixed inorganic substrate made with sand, gravel, and eggs. Cylinders were provided with a water circulation system, ensuring the constant flow of water with a stable quality, temperature, and O_2-saturation and allowing the measurement of inflowing and outflowing water chemistry and flux calculations. In order to quantify the contribution of eggs to the respiration of the whole system, eggs were also incubated in water alone (i.e., microcosms).

For the microcosm, a batch of eggs was kept in the trays of the vertical incubator and used to run closed respirometry incubations in order to quantify oxygen demand and N-NH_4^+ excretion from single individuals at the three main developmental stages (eyed egg, nearly hatched, and yolk sack absorbed). Respiration and excretion rates were measured by means of 22 mL glass chambers equipped with optode sensor spots (Politi et al., 2023; Figure 13 d) for continuous DO measurement (FireSting O_2, PyroScience GmbH) using the following formula:

$$Respiration\ or\ Excretion\ (mg\ or\ \mu g\ egg^{-1}h^{-1}) = \frac{\Delta C \times V}{n \times \Delta t}$$

where $\Delta C/\Delta t$ (mg or µg $L^{-1}h^{-1}$) is the change of DO or N-NH_4^+ concentration during the incubation time interval; V (L) is the volume of the incubation chamber; and n is the number of incubated eggs.

Chambers were equipped with a stirring device in order to keep water moving to avoid O_2 sags and stratification. Data was pooled from glass chambers in which each stage was incubated at four different densities (n = 5, 10, 15, and 20 per chamber) to account for any density-dependent mechanism changing DO consumption and N-NH_4^+ excretion. Final stage alevins were fitted in the chambers only at densities of 1, 3, and 5 individuals because of their larger size. Water at the same temperature and O_2 saturation as that supplying the cylinders was initially provided, then chambers were closed and fluxes measured in dark conditions. Each batch also had one control chamber with only water, to correct for egg respiration and excretion rates and to account for any change in the target parameters. In order to achieve results describing metabolic rates under optimal conditions, the experiments ended when the DO concentration reached 8–9 mg L^{-1}, from an initial DO concentration of 11.760 ± 0.003 mg L^{-1}. Incubation time was set to avoid the critical level of 7.2 mg L^{-1} defined by Alderdice et al. (1958) for O. keta that would affect the respiration rate. Above this threshold, respiratory rate is independent from the external DO concentration, thus keeping growth and metabolism rates unmodified (Alderdice et al., 1958). At the end of the experiment, a water sample was collected from each microcosm and filtered (Frisenette GF/F filters) into plastic test tubes to account for the N-NH_4^+ concentration using standard colorimetric methods (Ehrhardt, 1999), thus providing individual excretion rates.

For the mesocosm setup, the experiment was carried out in the recirculating aquaculture system (RAS) at the Fisheries and Aquaculture Laboratory of the Marine Research Institute, Klaipėda University (Lithuania). Rainbow trout (O. mykiss) eyed stage eggs at 270-degree days (dd) were supplied by the Dabie hatchery in Poland on 19 April 2023. Subsamples of the eggs were distributed in three control trays with 100 eggs each to assess the natural mortality of the strain, and to track the developmental stage. Meanwhile, 6 reconstructed trout egg pockets were built by packing spawning ground (pooled gravel and sand and silt) into 6 transparent plexiglass cylinders (cores with a height of 40 cm and an inner diameter of 8 cm; Figure 13, a). To comply with the security standard in the RAS plant, the substrate was initially sterilised with oxygen peroxide (concentration 30%). Once filled with water, the substrate and eggs were gently transferred into the cylinders and egg pockets were reconstructed by pooling larger stones together and then laying them on top the eggs by means of a plastic spoon in order to ensure an even distribution as much as possible (Figure. 13, b). Three cylinders were packed with 100 live eggs each, hereinafter called "L" (live), and three cylinders were packed with 50 live and 50 dead eggs (previously scarified), adequately mixed, hereinafter labelled as "L+D" (live and dead). This was done in order to simulate the processes occurring in a partly compromised environment with high rates of mortality.

In each cylinder, eggs were distributed in a ~25 cm column. The bottom lids of each cylinder, consisting of rubber bungs, were drilled and connected to 5 silicone tubes, ensuring constant and similar one-way water flow (from the bottom to the top) inside the reconstructed egg pockets. The upper end of each cylinder was sealed with another rubber bung that held a single hole and a pipe collecting the overflow. The water circulating in the six packed mesocosms was ensured by peristaltic pumps. All cylinders were submersed into a large aquarium, ensuring a constant temperature (Figure 14). The outflow pipe of each cylinder was discharging and overflowing water into a 50 mL falcon tube fixed on the outer cylinder wall, ensuring the possibility of subsampling the water.

The velocity of the peristaltic pumps was adjusted in order to provide a constant outflow of 187 ml min^{-1}. Pilot tests were previously carried out using dye as a tracer flowing into the same mixed substrate in order to reproduce a water velocity in the porous medium of 1440 ± 60 cm h^{-1} (average ± standard deviation). This velocity was selected in order to guarantee intragravel survival at the ranges of DO concentration supplied during the experiment (11.760 ± 0.003 mg L^{-1}). Such a velocity is far above the optimal range of 15–500 cm h^{-1} defined by Greig et al. (2007) in UK rivers to guarantee the optimal egg survival of Atlantic salmon (S. salar) nests. Such a range is, however, supported by Nika (2011), who found high rates of survival with a velocity range of 1000–10000 cm h^{-1} for local sea trout populations in nests with a substrate similar to the one used here.

After two days of acclimatization in the cylinders (eggs at 286 dd), periodical DO measurements were conducted by means of an optode sensor needle (FireSting O_2, PyroScience GmbH) inserted into the falcon receiving the outflowing water (Figure 13 c). Besides O_2, N-NH_4^+ and nitrate N-NO_3^- were also measured in the water samples via falcon spectrophotometry. DO demand and inorganic nitrogen fluxes were expressed on a per core basis (i.e., single core) and related to the main stage of development (i.e., "eyed egg", "alevin"), as recorded in the nearby trays, with the following formula:

$$\text{Fluxes (mg or µg } L^{-1} core^{-1}) = (C_{out} - C_{in}) \times Q$$

where C_{out} and C_{in} (mg or µg L^{-1}) are the concentrations of DO or dissolved inorganic N at the core outlet and inlet, respectively, and Q (L h^{-1}) is the water flow.

Following a previous definition (Alderdice et al., 1958), the term "embryo" is used to point out unhatched fish that remain inside the egg, while the term "larva" refers to hatched fish, namely "alevin", with yolk sacks. During the entire duration of the experiment, the gravel cores remained in the dark to prevent algal growth, and the RAS system supplied water at a constant temperature of 8 °C and 100% DO saturation. The experiment ended on May 7th, when the vitelline sack was completely adsorbed into the control trays for reference. Then, cylinders were opened and unpacked, and juveniles were counted to assess the mortality rate. The substrate was analysed to detect the percentage of fines (<2 mm and <0.063 mm) by means of different sieve diameters following the methodology of Nika (2011). Once cores were opened to remove and count the alevins, three cores (control) were prepared with a similar approach and re-incubated for one additional day to account for only the flux rates of the bare intragravel environment.

Respirometric measurements in closed chambers were tested for differences in DO demand (mg O_2 egg h^{-1}) and ammonium excretion (µg N-NH_4^+ egg^{-1} h^{-1}) among the three stages using the non-parametric Kruskal-Wallis test due to the absence of homoscedasticity and normality in the data. Being significant, pairwise comparisons using the post hoc Dunn's test were performed, and p values were corrected using the Bonferroni–Holm method. Gravel cores were checked for differences in fines (<2mm), silt (<0.063 mm), and survival percentages between the L and L+D setups using the independent sample t-test. Differences between the L and L+D setups with respect to the two stages, "eyed egg" and "alevin", were checked for DO demand, N-NH_4^+, and N-NO_3^- fluxes using the Wilcoxon Signed-Rank test as a non-parametric alternative to cope with the heteroscedasticity and non-normality of the data. For the same reasons, the "eyed egg" and "alevin" stages were tested for any difference in DO

Figure 13. The sequence of actions for the mesocosm apparatus and measurements: a) Preparation of cores with spawning gravel, b) Egg pocket construction and laying eggs, c) Falcon tube sampled with the needle oxygen logger, and d) Chamber used to incubate eggs for the microcosm assay (reprinted from Paper II).

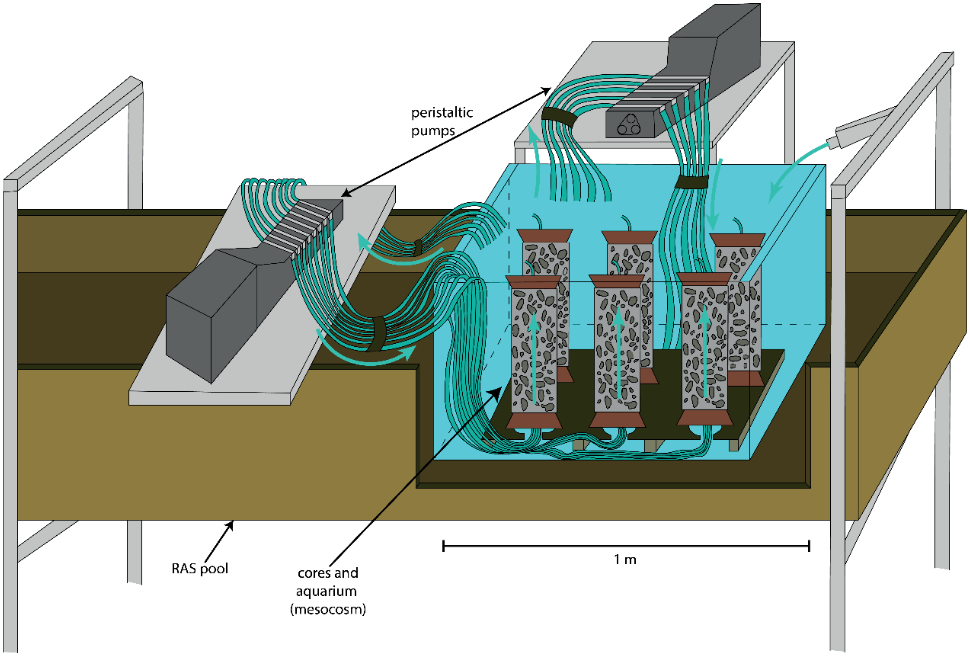

Figure 14. Mesocosm setup in which reconstructed trout nests were incubated. The aquarium was laid in one pool of the RAS system. Arrows indicate the direction of water circulation (reprinted from Paper II).

demand and NO_3^- fluxes using the Wilcoxon Signed-Rank test, while $N\text{-}NH_4^+$ fluxes were tested with the independent sample t-test. Finally, the Wilcoxon Signed-Rank test was also used to assess differences in $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations between all inflow and outflow sections grouped together from the mesocosms. All statistical tests were performed at an alpha level of 0.05. R software (R Core Team, 2023) was used to perform all statistical analyses and create charts using the rstatix (Kassambara, 2020) and ggplot2 (Wickham et al., 2016) packages, respectively.

4

Results and discussion

4.1. Surface water quality assessment

(material in section 4.1 from Paper I)

4.1.1. Land use as the main driver of water quality

Principal component analysis (PCA) was used to group stream water samples according to similarities/dissimilarities in physicochemical parameters for each season separately. From the PCA results, the layouts of the three watersheds are positioned in distinct areas of the charts, and this is usually related to the same array of vectors consistently across different seasons and hydrological conditions. This multivariate analysis leads to the clear differentiation of samples from the different basins in a consistent fashion over time. This suggests that such differences are driven by consistent features, i.e., the land use peculiarities of each watershed, rather than seasonal or short-lived phenomena (Figure 15).

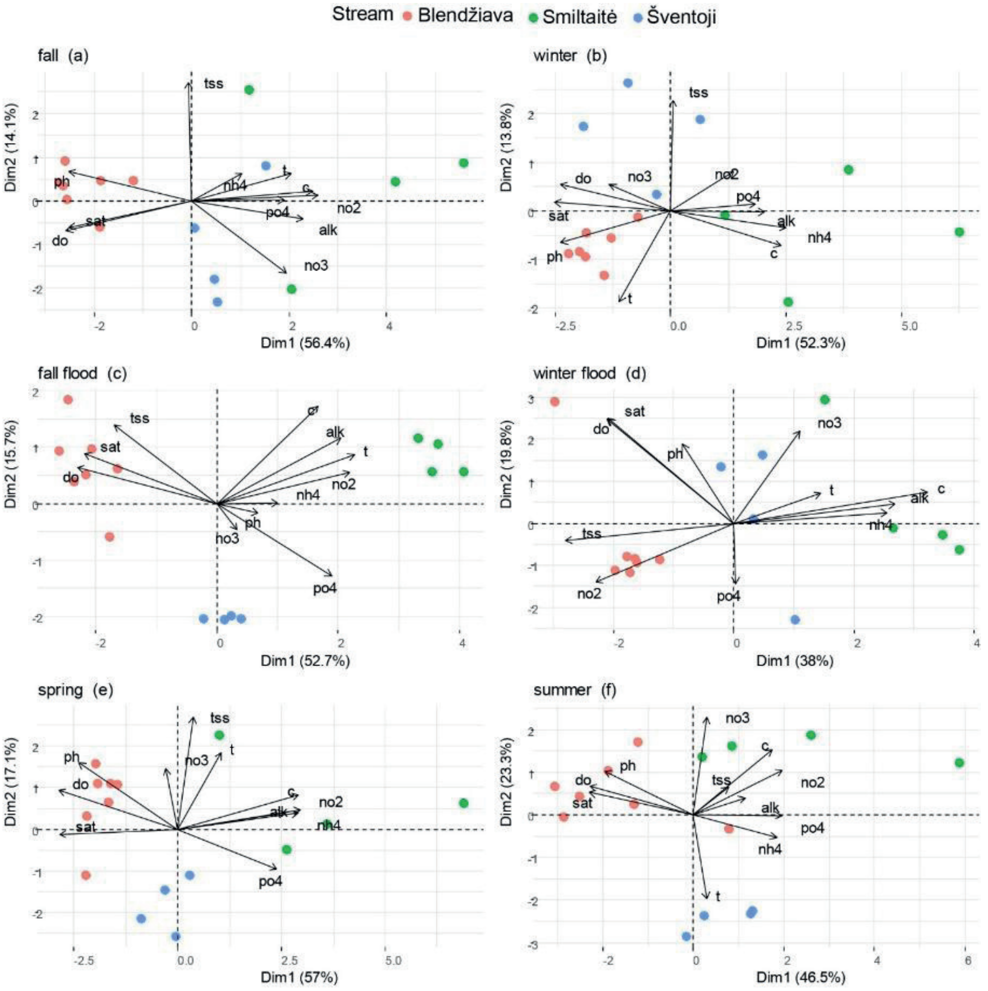

Figure 15. PCA biplots portraying the associations among the stations (dots) of the three different basins and the environmental variables across all seasons and the two flood periods. Environmental variables labels refer to: do – dissolved oxygen (DO, mg L^{-1}); sat – percentage oxygen saturation (% sat.); ph – pH; c – electrical conductivity (EC, µS cm^{-1}); t – temperature (T °C); nh4 – ammonium N-NH_4^+; no3 – nitrate N-NO_3^-; no2 – nitrite N-NO_2^-; po4 – soluble reactive phosphorous P-PO_4^{3-}, all expressed in mg L^{-1}; alk – alkalinity (mmol L^{-1}); and tss – total suspended solids (TSS, mg L^{-1}) (reprinted from Paper I).

In terms of land cover, the three drainage basins are mainly composed of agricultural areas (Blendžiava – 77%, Smiltaitė – 70%, and Šventoji – 58%). The Šventoji watershed has the highest percentage of forests (40%), while 13% of Smiltaitė consists of urban areas. Focusing on the 3rd CLC level, non-irrigated arable land is the primary feature of Šventoji and Smiltaitė, with 43% and 34% coverage, respectively. Blendžiava exhibits the highest percentage of land cover classified as complex cultivation patterns. CA confirmed that the three basins have different land uses ($\chi^2 = 110$, $p < 0.001$, df = 16), and the asymmetric biplot visually depicts the relationships in terms of land use for the three watersheds outlined in the contingency table (Figure 16).

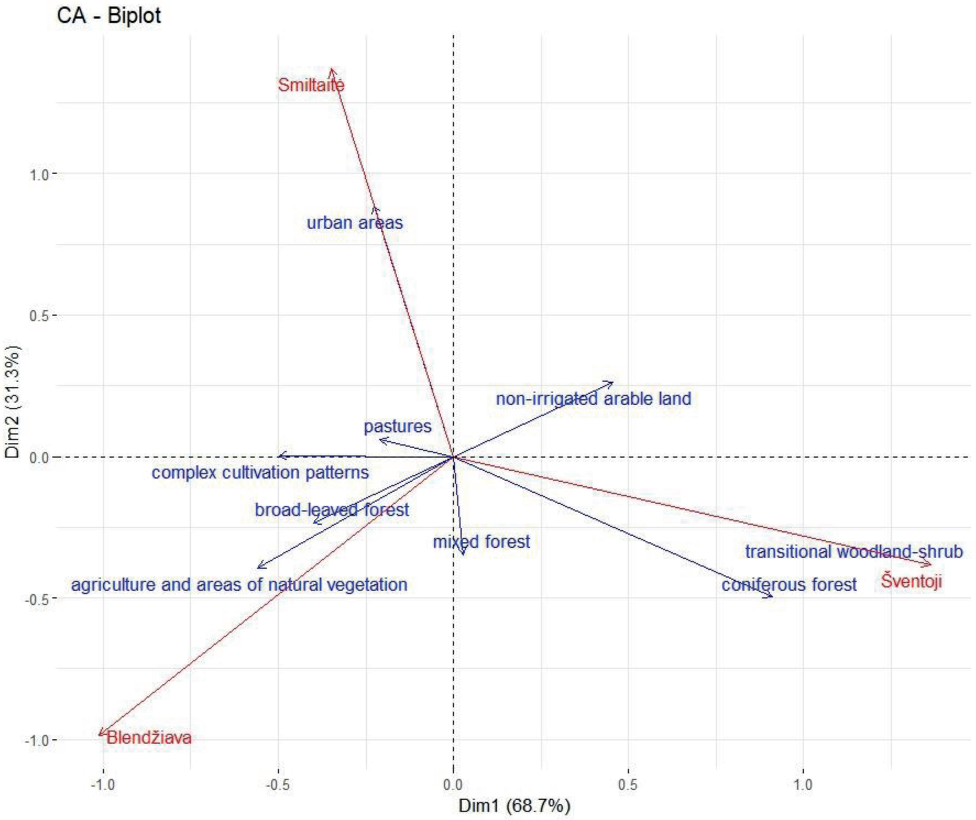

Figure 16. An asymmetric biplot CA portraying the relationship between different types of land use within the three investigated basins (reprinted from Paper I).

The first dimension of the asymmetric biplot (explaining 69% of the variability) is represented by complex cultivation patterns as opposed to coniferous forest or transitional woodland-shrub in terms of land use (blue arrows), and by Šventoji in terms of watersheds (red arrow). Indeed, the Šventoji watershed holds the highest share in terms of the coverage of these land use features. Their correlation is further supported by the reciprocal proximity in the asymmetric biplot. The dimension of complex cultivation patterns is in opposition to these two land features, and is typical of the Blendžiava and Smiltaitė basins. The second dimension (explaining 31% of the variability) is mainly composed of mixed forest and urban areas in terms of land use (blue arrows), and by Smiltaitė in terms of watershed (red arrow). The overlap between Smiltaitė and urban areas confirms the record for the Smiltaitė basin in terms of this feature (13%), while mixed forests are more typical of the other two watersheds. Non-irrigated arable land, broad-leaved forest, and agriculture and areas of natural vegetation equally contribute to the two dimensions, being the first feature shared by the Šventoji and Smiltaitė basins. On the other hand, the other two features characterize Blendžiava (15% of agriculture and areas of natural vegetation and 6% of broad-leaved forest) more than Smiltaitė and Šventoji, where their percentages are much smaller. Moreover, Blendžiava holds the lowest percentages of urban areas (2%) and non-irrigated arable land (8%) among the watersheds, as suggested by the strong contraposition of these vectors in the CA (Figure 16). Finally, pastures are similarly represented in all basins with small percentages, contributing minimally to both dimensions.

The Blendžiava stations are grouped on the left sides of the PCs and coupled with the DO, % sat., and pH vectors, which dominate these areas of the charts in all the sampled seasons and which always present as significantly higher than the values in Smiltaitė ($p < 0.05$, Figure 17 a, b). During the fall flood and summer low flow periods, the Blendžiava DO and % sat. values were also recorded as the highest in respect to the other two basins ($p < 0.05$, Figure 17 a, b). The pH vector usually lies on the left side of the axis of the first PC, and this is highly correlated with the Blendžiava stations and the PCA biplots, except during flood events. Indeed, Blendžiava exhibits significantly higher pH values during low flow periods, ranging from 8.1 to 9.2, throughout the year compared to the other two watersheds (mean ranges: Smiltaitė 7.8–8.2; Šventoji 7.6–8.3). These values are supported by the significance values of univariate tests during the low flow periods through all the seasons ($p < 0.05$, Figure 17 e). This can be partially explained by the clay loams in the Blendžiava streambed (Nika, 2011) and their buffer capacity due to the high presence of exchange sites (Jeon et al., 2019; Kumari et al., 2021).

N and P nutrients in the other two watersheds can trigger eutrophication events (McDowell et al., 2013; Sager, 1976), as previously ascertained for the Smiltaitė basin. Thus, the accumulation of decomposing algal mats and dead macrophytes following these eutrophication events leads to the release of carbon dioxide, principally responsible for the lowering of the pH in the water (Cai et al., 2011; Sunda et al., 2012). If not contrasted, this phenomenon can severely compromise the capacity of the ecosystem to host stenoecian fish species like brown trout, as already pointed out in boreal rivers subjected to strong acidification conditions (Sutela et al., 2010). Although the Blendžiava watershed features a consistent percentage of agricultural areas, the number of uncovered surfaces subjected to possible nutrient and organic matter leaching into the watercourse is low. The main cultivated layout is that of complex cultivation patterns, forming 42% of the watercourse and composing, according to the definition from the CLC Copernicus project (Copernicus Land Monitoring Service – https://land.copernicus.eu, accessed 10 November 2023), a “mosaic of arable and permanent crops and grasslands”. This, combined with the consistent presence of “agriculture and areas of natural vegetation”, creates a complex mix of cultivated patches with little presence of uncovered soil. As a result, these sheltered surfaces help buffer potential nutrient and organic matter inputs into the watercourse (Dabney et al., 2010; Delgado et al., 2007). Similar findings have already been ascertained in a multidecade study on boreal soils cultivated with perennial crops, which highlighted how these covers guarantee small N losses as they minimize the need for external N fertilizers (Ross et al., 2008).

As can be seen from Figure 15, Smiltaitė watershed sampling stations were closer to the nutrient (N and P forms), EC, and alkalinity vectors. These factors are considered indexes of a higher trophic status (Malcolm et al., 2004; McDowell et al., 2013; Wu et al., 2020), and pointed toward the PCA areas where the Smiltaitė stations lay. Indeed, through all the sampling events, Smiltaitė showed the highest values in EC ($p < 0.05$, Figure 17 c). This was also evident for $N\text{-}NO_3^{-}$, $N\text{-}NO_2$-, $N\text{-}NH_4^{+}$, $P\text{-}PO_4^{3-}$, while alkalinity values were always significantly higher in respect to Blendžiava ($p < 0.05$, Figure 17 g, i, j, k, h). Such separations in the PCs follow the differences in land use that these watersheds display. Higher N and P concentrations in the other two watersheds can trigger eutrophication events (McDowell et al., 2013; Sager, 1976), a situation already ascertained for the Smeltalė basin (HELCOM, 2021; Nemunas river basin district, 2010).

A parallel situation has already been observed in lentic boreal ecosystems surrounded by urbanized areas. This anthropogenic disturbance paired with rising temperatures has triggered unprecedented cyanobacteria blooms (Sivarajah et al., 2021). The Smiltaitė watershed holds a high share of non-irrigated arable land (34%) connected with urbanized areas (13%). These features lead to a higher share of uncovered soil subjected to the possible runoff and leaching of nutrients and organic matter to the stream. EC and alkalinity vectors usually share the same direction of the aforementioned variables in all PCA biplots. It has already been ascertained how high values in these two parameters are regarded as indicators of weathering processes (Malcolm et al., 2004), marking eutrophic conditions (Heathwaite et al., 1996; Wu et al., 2020). Similar values were also recorded in a spawning-salmonid watercourse salmonid production in the same context of an intensively exploited agricultural catchment area (Malcolm et al., 2003a). The situation is further worsened by the stronger presence of wastewater septic tanks connected with household leakages located in the Smiltaitė watershed (Nemunas river basin district, 2010). Thus, it is not surprising if the N and P vectors are generally correlated together through the first PCA axes and point

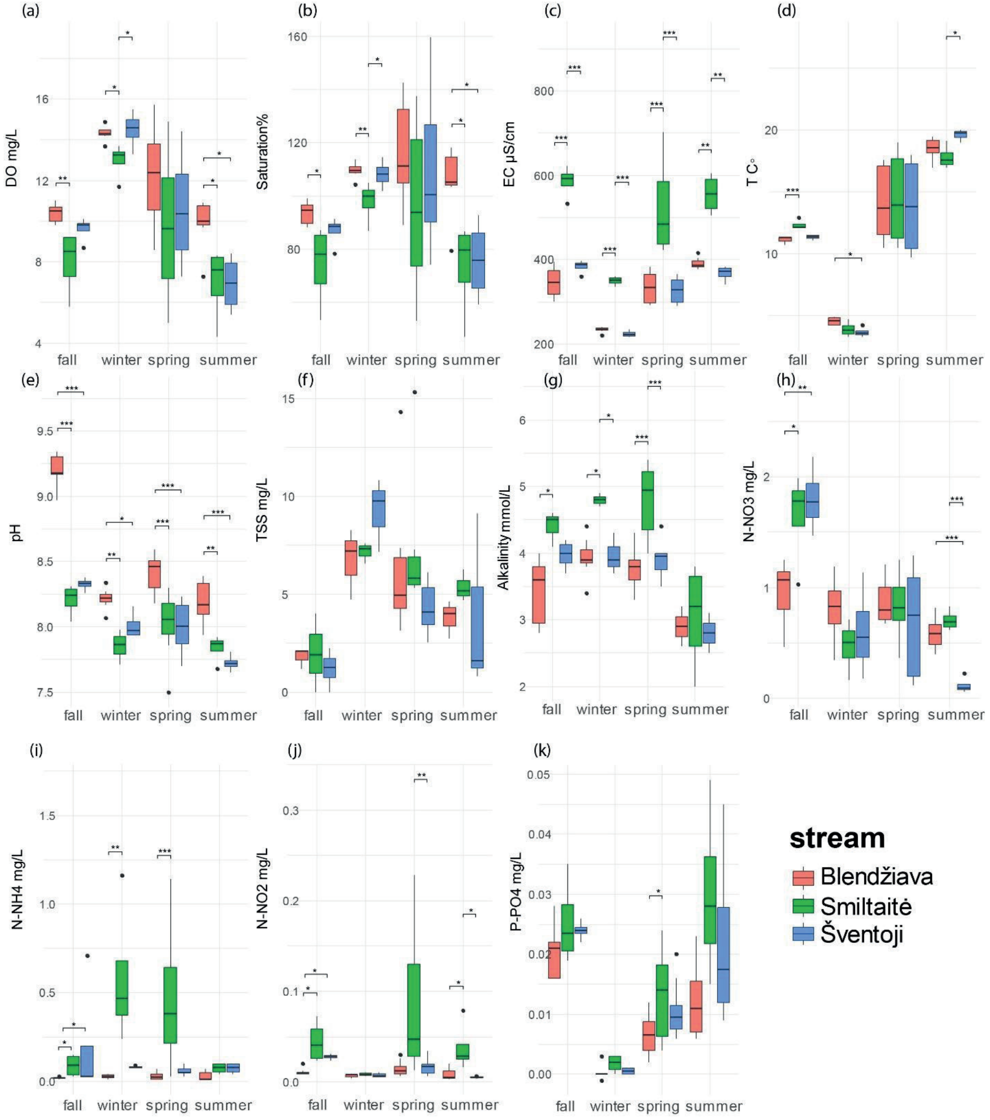

Figure 17. Seasonal variations in the 11 environmental variables across the three watersheds during low flow periods. Values presented as median, 75%, and 25% quartiles. ANOVA and Kruskal–Wallis tests were employed to detect any significant difference – $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (reprinted from Paper I).

toward the Smiltaitė stations. This supports the notion that Smiltaitė sampling stations are likely to have poorer water quality, as additionally confirmed by previous ecological assessments (HELCOM, 2021; Nemunas river basin district, 2010) under the Water Framework Directive.

The Šventoji stations hold an intermediate position on the PC maps, as they are either closer to the Blendžiava fall low flow or the Smiltaitė winter low flow, or equidistant from the other two basins during other events. This is confirmed by intermediate EC, TSS, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, and $N\text{-}NH_4^+$ values between the other two watersheds (Figure 17 f, g, i, j, k). Among the three basins, the Šventoji sees the highest percentage of forested areas (40%), a trait that might have contributed to keeping its station points off-centre with respect to the N– and P–nutrient, EC, and alkalinity vectors. Indeed, according to the CA, coniferous forest is a distinct trait of this basin that additionally shares the mixed forest vector with Blendžiava. However, Šventoji shares the non-irrigated arable land vector with Smiltaitė (Šventoji 43%, Smiltaitė 34%), which is the primary agricultural layout in this watershed. As a result, the Šventoji stations remain off-centre with respect to the Blendžiava points and their related vectors. In fact, the investigated transect falls in the Šventoji stretch classified as a water body at risk, lacking "good" ecological status due to the spread of agricultural pollution (Kesminas, 2011) and livestock farming (Venta river basin district, 2010). These two forms of point and non-point pollution can represent the main pool of nutrient input in lotic ecosystems with a similar agricultural background (Kato et al., 2009; Yang et al., 2016). Therefore, in terms of N and P vectors, the Šventoji samples hold an alternatively intermediate position between the other two basins (e.g., $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ in spring), or share similar values with the Smiltaitė samples ($N\text{-}NO_x^-$, $N\text{-}NH_4^+$, and $P\text{-}PO_4^{3-}$ in fall).

Vector zonation according to watershed land use is also reflected in the annual values of N and P nutrients as well as DO, marking the different degrees of pollution in the three systems. In these terms, Smiltaitė holds the highest mean values (or the lowest, if DO is considered) followed by Šventoji and Blendžiava. Nonetheless, $P\text{-}PO_4^{3-}$ concentrations fall in the range of "high" ecological status (0.05 mg L^{-1}, Table 1), and the same can be stated for $N\text{-}NO_3^-$ concentrations in the Smiltaitė stream. In the other two streams, $N\text{-}NO_3^-$ values fall within the "good" ecological status range, with concentrations lower than 2.3 mg L^{-1} – with the exception of one single station in Blendžiava and Šventoji during flooding events (Table 1).

Table 1. Annual averages (± sd) and respective ranges (min-max) of the parameters for which ecological status classes have been established for river types (1 – Blendžiava and Smiltaite, 2 – Šventoji) pursuant to the Water Framework Directive (WFD, 2000/60/EC) for Lithuania, summarized in the Nemunas and Venta river basin districts. Limits for salmonid protection are listed alongside (reprinted from Paper I).

			Ecological status classes					
Water course	Annual average ± sd (min-max)	River type	High	Good	Moderate	Poor	Limits for salmonid protection	Parameter
Sm	0.017 ± 0.015 (0–0.049)	1–2	<0.050	0.05–0.09	0.091–0.180	0.181–0.400	NA	$P\text{-}PO_4^{3}$ mg L^{-1}
Sv	0.015 ± 0.014 (0–0.045)							
Bl	0.01 ± 0.0092 (0–0.028)							
Sm	0.26 ± 0.31 (0.027–1.16)	1–2	<0.10	0.10–0.20	0.21 – 0.6	0.61–1.50	0.017–17 (Finn 2007, Brinkman, Woodling et al. 2009)*‡	$N\text{-}NH_4^{+}$, mg L^{-1}
Sv	0.079 ± 0.13 (0.015–0.71)							
Bl	0.03 ± 0.016 (0.009–0.07)							
Sm	1.01 ± 0.52 (0.17–2.11)	1–2	<1.30	1.3– 2.3	2.31–4.50	4.51 –10.00	20–34 (Kincheloe, Wedemeyer et al. 1979)‡	$N\text{-}NO_3^{-}$, mg L^{-1}
Sv	0.92 ± 0.75 (0.06–2.97)							
Bl	0.85 ± 0.39 (0.35–2.34)							

			Ecological status classes					
Water course	Annual average ± sd (min-max)	River type	High	Good	Moderate	Poor	Limits for salmonid protection	Parameter
Sm	9.65 ± 2.95 (4.33–14.94)	1	>8.50	8.50–7.50	7.49–6.00	5.99–3.00	7–9 (Greig, Sear et al. 2007, Crisp 2008)‡†	O_2 mg L^{-1}
Bl	11.92 ± 1.89 (7.35–15.7)							
Sv	10.59 ± 2.65 (5.38–15.48)	2	>7.50	7.5–6.5	6.49–5.00	4.99–2.00		
Sm	0.04 ± 0.05 (0.007–0.23)	NA					14 (Williams and Eddy 1989, Dumas, Bassenave et al. 2007)‡	$N–NO_2^–$ mg $L^{–1}$
Bl	0.11 ± 0.28 (0.003–1.43)							
Sv	0.01 ± 0.01 (0.004–0.034)							
Sm	10.46 ± 5.71 (1.9–19.1)	NA					0–11 (Crisp 2008)‡ 19–20 °C (Crisp 2008, Smialek, Pander et al. 2021)†	T °C
Bl	9.94 ± 5.9 (1.6–19.5)							
Sv	10.01 ± 6.21 (2.3–20)							

			Ecological status classes					
Water course	Annual average ± sd (min-max)	River type	High	Good	Moderate	Poor	Limits for salmonid protection	Parameter
Sm	8.01 ± 0.2 (7.5–8.35)	NA					>6.7 (Peterson, Daye et al. 1980)‡	pH
Bl	8.36 ± 0.43 (7–9.34)							
Sv	7.96 ± 0.42 (6.03–8.38)							
Sm	8.27 ± 5.33 (0–22)	NA					<25 increment in clear waters (Singleton 2001)‡§	TSS mg L^{-1}
Bl	19.3 ± 26.4 (1.2–86)							
Sv	6.25 ± 5.1 (0–22)							

Notes:
*relates to N-NH$_3$ (range from the three basins: 1 µL^{-1}–0.011 mg L^{-1})
‡ intragravel stages
† juvenile and adult stages
§ classified with <25 mg L^{-1} of TSS under average conditions (Singleton, 2001)
Detection limit: P-PO$_4^{3}$: 0.1 µL^{-1}, TSS: 0.5 mg L^{-1}
Sm: Smiltaitė Bl: Blendžiava and Sv: Šventoji
NA: the value is missing for that specific parameter

Therefore, $P\text{-}PO_4^{3-}$, and $N\text{-}NO_3^-$ may not be considered as primary variables affecting these three water courses. Conversely, $N\text{-}NH_4^+$ concentrations in Smiltaitė are remarkably higher during the winter and spring low flow periods, being up to one order of magnitude above the other two basins. In all streams, $N\text{-}NH_4^+$ values occasionally fall in the "moderate" and "poor" ecological status classes (Table 1), with more than 0.6 mg of $N\text{-}NH_4^+$ L^{-1}. Concentrations of this compound peak above 1 mg $N\text{-}NH_4^+$ L^{-1} in the stations in the proximity of urbanized areas in all three watersheds. This trend can be explained by the low efficiency of wastewater treatment plants and septic tanks, with low temperatures during the winter months slowing down the nitrification processes of this compound (Conallin, 2004). This situation is further worsened by the prolonged low flow period, lasting also into spring, that shrunk the water column, weakening its dilution effects (Peña-Guerrero et al., 2020). Additionally, early spring can be still a relatively cold season for these latitudes (water T in Smiltaitė: 14.4 ± 3.6 °C), which postpones and lowers the growth of primary producers and, thus, their nutrient sequestration capacity (Vybernaite-Lubiene et al., 2018).

The Blendžiava and Šventoji samples presented DO values that correspond to the "good" ecological status class for this parameter (Table 1), as confirmed by the arrangement of the vectors in the PCAs. This is partially true for the Smiltaitė stations, although alarming concentrations corresponding to the "moderate" and "poor" classes were recorded in late spring and summer. Here, the water peaks in temperature (18 ± 1° C), and this prolonged dry moment surely contributes to macrophyte and epiphyton growth leading to nocturnal DO depletion.

4.1.2. Accounting for shifts in seasonality and hydrological extremes

The strength of the relationships between environmental variables (arrows) and sample stations (points) varies in the PCAs according to the season and the occurrence of hydrological extremes . Indeed, low flow events amplify the effects of point source pollution (i.e., wastewater treatment plants), as has already been underlined in similar studies which assessed water quality based on land cover (Gorgoglione et al., 2020) and hydrological extremes (Peña-Guerrero et al., 2020). This is confirmed by the consistently significantly higher EC values during the low flow fall and winter periods ($p < 0.01$, Figure 18 c, k) compared to flood situations. Additionally, alkalinity rises during low flow periods in Blendžiava (fall $p < 0.01$, winter $p < 0.001$, Figure 18 k), for Šventoji in winter ($p < 0.05$), and partially for Smiltaitė in fall ($p < 0.05$).

Parallel findings suggest how the presence of urbanized areas plays a crucial role in the peak release of nutrients in the adjacent river stretch (Liu et al., 2023). Furthermore, the reduction in river discharge necessarily leads to lowered dilution followed by mass concentration (Mosley, 2015). This triggers an increase in EC and alkalinity (Malcolm et al., 2004) patterns, confirmed during the low flow events inside each watershed. In this regard, the presence of higher groundwater input during low flow events could be an additional explanation for augmented EC and alkalinity values (Malcolm et al., 2004). N– and P– nutrients presented only partial differences in their concentrations when low flow and flood events were compared inside each watershed, and there was no clear evidence that N–compound concentrations were influenced by flow conditions. $N\text{-}NO_3^-$ rose significantly only in Blendžiava during the winter low flow event, ($p<0.05$, Figure 18 g), while $N\text{-}NH_4^+$ increased only during the winter flood ($p < 0.05$, Figure 18 i) in the Blendžiava stream. $N\text{-}NO_2^-$ increased significantly only during the winter flood in Blendžiava ($p < 0.01$, Figure 18 h) and the fall low flow in Šventoji ($p < 0.01$).

$P\text{-}PO_4^{3-}$ consistently rose during the fall flood period in all three systems (Blendžiava $p < 0.05$, Smiltaitė and Šventoji $p < 0.01$, Figure 18 j). This finding has already been ascertained in similar watersheds with high agricultural activity in the same study region (Povilaitis, 2004) and during different hydrologic conditions (Trentman et al., 2021). Agricultural areas usually undergo fertilization practices (Povilaitis, 2004), which can provoke the release of P– forms during intense rainfall events as a result of soil leaching (Trentman et al., 2021). However, weak changes in nutrient concentrations during hydrological events may suggest limited control by non-point sources during the rainfall period or high baseflow concentrations delivered by point-source activities (e.g., wastewater in Smiltaitė).

A similar pattern was observed with the fluctuating DO patterns, leading to speculation that the cold season (4.2 ± 1 °C) during which the only two flood events were sampled favoured DO dissolution. This reduced the intensity of physical DO changes due to water level fluctuations. Prolonged low flow periods exacerbated differences among the three watersheds in terms of significantly higher $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, EC, and alkalinity values for the Smiltaitė stream. This contrasts with the remarkably higher DO, % sat., and pH values observed for Blendžiava. DO rises during low flow periods, with significant increases observed in the Blendžiava and Šventoji watersheds during winter ($p < 0.05$, Figure 18 a). This is further confirmed by a similarly significant increase in % sat. across all watersheds at low flow conditions in winter ($p < 0.05$, Figure 18 b).

These differences could be attributed to the Smiltaitė watershed having the highest share of urbanized areas (13%). These findings are further supported by the "poor" ecological status assigned during the last nationwide survey (List of water bodies at risk (in Lithuanian), 2017; Nemunas river basin district, 2010) under the Water Framework Directive, within the scope of actions to improve sea trout spawning habitats (HELCOM, 2021). These recent studies demonstrate that surface water pollution from household leakage is the primary concern in this watershed. The transport of reduced N– forms in the watercourse occurs when oxidation treatment is absent (Brinkman et al., 2009; Wong et al., 2003) or inhibited by low temperatures (Conallin, 2004; Zhou et al., 2018). This is confirmed by the significantly higher concentrations of $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ observed in winter and spring compared to in the other two systems.

Even a small number of rural dwellings not connected to a modern wastewater drainage system can heavily impact small freshwater environments given the untreated nature of their sewage waters (Bach et al., 2002). This situation has already been underlined for the Smiltaitė watershed, as its $N\text{-}NH_4^+$ and BOD7 values exceed the thresholds for "good" ecological status (Nemunas river basin district, 2010). Further evidence of these eutrophic conditions is supported by the EC and alkalinity values, which are consistently the highest for this watershed. In contrast, the Blendžiava basin features a low percentage of urbanized areas (2%), and those that do exist are located far from the sampled stretches. In situ investigations within the scope of this study confirm the almost total absence of point sources entering the Blendžiava sample area. These isolated sources include sewage water treatment plant outlets or rural dwellings not connected to the sewage drainage. Furthermore, during low flow periods, the nutrient inputs coming from agricultural runoff may be negligible (Chen et al., 1999; Schepers et al., 1980; Van Esbroeck et al., 2016).

With a small mean annual water discharge (1.06 m^3 s^{-1} Jablonskis et al., 1962) the concentration phenomena of polluting substances can be enhanced. This explain why low flow periods are always characterized by low $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ values. This is significant in comparison with the more urbanized Smiltaitė watershed. Furthermore, the well-expressed riparian belts along the river channel (Nika, 2011) contribute to the sequestration of pollutants leached from uncovered soils (Maraseni et al., 2016) and their degradation (Luke et al., 2007). A parallel study on boreal watercourses found how the presence of riparian belts reduces the conditions associated with enhanced nitrate removal rates (Luke et al., 2007). Therefore, nutrient scarcity resulting from the aforementioned factors likely contributed to maintaining low biochemical and chemical oxygen demand (BOD, COD). This, in turn, resulted in significantly higher levels of DO and % sat. in Blendžiava during low flow events compared to the two other water courses.

On the other hand, flood events enhance non-point source pollution via field leaching during rainfall (Binkley et al., 1993). This is confirmed by the higher $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$ concentrations in the Smiltaitė and Blendžiava basins, which have a greater portion of cultivated land (Blendžiava 70%, Smiltaitė 77%) compared to Šventoji (58%). Indeed, it has been previously stated that the conversion of pasture into cropland is the main driver of increased N– exports from the principal Lithuanian rivers (Bauer et al., 2015). Moreover, besides the exogenous contribution from runoff from cultivated areas (Green et al., 2004; Lawlor et al., 2005), high water periods are also characterized by endogenous inputs from metabolic processes. Endogenous inputs are driven by increased amounts of suspended solids and infiltration rates in the hyporheic zone (Burke, 2011; Greig et al., 2005). Therefore, higher shares of uncovered areas may favour the mobility of these reduced N– forms in the surface runoff. Nevertheless, it is unlikely that these compounds will be metabolized (e.g., oxidized into $N\text{-}NO_3^-$) by the system due to the slowing of biogeochemical processes caused by the low temperatures (4–5 °C) typical of the cold season (Conallin, 2004; Szewczyk et al., 2023; Vybernaite-Lubiene et al., 2018). This may help explain why N-NO_3^- trends were similar across the three watersheds as well as within each watershed – a similarity also registered between the flood and low flow events in the late fall and winter, when temperatures were low.

Besides leakage from agricultural areas, flooding can severely hinder the functioning of wastewater treatment plants and septic tanks by overloading them with increased flow rates or inundating their operational units (Olyaei et al., 2018; Zouboulis et al., 2015). This may lead to the release of sludge directly into the watercourse. Consequently, when the three watersheds were contrasted during flooding moments, N-NH_4^+, alkalinity, and EC were still higher for Smiltaitė. During flood events, differences in DO and % sat. were partially smoothed out among the three systems. This is due to its physical mixing in the water column aided by the low temperatures registered in fall and winter across the three systems.

T vectors are mostly relegated to the axes of the second PC, and are regarded as a minor variable explaining watershed differences. Indeed, there is no clear contraposition in terms of this parameter among the watersheds. T values hold higher records across the sampling moments and globally increase as the warm period advances. Temperatures increase during low flow periods in all the rivers in winter (Blendžiava $p < 0.001$, Smiltaitė $p < 0.05$, Šventoji $p < 0.01$, Figure 18 d), but only partially in the fall low flow period (Blendžiava $p < 0.05$, Šventoji $p < 0.001$). pH shows similar values between the two hydrologic conditions in winter in all the three watersheds. However, in Blendžiava ($p < 0.05$, Figure 18 e) and Šventoji ($p = 0.01$) pH fall low flow values are significantly higher than in the flood period. Nevertheless, T significantly rises in each watershed during low flow moments, even though these periods occur in the cold season. Low flow periods are characterized by a reduction in the wetted stream area, coupled with slower current velocity. These factors lead to increased retention times and, consequently, an increase in water insulation connected with higher T (White et al., 2023). Conversely, pH differences during flood events are less pronounced across the three watersheds. This is most probably due to the dilution effect of the high-water period and the acidifying impact of rainwater (Haapala et al., 1975).

TSS accounts for the amount of organic and inorganic particles carried by the water (Brils, 2008; Swietlik et al., 2003). In the indicative list of the main pollutants (annex VIII of the WFD 2000/60/EC), they are loosely defined as “materials in suspensions”, without specific guidance on their monitoring and ecological thresholds (Brils, 2008). To the author’s knowledge, Lithuania has not yet established such assessment criteria for running waters. In this appraisal, TSS vectors were laid on the axes of the second PC during the low flow periods, explaining little variability in the dataset. This is not surprising, since the three systems hold average values from 4.4 to 5.9 mg L^{-1} for all low flow events, classifying them as low turbidity systems (<25mg L^{-1}, Nika, 2011). Consequently, there are no significant differences among the systems during low flow events. On the contrary, TSS concentrations are more pronounced during flooding phases, with higher values observed at the Blendžiava stations. TSS significantly increases only during the fall flood event ($p < 0.05$, Figure 18 f) in the Smiltaitė samples. Also in Blendžiava TSS values rise (Figure 18 f), but the high variability of the data coupled with the low sample size did not allow any statistical significance to be derived.

A well-expressed dichotomy was registered during the two high water periods, with Smiltaitė and Šventoji having comparable mean values of 14.2 and 12.3 mg L^{-1}, respectively – from three to four times lower than Blendžiava. This is confirmed by statistically significantly higher TSS values for Blendžiava in comparison with the two other basins ($p < 0.05$). This is also reflected within each watershed, where TSS values for Smiltaitė and Šventoji are generally similar during both flood and low flow events. However, for Blendžiava they greatly increase during the flood period, although this is associated with high variability. Indeed, high runoff generated during rainy events has the potential to erode uncovered and cultivated soils, bringing high amounts of sediment inside the watercourse (Adjovu et al., 2023; Soulsby et al., 2001). These phenomena are more typical during cold seasons, where high soil humidity associated with low temperatures increases the amount of washed-off sediments (Gorgoglione et al., 2020).

It is speculated that the higher TSS loads found in Blendžiava could be related to its higher share of cultivated features – 70%, which, although slightly lower than the 77% found in Smiltaitė, rely on a drainage basin that is more than twice the size of Smiltaitė. A scenario that is worsened given the pastures conversion in Lithuania to cultivated land (Bauer et al., 2015). This similar situation already occurred for other salmonid-spawning waters in central Europe (Malcolm et al., 2004; Sternecker et al., 2013; Sutherland et al., 2002). Similar studies focusing on the effects of land cover on water quality in sustaining salmonid populations have pointed out how crop areas are land features vulnerable to soil erosion (Opperman et al., 2005; Sutherland et al., 2002). This stressor can deliver high amounts of fine sediments (<0.85 mm) to spawning grounds, reducing their suitability (Opperman et al., 2005; Sutherland et al., 2002).

Moreover, the steeper bed slope of Blendžiava (0.33%, Gailiušis et al., 2001) compared to the gentler incline of Smiltaitė 0.007% and Šventoji 0.006% (Venta river basin district, 2010) surely contributes to increased velocity in surface runoff, leading to higher soil erosion and, thus, higher TSS delivery to the stream. On the other hand, the presence of forests dampens TSS loads in the watercourse, as is evident from the Šventoji values (forest coverage: Šventoji: 40%, Blendžiava: 28%, Smiltaitė 10%). Šventoji holds the lowest TSS values in most of the sampling events, and always during flooding periods. Since the three systems respond differently in terms of TSS loads during flooding events, it is speculated that the origin of TSS would be more exogenous (i.e., brought by runoff) rather than endogenous (i.e., resuspended by high flow), and thus connected to land cover composition. Similar studies have already ascertained how the presence of forested areas can sequester the suspended solids entering streams during intense runoff events (Billi et al., 2022; Opperman et al., 2005; Sutherland et al., 2002).

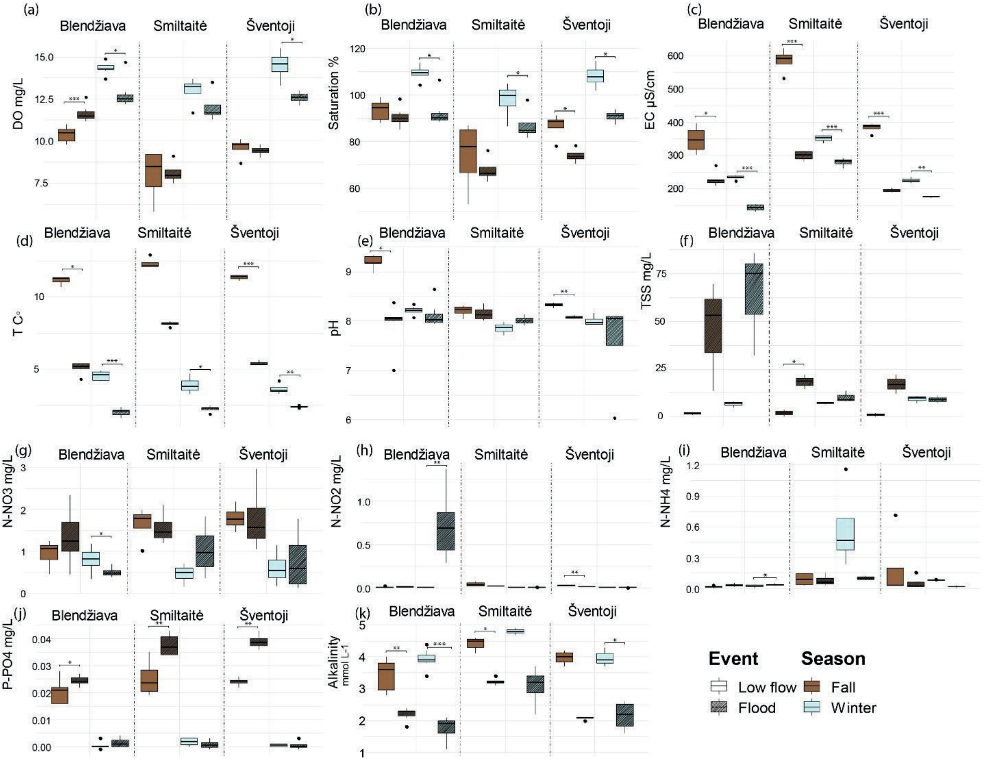

Figure 18. Variations between the flooding and low flow phases during the fall and winter seasons for the 11 environmental variables in the three watercourses. Values presented as median, 75%, and 25% quartiles. Paired t-test and non-parametric Wilcoxon Ranked Sum test were used: $p \leq 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (reprinted from Paper I).

4.2. Incubation time: hyporheic water quality

(material in section 4.2 from Manuscript II)

The two resemblance matrices built using only the intragravel water data (Figure 19, a, b, c) and coupled data from intragravel and surficial water samples (Figure 19, d) generated two dimensional (2D) NMDS ordinations with stress values ranging from 0.14 to 0.17, respectively. “Stress” is a measure of the quality of representation from the resemblance matrix in the reduced NMDS space, and values between 0.1 and 0.2 represent a satisfactory ordination (Clarke, 1993; Clarke et al., 2001).

Clusters appearing from the NMDS ordination are supported by the significant ANOSIM results (Table 2) when the factors “stream”, “month”, and “type” are considered ($p < 0.001$). Moreover, the ISA analysis (Table 3) indicated a specific set of environmental variables that significantly ($p < 0.05$) drove the difference for the stream (pH, EC, N-NH_4^+), month (N- NO_3^-, T, EC, O_2, N-NH_4^+) and type factors (O_2, pH, N-NH_4^+) supported by statistically significant results from the univariate tests (Tables 5, 6).

Table 2. Results of the multivariate ANOSIM analysis to assess differences among the stream, month, site, and type factors according to the normalized values of all variables accounted for in this survey. R is defined as the difference between the similarities between groups and within each group divided by the total number of samples. R ranges from 1, representing high differences between groups, to 0, representing low to null differences between groups. $p \leq 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (Manuscript II, in preparation).

Factor levels	R	p
Global test: streams	0.2	<0.001***
Blendžiava vs Smiltaitė	0.3	0.002**
Blendžiava vs Šventoji	0.08	0.05*
Smiltaitė vs Šventoji	0.2	0.002**
Global test: months	0.7	<0.001***
March vs April	0.4	0.001**
March vs May	0.9	0.001**
April vs May	0.7	0.001**
Global test: site (nest vs near nest)	0	0.5
Global test: type (hyporheic vs surface)	0.1	<0.001***

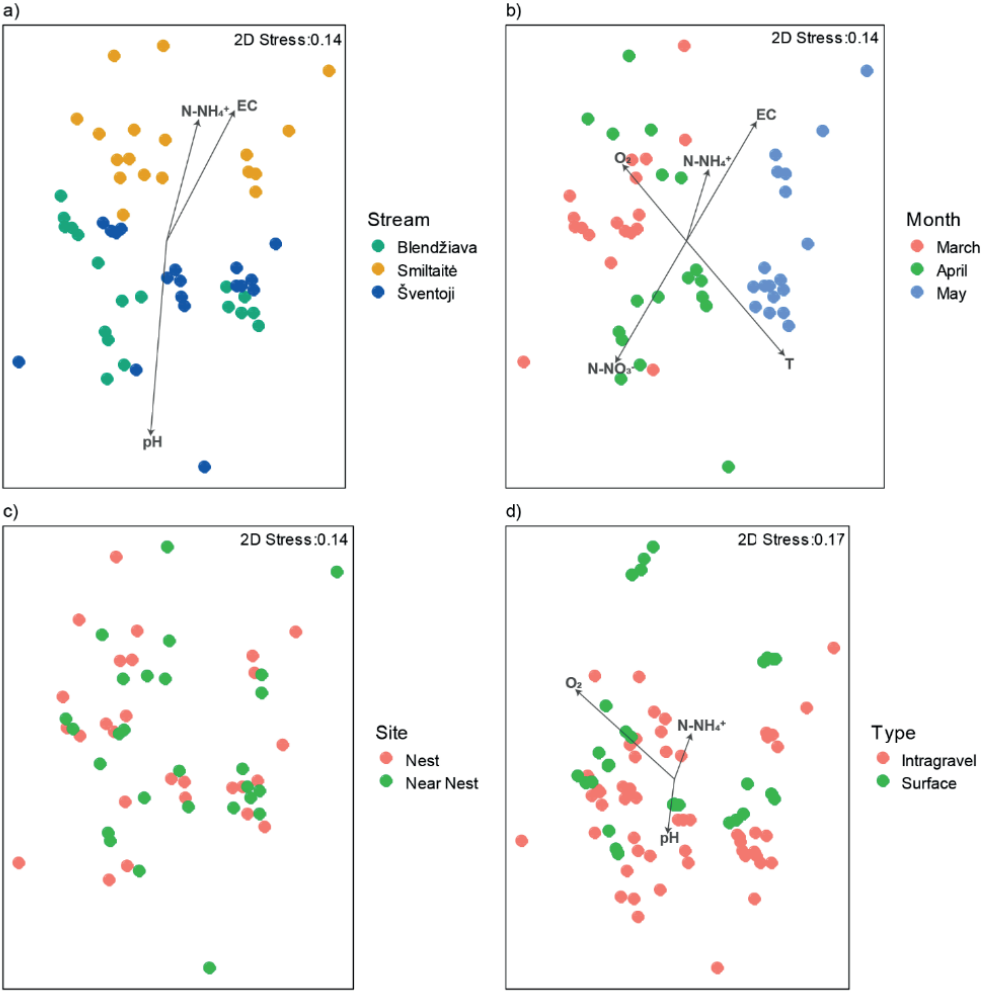

Figure 19. NMDS plots portraying the separation between the intragravel water parameters according to the a) stream, b) month, and c) site factors, and d) against the surface water. Plots display only the variables that significantly contributed to the differentiation of each factor according to the ISA results. Arrow length of each variable is proportional to the strength of the contribution of that variable to that factor (ISA statistics, see Table 3) (Manuscript II, in preparation).

Table 3. Results of Indicator Species Analysis (ISA) portraying which of the intragravel water parameters drive significant differences among the stream, month, site, and type factors. For each factor, all variables are uniquely distributed among its levels or a combination thereof (i.e., level A + level B). Then, each variable-factor level association is marked as significant ($p < 0.05$) or non-significant ($p > 0.05$) and correlated to an ISA statistic. The ISA statistic indicates the correlation strength from 0, representing the absence of correlation, to 1, meaning highly correlated. $p \leq 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (Manuscript II, in preparation).

Variable	Stream	ISA statistic	p
pH	Blendžiava	0.6	<0.001***
N-NO_3^- mg L^{-1}		0.2	0.3
EC µS cm^{-1}	Smiltaitė	0.6	<0.001***
N-NH_4^+ mg $^{L-}$1		0.4	<0.001***
T °C	Šventoji	0.3	0.1
N-NO_2^- mg L^{-1}		0.2	0.3
O_2 mg L^{-1}	Blendžiava + Smiltaitė	0.2	0.3
Variable	Month	Statistic	P
N-NO_3^- mg $^{L-}$1	April	0.7	<0.001***
T °C	May	0.8	<0.001***
EC µS cm^{-1}		0.7	<0.001***
O_2 mg L^{-1}	March+April	0.5	<0.001***
N-NO_2^- mg L^{-1}		0.1	0.6
N-NH_4^+ mg L^{-1}	March+May	0.3	0.03*
pH	April+May	0.2	0.2
Variable	Site	Statistic	P
O_2 mg L^{-1}	Nest	0.2	0.07
N-NO_3^- mg L^{-1}		0.2	0.09
EC µS cm^{-1}		0.01	0.9
pH	Near Nest	0.06	0.6
N-NH_4^+ mg L^{-1}		0.03	0.8
N-NO_2^- mg L^{-1}		0.02	0.9
T °C		0.005	1
Variable	Type	Statistic	P
O_2 mg L^{-1}	Surface	0.7	<0.001***
pH		0.2	0.008**
N-NH_4^+ mg L^{-1}		0.2	0.02*
N-NO_2^- mg L^{-1}		0.1	0.3
N-NO_3^- mg L^{-1}		0.04	0.6
EC µS cm^{-1}		0.007	0.9
T °C		0.002	1

4.2.1. Spatiotemporal differentiation

Stream differentiation is primarily driven by higher EC and $N\text{-}NH_4^+$ (Figure 20 b, f) values for Smiltaitė contrasted with Blendžiava and Šventoji ($p < 0.001$ for Blendžiava, $p < 0.05$ for Šventoji, Table 4). Indeed, the average EC value in Smiltaitė was found to be above 350 µS cm^{-1}, while for the other two basins average values ranged from 250 to 270 µS cm^{-1}; similarly, $N\text{-}NH_4^+$ values were from two to three times higher in Smiltaitė, which attained an average concentration of 0.1 mg L^{-1}. Nevertheless, such parameters present similar values when Blendžiava and Šventoji are compared. Finally, Blendžiava holds an average pH value of 8, significantly higher by 0.3 units than the averages of the other two basins ($p < 0.001$, Figure 20 d).

Table 4. Comparisons of the intragravel water parameters from the three different streams (Blendžiava, Smiltaitė, and Šventoji) grouped for all the sampling months using the one-way ANOVA (F) and Tukey tests for post-hoc comparisons. The Kruskall Wallis test (H) and pairwise comparisons using Dunn tests were employed for non-normally distributed data. $p \leq 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Df between groups – 3; Df within groups – 18 (Manuscript II, in preparation).

Variable	*F (H)*	*p*	Post-hoc comparison
O_2 mg L^{-1}	1.0	0.4	
T °C	2.5	0.09	
EC µS cm^{-1}	28.4	<0.001***	Smiltaitė - Blendžiava < 0.001*** Šventoji - Smiltaitė < 0.001***
$N\text{-}NH_4^+$ mg ^{L}-1	17.2	<0.001***	Smiltaitė - Blendžiava < 0.001*** Šventoji - Smiltaitė =0.04*
$N\text{-}NO_3^-$ mg L^{-1}	1.6	0.4	
$N\text{-}NO_2^-$ mg L^{-1}	2.4	0.3	
pH	26.6	<0.001***	Smiltaitė - Blendžiava <0.001*** Šventoji - Blendžiava <0.001***

The distinction between the streams following differences in intragravel physicochemical water parameters has already been confirmed by investigations into surficial water quality (see Chapter 4.1). The Smiltaitė data points lay far away from those of Blendžiava and Šventoji on the NMDS plot, presenting higher EC and $N\text{-}NH_4^+$ values which serve as proxies of the higher trophic status and weathering processes occurring in intensively cultivated catchments (Binkley et al., 1993; Green et al., 2004; Lawlor et al., 2005), or perhaps representing improperly functioning wastewater treatment plants (Conallin, 2004; Olyaei et al., 2018; Zouboulis et al., 2015). Indeed, the mean Smiltaitė conductivity value is 360 ± 74 µS cm^{-1}, which is higher than the values ranging from 280 to 310 EC µS cm^{-1} that were reported by Malcolm et al. (2003a) in a

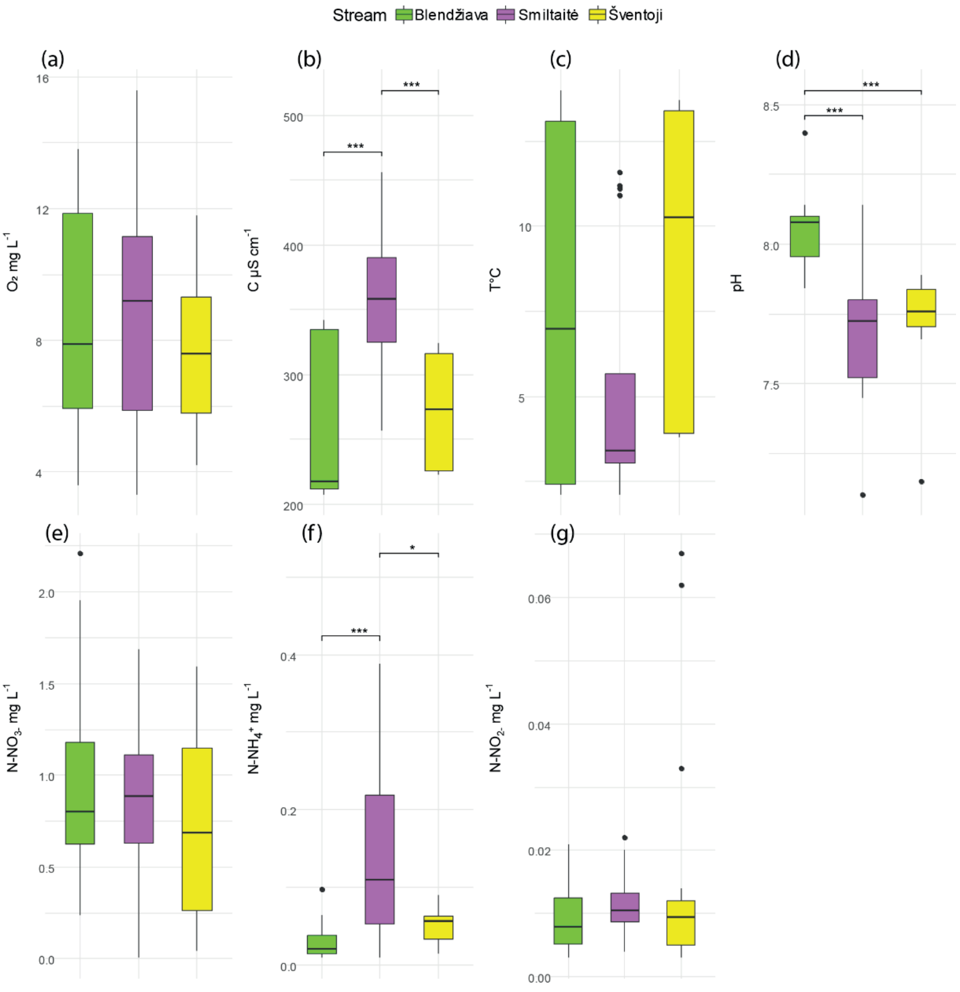

Figure 20. Variations of the 7 intragravel water parameters across the three streams, with data gathered for all sampling months. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (Manuscript II, in preparation).

highly exploited agricultural catchment of a similar size and latitude in the context of an appraisal of salmonid incubation.

Chapter 4.1 observed that, among the three watersheds, Smiltaitė holds the highest share of urbanized and uncovered soil areas, which are more prone to leaching. Additionally, the presence of isolated septic tanks directly connected to the stream (Nemunas river basin district, 2010) contributes to the leakage of untreated water. Therefore, such processes will likely increase the delivery of organic matter into this watercourse, in turn affecting EC values. Previous studies have already confirmed the "poor" ecological status of the Smiltaitė stream (Nemunas river basin district, 2010; project RETROUT, 2021) in respect to N-NH4 $^+$ and BOD7 values outside the "good" ecological status range (in terms of the Water Framework Directive 2000/60/EC). Household leakage is attributed as the primary concern and reason for the poor quality of surficial water in Smiltaitė (List of water bodies at risk (in Lithuanian), 2017). Furthermore, given the small size of the stream and its discharge of only a few litres per second (see chapter 4.1), even a small amount of untreated sewage water can significantly impact the overall water quality of this ecosystem (Bach et al., 2002).

On the contrary, the presence of well-expressed riparian belts and sheltered soil areas in the Blendžiava and Šventoji basins (Nika, 2011) helps in dampening the release of organic nutrients (Maraseni et al., 2016), keeping their values away from the EC and N-NH4$^+$ vectors. The Blendžiava values are related to the pH vector, since such values appear higher in respect to the other two rivers. Higher pH values in the intragravel water are in line with previous findings (chapter 4.1), and can be related to the sequestering effect (Jeon et al., 2019; Kumari et al., 2021) of hydrogen ions from a streambed mostly made from clay loam (Nika, 2011). Nevertheless, the mean range of pH found in these watersheds, from 7.7 to 8.0, is much higher in respect to the critical values of 4.7 and 5 which, in Nova Scotia rivers, displayed a significant decrease in their salmon runs (Stanley et al., 1995). The pH values in this study are also above the critical threshold of 6.7, above which the normal development of eggs is registered (Peterson et al., 1980). $N\text{-}NH_4^+$ means ranged from 0.03 to 0.1 mg L^{-1} in the intragravel water, lower than the range of 0.1-0.2 mg L^{-1} which represents the threshold for a "good" ecological classification (Water Framework Directive 2000/60/EC).

The differentiation of intragravel water parameters follows a monthly gradient, with points representing the early incubation season, March, spread on the left side of the NMDS ordination. Approaching the ending of the incubation period towards fry emergence, in April–May, points are clustered toward the middle and right side of the ordination plot (Figure 20 b). This is confirmed by the ANOSIM pairwise comparisons, which point out significant differences when all the three sampled months are pairwise contrasted ($p = 0.001$, Table 5). The main partition in NMDS points is created by the opposition of the O_2 vector, pointing towards the March–April cluster. This is in contrast to the T vector, which is directed toward the May points.

In all three watersheds, O_2 values decrease with the advancing of the incubation season, being significantly higher (Figure 21 a) in March for Blendžiava and Šventoji ($p < 0.05$) and April for Smiltaitė ($p < 0.05$) in respect to May. Among the three watersheds, O_2 averages ranged from 9 to 12 mg L^{-1} in March, from 7 to 11 mg L^{-1} in April, and from 5 to 7 mg L^{-1} in May. In parallel, the surficial DO values of earlier periods also underwent a significant ($p < 0.01$) drop from their 14 to 12 mg L^{-1} averages when passing into May. Meanwhile, T values significantly increase in each sampling month,

Table 5. Comparisons of the intragravel water parameters from the three different sampling months (March, April, May) for each stream using one-way ANOVA (F) or the Kruskal–Wallis (H) test if data is normally or non-normally distributed, respectively. $p \leq 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Df between groups – 3; Df within groups – 6 (Manuscript II, in preparation).

Variable	Water course	F (H)	p
O_2 mg L^{-1}	Blendžiava	12.4	0.002**
	Smiltaitė	5.0	0.02*
	Šventoji	4.3	0.03
EC µS cm^{-1}	Blendžiava	5315.7	<0.001***
	Smiltaitė	1001.2	<0.001***
	Šventoji	2245.2	<0.001***
T °C	Blendžiava	763.7	<0.001***
	Smiltaitė	12.3	0.002**
	Šventoji	15.5	<0.001***
$N\text{-}NH_4^+$ mg L^{-1}	Blendžiava	2.0	0.4
	Smiltaitė	11.4	0.003**
	Šventoji	0.2	0.8
$N\text{-}NO_3^-$ mg L^{-1}	Blendžiava	18.7	<0.001***
	Smiltaitė	3.8	0.04*
	Šventoji	32.7	<0.001***
$N\text{-}NO_2^-$ mg L^{-1}	Blendžiava	2.1	0.2
	Smiltaitė	4.1	0.04*
	Šventoji	11.9	0.003**
pH	Blendžiava	0.5	0.6
	Smiltaitė	3.3	<0.001***
	Šventoji	1.8	0.4

with the highest values approaching the end of the incubation period (Figure 21 c) in Blendžiava ($p < 0.001$) and Šventoji (March vs May $p < 0.001$, April vs March/May $p = 0.05$). This is only partially true for Smiltaitė where an appreciable increase was registered only in May (Table 5, $p < 0.01$). For the three watersheds, averages ranged from 2 to 4 °C in March, from 3 to 10 °C in April and from 11 to 14 °C May.

In defining monthly diversity, the second gradient is created by the $N\text{-}NO_3^-$ vector, pointing towards the April cluster. This is in contrast to EC and the slight contribution of $N\text{-}NH_4^+$, which is directed toward the May group. As for the T values, EC also significantly rises for all three watersheds (Table 5, $p < 0.001$), with averages ranging from 210 to 270 μS cm^{-1} in March, from 220 to 360 μS cm^{-1} in April, and from 320 to 450 μS cm^{-1} in May. This trend is partially followed by the N-NH$_4^+$ values, which significantly increased only for the Smiltaitė stream ($p < 0.05$) and peaked at 0.2 mg L^{-1} in May with the advancing of the incubation period (Figure 21 f). Finally, N-NO$_3^-$ presents April averages for the three watersheds ranging from 1 to 1.7 mg L^{-1}. This is higher in respect to the two other months, the average values of which fall below 1 mg L^{-1}. This is significant for Blendžiava and Šventoji ($p < 0.01$, Figure 21 e) in respect to the two other months, but only partially for Smiltaitė in respect to March ($p < 0.05$).

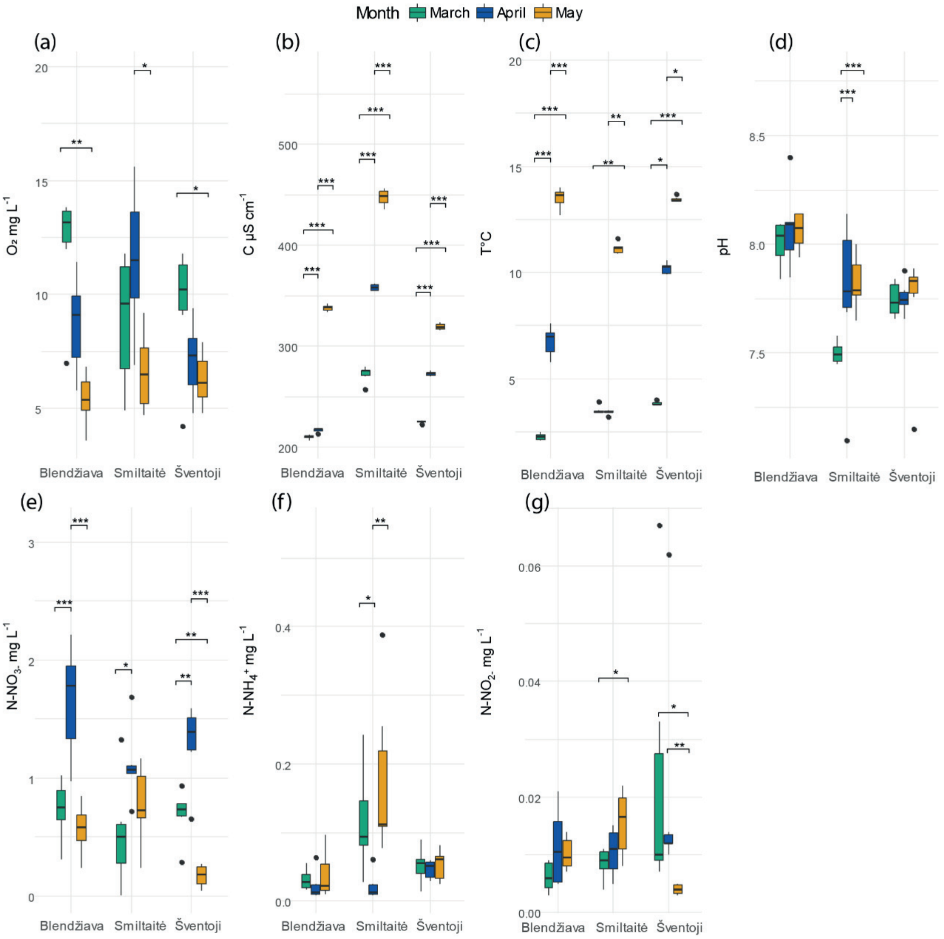

Figure 21. Variations in the 7 intragravel water parameters for each stream across the three sampled months. Post-hoc comparisons used Tukey tests or Dunn's tests if the data followed a normal or non-normal distribution, respectively. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), (Manuscript II, in preparation).

Salmonids rely on cold-water environments and, with regard to the early ontogenetic phases, incubation temperatures should not exceed 11–12 °C to guarantee hatching rates above 50% (Crisp, 2008), while the intragravel oxygen levels should be higher than 8–9 mg L^{-1} (Greig et al., 2007; Malcolm et al., 2003a). As highlighted in the NMDS ordination, surficial and intragravel O_2 values significantly decrease for all three watersheds in the final incubation phases toward the end of April and the beginning of May. The mean ranges from 9 to 12 mg L^{-1} registered in March and from 7 to 11 mg L^{-1} recorded in April can ensure high survival rates of 50%–70% (Crisp, 2008; Greig et al., 2007). However, as the incubation season advances, the mean rates of O_2 concentration sharply drop, with means ranging from 5.4 to 6.6 mg L^{-1} (Figure 22 a). This can severely hinder the post-hatching stages that are still dwelling in the intragravel matrix, which can lead to anything from moderate (25%) to negligible (5%) survival rates (Greig et al., 2007). Although the literature on salmonids presents great variation when it comes to critical thresholds, ranging from 5 (Bjornn et al., 1991) to 10 (Rubin et al., 1996) mg L^{-1}, the majority of authors report 7–8 mg L^{-1} as the critical range below which survival becomes unlikely (Crisp, 1996; Greig et al., 2007; Ingendahl, 2001; Malcolm et al., 2004). This scenario is further worsened by the higher O_2 consumption rates of post-hatching stages (Alderdice et al., 1958).

T values significantly rise among the watersheds from a mean range of 2–4 °C in March to 3–10 °C in April, before peaking between 11 and 13.5 °C in May. The optimal range to ensure a more than 50% survival rate (sensu Crisp, 2008) has been observed around 2–11/12 °C (Burke, 2011; Crisp, 2008; Danner, 2008; Takle et al., 2005). Thus, a concerning situation can be observed in May, with temperatures around and above the upper lethal limit (Figure 22 b). Additionally, a temperature increase of 2–3 °C beyond 10 °C will increase the metabolic rate and oxygen demand by several milligrams (Chapman, 1988; WDOE, 2002), and will affect the O_2 pressure in the microenvironment surrounding the egg by lowering the amount available (Alderdice et al., 1958). However, it should be remembered that in this last period of incubation, eggs have already hatched, and the alevins have the capacity to move and then to avoid unfavourable patches (Cope, 1996; Spoor, 1990), as was already reported for Sockeye salmon sac-fry that display active avoidance due to unfavourable temperatures (Cope, 1996).

Temperature increases during the springtime boost the breakdown of organic matter previously accumulated in winter. In a regime of persistent lean flows such as that which was registered toward the final sampling period there will be a lack of mechanical action removing organic matter. This will lead to DO sags in the intragravel environment since the organic matter breakdown process is DO dependent (Conallin, 2004; Rubin et al., 1996). This situation, if paired with the low hypoxic values reported for this final period, can severely compromise offspring survival. Even if it does not directly kill the eggs, it can result in the retardation of alevin development and lead to smaller body size, which will likely increase the frequency of predation events during the early breeding stages (Dumas et al., 2007; Malcolm et al., 2003a; Massa et al., 2000).

Nitrate values ranged across the months and the watersheds from the detection limit to 1.7 mg L^{-1}. This is indicative of waters from the "high" to "good" ecological status classes related to this nitrogenous compound (Water Framework Directive 2000/60/EC), and these values are much lower than the values from 6 to 8 mg L^{-1} found in the intragravel water of a similar spawning-salmonid stream of comparable size in a similar land use context (Malcolm et al., 2003a). A significant increase was registered during April in the three watersheds, a trend that can be explained by the higher water level conditions after the flooding phase. It has been ascertained elsewhere how nitrogenous inputs can increase with runoff episodes connected to leaching events from agriculture – an exogenous input (Green et al., 2004; Lawlor et al., 2005; Lord et al., 2000; Withers et al., 2002). Also, higher sediment infiltration rates in the hyporheic zone are associated with stagnation episodes which enhance oxidative pathways – increasing the endogenous input of nitrate (Dahm et al., 1998; Greig et al., 2005; Hedin et al., 1998). Nevertheless, even during this sampling event the nitrate mean, which ranged from 1.1 to 1.7 mg L^{-1}, did not directly represent a threat for the incubating eggs as it was under the critical thresholds of 20 and 34 mg L^{-1} that affect the development of cutthroat trout (Oncorhynchus clarkii) and Chinook salmon (Oncorhynchus tshawytscha) fry, respectively (Kincheloe et al., 1979).

EC registered a steady increase for all the three watersheds as the end of the incubation season approached. This is most probably related to the decrease in surficial water flow which followed a period that saw the absence of precipitation. During periods of declined baseflows, the presence of groundwater becomes more prominent, and this can partially explain the EC increase (Malcolm et al., 2004) due to stronger weathering processes and the residence time of this water (Malcolm et al., 2008; Malcolm et al., 2005; Silliman et al., 1993). The shrinkage of the wetted area leads to lower dilution capacity, provoking a rise in the concentration in the pool of suspended and particulate organic matter (Conallin, 2004). This is more evident for Smiltaitė, where a value of 447 ± 7.8 is markedly higher in respect to the Blendžiava (338.0 ± 3.0) and Šventoji (319.5 ± 3.4) values registered during the final sampling period. This situation is confirmed by previous studies evaluating the surficial water quality of these three watersheds (see Chapter 4.1). Higher conductivity values are typical of exploited watersheds in terms of high agricultural and urbanized surfaces (Malcolm et al., 2003a), and this is in line with the higher share of such land features in the Smiltaitė basin (see Chapter 4.1).

The $N\text{-}NH_4^+$ pool into the stream comes from both point (sewage treatment plants and septic tanks) and non-point (agricultural runoff) sources (Brinkman et al., 2009). Like EC, $N\text{-}NH_4^+$ also registers an increase across the sampling period, but this is visible only in the Smiltaitė basin. This is attributable to the lower water discharge in respect to the two other basins (see Chapter 4.1), and at the same time to the increased presence of uncovered soil surfaces due to the greater coverage of agriculture and urban areas, which are more prone to leaching events. Thus, even after a short period without precipitation, the water level in Smiltaitė significantly drops, leading to the concentration of inorganic nutrients and organic matter. The characteristics of this basin can therefore easily increase the N-NH_4^+ pool during lean flow periods. This is not only reflected in higher N-NH_4^+ concentrations in Smiltaitė in respect to Blendžiava and Šventoji, but also in the markedly higher values during lean periods overlapping with the last incubation phases (i.e., the beginning of May). Thus, it is no surprise that, at the end of the incubation period, Smiltaitė reported values from 0.25 to 0.4 mg L^{-1}, pertaining to the "Moderate" WFD classification, along with 0.21–0.6 mg L^{-1} for N-NH_4^+. Of the total N-NH_4^+ pool, the unionized fraction (i.e., ammonia, N-NH_3) represents the real concern as it is capable of penetrating through biological membranes.

Variations in pH and temperature are the main drivers dictating the presence of the unionized fraction, the toxicity of which increases with the increase in T and the reduction of DO (Randall et al., 2002). Given that the average pH ranges from 7.7 to 8 and drawing calculations considering the highest possible temperatures registered during this investigation (14–15.5 °C), the calculated N-NH_3^+ concentrations range from 3 to 5 μL^{-1}, which is far below the toxicity thresholds of 17 (Finn, 2007; Randall et al., 2002) and 21 μL^{-1} (Eddy, 2005) that protect the adult stages in natural environments. The N-NH_3^+ values reported in this study become negligible when considered against the thresholds from 16.8 (Brinkman et al., 2009) to 58 mg L^{-1} (Burkhalter et al., 1977) for the early ontogenetic stages. Indeed, while the embryo is still inside the egg, the sheltering effect of the chorion impedes osmotic processes to a higher extent. This does not occur for hatched fries (i.e. alevin Calamari et al., 1981; Williams et al., 1989), making this stage one of the most vulnerable phases in the lives of teleost fish (Von Westernhagen, 1988). However, it must be remembered that laboratory bioassays miss the conditions faced in a natural environment, minimizing stress and thus underestimating toxic effects (Brinkman et al., 2009; Ip et al., 2001). Indeed, stress conditions (i.e., oxygen shortage, high temperatures) lead to an increase of cortisol levels that in turn increases internal ammonia levels, leading the fish to be more vulnerable to externally present ammonia (Brinkman et al., 2009; Eddy, 2005).

If the presence of N-NH_4^+ cannot directly constitute a threat for incubating embryos, it can be strongly associated with the presence of unprocessed organic matter that can serve as fuel for the growth of bacterial mats, leading to biological clogging (Battin et al., 1999; Chen et al., 1999; Greig et al., 2005) as well as DO depletion episodes (Bjornn et al., 1991; Finn, 2007; Massa et al., 2000).

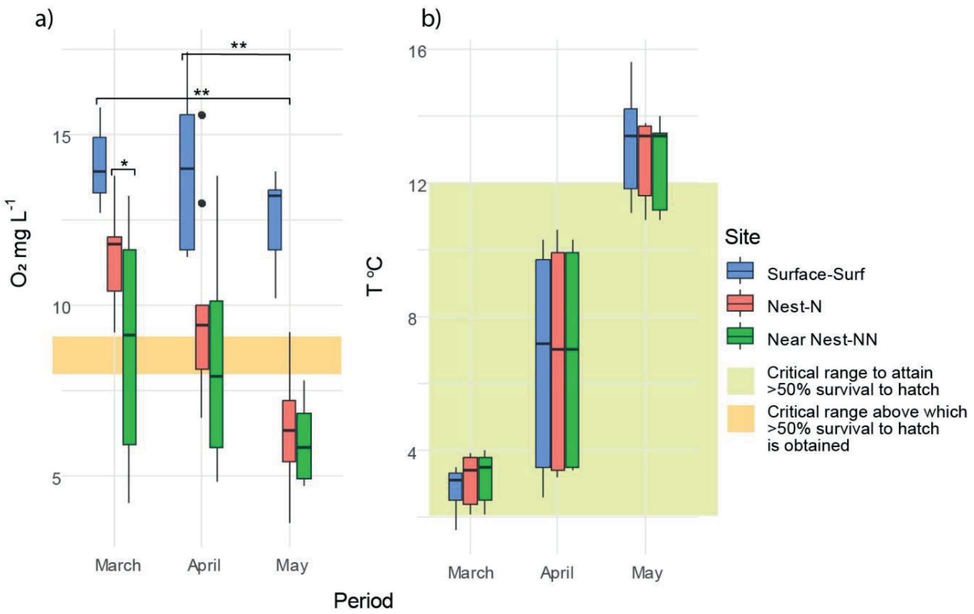

Figure 22. a) Surficial and intragravel DO patterns cumulated for the three watersheds across the three sampled months. Changes across the months are investigated for surficial DO, while changes between Nest and Near Nest are investigated for intragravel DO for each month. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). b) Surficial and intragravel T patterns are cumulated for the three watersheds across the three sampled months. Coloured areas represent the critical range above which, for oxygen, and inside which, for temperature, a > 50% in situ hatching survival rate is ensured (see text for specifications) (Manuscript II, in preparation).

4.2.2. Intragravel and surface patterns

Spatial differentiation in the NMDS ordination is also visible when sampled points are grouped according to the “type” factor (Figure 23 d). Indeed, the global ANOSIM statistic displays a significant difference ($p < 0.001$) for intragravel water samples when contrasted with surficial samples. Among the three watersheds, diversity is mainly driven by the O_2 vector. This was found to be significantly higher (Table 6, $p < 0.001$) for the surficial water samples, which attained average values from 12 to 14 mg L^{-1}, while the intragravel samples averaged from 7 to 10 mg L^{-1} (Figure 23 a). This trend is also partially followed by pH for Blendžiava and Šventoji ($p < 0.05$), where intragravel averages ranged from 7.7 to 8 compared to 7.9 and 8.2 as an average range from the intragravel samples. Additionally, N-NH_4^+ in Smiltaitė presented double the concentration in respect to the intragravel water values, which had averages from 0.12 to 0.14 mg L^{-1} ($p < 0.001$; Figure 23 f).

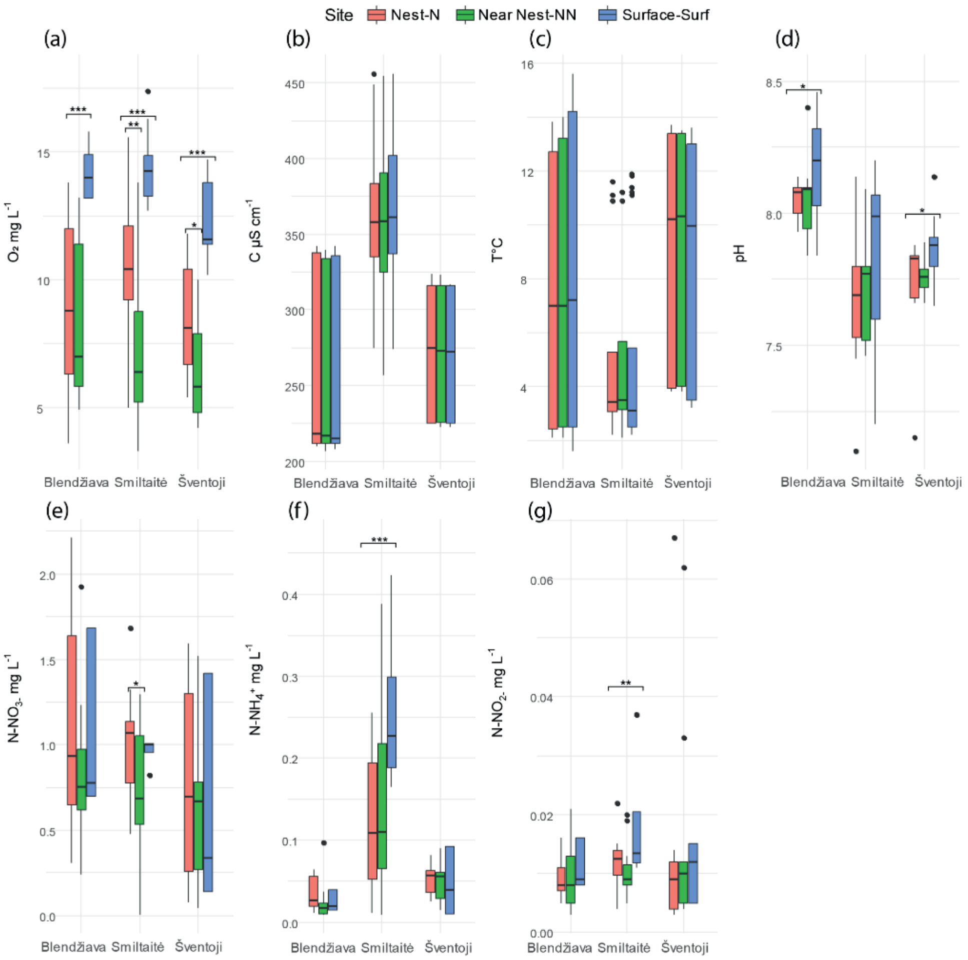

Figure 23. Variation of the 7 intragravel water parameters for each stream according to the sampling position in the nest (near the nest – NN; nest centre – N) and the reference describing the surface water. Data was gathered for the entire incubation period. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (Manuscript II, in preparation).

Table 6. Comparisons between the intragravel water types sampled near the nest (NN) and the in the centre of it (N) using a paired t-test (t) for each stream system with data coupled for the entire incubation period. Paired Wilcoxon tests (T) were employed for non-normally distributed data, while paired Welch t-tests (V) were used for data presenting unequal variance. Unpaired versions of the above tests were used instead to test for differences between the intragravel (coupled samples N + NN) and surface waters (Surf). $p \leq 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (Manuscript II, in preparation).

Variable	Water course	N *vs* NN		Int *vs* Surf	
		t (T)	*p*	*T (V)*	*p*
O_2 mg L^{-1}	Blendžiava	0.2	0.9	15.0	<0.001***
	Smiltaitė	3.4	0.006**	37.5	<0.001***
	Šventoji	2.8	0.02*	22.5	<0.001***
EC µS cm^{-1}	Blendžiava	33	0.2	165.0	0.9
	Smiltaitė	52	0.3	254.5	0.5
	Šventoji	0.9	0.4	183.5	0.5
T °C	Blendžiava	0.9	0.4	140.0	0.5
	Smiltaitė	24.5	0.8	338.5	0.3
	Šventoji	13.0	0.7	192.5	0.3
N-NH_4^+ mg L^{-1}	Blendžiava	31.0	0.4	165.0	0.9
	Smiltaitė	0.5	0.7	96.0	<0.001***
	Šventoji	0.3	0.8	180.0	0.6
N-NO_3^- mg L^{-1}	Blendžiava	1.7	0.1	138.0	0.4
	Smiltaitė	2.8	0.02*	270.0	0.7
	Šventoji	0.9	0.4	180.0	0.6
N-NO_2^- mg L^{-1}	Blendžiava	0.04	1	105.0	0.07
	Smiltaitė	45.5	0.3	144.0	0.003**
	Šventoji	9.5	0.5	129.0	0.3
pH	Blendžiava	0.1	0.9	2.4	0.02*
	Smiltaitė	26.0	0.7	111.0	0.1
	Šventoji	23.0	1.0	82.0	0.01*

It is not surprising that O_2 displays significantly higher values in the surficial water conditions when contrasted with the intragravel (pooled nest and near nest) records; this is in line with other similar investigations (Calles et al., 2007; Malcolm et al., 2003a). Indeed, when passing through the hyporheic zone, the O_2 pool will be likely tapped by all aerobic processes, such as respiration and growth (Cope, 1996; Malcolm et al., 2004), as well as mixing with hypoxic groundwater seeps (Malcolm et al., 2010). This translates into an O_2 differential of several mg L^{-1} when the two sample types are compared (Chapman, 1986; WDOE, 2002). This is why, when applying criteria to determine adequate surficial DO levels for salmonid survival, at least 3 mg L^{-1} should be subtracted when back-calculating the appropriate intragravel DO (Chapman, 1986; WDOE, 2002).

A similar decrease was also registered for $N\text{-}NH_4^+$ values which, for Smiltaitė, halved for the intragravel records in respect to the surficial ones. This pattern can be attributed to the higher $N\text{-}NH_4^+$ concentration found in the surficial waters in Smiltaitė when compared to the other two watersheds due to the land use conditions (see chapter 4.1). This excess can easily be taken up by the growth and metabolism of biofilms dwelling in the hyporheic zone matrix and acting as a sink for organic nutrients (Dahm et al., 1998; Hedin et al., 1998; Triska et al., 1993). A pH decrease was partially reported for Blendžiava and Šventoji when the hyporheic waters were contrasted with their surficial counterparts. This can be explained by the higher retention time in the intragravel environment, leading to the accumulation of biogenic carbon dioxide coming from the oxidative reactions of organic matter decomposition, which were mainly responsible for lowering the pH in the water (Cai et al., 2011; Chevalier, 1984; Greig et al., 2005; Sunda et al., 2012).

The overlapping of points in the NMDS ordination suggests low differentiation in the environmental variables describing these two intragravel water types. This is statistically confirmed by the non-significance of the ANOSIM test applied for the "site" factor ($p > 0.05$), and by the absence in the ISA analysis of any environmental variable that could significantly drive differences between the two intragravel water types. Nevertheless, in the ISA output, the O_2 and $N\text{-}NO_3^-$ variables had p values approaching significance ($p < 0.1$). Indeed, from the univariate tests it appeared that the O_2 concentrations found in the pore water coming from the nest centrum were significantly higher in respect to those coming from the nest periphery (Figure 23 a) for the Smiltaitė (N: 10.5 ± 2.8; NN: 7.3 ± 3.1, $p < 0.01$) and Šventoji (N: 8.6 ± 2.3; NN: 6.6 ± 2.0, $p < 0.05$) basins. Similarly, this also holds true for the significantly higher $N\text{-}NO_3^-$ values in the nest centrum for Smiltaitė (Figure 23 e. N: 1.0 ± 0.3, NN: 0.7 ± 0.4, $p < 0.05$). As the incubation season advanced, differences between the intragravel DO from the nest centrum and periphery began to smooth out: while in March the intragravel DO coming from the nest centrum was significantly higher compared to that coming from the periphery ($p = 0.02$, N: 11.6 ± 1.6, NN: 8.7 ± 3.4), in April (N: 9.7 ± 2.9, NN: 8.5 ± 2.9) and May (N: 6.3 ± 1.6, NN: 5.9 ± 1.2) no significant variation was detected.

Intragravel DO drops consistently from the nest centrum to its periphery, and this is significant for Smiltaitė and Šventoji. This can be explained by the unique structure of the egg pockets and the core part of the nest, in which the majority of eggs are laid down (Chapman, 1988; Rubin et al., 1996). The gravel forming the egg pockets is of a larger size, and the mechanical action of the female while digging the nest ensures low fine content in such places (Chapman, 1988; Rubin et al., 1996). These areas hold higher permeability values in respect to their surroundings, implying more water circulation and thus greater oxygen delivery (Chapman, 1988). It has already been ascertained how a well-connected hyporheic environment can act as a powerful oxidative hotspot for nutrient oxidation (Dahm et al., 1998; Hedin et al., 1998). Therefore, is not surprising that the $N\text{-}NO_3^-$ content coming from the egg pocket area was greater than that coming from the nest periphery. Increases in intragravel DO and $N\text{-}NO_3^-$ from the nest periphery to the nest centrum were more prominent and statistically significant in Smiltaitė, and partially in Šventoji. This is because, in respect to the more pristine situation in Blendžiava, these watersheds suffer from point (Smiltaitė) and non-point (Šventoji) pollution problems (see Chapter 4.1) (Gailiušis et al., 2001). This combination of factors leads to biofilm formation, slower current velocities, and thus a higher likelihood of stagnation episodes. This enhances the differences in percolation between the moved and cleaned gravel areas due to spawning activity, contrasted with the untouched substratum at the nest border.

As incubation time goes on, nests are prone to trap sediments that lodge and cake on the surface, forming a "silt crust" (Hobbs, 1937) that eventually returns the egg pocket environment to how it appeared before the spawning activities (Conallin, 2004; Crisp, 1989), thus homogenizing the conditions with the nest periphery. These processes are confirmed by the similarities in intragravel DO values coming from the nest centrum and the near-nest area during the final incubation phases (i.e., April and May), while in March such values present significant differences (Figure 23 a). This aspect is cross-confirmed by the general decline of intragravel DO and $N\text{-}NO_3^-$ across the incubation period (Figure 23 a, e), a pattern that can highlight an incipient clogging situation in the nest area and thus oxidation processes weakening. The patchy conditions encountered in the redd environment elicit concerns about the real effect of ammonia and the appropriate intragravel DO concentrations, since they can dramatically vary in the microenvironment surrounding the eggs. Indeed, around the egg clusters where high density is registered, the likelihood that ammonia levels will increase is high due to the release of ammonia from the many closely incubated eggs. In parallel, this can create a reduction in intragravel DO due to the higher respiration rates associated with higher egg density. If such a situation is connected to a moment of low waterflow, clogging after a spate event, or the overgrowth of biofilms, this can impede water circulation, leading to stagnation episodes and thus rapid ammonia accumulation and intragravel DO depletion (Crisp, 2008). This aspect is also connected with the practical impossibility of locating the exact egg pocket position and therefore sampling the specific water parcel circulating through the eggs (Chapman, 1988).

4.3. SW-GW interactions and their impact on salmonid spawning and emergence times in three boreal streams

(material in section 4.3 from Manuscript III)

4.3.1. The coupled use of dissolved gases and ions to trace groundwater inputs into nests

In this study area, groundwater inputs were hypothesised to have several positive effects on the eggs, including physical (i.e., temperature), chemical (i.e., oxygen supply), and hydrodynamic (i.e., intragravel water flow) benefits. The presence of groundwater is discussed in respect to similarities in ions and gases between the nest waters and proper groundwater sampled from the wells. The importance of groundwater for the incubating salmonid stages in these systems is therefore discussed. During the two campaigns, the same groundwater level was measured, with 60.99 m asl in December and 60.89 m asl in May, resulting in a similar groundwater input to the rivers (Figure S1). Nevertheless, changes in groundwater heads highlighted the significant recharge of the aquifer between the two sampling campaigns, which is consistent with the rainiest period of the year in this region (Benetti et al., 2024; Čerkasova et al., 2024; Nika, 2011) and may have affected groundwater chemistry. All of the average concentrations (average ± standard deviation, pooled among three riverine systems) of the parameters analysed for the three groups (nests, well, and surface water) are reported in the Supplementary Material as Table S1, S2, and S3. To the author's knowledge, no previous assessments of SW-GW interactions have been carried out in these three systems.

EC, pH, and temperature are classical water parameters employed in the recognition of SW-GW interactions (Fell et al., 2017; Geist et al., 2002; Malcolm et al., 2006; Malcolm et al., 2004). However, surface water intrusion into the hyporheos, seep residence time, and dilution phenomena with surficial water may erase differences in these parameters (Coluccio et al., 2019; Neumann et al., 2016). For example, clay loams dominating these watercourses (Nika, 2011) feature the elevated presence of exchange sites eliciting a pH buffer capacity (Jeon et al., 2019; Kumari et al., 2021). This may have contributed to keeping surficial and hyporheic pH values higher in respect to the groundwater samples. Therefore, it was felt that a mix of physiochemical parameters would best aid in tracing the possible contribution of groundwater in respect to the hyporheic seeps. It has already been proven how the usage of the main water ions (Neumann et al., 2016; Soulsby et al., 2012) and dissolved nutrients (Geist et al., 2007; Sternecker et al., 2013) can be successful in tracking the origin of hyporheic water seeps. Percolation paths through the soil layers and longer retention times allow groundwater to leach minerals (Malcolm et al., 2006; Soulsby et al., 2012) and nutrients (Heppell et al., 2014; Zarnetske et al., 2011). Groundwater samples consistently overlaid the nest samples in the nMDS plots (Figures 24 a and 25 a), highlighting possible similarities. The linear mixed effect models were then able to highlight differences among groups of samples.

During winter, linear mixed effect models revealed a significant difference ($p < 0.05$) among the three water types (nest, surface, well) in terms of temperature, NH_4^+, O_2, and F^- in respect to all the watersheds (Figure 24 b). Pairwise tests were then performed to further investigate differences among each group. Groundwater temperatures appeared to be significantly higher when compared to the nests ($t = 20.8$, $p < 0.001$), and river samples ($t = 21.3$, $p < 0.001$). River and nests temperature values were similar held similar values (Table 7).

Table 7. Mean and standard deviations of the physicochemical variables selected through the linear mixed effect models for winter and spring sampling campaigns individually (Manuscript III, in preparation).

Season	Group	T (°C)	F^- (mg L^{-1})	N-NH$_4$ (mg L^{-1})	O_2 sat (%)	
winter	nests	1.1 ± 0.3	0.4 ± 0.1	0.3 ± 0.4	81.9 ± 46.6	
	river	0.9 ± 0.5	0.3 ± 0.1	0.4 ± 0.5	102.7 ± 4.6	
	groundwater	7.5 ± 1	0.5 ± 0.2	0.2 ± 0.3	54.5 ± 25.8	
		T (°C)	Ar sat (%)	pH	O_2 sat (%)	N_2 sat (%)
spring	nests	14.9 ± 0.8	16.1 ± 1.1	7.4 ± 0.6	68.5 ± 27.6	106.0 ± 4.3
	river	14.7 ± 0.9	14.9 ± 0.8	7.5 ± 0.2	100.7 ± 16.9	103.5 ± 0.7
	groundwater	10.9 ± 3.8	16.4 ± 0.8	6.9 ± 0.4	51.3 ± 32	108.8 ± 4

F^- appeared to be significantly lower in the river samples when compared to the groundwater ($t = 3.3$, $p < 0.01$), whereas similar concentrations were observed between the nests and groundwater samples (Table 7, $t = 2.2$, $p = 0.09$). NH_4^+ and O_2 values were found to be significantly lower for the groundwater samples when compared to the river ($t = 2.9$, $p < 0.05$ for NH_4^+; $t = 3.8$, $p < 0.01$ for O_2), while nest samples were not significantly different in respect to the groundwater and river samples.

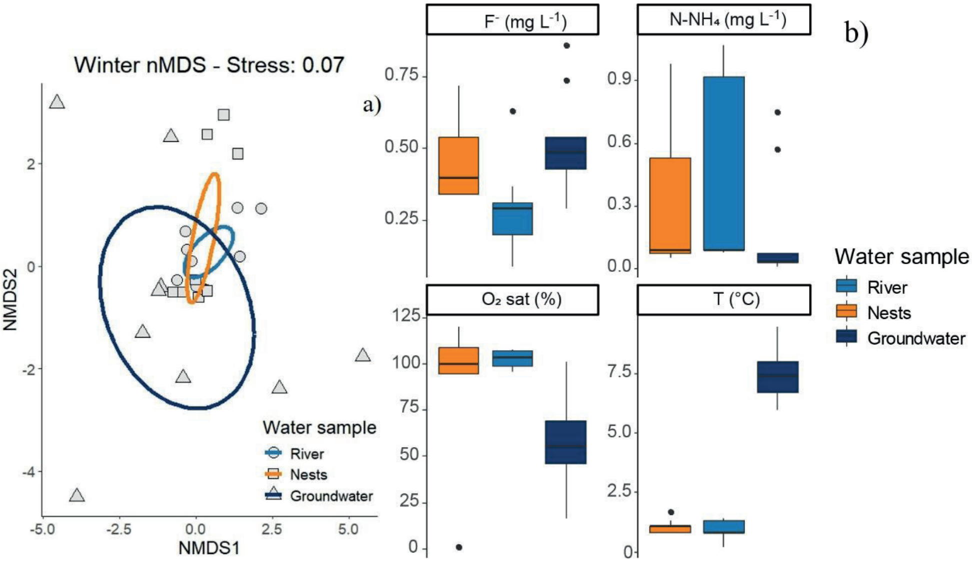

Figure 24. a) nMDS plot describing the spatial relationships among the three water types during winter sampling. b) Boxplots showing the variation of winter physicochemical variables selected through linear mixed effect models. Values are pooled for the three riverine systems – Šventoji, Blendžiava and Smeltalė (Manuscript III, in preparation).

In winter, groundwater and surficial samples displayed different concentrations in terms of NH_4^+ and F^-. However, while F^- appeared to be higher in the groundwater samples, NH_4^+ showed the opposite trend, with groundwater samples holding the lowest concentrations. NH_4^+ results may be explained by the influence of factors such as the manure application period, low water temperatures, and the presence of point sources pollution. First, organic fertilization practices in Lithuania operate from April to mid-November (Ruzgienė, 2015; State Plant Service, 2019), and manure is rich in ammonium which can leak into the aquifer beneath. However, given adequate oxygen content and reasonable time, ammonium is one of the first chemicals to be oxidized in the more recalcitrant nitrate (Klimas et al., 1993; Severini et al., 2022; Severini et al., 2023). Therefore, since fertilization practices occurred several months before the sampling event, the majority of the ammonium pool in the groundwater environment had likely been oxidized into nitrate. This hypothesis is supported by the evidence of an oxidative environment in the wells. Indeed, mean winter oxygen saturations were found to be higher (35%–68%) than the anoxic (0%) and hypoxic (0%–30%) thresholds (Breitburg et al., 2018). Second, the slowing down of biogeochemical processes during the cold season prevents the ammonium pool in the surficial water column from being oxidized (Sternecker et al., 2013). Therefore, ammonium can be transported, unaltered, through the watercourse, as has already been proven in nearby running water environments (Vybernaite-Lubiene et al., 2018). Third, watercourse systems can be affected by ammonium entering via point source pollution such as wastewater treatment plants or domestic ditches not connected to the treatment system (Brinkman et al., 2009; Conallin, 2004). This is especially true for the Smeltalė River, which suffers from point source pollution problems (Nemunas river basin district, 2010).

When nests samples are considered in terms of NH_4^+, they fall in between the surficial and groundwater categories without clear differentiation. This could be explained by the biogeochemical processes happening inside the hyporheic zone. Here, NH_4^+ production can be the result of organic matter decomposition from biofilms (Burke, 2011; Conallin, 2004) and incubating eggs (Crisp, 2008). Additionally, potential mixing with surficial water rich in NH_4^+ locally infiltrated into the hyporheos could have further contributed to the removal of any clear difference between the nests and groundwater samples. In contrast, F^- values were similarly high for the groundwater and nest samples in respect to the surface areas, suggesting the effective contribution of groundwater to the spawning area. F^- has already been proven to be a conservative tracer: namely F^- concentration will likely remain unaltered through short percolation paths (Brunt et al., 2004; Lin et al., 2013; Martinez et al., 2024). F^- is usually more abundant in groundwater since the long residence time allows for longer interactions with potential sources (Bove et al., 2009; Brunt et al., 2004). Additionally, F^- is unlikely to be employed in most of the common biogeochemical reactions inside the hyporheos since it has well-established bacteriostatic (Pandey, 2024; Zhang et al., 2019; Zhu et al., 2022) and phytotoxic (Katiyar et al., 2020) effects.

In spring, linear mixed effect models again revealed a significant difference ($p < 0.05$) among the three water groups, but the parameters shaping these differences changed with respect to winter. Differences among groups were driven by temperature, pH, O_2, N_2, and Ar (Figure 25 b). Groundwater temperatures and pH values appeared to be significantly lower when compared to the nests ($t = 3.6$, $p < 0.01$ for temperature; $t = 2.9$, $p < 0.05$ for pH) and river samples ($t = 3.4$, $p < 0.01$ for temperature; $t = 3.3$, $p < 0.01$ for pH), which held similar values between each other (Table 7). Groundwater samples held significantly higher N_2 saturations ($t = 3.3$, $p < 0.01$) when contrasted with the river samples, whereas nests had intermediate concentrations. Finally, nests and groundwater showed similarities in some parameters, which were also statistically different from surface water samples. The O_2 saturation in river samples was higher (Table 7) than in nests ($t = 2.8$, $p < 0.05$) and groundwater samples ($t = 4.2$, $p < 0.01$). Similarly, Ar concentrations in river samples were lower than those in groundwater ($t = 4.1$, $p < 0.01$) and nests ($t = 3.1$, $p < 0.05$).

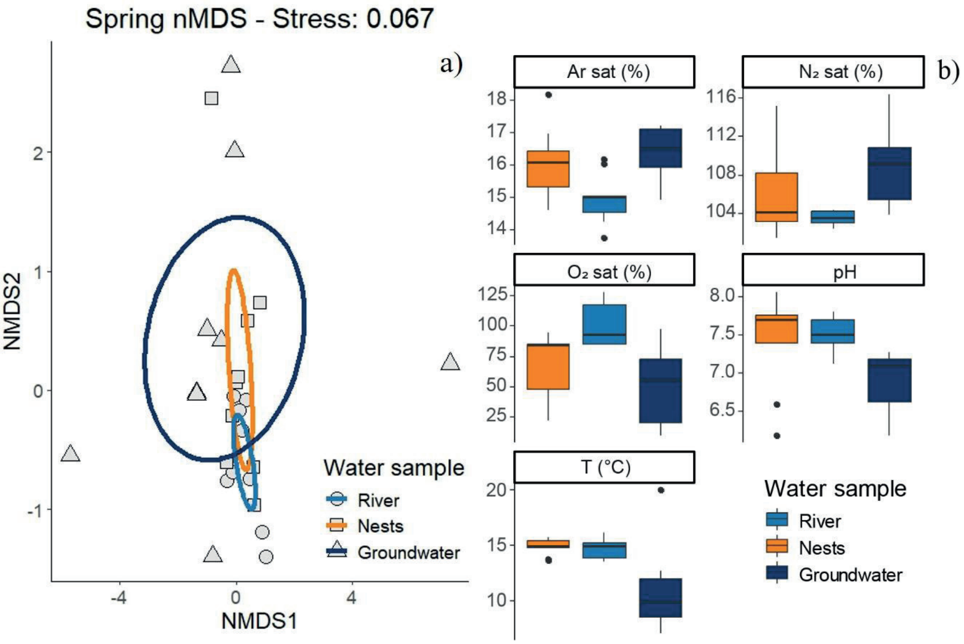

Figure 25. a) nMDS plot describing the spatial relationships among the three water types during spring sampling. b) Boxplots showing the variation of spring physicochemical variables selected through linear mixed effect models. Values are pooled for the three riverine systems – Šventoji, Blendžiava and Smeltalė (Manuscript III, in preparation).

Spring sampling was performed after a consistent period of aquifer recharge (Figure S1). During this phase, groundwater flow increased and the lower retention time diluted the ions present in the groundwater (García-Menéndez et al., 2021; Mehner, 2009; Whittemore et al., 1989). These factors can explain why linear mixed effect models selected none of the ions during spring sampling. However, gas measurements were also included in the dataset, as they have been revealed to be a useful tool in detecting streambed connectivity (Heilweil et al., 2016) and groundwater-gaining zone entities (Brunner et al., 2017; Jurado et al., 2018). Confinement in respect to the atmosphere leads to the accumulation of inert gases like Ar, which, due to its chemically inert nature, does not undergo oxidation and reduction processes (Heaton, 1981; Stute et al., 2000). Also, anaerobic conditions in these environments can lead to denitrification processes with the development of N_2 (Jurado et al., 2018). This is confirmed, in spring, by higher groundwater Ar and N_2 saturations in respect to the surface. However, N_2 nest samples held an intermediate position between those belonging to the groundwater and river. This may be explained by denitrification processes happening not only in the underlying aquifer, but also in the hyporheic zone belonging to the nest area. In other words, salmonid nests can act as an N_2 source for the watercourse. After the construction phase, the nest acts as a sediment trap (Conallin, 2004), where fines cake and consequently penetrate the intragravel environment (Crisp, 1989; Hobbs, 1937). Towards the end of the incubation period, the nest will likely present the highest silt content, with the condition of the bed as it was prior to spawning activity (Lisle et al., 1992; McHenry et al., 1994).

In the study area, previous assessments highlighted critical levels of fine sediments (<2mm) in the nests (Nika, 2011). Additionally, spawning grounds in such systems are vulnerable to siltation events following intense rainfall (Benetti et al., 2024). It is apparent that in aged nests the extent of clogging can hamper water circulation (O'CONNOR et al., 1998; Peterson et al., 1996). This can lead to the formation of hypoxic patches where nitrate is reduced into N_2 (Harvey et al., 2013; Kessler et al., 2012; Strong et al., 2002), a situation already detected in boreal streams (Pinay et al., 2003; Pinay et al., 2009). It should also be noted that the likelihood of denitrification reactions will increase following the spring temperature increase (Bartoli et al., 2012; Bartoli et al., 2021). In parallel, incipient clogging conditions can be coupled with a hyporheic O_2 decrease (Harvey et al., 2013; Kessler et al., 2012; Strong et al., 2002). Interestingly, hyporheic O_2 depletion is not reported in winter, where the freshly excavated nest environment allows elevated surface water intrusion and proper seep mixing. This creates a situation in which the hyporheic O_2 values fell in between the surficial and groundwater categories in winter. On the other hand, this would explain why the spring O_2 values were similar between the nests and groundwater samples but were clearly distinguished from the surface water. Thus, similarities between the nest and groundwater O_2 and N_2 samples in spring would represent the sum of groundwater seeps naturally depleted in O_2 and enriched in N_2, with the local oxidation and denitrification processes happening in a hyporheos with a low level of permeability.

4.3.2. The ecological effects of groundwater inputs on salmonid nests

At boreal latitudes, one of the main advantages offered by stretches that gain groundwater is the stable thermal regime (Mouw et al., 2014; Zimmerman et al., 2012). During the coldest periods, this can prevent the formation of anchor ice and related egg freezing (Baxter et al., 2000; Bjornn et al., 1991). Also, throughout the incubation season, stable temperatures guarantee the steady accumulation of degree days which are connected to optimal fry emergence time (Fell et al., 2017; Lorenz et al., 1989). However, within the scope of this appraisal, the groundwater displayed a thermal regime apparently disconnected from that occurring in the nests. In winter, groundwater samples were marked by higher temperatures in respect to the river and nests samples, which showed similar values. In spring, the groundwater displayed significantly lower temperatures in respect to the nests and river samples, which were both similar.

The confinement and related isolation of groundwater from the atmosphere cause it to hold a stable temperature trend over the year (Burke, 2011; Cope, 1996). This translates into higher temperatures during the winter months contrasted to lower temperatures during the spring period when compared to the surface water (Fell et al., 2017; Malcolm et al., 2004; Neumann et al., 2016). However, pairwise comparisons on the temperature linear mixed effect model were not able to detect any significant differences between surface and nest samples. Many studies which have dealt with the utilization of groundwater patches by spawning salmonids have employed temperature data to detect the presence of stretches of water that benefit from upwelling (Garrett et al., 1998; Geist et al., 2002; Lorenz et al., 1989). However, according to the areas where groundwater seeps into the river stretch, it is not always possible to rely on temperature data to determine the presence of groundwater (Irvine et al., 2017; Neumann et al., 2016). Even though groundwater intrusion can be consistent, dilution effects may have masked temperature differences (Coluccio et al., 2019; Johnson, 2003). Additionally, continuously analyses are generally recommended to detect temperature differentials (Irvine et al., 2017). However, as first outlined in the NMDS plots and secondly confirmed through the linear mixed effect models, the presence of groundwater inside the hyporheic zone can still be recognized through the two sampling occasions. This is linked to trace ions like F^- and the chemically inert Ar gas, as previously discussed. The temporal limitations of the sampling effort may be another aspect responsible for missing temperature differences. The use of only two sampling occasions may not be sufficient to judge thermal fluctuations across the entire salmonid incubation period (Garrett et al., 1998; Lorenz et al., 1989), which in these systems can last up to 6 months (Nika, 2011). Additionally, day-night variability (Malcolm et al., 2004) was not accounted for.

The issuing of groundwater from the riverbed adds a vertical flow dimension, the results of which are beneficial for the improvement of intragravel circulation (Casas-Mulet et al., 2015; Mouw et al., 2014). This translates into higher oxygen delivery rates if seeps hold a low residence time (Geist et al., 2002) and percolation paths happen through an inorganic matrix (Malcolm et al., 2004). In this investigation, oxygen content in the wells was revealed to always be lower than at the surface. This may be explained by the oxidation processes occurring in a shallow aquifer matrix where water percolates through organic-rich layers (Severini et al., 2023). Intensively cultivated lands (Benetti et al., 2024) and the spread of related non-point source pollution in these systems (Nemunas river basin district, 2010) may have aided the oxygen sequestration of percolating water across the first soil layer (Severini et al., 2023). Given the alimentation of groundwater, it is not surprising that O_2 nest values fall in between the surface and groundwater categories in winter. However, the extent of this similarity will vary accordingly to hyporheic connectivity and the aquifer recharge phase, as previously discussed. Overall, nests experience O_2 saturations of 82% in winter and 68% in spring, which would not constitute a constraint for the developing larvae assuming a mean of 50% saturation as a critical threshold when calculated in similar environments (Malcolm et al., 2010).

Besides O_2 provision, augmented intragravel currents aid in the faster removal and dilution of waste substances coming from the metabolism of the hyporheic matrix (Bjornn et al., 1991) and incubating eggs (Crisp, 2008). The presence of upwelling seeps has also been proven to have a desilting and loosening effect inside the redd environment (Casas-Mulet et al., 2015; Mouw et al., 2014). This enhances the movement of larvae into the intragravel matrix, facilitating swim-up during the emergence phase (Zimmerman et al., 2012). It is apparent that in systems subjected to high siltation events, either for their intrinsic nature (Nika, 2011) or due to ongoing land use changes (Benetti et al., 2024), the presence of patches of upwelling groundwater can undoubtedly relieve clogging situations. This becomes even more critical during the fry emergence phase, where the extent of clogging is at its maximum due to the accumulation of sediment throughout the incubation period.

4.4. Salmonid nests under laboratory conditions

(material in section 4.4, from Paper II)

A pilot investigation into the functioning of simulated salmonid egg pockets in terms of O_2 demand and ammonium and nitrate net fluxes was carried out. According to the wider literature, this is the first attempt in which fluxes from reconstructed salmonid egg pockets have been measured in an intact substrate setting. Biogeochemical processes are traditionally studied with mesocosm systems in a static water environment, presenting a clear air-water-sediment interface (Benelli et al., 2021; Naldi et al., 2020; Nika et al., 2021). The innovation of this study was to adapt these tools to characterize the free-flowing conditions occurring in a lithophilic fish nest – namely, a parcel of the hyporheic zone. In terms of O_2 demand, the increase in the cores (mesocosms) follows a trend that parallels that which observed the chambers (microcosms). This is because of the increase in metabolic intensity with the advancement of the developmental stage. Additionally, biofilm growth increases respiration in the cores, becoming predominant in the final phase of incubation, as highlighted by the bare cores (control).

Overall, reconstructed nests acted as an ammonium source, reflecting the increase in metabolic excretion of larvae in the final stages. Nevertheless, as incubation progresses, the cores become ammonium sinks, showing a contrasting trend in respect to the eggs alone. This is because of the formation of biofilm which consumes the nutrients needed for their growth. The same explanation can also be used to justify the increase in nitrate removal if it is assumed that the formation of hypoxic spots led to denitrification.

4.4.1. Microcosms

The Kruskal-Wallis test indicated that the fish stage significantly affected DO demand (H(2) = 31, $p < 0.001$) and N-NH_4^+ excretion (H(2) = 30, $p < 0.001$) (Figure 26). Pairwise comparisons using Dunn's test showed that DO demand (mg O_2 egg h^{-1}) in the eyed eggs (294–310 dd) was 0.002 ± 0.0004 mg O_2 egg^{-1} h^{-1} – significantly lower (p = 0.001) than the nearly hatched alevins (350–358 dd), which respired at 0.016 ± 0.003 mg O_2 egg^{-1} h^{-1}. In turn, the stage with completely absorbed yolk sacks (454–462 dd) had a DO demand of 0.07 ± 0.007 mg O_2 egg^{-1} h^{-1}, itself significantly higher (p = 0.03) than the previous stage. This marked increase of metabolic activity from the embryo to the larval stage is not surprising since it was earlier observed by Wickett (1954) for pink (O. gorbuscha) and coho (O. kisutch) salmon reared at 8 and 5 °C, respectively. Wickett (1954) reported metabolic rates for eyed eggs ranging from 0.0006 to 0.0002 mg O_2 egg^{-1} h^{-1}, sharply increasing by one order of magnitude (0.009–0.01 mg O_2 egg^{-1} h^{-1}) for newly hatched alevins. Further evidence comes from Alderdice et al. (1958), who found that freshly fertilized chum (O. keta) salmon eggs at 10 °C were respiring at 0.00093 mg O_2 egg^{-1} h^{-1}, while hatching larvae respiration peaked at 0.0052 mg O_2 egg^{-1} h^{-1}. Rombough (1988) found that the metabolic rates of pooled rainbow trout embryos and alevins ranged from 0.028 to 0.078 mg O_2 egg^{-1} h^{-1} when reared at 6 and 15 °C, respectively. The triploid stages of the same species presented a similar trend, spanning from 0.001 (eyed eggs), to 0.004 (hatched alevins), to 0.03 mg O_2 $egg^{-1}h^{-1}$ (yolk sack absorbed) (Oliva-Teles et al., 1987). Finally, for Atlantic salmon, DO demand ranged from 0.0067 (Lindroth, 1942) to 0.0048 mg O_2 egg^{-1} h^{-1} (Hayes et al., 1951) for hatching larvae under temperatures of 17 and 10 °C, respectively.

The variability in DO fluxes is related to different ontogenetic phases, reflecting different mass and metabolic activity which, if not clearly highlighted, prevents meaningful comparisons among different works (Rombough, 1988; Smith, 1947). Metabolic rates should be compared when eggs belong to the same stage and when they are exposed to the same level of stress – something that is not guaranteed given the different scopes of studies. Indeed, it is well known that activity and stress, though not easily quantifiable or controllable, can greatly affect the baseline metabolic rates attained under ideal conditions (Fry, 1957; Fry, 1971). For this reason, the higher variability of DO respiration rates among alevins, especially those in the last batch, can be attributed to the larger size and increased mobility of the larvae in the restrained closed respirometry chambers with respect to the embryos. Similar problems arise when burrowing macrofauna or larvae are incubated in the absence of sediments, where they feel uncomfortable and try to dig across the glass walls of the incubation chambers leading to unrealistic, overestimated metabolic activity, largely exceeding the baseline respiration rate measured when they are within sediments (Nika et al., 2021). Other sources of variability can be attributed to the use in some works (Alderdice et al., 1958; Rombough, 1988) of mass units instead of egg units, leading to an underestimation of metabolic rates if the inactive mass of the yolk sack is considered in the calculations. This is especially true for the early stages, when the inactive mass of the yolk sack can represent a major proportion of the mass of the embryo. However, for those works that report metabolic activity in relation to the whole embryo, inter-genera differences in egg size (Burgner, 1991; Healey, 1991; Rubin et al., 1996) can explain the variability among respiration rates. Finally, different incubation temperatures inevitably affect metabolic rates and thus DO demands; for example, Atlantic salmon eggs incubated at 17 and 5 °C nearly halved their metabolic activity at the hatching stage, where it shifted from 0.0067 to 0.0039 mg O_2 egg^{-1} h^{-1} (Lindroth, 1942).

Ammonium excretion at the eyed stage averaged 0.23 ± 0.07 µg N-NH_4^+ egg h^{-1}, being substantially lower ($p = 0.001$) than the 0.69 ± 0.20 µg N-NH_4^+ egg^{-1} h^{-1} of the alevins with yolk sacks. This, in turn, differed significantly from the excretion of the stage with completely absorbed yolk sacks (3.60 ± 2.0 µg N-NH_4^+ egg^{-1} h^{-1}, $p = 0.04$). Increases in metabolic rates, following the ongoing developmental process and the increase of biomass, explain the increased excretion rate in the later stages (Wright et al., 2001). It is therefore speculated that the variability among rates can be justified by the same reasons discussed earlier for DO fluxes, which are related to experimental conditions, size, and stress increases along with the different stages. Smith (1947) was a pioneer in evaluating N-NH_4^+ excretion rates from rainbow trout eggs, with observations ranging from 0.1 for early stages to 0.7 µg N-NH_4^+ egg^{-1} h^{-1} for alevins. Such a range of values includes the excretion rate reported for the pre-hatching stages of the same species by Noronha et al. (Rahaman-Noronha et al., 1996) (0.16 µg N-NH_4^+ egg^{-1} h^{-1}). Oliva-Teles et al. (1987), incubating diploid and triploid rainbow trout eggs, measured N-NH_4^+ excretion rates from 0.08 (early eyed) to 0.25 (hatching phase), to a peak of 1.5 µg N-NH_4^+ egg^{-1} h^{-1} (eggs with exhausted yolk sacks). Besides the aforementioned factors dictating diversity in DO respiration activity, an additional variable for N-NH_4^+ is pH changes. Indeed, N-NH_4^+ excretion is primarily driven by the diffusion of the unionized ammonia form (N-NH_3) owing to a partial pressure gradient, while the ionized ammonium form (NH_4^+) cannot diffuse across the almost impermeable chorion (Rahaman-Noronha et al., 1996). Due to its pK of 9.5, NH3 will more readily diffuse into acidic compartments (Pitts, 1973), and this can double the excretion rates from eggs incubated at pH 10 when they are incubated at lower pH values (e.g., 6 to 8) (Rahaman-Noronha et al., 1996).

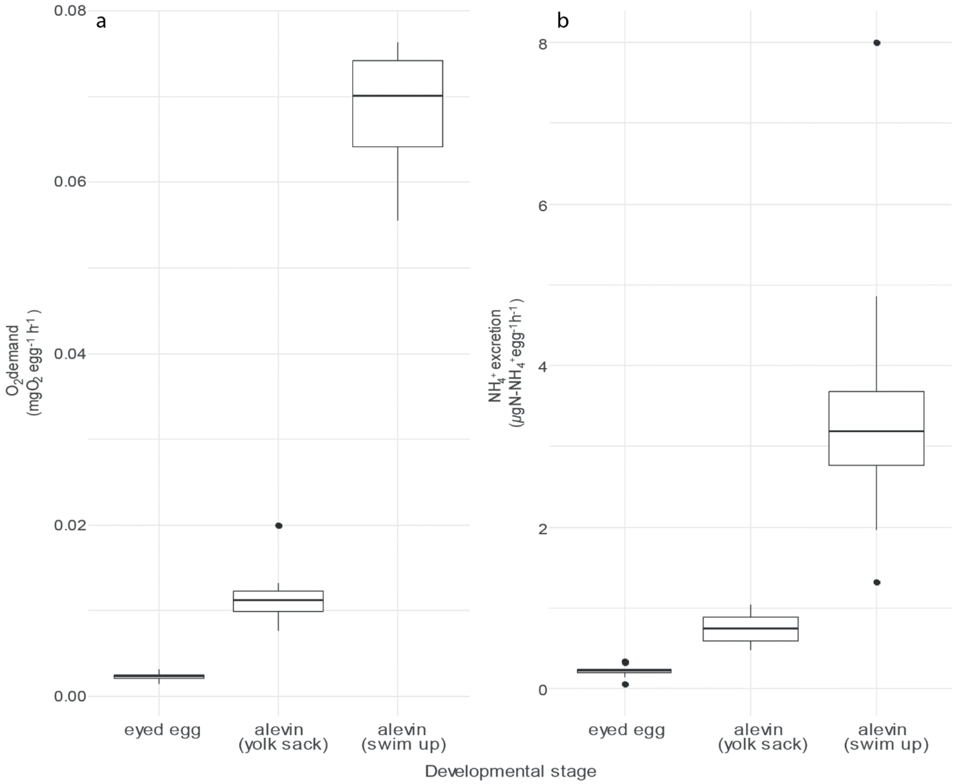

Figure 26. Boxplot portraying (a) DO demand (mg O_2 egg^{-1} h^{-1}) and (b) N-NH_4^+ excretion rates (µg N-NH_4^+ egg^{-1} h^{-1}) of rainbow trout eggs at the three main developmental stages: eyed egg (294–310 dd), nearly hatched alevin with yolk sack (350–358 dd), and prior to emergence time (swim up, 454–462 dd). The three egg stages were incubated in closed glass chambers (see text for more details) (reprinted from Paper II).

4.4.2. Mesocosms

4.4.2.1. Setups

With respect to the comparison of L and L+D treatments, the respiration, net ammonium, and nitrate fluxes of the simulated egg pockets during both the egg and alevin stages were not significantly different (for DO fluxes: $Z_{egg} = 192$, $p = 0.36$; $Z_{alevin} = 212$, $p = 0.83$; for N-NH_4^+ fluxes: $Z_{egg} = 75$, $p = 0.86$; $Z_{alevin} = 92$, $p = 0.25$; for N-NO_3^- fluxes: $Z_{egg} = 75$, $p = 0.89$; $Z_{alevin} = 90$, $p = 0.32$). This result can be justified by the "net" nature of the fluxes measured in the gravel cores, integrating the metabolic activity of live eggs, the heterotrophic activity of microbes growing within the substratum matrix or decomposing dead eggs, and the different levels of mortality in the different mesocosms. The similarity of the respiration and ammonium fluxes in the two treatments (L and L+D) may therefore result from the higher metabolic activity of eggs in treatment L and the higher microbial contribution to the total mesocosm metabolism in treatment L+D.

Nevertheless, during both stages, DO demand and N-NH_4^+ fluxes tended to be higher in the L (DO demand$_{egg}$ = 6.7 ± 4.6 mg O_2 mecocosms^{-1} h^{-1}; N-NH_4^+ fluxes$_{egg}$ = 0.12 ± 0.13 mg N-NH_4^+ mecocosms^{-1} h^{-1}; DO demand$_{alevin}$ = 11.1 ± 2.4 mg O_2 mecocosms^{-1} h^{-1}; N-NH_4^+ fluxes$_{alevin}$ = −0.16 ± 0.07 mg N-NH_4^+ mecocosms^{-1} h^{-1}) than the L+D (DO demand$_{egg}$ = 5.3 ± 4.2 mg O_2 mecocosms^{-1} h^{-1}; N-NH_4^+ fluxes$_{egg}$ = 0.08 ± 0.11 mg N-NH_4^+ mecocosms^{-1} h^{-1}; DO demand$_{alevin}$ = 11 ± 2.2 mg O_2 mecocosms^{-1} h^{-1}; N-NH_4^+ fluxes$_{alevin}$ = −0.25 ± 0.2 mg N-NH_4^+ mecocosms^{-1} h^{-1}) setup. It is speculated that the higher number of live eggs and associated metabolic rates in the L setup, together with the undeveloped biofilm at the beginning of the incubation process, were the main causes of such differences.

Nitrate fluxes showed a rather erratic trend, with a tendency toward higher values during the egg stage for the L+D setup (N-NO_3^- fluxes$_L$ = −0.6 ± 3.0 mg N-NO_3^- mecocosms^{-1} h^{-1}; N-NO_3^- fluxes$_{L+D}$ = −0.1 ± 5.0 mg N-NO_3^- mecocosms^{-1} h^{-1};). However, this trend was reversed during the alevin phase, when N-NO_3^- fluxes tended to be higher for the L setup (N-NO_3^- fluxes$_L$ = −0.1 ± 6.3 mg N-NO_3^- mecocosms^{-1} h^{-1}; N-NO_3^- fluxes$_{L+D}$= −3.0 ± 3.4 mg N-NO_3^- mecocosms^{-1} h^{-1}).

Nitrate fluxes were mostly negative, suggesting the consumption of this oxidized ion, even if they were characterised by large variability. It is speculated that hypoxic pockets forming around dead eggs collated with biofilm mass may act as local sinks for electron acceptors, depleting DO and promoting nitrogen loss via denitrification (Burke, 2011; Greig et al., 2005).

Reconstructed trout egg pockets generally acted as a moderate N-NH_4^+ source, although not in a statistically significant manner ($p > 0.05$). Indeed, data pooled from the whole incubation period presented concentrations ranging from 0.290 ± 0.015 to 0.310 ± 0.003 mg L^{-1} in the input and output sections, respectively. In contrast, they tended to act as a sink for N-NO_3^-, with concentrations at the inflow (0.16 ± 0.02 mg L^{-1}) decreasing to 0.130 ± 0.002 mg L^{-1} at the outflow in a statistically significant manner ($p = 0.03$). Though evidence (Burke, 2011; Kincheloe et al., 1979; McGurk et al., 2006) points to N-NO_3^- as a harmful molecule for the early ontogenetic stages of salmonids, the present levels are far below thresholds of 20 and 34 mgL-1 proved to be detrimental for species belonging to the same genus as those studied here, such as cutthroat trout (Oncorhynchus clarkii) and Chinook salmon (Oncorhynchus tshawytscha) fry (Kincheloe et al., 1979).

4.4.2.2. Survival and substratum composition

Oxygen concentrations measured at the gravel core outflow averaged 9.7 ± 0.3 mg L^{-1}, a value which is considered highly protective for the intragravel stage under natural conditions (Greig et al., 2007; Malcolm et al., 2004). However, at the beginning of the experiment, transient, lower concentrations (6.6 mg L^{-1}) were recorded, coinciding with the clogging of some tubes of the peristaltic pump due to the accumulation of fine material exported from the cores. These tubes were promptly cleaned, ensuring homogeneous water flow, and similar episodes did not occur during the remainder of the experiment.

It cannot be excluded that this initial shortcoming, resulting in the transient decrease of water velocity within the simulated egg pockets, could partially explain the variable survival rates of the eggs (6% to 64%) in comparison with the control trays (99 ± 1%). Indeed, it was previously noticed that swim up from natural redds, following successful incubation, occurred at DO values around 7 (Dumas et al., 2007; Ingendahl, 2001), even for those with a sediment composition similar to the one used here (Nika, 2011). Additionally, in these same environments flow velocities lower than 500–600 cm/hr severely compromised survival chances (Nika, 2011). Although the period of reduced velocity and DO levels lasted only few hours in the present experiment, multiple sources (Greig et al., 2007; Malcolm et al., 2003a; Malcolm et al., 2004) suggest that even transient periods of hypoxia can weaken the fitness of embryos and lead to post-hatching mortality.

Another explanation for poor survival can be found in fines (<2 mm) content, which ranged from 14% to 29%, and silt (<63 μm) content, which varied from 0.16% to 0.38%. Indeed, the literature on salmonids already considers 15%–18% fine content to be unsuitable for egg survival (Chapman, 1988; Crisp, 2008; Greig et al., 2005; Pulg et al., 2013).

Overall, there was no significant difference between the two setups in terms of fines ($t(4) = -0.76$, $p = 0.49$) or silt ($t(4) = -0.28$, $p = 0.79$), despite the fact that the L setup showed a lower amount of such fine fractions (<2 mm = 20 ± 6.3; <63 μm = 0.2 ± 0.07) than the L+D setup (<2 mm = 23 ± 5 < 63 μm = 0.24 ± 0.13). This slight discrepancy can partially support the answer to the question of why the L setup presented significantly higher ($t(4) = 3.64$, $p = 0.02$) survival percentages than the L+D setup ($\text{survival}_{\text{live}}$: 51% ± 11%; $\text{survival}_{\text{live+dead}}$: 11% ± 6%), assuming that variations of even a few percent in these fractions can dramatically decrease incubation success (Casas-Mulet et al., 2015; Lapointe et al., 2004).

Additionally, it must be remembered that half of the eggs in the L+D setup were dead: decaying embryos constitute an additional DO sink, acting also as a source of toxic nitrogen compounds (Chevalier, 1984; Eddy, 2005) such as NH3. Moreover, dead eggs can represent a fuel for algae and bacterial mats, which can synergistically act with fines to cleave the intragravel matrix and thus prevent the proper respiration or upward movement of the embryos and alevins (Greig et al., 2005). These speculations are supported by the fact that during the opening procedure no alevins were recorded on the top layer of the cores, suggesting that clogged interstices prevented swim up (Chapman, 1988; Ingendahl, 2001). Such hypoxic bags lead to a cascade effect, since larvae excrete but accumulate $N\text{-}NH_4^+$ and other waste substances in these clogged spaces, provoking detrimental conditions and egg death in the long run (Battin et al., 1999; Battin et al., 2003; Chen et al., 1999; Chevalier, 1984). It is widely recognised that biological membranes, like the egg chorion, are highly permeable to the unionized form ($N\text{-}NH_3$), which constitutes the real threat of ammonia+ammonium pooling ($NH_3 + NH_4^+$) and which increases with a more basic pH (Finn, 2007; Rahaman-Noronha et al., 1996; Wright et al., 2001). However, according to the pH value measured in the aquarium (nearly 7.4 units), the $N\text{-}NH_3$ quantity was 1.2 µg L^{-1} – far below the toxicity level (96h LC50) of 17 µg L^{-1} quantified for salmonids (Eddy, 2005; Finn, 2007). Nonetheless, it is likely that conglomerations of fine particles aided by the presence of biofilm led to uneven waterflow and O_2 distribution, as well as hindering the removal of metabolic waste (Battin et al., 1999; Burke, 2011). This is in spite of the fact that O_2 and ammonium levels at the outflowing sections were within the safety ranges. This hypothesis remains unproven, as it would necessitate the reconstruction of O_2 distribution and flow maps inside the simulated egg pockets at a high spatial scale (Cardenas et al., 2016).

4.4.2.3. Stages

From a comparative perspective, DO demand changed significantly across egg stages ($Z = 276$, $p < 0.001$), increasing from 6.0 ± 4.4 mg O_2 mesocosm^{-1} h^{-1} for the eyed eggs to 11.0 ± 2.3 mg O_2 mesocosm^{-1} h^{-1} for the alevin stage (Figure 27). This increase is partially explained by the augmented metabolic rates during embryo development (see comments on the results of the microcosm experiment), and by the metabolic activity of growing microbial biomass within the substratum. Indeed, DO demand from the bare gravel re-incubated without eggs was 8.6 ± 1.3 mg O_2 mesocosm^{-1} h^{-1}. Although not directly comparable with the entire series, this accounted for 78% of the total respiration of the mesocosm during the final period of incubation.

Ammonium fluxes changed markedly along the course of the experiment ($t(45) = 8$, $p < 0.001$), decreasing form 0.10 ± 0.12 mg $N\text{-}NH_4^+$ mesocosm^{-1} h^{-1} at the eyed egg stage to −0.21 ± 0.14 mg $N\text{-}NH_4^+$ mesocosm^{-1} h^{-1} at the alevin stage. Although the excretion of nitrogenous wastes is also expected to increase along with embryo development, it is speculated that other processes consuming the produced ammonium can contrast its net release outside the system. These processes can include microbial uptake or nitrification. As $N\text{-}NH_4^+$ excretion averaged −0.090 ± 0.014 mg $N\text{-}NH_4^+$ mesocosm^{-1} h^{-1} when only bare gravel was incubated, it is speculated that the presence of live or dead eggs, together with their biomass supporting heterotrophic activity or their excretion supporting nitrification, contributed to large microbial growth in the proximity of the egg pocket (Burke, 2011; Greig et al., 2005).

Nitrate followed a similar and significant trend ($Z = 389$, $p < 0.04$), with higher fluxes measured for the eyed egg stage of −0.4 ± 3.9 mg N-NO$_3^-$ mesocosm^{-1} h^{-1} as compared to −1.5 ± 5.2 mg N-NO$_3^-$ mesocosm^{-1} h^{-1} measured at the alevin stage. This suggests that the incorporation of nitrates into microbial biomass or denitrification processes was higher during the final phase of incubation, when maximum biofilm growth and the presence of hypoxic or anoxic microzones within the mesocosms was hypothesized. In natural environments, anaerobic-aerobic interfaces in the hyporheic zone act as powerful hotspots for oxidative and reductive processes (Dahm et al., 1998; Hedin et al., 1998). Thus, an ammonium pool that is successfully oxidized into nitrate in the oxic matrix can fuel denitrification processes in such hypoxic niches. Net nitrate fluxes in the control cores decreased to −1.15 ± 0.85 mg N-NO$_3^-$ mesocosm^{-1} h^{-1}, compared to the previous period. Lower biofilm performance can be attributed to the substratum mixing, the oxygenation of the hypoxic niches, and the reduction of nitrate removal processes.

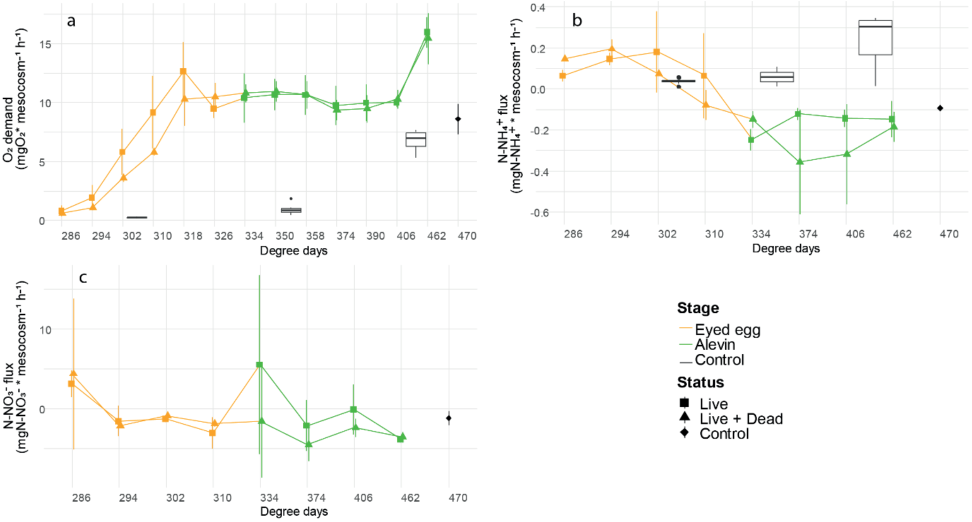

Figure 27. Line plots (average + standard errors, n = 3) depicting a) oxygen (mg O$_2$ mesocosm^{-1} h^{-1}) demand, b) ammonium (mg N-NH$_4^+$ mesocosm^{-1} h^{-1}), and c) nitrate fluxes (mg N-NO$_3^-$ mesocosm^{-1} h^{-1}) for the simulated salmonid egg pockets (cores), for the entire incubation period until complete alevin yolk sack absorption. Different colours represent the two main stages of "eyed egg" and "alevin", while different geometries stand for the Live (L) and Live + Dead (L+D) setups and the control (bare sediments). Boxplots upgraded to 100-egg respiration and excretion data from the incubation of microcosms are inserted for comparison. Please note that the position of the boxplot on the degree days axis is representative of the degree days intervals in which fluxes were measured from microcosm experiments (reprinted from Paper II).

5

Recommendations

The approach taken in this thesis demonstrates how the integration of different spatial scales can provide a complete picture when assessing a complex problem like the evaluation of salmonid spawning habitats. A multi-layered investigation is needed to consider the threads encountered at the mesoscale, e.g., hypoxia events in spawning areas, alongside the information collected at the macroscale, e.g., eutrophic conditions due to prolonged drought periods in urbanized watersheds. The microscale, i.e., egg pocket biogeochemistry, can promote different survival hypotheses by exploring the incubation period under controlled laboratory conditions. The fine-scale manipulation of the contours of physicochemical variables can be used to reproduce the specific in situ conditions of the object of investigation. Additionally, flexibility during sampling when seeking to investigate hydrological variations allows transient conditions regarding physicochemical water quality to be observed. Physicochemical values outside the WFD's definition of GES can inevitably hamper an entire brood year. Despite this, they could remain hidden if a traditionally rigid sampling protocol were to be applied. Therefore, the following recommendations are made for future studies:

1. When assessing the suitability of a watercourse in relation to salmonid spawning grounds, it is essential to contextualize the information collected through sampling (e.g., egg incubation conditions using physicochemical parameters) in terms of watershed layout (e.g., coverage of urbanized and agricultural areas in the watershed) and the position and intensity of point and non-point pollution (e.g., sewage water intrusion can lead to high trophic status, with cascading effects on hyporheic water for incubating eggs).

2. It is crucial to match qualitative information about the thresholds of the physicochemical water components responsible for the survival of the organism being investigated with quantitative information derived from different hydrological scenarios (e.g., how the system behaves during drought and flood events) and seasons (e.g., whether the system behaves differently across the season and year of sampling). This will allow practitioners to confidently interpret values outside the WFD's definition of GES or the requirements of salmonid incubation in relation to their duration, hydrological condition, and seasonal and inter-annual variability (e.g., by asking questions such as: When are hypoxia events more likely to happen? or What is their duration?). Repeated sampling allows practitioners to determine whether conditions unsuitable for the survival of the targeted organism can be interpreted as outlier episodes due to the specific year of sampling or conditions resulting from chronic deficiencies in the system.
3. Manipulative experiments can provide a robust technique with which to consistently test in situ physicochemical conditions. This can generate predictions about the suitability of a given environment for the development and survival of the salmonid larvae subject to conservation (e.g., by asking questions such as what is the salmonid larvae hatching rate in a pristine or polluted spawning area.
4. After considering and applying points 1) and 2) (and potentially with the aid of point 3), researchers and practitioners will be able to create a comprehensive picture of system-related stressors. From this platform, it will then be possible to develop multi-layer and multi-year restoration projects. The first layers may directly target the augmentation (e.g., by adding spawning gravel) or improvement (e.g., by cleaning spawning gravel) of the habitat directly connected to the lifecycle of the species. At the watershed level, the land use layout can be restored (e.g., by increasing buffer zones along the watercourse) or dampened (e.g., by constructing wetlands in the proximity of wastewater inlets). A multi-year approach will allow the settlement and integration of so-called nature-based solutions, which yield the most potential from emerging interactions between human-based improvements and the inherited resilience of ecosystems (e.g., increasing the salmonid population in the restored watershed and consequently increasing the resilience of the population against other anthropogenic pressures, like commercial fisheries or post-migration smolt mortality).
5. The present approach should be considered in relation to the potential of many Lithuanian watercourses to sustain populations of lithophilic (i.e., which spawn in gravel areas) endangered fish species (e.g., Atlantic salmon, sea trout, and river lamprey). The workflow scaffold presented in this thesis can then be used as a template to enhance preliminary status assessments followed by habitat restoration, and then augment the population statuses of many fish species dwelling in and exploiting Lithuanian watercourses.

6

Conclusions

The outcomes of this thesis can be integrated into the following conclusions:

1. The Blendžiava, and Šventoji watersheds feature significantly different land use environments and varying physicochemical water qualities. The Blendžiava Stream features high shares of forests and complex cultivation patterns related to high DO levels in its watershed. The Smiltaitė Stream watershed has high shares of uncovered arable land and urban areas related to high levels of N-compounds, EC, and alkalinity. The watershed of the Šventoji River presents significant forests and arable land coverage, with a physicochemical water quality profile that falls between that of the two other watercourses.
2. The three systems respond differently during extreme hydrological events. In the Smiltaitė Stream, prolonged low water periods amplify the concentration of N–compounds, alkalinity, and EC values. In the Blendžiava Stream, intense rainfall events connected to its higher bed slope lead to peaks in the concentration of total suspended solids. In the Šventoji River, the abundance of forest dampens the erosive potential of surface runoff, leading to lower suspended solid values.
3. The systematic literature review highlighted how spatiotemporal diversity, physicochemical quality, and intra- and interspecific variation drive diversity in the use of SW-GW interactions by spawning salmonids. The Salvelinus

and Oncorhynchus genera displayed the widespread use of spawning areas in groundwater conditions related to marginal habitats inside expansive floodplains. On the other hand, the Salmo genus prefers to spawn in pool-riffle transitional zones where the surface water intrudes into the streambed. The ubiquitous use of upwelling and downwelling zones is simultaneously justified by the multiple scales of investigation used in this study, which are capable of catching localized downwelling currents nested within regional upwelling conditions.

4. The presence of groundwater is consistent in the hyporheic seeps of the nest environment in the Blendžiava, Šventoji, and Smiltaitė watercourses. This was highlighted by common values in F^- and Ar, which were similar between nest and well samples but different with respect to those from the surface. Groundwater supply in these systems increases intragravel flow into the hyporheos. This can elicit a desilting action and aid the larvae in moving across the intragravel matrix.
5. The assessment of hyporheic water across the salmonid incubation period highlighted hyporheic DO values below the critical limits for embryo survival. These unsuitable values are worsened by temperatures which are rising toward the upper incubation limits. This not only decreases O_2 dissolution, but also increases the metabolic activity of organisms.
6. The reconstructed salmonid nests registered an increase in O_2 demand because of the advancement of the developmental stage. Additionally, biofilm growth increases overall respiration in the nest cores, and this is more prevalent in the final incubation phase. As incubation progresses, the cores become sinks for ammonium and nitrate because of the formation of biofilm and the presence of oxic-anoxic microzones.

7

Summary in Lithuanian

7.1. Disertacijos aktualumas

Lašišinės žuvys yra viena labiausiai ištirtų kaulinių žuvų šeimų pasaulyje (Bjornn & Reiser, 1991; Crisp, 2008; Louhi et al., 2008; Malcolm et al., 2003). Daugelis lašišinių žuvų rūšių turi didelę socialinę ir ekonominę reikšmę (Bloomer et al., 2016), susijusią su mėgėjiška ir versline žvejyba (Geist et al., 2002; Liu et al., 2019; Malcolm et al., 2003). Be to, būdamos mitybos grandinės viršūnėje, jos atlieka svarbų vaidmenį tiek jūrų, tiek gėlo vandens ekosistemose, pavyzdžiui, reguliuodamos kitų žuvų populiacijas (Bottom et al., 2008; Rudstam et al., 1994; Wipfli & Baxter, 2010). Visgi daugelis lašišinių rūšių patiria bendrą populiacijų mažėjimą dėl pergaudymo (Williams et al., 2024), buveinių nykimo ir degradacijos (Conallin, 2004; Malcolm et al., 2004).

Atlanto lašišos (Salmo salar) ir šlakiai (Salmo trutta), kurie maitinasi ir auga Baltijos jūroje, plaukia neršti į upes, taip pat ir į Lietuvos vandens telkinius. Tačiau Lietuva susiduria su sparčiais ir radikaliais žemės naudojimo pokyčiais, kurie gali turėti didelės įtakos gėlo vandens ekosistemų būklei (Gorgoglione et al., 2020; Pulg et al., 2013; Sutherland et al., 2002). Šiame kontekste, siekiant užkirsti kelią lašišinių išteklių prastėjimui, turėtų būti svarstomos apsaugos priemonės, nukreiptos į įvairius kritinius šių vertingų žuvų gyvenimo etapus (Smialek et al., 2021; Sternecker et al., 2013).

Yra žinoma, kad tinkamų nerštaviečių skaičiaus ir ploto didinimas bei atkūrimas stiprina lašišinių žuvų populiacijas (Kondolf et al., 2008; Smialek et al., 2021; Sternecker et al., 2013). Tačiau šios priemonės turėtų būti įgyvendinamos atsižvelgiant į restauruojamų upių baseinų ypatumus, kad būtų pasiekti ilgalaikiai ir veiksmingi rezultatai (Malcolm et al., 2005; I. Malcolm, A. Youngson et al., 2003; Soulsby et al., 2001).

Šiame darbe siekiama integruoti tyrimus trimis erdviniais masteliais: makromasteliu – kuomet vertinama upės vagos ir baseino dalis kilometrais, mezomasteliu – metrai upės vagos ir mikromasteliu – analizuojant konkrečias mikrobuveines, pvz., neršto lizdus, siekiant pateikti išsamią ekosistemos funkcionavimo analizę, įvertinant nerštaviečių tinkamumą ir galimus stresorius besinkubuojantiems lašišinių žuvų embrionams. Darbe pristatomas paviršinio ir gruntinio vandens sąveikos hiporėjinėje zonoje analizės modelis padės kurti panašius vertinimus, susijusius su buveinių apsauga ir atkūrimu bei gėlo vandens litofilinių žuvų rūšių išsaugojimu.

7.2. Tyrimo tikslas ir uždaviniai

Šiame tyrime nagrinėjama, kaip hidrologiniai svyravimai, paviršinio ir gruntinio vandens sąveika bei žemėnauda veikia upių ekosistemų funkcionavimą ir susijusius fizikocheminius veiksnius, lemiančius lašišinių žuvų neršto ir inkubacijos sėkmę.

Darbe buvo iškelti šie uždaviniai:

1. išanalizuoti fizikocheminius vandens parametrus trijose Lietuvos upėse ir susieti jų kaitą su baseino žemėnauda bei hidrologiniu režimu;
2. atlikti sisteminę mokslinės literatūros analizę apie paviršinio ir gruntinio vandens sąveikos vaidmenį formuojant lašišinių žuvų (Salmo, Oncorhynchus ir Salvelinus) nerštaviečių pasirinkimo mechanizmus lotinėse ekosistemose;
3. ištirti paviršinio ir gruntinio vandens sąveikos specifiką skirtingais erdviniais masteliais ir susieti ją su lašišinių žuvų neršto bei mailiaus išsiritimo laikotarpiais;
4. analizuoti vidugruntinio vandens kokybę tyrimo teritorijų nerštavietėse ir susieti ją su lašišinių žuvų inkubacijos sąlygomis;
5. laboratorinėmis sąlygomis įvertinti rekonstruotų ikrų dėčių metabolinę raidą, vertinant kvėpavimo ir ekskrecijos rodiklius skirtingais embrionų vystymosi etapais.

7.3. Darbo naujumas

Šioje disertacijoje buvo taikytas konceptualiai naujas integruotas tyrimas, apimantis skirtingus mastelius, taikant daugiadalykį požiūrį, kuris apima įvairius metodus. Makrolygmens analizei naudoti paviršinio vandens mėginiai ir GIS įrankiai, o mezolygmeniu taikyta vidugruntinio vandens cheminė analizė, siekiant ištirti paviršinio ir požeminio vandens sąveiką lašišinių žuvų lizduose. Galiausiai, mikrolygmeniu panaudoti mezokosmosai – inkubacinės kolonėlės su dirbtine ikrų dėties struktūra ir kiekybiškai įvertinti medžiagų srautai. Darbas siekia konceptualiai suprasti lotinių ekosistemų, naudojamų lašišinių žuvų reprodukcijai, funkcionavimą, tarpusavyje susiejant skirtingus tyrimo mastelius. Įvairaus erdvinio padengimo tyrimų integracija turėtų užtikrinti visapusišką požiūrį, apibendrinantį skirtingus sėkmingos lašišinių žuvų reprodukcijos aspektus.

7.4. Rezultatų mokslinė ir praktinė reikšmė

Mokslinė rezultatų reikšmė yra susijusi su naujoviškais metodais, taikytais tiek in situ lauko tyrimuose, tiek laboratoriniuose eksperimentuose. Kalbant apie in situ matavimus, ankstesni moksliniai tyrimai toje pačioje tiriamojoje teritorijoje (Nika, 2011) analizavo vidugruntinio vandens chemiją, siejant ją su šlakių embrionų išgyvenamumu. Tačiau pirmą kartą šiame darbe buvo kiekybiškai vertinta paviršinio ir požeminio vandens sąveika nerštavietėse. Tam buvo analizuotos pagrindinių vandens jonų (pvz., K^+, Ca^{2+}, F^-, Cl^- ir kt.) bei biologinei aplinkai svarbių dujų (N_2, Ar) koncentracijos, siekiant nustatyti tinkamiausius gruntinio vandens indikatorius hiporėjinėje zonoje. Laboratoriniu lygmeniu pratekančio srauto (flow-through) sistemos jau anksčiau buvo naudojamos biogeocheminių ciklų sekimui Klaipėdos universitete (Politi, 2022). Tačiau šįkart jos pritaikytos stambiagrūdžio substrato – žvirgždo terpės sąlygoms, kurios yra žymiai intensyvesnės nei tradiciniuose smulkiagrūdžio substrato inkubacinių sistemų eksperimentuose. Be to, buvo atlikta sisteminė literatūros apžvalga, nagrinėjanti paviršinio ir gruntinio vandens sąveikos vaidmenį lašišinėms žuvims (Oncorhynchus, Salmo ir Salvelinus) pasirenkant neršto vietas. Taikomąją rezultatų reikšmę turi daugiapakopio tyrimų metodo taikymas sudėtingų lotinių ekosistemų analizei. Kiekvienas tyrimo mastelis turi savo specifinius praktinius aspektus, padedančius geriau suprasti ekosistemų veikimą ir nustatyti galimus stresorius bei tinkamiausias ekosistemų atkūrimo priemones skirtingais lygmenimis. Makrolygmeniu hidrologinių svyravimų metu imami mėginiai turėtų būti derinami su žemės dangos specifika; mezolygmeniu turėtų būti taikomos hiporėjinio vandens stebėsenos ir vertinimo metodikos lašišinių žuvų lizduose ir tokių rezultatų pagrindu konstruojamos hiporėjinės zonos litofiliniams gyvūnams; mikrolygmeniu – laboratoriniais tyrimais įvertinta vidugruntinės aplinkos įtaka žuvų ikrams ar kitiems sėsliems litofiliniams gyvūnams leidžia parinkti ir maksimaliai tiksliai projektuoti buveines. Taigi, daugiapakopis integruotas vertinimas leidžia pritaikyti upių baseinams tinkamiausius specifinius sprendimus – nuo ekologinio kraštovaizdžio valdymo baseino lygmeniu iki konkrečių mikrobuveinių atkūrimo darbų. Tai turėtų padėti įgyvendinti Vandens pagrindų direktyvos (WFD) gaires, kurios per konkrečius upių baseinų valdymo planus ir priemonių programas yra pagrindiniai ES instrumentai siekiant geros ekologinės būklės (GES). Galiausiai tokia integruota stebėsena ir pagal ją parinktos valdymo priemonės padidintų ekosistemų atsparumą, siekiant sumažinti su buveinių degradacija susijusias grėsmes, galinčias trukdyti lašišinių žuvų sėkmingai reprodukcijai Lietuvos vandenyse.

7.5. Rezultatų aprobacija

Šio tyrimo rezultatai buvo pristatyti sekančiuose mokslo renginiuose:

1. Baltijos jūros mokslo kongresas 2023, Helsinkis, Suomija. Benetti, R.; Nika, N.; Bartoli, M. „Responsiveness to surface-groundwater interactions by spawning salmonids: site selection patterns and control mechanisms in Oncorhynchus, Salmo and Salvelinus genera.“ Stendinis pranešimas.
2. Laisvai tekančių upių kongresas 2024, Groningenas, Nyderlandai. Benetti, R.; Nika, N.; Bartoli, M. „Responsiveness to surface-groundwater interactions by spawning salmonids: site selection patterns and control mechanisms in Oncorhynchus, Salmo and Salvelinus genera.“ Stendinis pranešimas.
3. Jūros ir krantų tyrimai 2024: Nacionalinė jūros mokslų ir technologijų konferencija. Benetti, R.; Politi, T.; Bartoli, M.; Nika, N. „Metabolic Rates of Rainbow Trout Eggs in Reconstructed Salmonid Egg Pockets.“ Žodinis pranešimas.
4. AIIAD (Italijos gėlavandenių žuvų ichtiologijos asociacija) nacionalinis simpoziumas 2024, Parma, Italija. Benetti, R.; Severini, E.; Nika, N.; Cerkasova, N.; Magri, M.; Bartoli, M. „Water quality in the framework of climate change- and land use-driven pressures: lessons from three boreal streams.“ Žodinis pranešimas.

7.6. Baigiamojo darbo struktūra

Disertaciją sudaro 10 skyrių: Įvadas, Literatūros apžvalga, Medžiaga ir metodai, Rezultatai ir jų aptarimas, Rekomendacijos, Išvados, Padėkos, Santrauka lietuvių kalba, Papildoma medžiaga ir Bibliografija. Medžiaga pateikiama 120 puslapių, su 27 paveikslais ir 7 lentelėmis. Disertacijoje remiamasi 331 literatūros šaltiniu.

7.7. Tyrimų medžiaga ir metodai

7.7.1. paviršinio vandens kokybės vertinimas

Trys tirti vandens telkiniai (Blendžiava, Smeltaitė ir Šventoji) yra vakarinėje Lietuvos dalyje ir laikomi lygumų tipo upėmis, kurių nuolydis svyruoja nuo 0,1 % iki

0,3 %, o vagos struktūra pasižymi periodišku duburių ir rėvų pasiskirstymu (Jablonskis & Lasinskas, 1962). Norint įvertinti skirtingos žemėnaudos pasiskirstymo panašumus ir skirtumus tarp trijų baseinų, buvo taikyta korespondencinė analizė (CA). Žemės dangos skirtumai tarp trijų baseinų buvo vertinami naudojant Europos projekto CORINE Land Cover (CLC) trečiojo tikslumo lygio 2018 duomenis (Copernicus Land Monitoring Service – https://land.copernicus.eu, prieiga gauta 2023 m. lapkričio 10 d.). Vandens mėginiai buvo imami nuo 2021 m. spalio mėn. iki 2022 m. rugpjūčio mėn. siekiant užfiksuoti vandens kokybę ir fizikocheminius pokyčius skirtingais metų laikais, atsižvelgiant į baseino žemėnaudą (Gorgoglione et al., 2020; Pinardi et al., 2022). Kiekvienu sezonu prioritetas buvo teikiamas hidrologiniams ekstremumams, t. y. aukšto vandens lygio laikotarpiams (potvyniams) ir mažo vandeningumo sąlygoms. Tai atitinka ankstesnius tyrimus, kuriuose buvo analizuojami maistinių medžiagų koncentracijų pokyčiai priklausomai nuo hidrologinių sąlygų (Racchetti et al., 2019; Severini et al., 2023). Potvynių ir žemo vandens lygio laikotarpiai buvo nustatyti remiantis realaus laiko duomenimis iš Lietuvos hidrometeorologijos tarnybos (https://www.meteo.lt/) ir patvirtinti vietiniais in situ stebėjimais. Norint apibūdinti vandens fizikocheminę būklę, buvo pasirinkta vienuolika aplinkos kintamųjų: ištirpusio deguonies koncentracija (DO, mg L^{-1} ir prisotinimas %), pH, elektros laidis (EC, µS cm^{-1}), temperatūra (T, °C), amonio jonai ($N\text{-}NH_4^+$, mg L^{-1}), nitratai ($N\text{-}NO_3^-$, mg L^{-1}), nitritai ($N\text{-}NO_2^-$, mg L^{-1}), tirpusis reaktyvusis fosforas ($P\text{-}PO_4^{3-}$, mg L^{-1}), šarmingumas (mmol L^{-1}) ir bendroji suspenduota medžiaga (TSS, mg L^{-1}). Šie 11 aplinkos kintamųjų buvo apibendrinti ir vizualizuoti naudojant pagrindinių komponentų analizę (PCA). Kiekviename baseine vandens cheminės sudėties pokyčiai buvo vertinami rudenį ir žiemą, kai pasitaikė hidrologiniai ekstremumai. Tam buvo taikomi susietų imčių t-testas ir Welcho t-testas. Sezoniniai bei vandeningumo skirtumai tarp trijų baseinų buvo įvertinti vienfaktorinės dispersinės analizės (ANOVA) ir Kruskalio–Valiso testais su papildomomis post-hoc palyginimo procedūromis. Visi statistiniai testai buvo atliekami taikant 0,05 reikšmingumo lygį.

7.7.2. Lašišinių žuvų inkubacijos fazė: vidugruntinio vandens vertinimas

Lauko tyrimai buvo atlikti 2021–2022 metų inkubacijos sezono metu nuo gruodžio vidurio iki gegužės pradžios. Tyrimai vyko Šventosios upėje, Blendžiavos ir Smiltaitės upelių atkarpose, kuriose gausiai aptinkama šlakių (Salmo trutta) (Šventojoje – ir Atlanto lašišų (Salmo salar)) populiacijos (9 pav.; Kesminas, 2011; Kontautas & Rauckis, 1994). Kiekvienoje vandens sistemoje buvo atrinktos trys nerštavietės kiekvienoje tiriamoje atkarpoje, kuriose per visą inkubacijos laikotarpį buvo įrengti minipiezometrai vidugruntinio (hiporėjinio) vandens mėginiams imti. Vidugruntinis ir paviršinis vanduo buvo tiriamas lizdo centre (lizdo centrinė dalis), orientuojantis į ikrų dėties vietas, ir lizdo pakraštyje (lizdo periferija). Mėginiai buvo imami kas mėnesį, tačiau dėl aukšto vandens lygio ir mažo vandens skaidrumo nuo gruodžio iki kovo pradžios pavyko sekti tik paskutinę inkubacijos periodo dalį. Paviršiniame ir vidugruntiniame vandenyje buvo matuojamas ištirpęs deguonis (DO; mg L^{-1}), elektrinis laidumas (EC; µS cm^{-1} prie 25°C), temperatūra (°C), pH, amonis ($N\text{-}NH_4^+$, mg L^{-1}), nitratai ($N\text{-}NO_3^-$, mg L^{-1}) ir nitritai ($N\text{-}NO_2^-$, mg L^{-1}). Neparametrinė daugiamatė skalių analizė (NMDS) buvo naudojama rezultatams suskirstyti pagal šiuos veiksnius: 1) upelis, 2) mėnuo, 3) mėginių ėmimo vieta lizde ir 4) skirtumas tarp vidugruntinio (hiporėjinio) ir paviršinio vandens tipų. Bet kokie reikšmingi skirtumai tarp kiekvieno iš keturių veiksnių lygių buvo vertinami vienpusės panašumų analizės (ANOSIM, Clarke & Warwick, 2001) metodu, naudojant NMDS analizėje taikytas panašumų matricas. Indikatorinių rūšių analizė (ISA) buvo pasitelkta nustatyti aplinkos kintamuosius, kurie geriausiai atitiko ($p<0,05$) NMDS grafikuose matomas klasterizacijas. Daugiaparametriniai rezultatai buvo papildyti vieno kintamojo testais. Daugiavariaciniai ir vieno kintamojo testai buvo atliekami su $p<0,05$ reikšmingumo lygiu.

7.7.3. Paviršinio ir požeminio vandens sąveika lašišinių žuvų neršto upėse

In situ matavimai buvo atlikti siekiant ištirti galimą požeminio vandens indėlį į upėtakių (Salmo trutta) ir Atlanto lašišų (Salmo salar) nerštavietes trijuose borealiniuose vandens telkiniuose: Smeltaitėje, Blendžiavoje ir Šventojoje. Hiporėjinis vanduo buvo paimtas iš neseniai sukurtų lizdų 2022 m. gruodžio mėn., atspindinčių sąlygas neršto vietos pasirinkimo metu. Antrasis mėginys buvo imtas inkubacijos laikotarpio pabaigoje, 2023 m. gegužę, sutampant su didžiausiu mailiaus išsiritimo piku šiose sistemose (Nika, 2011). Hiporėjinis vanduo buvo paimtas iš kiekvienos nerštavietės naudojant vidugruntinio vandens mėginių ėmiklį. Tuo pačiu metu trys paviršinio vandens mėginiai (toliau vadinami „upės“) buvo paimti šalia nerštaviečių. Galiausiai buvo paimti trys buitinių šulinių vandens mėginiai (toliau vadinami „požeminis vanduo“), esantys šalia kiekvienos tyrimo atkarpos. Šulinio vanduo turėtų reprezentatyvai atspindėti regioninio požeminio vandens savybes (Neumann & Curtis, 2016; Severini et al., 2022; Severini et al., 2023).

Hiporėjinio, paviršinio ir požeminio vandens mėginiai buvo analizuojami pagal šiuos parametrus: ištirpusio deguonies (DO; % prisotinimas), elektros laidumo (EC; µS cm^{-1} prie 25°C), temperatūros (°C), pH, pagrindinių katijonų (Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, mg L^{-1}) ir anijonų (F^-, Cl^-, $N\text{-}NO_3^-$, HCO_3^-, SO_4^{2-}, PO_4^{3-}, mg L^{-1}) koncentracijas bei ištirpusių dujų (N_2, µM), (Ar, µM), (O_2, µM) ir (CH_4, nM) kiekius. N_2, Ar ir O_2 prisotinimas vandens mėginiuose buvo apskaičiuotas lyginant išmatuotas ir teorines koncentracijas, gautas pagal dujų tirpumą in situ sąlygomis (Sander, 1999).

Erdviniai ryšiai tarp trijų vandens tipų buvo analizuojami naudojant nMDS diagramas ir panašumo matricą, pagrįstą euklidiniais atstumais (Oksanen et al., 2007). Kiekvienam fizikocheminiam parametrui kiekvieno mėginių ėmimo metu buvo sudaryti linijiniai mišraus poveikio modeliai (Bates et al., 2007), apimantys duomenis iš visų tyrimo baseinų. „Baseino“ terminas buvo naudojamas kaip atsitiktinis veiksnys modeliuojant fizikocheminį kintamumą tarp baseinų. Jei linijiniai mišraus poveikio modeliai parodė reikšmingus skirtumus, buvo atliekami poriniai Tukey testai. Statistinė analizė buvo atlikta esant reikšmingumo lygiui $\alpha = 0{,}05$..

7.7.4. Lašišinių žuvų lizdo vandens ir medžiagų apykaitos procesų tyrimas taikant laboratorinę mezokosmų inkubacinę sistemą

Ištirpusio O_2 ir neorganinio azoto (amonio – N-NH_4^+ ir nitratų – N-NO_3^-) srautai buvo tiriami rekonstruotose ikrų dėtyse kontroliuojamomis laboratorinėmis sąlygomis (t. y. mezokosmuose). Siekiant kiekybiškai įvertinti ikrų indėlį į visos sistemos kvėpavimą, ikrai taip pat buvo inkubuojami vien tik vandenyje (t. y. mikrokosmuose). Mikrokosmo eksperimento metu srautai buvo apskaičiuoti pagal šią formulę:

$$Kvėpavimas\ arba\ ekskrecija\ (mg\ arba\ \mu g\ ikreliui^{-1}h^{-1}) = \frac{\Delta C \times V}{n \times \Delta t}$$

kur ΔC/Δt\Delta C / \Delta tΔC/Δt (mg arba µg $L^{-1}h^{-1}$) yra DO arba N-NH_4^+ koncentracijos pokytis inkubacijos laikotarpiu Δt\Delta tΔt, V (L) yra inkubacinės kameros tūris, o n – inkubuotų ikrelių skaičius. Bet koks skirtumas tarp DO poreikio (mg O_2 ikreliui^{-1} h^{-1}) ir amonio ekskrecijos (µg N-NH_4^+ ikreliui^{-1} h^{-1}) tarp trijų vystymosi stadijų („akutės“ stadijos ikrelis, „beveik išsiritęs“, „trynio maišelis absorbuotas“) buvo įvertintas naudojant neparametrinį Kruskalo–Valiso testą.

Laboratorinė mezokosmų sistema buvo sudaryta iš 6 skaidraus organinio stiklo inkubacinių kolonėlių, užpildytų nerštiniu substratu ir vaivorykštinių upėtakių ikreliais. Trys cilindrai buvo pripildyti po 100 gyvų ikrelių, toliau vadinamų „L“ (gyvi); tuo tarpu trys cilindrai buvo pripildyti po 50 gyvų ir 50 negyvų ikrelių, toliau žymimų „L+D“. Vandens cirkuliaciją šešiose inkubacinėse kolonėlėse užtikrino peristaltiniai siurbliai, panardinti į didelį akvariumą, palaikantį pastovią temperatūrą. DO poreikis ir neorganinio azoto srautai buvo išreikšti vienai inkubacinei kolonėlei ir susieti su pagrindinėmis vystymosi stadijomis („akutės“ stadijos ikrelis, mailius), naudojant šią formulę:

$$\text{Srautai (mg arba µg } L^{-1} branduoliui^{-1}) = (C_{out} - C_{in}) \times Q$$

kur Cout ir Cin (mg arba µg L^{-1}) yra ištirpusio deguonies (DO) arba neorganinio azoto koncentracijos atitinkamai branduolio ištekėjimo ir įtekėjimo taškuose, o Q (L h^{-1}) – vandens srauto greitis. Skirtumai tarp L ir L+D eksperimentinių sąlygų buvo tikrinami naudojant neparametrinį Wilcoxono rangų sumos kriterijų (Wilcoxon Signed-Rank test). Dviejų vystymosi stadijų – „akutės“ stadijos (eyed egg) ir lervutės (alevin) – skirtumai taip pat buvo vertinami naudojant Wilcoxono rangų sumos kriterijų. Galiausiai, šiuo metodu buvo patikrinti skirtumai tarp $N\text{-}NH_4^+$ ir $N\text{-}NO_3^-$ koncentracijų visose mezokosmų įtekėjimo ir ištekėjimo sekcijose, sujungtose į vieną grupę. Visi statistiniai testai buvo atliekami esant reikšmingumo lygiui $\alpha = 0{,}05$.

7.8. Rezultatai ir jų aptarimas

7.8.1. Žemėnaudos tipas kaip pagrindinis vandens kokybės veiksnys

PCA rezultatai parodė, kad trys baseinai išsidėstę skirtingose diagramos vietose dažniausiai yra susiję su tais pačiais vektoriais, nepriklausomai nuo sezono ar hidrologinių sąlygų. Tai leidžia daryti prielaidą, kad šie skirtumai yra nulemti pastovių veiksnių, t. y. kiekvieno baseino žemės naudojimo ypatumų, o ne sezoninių ar vandeningumo pokyčių. Iš tiesų, korespondencinė analizė (CA) patvirtino, kad trijų baseinų žemėnauda skiriasi (chi-kvadratas: 110, $p<0{,}001$, df: 16).

Blendžiavos stotys yra išsidėsčiusios kairėje PC pusėje ir susietos su ištirpusiu deguonimi (DO), prisotinimo procentine dalimi (% sat.) ir pH vektoriais, kurie dominuoja šioje diagramų dalyje visais metų laikais ir visada buvo reikšmingai aukštesni nei Smeltaitės. Atvirų plotų, kurie gali būti maistinių medžiagų ir organinių medžiagų išplovimo šaltinis į Blendžiavos vandens telkinį, yra mažai. Pagrindinis žemės ūkio naudmenų tipas yra „sudėtingi žemės ūkio raštai“ (42%), kurie, remiantis CLC Copernicus projekto apibrėžimu (Copernicus Land Monitoring Service – https://land.copernicus.eu, prieiga: 2023 m. lapkričio 10 d.), reiškia „ariamosios ir nuolatinės kultūros bei pievų mozaiką“. Tai, kartu su nuolatine „žemės ūkio ir natūralios augmenijos plotų“ buvimu, sudaro sudėtingą kultūrinių plotų mozaiką su nedideliu atvirų dirvožemio plotų kiekiu. Todėl šie apsaugoti paviršiai padeda sumažinti galimą maistinių medžiagų ir organinių medžiagų patekimą į vandens telkinį (Dabney et al., 2010; Delgado et al., 2007).

Smeltaitės baseino matavimo stotys yra arti maistinių medžiagų (N- ir P- formų), EC ir šarmingumo vektorių. Šie rodikliai laikomi aukštesnio trofinio lygmens indikatoriais (Malcolm et al., 2004; McDowell & Hamilton, 2013; Wu et al., 2020) ir nukreipia į dešiniąją PC diagramų dalį, kur yra Smeltaitės stotys. Iš tiesų, per visus mėginių ėmimo laikotarpius Smeltaitė pasižymėjo aukščiausiomis EC reikšmėmis. Tai taip pat akivaizdu ir N-NO_3^-, N-NO_2^-, N-NH_4^+, P-PO_4^{3-} bei šarmingumo reikšmėms, kurios visada buvo reikšmingai didesnės nei Blendžiavoje. Smeltaitės baseinas pasižymi didele „nedrėkinamos ariamosios žemės“ dalimi (34%), kuri yra susijusi su „urbanizuotomis teritorijomis“ (13%). Šios savybės lemia didesnį atvirų dirvožemio plotų, kurie gali būti maistinių medžiagų ir organinių medžiagų išplovimo į upelį šaltinis, kiekį. EC ir šarmingumo vektoriai dažniausiai sutampa su aukščiau minėtais kintamaisiais visuose PCA dvimačiuose grafikuose.

Šventosios upės stotys užima tarpinę poziciją PC diagramose. Tai patvirtina ir tarpinės EC, TSS, N-NO_3^-, N-NO_2^- bei N-NH_4^+ reikšmės, palyginti su kitais dviem baseinais. Tarp trijų baseinų Šventoji turi didžiausią miškingų teritorijų dalį (40%), o tai galėjo prisidėti prie to, kad jos taškai yra nutolę nuo N- ir P-maistinių medžiagų, EC ir šarmingumo vektorių. Tačiau Šventoji dalijasi „nedrėkinamos ariamosios žemės“ vektoriumi su Smeltaite (Šventoji: 43%, Smeltaitė: 34%), kuris yra pagrindinis žemės ūkio paskirties naudmenų tipas šiame baseine.

7.8.2. Sezoniškumo ir hidrologinių ekstremumų poveikis

Mažo upės nuotėkio laikotarpiai sustiprina taškinės taršos (pvz., nuotekų valymo įrenginių) poveikį, kaip jau buvo pabrėžta panašiuose tyrimuose, kuriuose vandens kokybė buvo vertinama atsižvelgiant į žemės dangą (Gorgoglione ir kt., 2020) ir hidrologinius ekstremumus (Peña-Guerrero ir kt., 2020). Tai patvirtina visuomet reikšmingai didesnės EC reikšmės mažo nuotėkio rudens ir žiemos laikotarpiais, palyginti su atitinkamais potvyniais. Be to, mažo nuotėkio momentais didėja šarmingumas – Blendžiavoje ir iš dalies Smeltaitėje rudenį, o Šventojoje žiemą. Panašūs tyrimai rodo, kad urbanizuotos teritorijos vaidina lemiamą vaidmenį didelio kiekio maistinių medžiagų išsiskyrimui į gretimą upės ruožą (Liu ir kt., 2023). Be to, sumažėjęs upės debitas neišvengiamai lemia mažesnį atskiedimą ir medžiagų koncentracijos padidėjimą (Mosley, 2015), kas skatina elektrinio laidumo ir šarmingumo didėjimą (Malcolm ir kt., 2004).

Potvyniai sustiprina išsklaidytos taršos poveikį dėl maistmedžiagių išplovimo iš laukų kritulių metu (Binkley & Brown, 1993). Tai patvirtina didesnės N-NO_2^- ir N-NH_4^+ koncentracijos Smeltaitės ir Blendžiavos baseinuose, kuriuose ariamosios žemės dalis yra didesnė (Blendžiava: 70 %, Smeltaitė: 77 %), palyginti su Šventąja (58 %). Jau anksčiau buvo nustatyta, kad pievų pavertimas ariama žeme yra pagrindinis azoto išplovimo didėjimo veiksnys pagrindinėse Lietuvos upėse (Bauer & Weert, 2015).

TSS (bendrasis suspenduotų dalelių kiekis) atspindi neorganinių ir organinių dalelių kiekį vandenyje (Brils, 2008; Swietlik ir kt., 2003). Pagrindinių teršalų sąraše (ES Vandens pagrindų direktyvos 2000/60/EB VIII priedas) jos apibrėžiamos kaip „suspensijos medžiagos“, tačiau nėra pateiktos konkrečios jų stebėsenos ir ekologinių ribų gairės (Brils, 2008). Kiek žinoma, Lietuva dar nėra nustačiusi tokio vertinimo kriterijų tekančiam vandeniui. Mažo nuotėkio laikotarpiais TSS vektoriai išsidėstė pagal antrąją pagrindinių komponentų ašį, rodydami mažą duomenų rinkinio kintamumą. Tačiau potvynių metu TSS koncentracijos išaugo visose trijose sistemose, o Blendžiavoje reikšmės buvo reikšmingai didesnės nei Smeltaitėje ir Šventojoje. Didelio nuotėkio įvykiai gali išplauti atviras ir ariamosios žemės teritorijas, įnešdami didelius nuosėdų kiekius į vandens telkinius (Adjovu ir kt., 2023; Soulsby ir kt., 2001).

Tokie reiškiniai būdingesni šaltuoju metų laiku, kai dėl aukšto dirvožemio drėgnumo ir žemų temperatūrų padidėja nuplautų nuosėdų kiekis (Gorgoglione ir kt., 2020). Manoma, kad didesnis nešmenų kiekis Blendžiavoje gali būti susijęs su dideliu ariamosios žemės plotu (70 %), kuris, nors ir šiek tiek mažesnis nei Smiltaitėje (77 %), yra dvigubai didesnio baseino plote nei pastaroji. Šią situaciją dar labiau blogina pievų pavertimas ariama žeme Lietuvoje (Bauer & Weert, 2015). Be to, Blendžiavos upės dugno nuolydis (0,33 %, Gailiušis ir kt., 2001), palyginti su mažesniais Smeltaitės (0,007 %) ir Šventosios (0,006 %) nuolydžiais (Ventos upės baseino rajonas, 2010), neabejotinai didina paviršinio nuotėkio greitį, skatina didesnę dirvožemio eroziją ir taip padidina suspenduotų dalelių patekimą į upę. Kita vertus, miškų buvimas mažina suspenduotų dalelių kiekį vandens telkiniuose, ką patvirtina ir Šventosios rezultatai (miškų dengiamas plotas: Šventoji – 40 %, Blendžiava – 28 %, Smeltaitė – 10 %).

7.8.3. Hiporėjinio vandens kokybė inkubacijos laikotarpiu

Hiporėjinio sluoksnio vandens O_2 reikšmės reikšmingai mažėja visuose trijuose baseinuose tęsiantis inkubacijos sezonui. O_2 vidutinės koncentracijos kovo mėnesį siekė 9–12 mg L^{-1}, balandį – 7–11 mg L^{-1}, o gegužę – 5–7 mg L^{-1}. Vidutinės kovo ir balandžio reikšmės gali užtikrinti aukštus išgyvenamumo rodiklius (50–70 %) iki išsiritimo (Crisp, 2008; Greig et al., 2007). Tačiau paskutiniame inkubacijos etape vidutinės O_2 koncentracijos gali lemti nuo vidutinio (25 %) iki nereikšmingo (5 %) išgyvenamumo lygio (Greig et al., 2007). Daugelis autorių kritinę ribą, žemiau kurios išgyvenamumas tampa nereikšmingas, nurodo kaip 7–8 mg L^{-1} (Crisp, 1996; Greig et al., 2007; Ingendahl, 2001; Malcolm et al., 2004). Šią situaciją dar labiau apsunkina padidėjęs O_2 suvartojimas po išsiritimo (Alderdice et al., 1958).

Hiporėjinio vandens O_2 koncentracijos, išmatuotos iš lizdo centro, buvo reikšmingai didesnės nei iš lizdo pakraščių Smeltaitės ir Šventosios baseinuose. Tai galima paaiškinti ikrelių sankaupų buvimu lizdo šerdyje, kur dedama didžioji ikrų dėties dalis (Chapman, 1988; Rubin & Glimsäter, 1996). Žvyras lizduose ikrų dėties aplinkoje yra stambesnis, o mechaninis patelių kasimo veiksmas užtikrina mažesnį smulkių dalelių kiekį šiose vietose (Chapman, 1988; Rubin & Glimsäter, 1996). Tokios zonos pasižymi didesniu vandens pralaidumu nei aplinkinės sritys, o tai reiškia intensyvesnę vandens cirkuliaciją ir geresnį deguonies tiekimą (Chapman, 1988). Inkubacijos sezonui einant į priekį, skirtumai tarp vidugruntinio vandens O_2 reikšmių lizdo centre ir pakraščiuose išsilygino. Kovo mėn. vidugruntinis O_2 lizdo centre buvo reikšmingai didesnis nei pakraščiuose, tačiau balandį ir gegužę reikšmingų skirtumų nebebuvo. Ilgainiui lizdai linkę kaupti nuosėdas, kurios formuoja paviršinę „dumblinę plutą“ (Hobbs, 1937), galiausiai suvienodindamos sąlygas su lizdo pakraščiais (Conallin, 2004; Crisp, 1989). Tokius procesus patvirtina kovo mėnesio vidugruntinio O_2 reikšmių skirtumai tarp lizdo centro ir pakraščių, o balandį ir gegužę šie skirtumai išnyksta.

Temperatūra reikšmingai didėjo kiekvieną mėnesį visuose trijuose baseinuose. Vidutinės temperatūros svyravo nuo 2 iki 4 °C kovo mėn., nuo 3 iki 10 °C – balandį ir nuo 11 iki 14 °C – gegužę. Optimalus intervalas, užtikrinantis daugiau nei 50 % ikrelių išgyvenamumą iki išsiritimo (sensu Crisp, 2008), yra apie 2–12 °C (Burke, 2011; Crisp, 2008; Danner, 2008; Takle et al., 2005). Gegužę užfiksuota nerimą kelianti situacija – temperatūra artima arba viršija viršutinę mirtiną ribą. Be to, temperatūros padidėjimas 2–3 °C virš 10 °C dar labiau padidina metabolizmo intensyvumą ir deguonies poreikį keliais miligramais (Chapman, 1988; WDOE, 2002) bei sumažina O_2 dalelinį slėgį mikroaplinkoje aplink ikrelį, mažindamas prieinamą deguonies kiekį (Alderdice et al., 1958).

7.8.4. Paviršinio ir požeminio vandens sąveika lašišinių žuvų neršto ir išsiritimo laikotarpiais tirtuose upeliuose

Pagal šiuo metu prieinamą informaciją, ankstesnių paviršinio ir požeminio vandens sąveikos vertinimų šiose trijose sistemose neatlikta. Per abu mėginių ėmimo laikotarpius požeminio vandens mėginiai nMDS diagramose nuosekliai persidengė su lizdų vudugruntinio vandens mėginiais, pabrėždami galimus panašumus. Linijiniai mišrūs efektų modeliai padėjo išryškinti skirtumus tarp mėginių grupių. Žiemą F^- reikšmės požeminio vandens ir lizdų mėginiuose buvo panašiai aukštesnės nei paviršinio vandens mėginiuose, o tai rodo efektyvų požeminio vandens indėlį į neršto vietas. Jau įrodyta, kad F^- yra konservatyvus pėdsakas, kurio koncentracija nesikeičia per trumpus perkoliacijos kelius (Brunt et al., 2004; Lin et al., 2013; Martinez & Widdowson, 2024). Jo daugiau randama požeminiame vandenyje, nes ilgas cirkuliacijos laikas leidžia jam ilgiau sąveikauti su galimais F^- šaltiniais (Bove et al., 2009; Brunt et al., 2004). Be to, F^- retai dalyvauja biogeocheminėse reakcijose hiporėjinėje zonoje, nes pasižymi bakteriostatiniu (Pandey, 2024; Zhang et al., 2019; Zhu et al., 2022) ir fitotoksiniu (Katiyar et al., 2020) poveikiu.

Pavasarį Ar koncentracijos upės mėginiuose buvo mažesnės nei požeminiame vandenyje ir lizduose. Dujų matavimai jau buvo pripažinti naudinga priemone nustatant upės dugno sujungtumą (Heilweil et al., 2016) ir požeminio vandens papildymo zonų dydį (Brunner et al., 2017; Jurado et al., 2018). Dėl atskyrimo nuo atmosferos inertinės dujos, tokios kaip Ar, kaupiasi, nes dėl savo cheminio inertiškumo jos nepatiria oksidacijos ar redukcijos procesų (Heaton, 1981; Stute & Schlosser, 2000). Be to, anaerobinės sąlygos tokiose aplinkose gali skatinti denitrifikacijos procesus, kurių metu susidaro N_2 (Jurado et al., 2018). Tai patvirtina didesnis N_2 prisotinimas požeminiame vandenyje nei paviršiniame.

Be deguonies tiekimo, padidėję hiporėjiniai srautai prisideda prie greitesnio atliekų, susidarančių hiporėjinėje matricoje (Bjornn & Reiser, 1991) ir ikruose (Crisp, 2008), pašalinimo ir praskiedimo. Taip pat įrodyta, kad išsikraunančio gruntinio vandens šaltinių buvimas lizdų aplinkoje turi desiltuojantį ir grunto atlaisvinantį poveikį (Casas-Mulet et al., 2015; Mouw et al., 2014). Tai palengvina lervų judėjimą hiporėjnėje matricoje ir pagerina jų išėjimą per išsiritimo fazę (Zimmerman & Finn, 2012). Akivaizdu, kad sistemose, kuriose būdingas stiprus dumblėjimas – tiek dėl natūralių ypatybių (Nika, 2011), tiek dėl vykstančių žemės naudojimo pokyčių (Benetti et al., 2024) – išsikraunančio požeminio gruntinio vandens zonos gali neabejotinai sumažinti nerštinio substrato uždumblėjimo problemas. Tai tampa ypač svarbu jauniklių išsiritimo fazėje, kai dėl viso inkubacijos laikotarpio metu nerštiniame žvirgžde sukauptų nuosėdų mastas yra didžiausias.

7.8.5. Lašišinių žuvų lizdų biogeocheminiai procesai laboratorinėmis sąlygomis

Buvo atliktas bandomasis tyrimas, skirtas įvertinti lašišinių žuvų ikrelių dėčių funkcionavimą, atsižvelgiant į deguonies (O_2) poreikį, amonio ir nitratų srautų balansą. Kiek žinoma, tai yra pirmasis bandymas, kurio metu rekonstruotų lašišinių žuvų ikrelių dėčių metabolizmo medžiagų srautai matuojami vientiso substrato sąlygomis. Biogeocheminiai procesai tradiciškai tiriami mezokosmų sistemose su statinio vandens aplinka, kur aiškiai išskiriama oro, vandens ir nuosėdų sąsaja (Benelli & Bartoli, 2021; Naldi et al., 2020; Nika et al., 2021). Šio tyrimo naujovė – tokių įrankių pritaikymas laisvai tekančioms sąlygoms, būdingoms litofilinių žuvų lizdui, t. y. hiporėjinės zonos daliai.

Kalbant apie O_2 poreikį, inkubacinių kolonėlių (mezokosmų) vandens deguonies suvartojimo didėjimo tendencija buvo lygiagreti biologiniams ikrelių (mikrokosmuose) pokyčiams, nes metabolinis aktyvumas intensyvėjo vystymosi stadijoms progresuojant. Be to, bioplėvelės augimas padidino bendrą kvėpavimo intensyvumą mėginiuose, tapdamas vyraujančiu veiksniu paskutinėje inkubacijos fazėje, kaip tai patvirtina ir kontroliniai mėginiai be ikrelių.

Apskritai, rekonstruoti lizdai veikė kaip amonio šaltinis, atspindėdami lervų metabolinės ekskrecijos padidėjimą vėlesnėse vystymosi stadijose. Tačiau inkubacijai tęsiantis, rekonstruotos ikrų dėtys su inkubaciniu žvirgždu tapo amonio kaupikliais, o tai rodo priešingą tendenciją nei laisvų ikrų matavimo rezultatai. Taip nutiko dėl bioplėvelės formavimosi, kuris naudoja maistines medžiagas savo augimui. Tokiu pačiu principu galima paaiškinti ir nitrato pašalinimo intensyvėjimą, jei laikoma, kad susidarė hipoksinės zonos, skatinančios denitrifikaciją.

7.9. Rekomendacijos

Darbe panaudotas metodas parodo, kaip integruojant skirtingus erdvinius mastelius galima gauti visapusišką vaizdą, vertinant sudėtingą sistemą, tokią kaip lašišinių žuvų neršto buveinės. Siekiant atsižvelgti į įvairaus masto iššūkius, reikia daugiasluoksnio tyrimo: mezomasteliu galima nagrinėti, pavyzdžiui, hipoksijos reiškinius nerštavietėse, o makromasteliu – eutrofines sąlygas dėl užsitęsusių sausros laikotarpių urbanizuotuose vandens telkinių baseinuose. Mikromasteliu tiriant ikrų dėties biogeochemiją, galima iškelti įvairias išgyvenimo hipotezes, tyrinėjant inkubacijos periodą kontroliuojamomis laboratorinėmis sąlygomis. Tikslus fizikocheminių sąlygų modeliavimas leidžia atkurti specifines in situ sąlygas. Be to, lanksčiai derinant mėginių ėmimą su hidrologinėmis variacijomis, galima užfiksuoti laikinus fizikocheminės vandens kokybės pokyčius. Fizikocheminės charakteristikos, neatitinkančios geros ekologinės būklės pagal BVPD, gali lemti ištisos kohortos žūtį, tačiau šis poveikis gali likti nepastebėtas, jei būtų taikoma tradicinė, bet nelanksti mėginių ėmimo metodika. Todėl ateities tyrimams siūloma vadovautis šiomis rekomendacijomis:

1. Vandens telkinio tinkamumo lašišinių žuvų nerštavietėms vertinimas turi būti atliekamas kontekstualizuojant surinktą informaciją. Tai reiškia, kad būtina susieti ikrų inkubacijos sąlygas su fizikocheminiais parametrais ir baseino savybėmis (pvz., urbanizuotų bei žemės ūkio naudmenų plotais), taip pat su taškinės ir netaškinės taršos šaltiniais (pvz., nuotekų infiltracija gali padidinti trofinį statusą, kuris savo ruožtu turės kaskadinį efektą hiporėjinėje vandens zonoje besiinkubuojantiems ikreliams).
2. Fizikocheminių vandens rodiklių ribinės vertės turi būti derinamos su kiekybiniais duomenimis, gautais įvairių hidrologinių situacijų metu. Tai reiškia, kad būtina įvertinti sistemos elgseną tiek sausros, tiek potvynių metu, taip pat skirtingais metų laikais. Tokia analizė leis tyrėjams geriau suprasti ribas, viršijančias geros ekologinės būklės kriterijus pagal BVPD, ir įvertinti jų trukmę, hidrologines sąlygas bei sezoninį ir ilgalaikį kintamumą. Pakartotiniai mėginiai padės atskirti, ar nepalankios sąlygos buvo atsitiktinės, ar sisteminės.
3. Manipuliaciniai eksperimentai gali suteikti patikimą metodą nuolatiniam fizikocheminių sąlygų testavimui. Tokie tyrimai leidžia prognozuoti aplinkos tinkamumą lašišinių žuvų lervų vystymuisi ir išgyvenimui apsaugos priemonių kontekste (pvz., kokie lašišinių lervų išsiritimo rodikliai būdingi natūralioms ir užterštoms nerštavietėms).
4. Sistemos stresorių išsamus įvertinimas turėtų būti naudojamas kaip pagrindas daugiapakopiams ir daugiamečiams atkūrimo projektams įgyvendinti. Pirmasis atkūrimo sluoksnis gali būti tiesiogiai susijęs su buveinės pagerinimu, pavyzdžiui, papildant nerštavietes žvirgždu ar jas valant. Vandens telkinio baseino mastu galima atkurti arba sumažinti tam tikras žemėnaudos charakteristikas,

pvz., plėsti apsaugines juostas palei upių krantus ar įrengti dirbtines šlapynes prie nuotekų išleidimo vietų. Daugiamečio atkūrimo metodo taikymas leistų įdiegti gamtiniais sprendimais (nature-based solutions) paremtas priemones, kurios skatintų pusiausvyrą tarp žmogaus veiklos ir ekosistemų atsparumo. Tai galėtų padidinti lašišinių žuvų populiacijas atkurtuose baseinuose ir sustiprinti jų atsparumą kitoms antropogeninėms grėsmėms, pvz., komercinei žvejybai ar migracijos metu patiriamam mirtingumui.

5. Šis metodas turėtų būti taikomas vertinant daugelio Lietuvos vandens telkinių potencialą palaikyti jautrias litofilines žuvų rūšis. Tai apima Atlanto lašišą, šlakį, upinę nėgę (*Lampetra fluviatilis*). Šio tyrimo metu pasiūlytas metodologinis karkasas gali būti naudojamas kaip šablonas pradiniam būklės vertinimui atlikti, po kurio galėtų būti įgyvendinamos buveinių atkūrimo priemonės, siekiant padidinti šių žuvų populiacijas ir jų išlikimo galimybes Lietuvos vandens telkiniuose.

7.10. Išvados

1. Trys tirtos upių baseinų sistemos pasižymi reikšmingai skirtingomis žemėnaudos struktūromis ir fizikocheminėmis vandens savybėmis. Blendžiavos upelis turi didelę miškų ir sudėtingų žemės ūkio naudmenų dalį, kas siejama su aukštu ištirpusio deguonies (DO) kiekiu. Smeltaitės upelis pasižymi didelėmis neapaugusių dirbamų žemių ir urbanizuotų teritorijų dalimis, kas susiję su aukštomis azoto junginių koncentracijomis, elektros laidumu (EC) ir šarmingumu. Šventosios upė turi tiek miškų, tiek ariamų žemių plotų, o jos fizikocheminės vandens savybės yra tarpinės tarp dviejų kitų vandens telkinių.
2. Trys upių sistemos skirtingai reaguoja į hidrologinius ekstremalius įvykius. Smeltaitės upelyje ilgalaikiai mažo vandens lygio periodai padidina azoto junginių koncentraciją, šarmingumą ir elektros laidumo vertes. Blendžiavos upelyje intensyvūs lietūs, sąlygojami didelio upės nuolydžio, sukelia bendrųjų suspenduotų dalelių koncentracijos šuolius. Šventosios upėje miškų gausa slopina paviršinio nuotėkio erozinį potencialą, todėl čia bendrųjų suspenduotų dalelių kiekiai yra maži.
3. Sisteminga literatūros apžvalga parodė, kad erdvinė-laiko įvairovė, fizikocheminė vandens kokybė bei tarprūšiniai ir vidurūšiniai skirtumai lemia lašišinių žuvų neršto vietų pasirinkimą ir jų sąveiką su gruntiniu vandeniu. Salvelinus ir Oncorhynchus gentys dažnai naudoja gruntinio vandens veikiamas nerštavietes. Salmo genties atstovai pirmenybę teikia rėvų-duburių perėjimo zonoms, kur paviršinis vanduo infiltruojasi į upės dugną. Upeliuose tiek paviršinio vandens infiltracijos, tiek gruntinio vandens išsikrovimo vietų lygiagretus naudo-

jimas nerštui gali būti paaiškinamas daugiapakope erdvine analize, kuomet paviršinio vandens infiltracijos vietos naudojamo nerštui lokaliai mikrobuveinės lygiu, didesnio regioninio mezo- ar makromastelio gruntinio vandens iškrovos ruožuose.

4. Gruntinio vandens buvimas yra būdingas Blendžiavos, Šventosios ir Smeltaitės vandens telkinių nerštaviečių hiporėjiniam vandeniui. Tai patvirtina F^- ir Ar koncentracijos, kurios buvo panašios nerštaviečių ir šulinių mėginiuose, bet skyrėsi nuo paviršinio vandens mėginių. Gruntinio vandens patekimas į šias sistemas sustiprina hiporėjinį srautą, kas gali prisidėti prie nuosėdų išsivalymo ir palengvinti lervų judėjimą smulkių nuosėdų terpėje.
5. Hiporėjinio vandens kokybės vertinimas lašišinių žuvų inkubacijos laikotarpiu parodė, kad DO reikšmės buvo mažesnės nei kritinės ribos, reikalingos embrionų išgyvenimui. Šias nepalankias sąlygas dar labiau blogina temperatūros didėjimas, kuris ne tik sumažina deguonies tirpumą, bet ir pagreitina organizmų metabolizmą, didindamas deguonies poreikį.
6. Rekonstruotose lašišinių žuvų ikrų dėtyse buvo užfiksuotas didėjantis O_2 suvartojimas progresuojant vystymuisi. Be to, bioplėvelės augimas padidino bendrą kvėpavimo intensyvumą eksperimentinėse inkubacinėse kolonėlėse, tapdamas dominuojančiu veiksniu inkubacijos pabaigoje. Inkubacijos metu ikrų dėtys su inkubaciniu substratu iš pradžių veikė kaip amonio šaltinis, tačiau vėliau tapo amonio ir nitratų siurbliais, dėl bioplėvelės formavimosi ir oksidacinių-redukcinių mikrozonų susidarymo.

8

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9

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CURRICULUM VITAE

Biography: Rudy Benetti was born on 1996 January 20, in Spilimbergo, Italy. In 2018, he graduated with a Bachelor's Degree in Environmental Sciences from the University of Trieste (110/110 and prize) in Italy with a thesis on the history and use of macroinvertebrates for the ecological status assessment of running waterbodies pursuant to the Water Framework Directive (Supervisor: Prof. Elisabetta Pizzul; Advisor: Dr. Marco Bertoli). The theoretical part of the thesis is applied to a case aimed at identifying the potential stressors responsible for the decline of the endangered marble trout (S. marmoratus) population in a stretch of Brenta River, Italy. In 2020, he graduated with a Master's in Sciences and Technologies for the Environment and Resources (Department of Life Sciences) from Parma University, Italy. The thesis subject was the ecosystem services improvement related to the fishways establishment in a Toce River stretch in Italy, falling in a protected Natura 2000 area (Supervisor: Prof. Marco Bartoli; Advisors: Dr. Rossano Bolpagni and Dr. Pietro Volta). In 2020, in cooperation with the Department of Life Sciences, he started a PhD position at the Marine Research Institute of Klaipėda University (Lithuanian state-funded scholarship) (Supervisor: Prof. Marco Bartoli; Advisors: Dr. Nerijus Nika and Edoardo Severini).

Research interests: Salmonids spawning habitats assessments, ecosystem services.

Biografija: Rudy Benetti gimė 1996 m. sausio 20 d. Spilimberge, Italijoje. 2018 m. jis baigė Triesto universitetą, įgijęs aplinkos mokslų bakalauro laipsnį (110/110 ir premija) su baigiamuoju darbu apie makrobestuburius ir jų naudojimą tekančių vandens telkinių ekologinės būklės vertinimui pagal Vandens pagrindų direktyvą (darbo vadovė – prof. Elisabetta Pizzul; konsultantas – dr. Marco Bertoli). Teorinė darbo dalis buvo pritaikyta tyrime, siekiant nustatyti galimus veiksnius, lemiančius nykstančio marmurinio upėtakio (Salmo marmoratus) populiacijos mažėjimą viename Brentos upės ruože Italijoje. 2020 m. jis baigė Aplinkos ir išteklių mokslų bei technologijų magistrantūros programą (Gyvybės mokslų fakultetas) Parmos universitete, Italijoje. Jo magistrinis darbas buvo skirtas ekosisteminių paslaugų gerinimui, susijusiam su žuvitakių įrengimu Točės upės ruože Italijoje, kuris priklauso saugomai „Natura 2000" teritorijai (darbo vadovas – prof. Marco Bartoli; konsultantai – dr. Rossano Bolpagni ir dr. Pietro Volta). 2020 m., bendradarbiaudamas su Gyvybės mokslų fakultetu, jis pradėjo doktorantūros studijas Klaipėdos universiteto Jūrų tyrimų institute (finansuojamas Lietuvos valstybės stipendija) (vadovas – prof. Marco Bartoli; konsultantai – dr. Nerijus Nika ir dr. Edoardo Severini).

Moksliniai interesai: Lašišinių žuvų neršto buveinių vertinimas, ekosistemų paslaugos.

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10

Supplementary materials

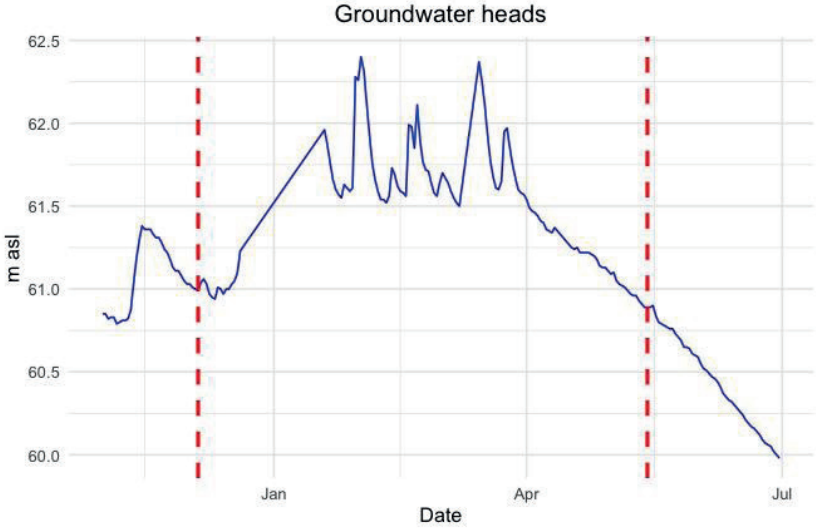

Figure S1 – Groundwater heads (meters above sea level) during the sampling campaigns. Sampling events are highlighted by the red line.

Table S1 – Average values of groundwater quality parameters during winter 2022 and spring 2023. Values are pooled from all three investigated systems.

Parameter	Winter 2022 Average	Spring 2023 Average	Winter 2022 SD	Spring 2023 SD
Ar (µM)	16.78	16.45	1.04	0.76
Ca^{2+} (mg L^{-1})	92.08	79.14	26.55	40.62
CH_4 (mg L^{-1})	3973.75	2763.07	11499.05	6834.09
Cl^- (mg L^{-1})	39.37	10.04	23.96	5.69
F^- (mg L^{-1})	0.52	0.11	0.18	0.04
HCO_3^- (mg L^{-1})	236.86	257.22	138.25	153.02
K^+ (mg L^{-1})	8.88	4.19	12.63	5.65
Mg^{2+} (mg L^{-1})	11.74	10.67	7.82	11.37
N_2 (sat %)	107.03	108.76	4.68	4.05
Na^+ (mg L^{-1})	10.98	0.87	7.06	0.64
N-NH_4 (mg L^{-1})	0.17	0.10	0.28	0.17
N-NO_3 (mg L^{-1})	0.99	0.96	1.27	1.23
O_2 (mg L^{-1})	54.55	51.26	25.83	32.02
pH (mg L^{-1})	7.51	6.86	0.37	0.40
PO_4^{3-} (mg L^{-1})	0.55	0.55	1.10	1.06
SO_4^{2-} (mg L^{-1})	49.04	9.64	21.47	9.05
Temp (°C)	7.48	10.92	1.05	3.84

Table S2 – Average values of surface water quality parameters during winter 2022 and spring 2023. Values are pooled from all three investigated systems.

Parameter	Winter 2022 Average	Spring 2023 Average	Winter 2022 SD	Spring 2023 SD
Ar (µM)	16.53	14.94	0.38	0.78
Ca^{2+} (mg L^{-1})	74.56	75.80	9.04	20.35
CH_4 (mg L^{-1})	321.70	314.70	170.08	153.48
Cl^- (mg L^{-1})	31.25	17.77	24.24	16.76
F^- (mg L^{-1})	0.30	0.11	0.15	0.04
HCO_3^- (mg L^{-1})	195.28	235.52	63.66	45.78
K^+ (mg L^{-1})	2.65	2.30	0.62	0.82
Mg^{2+} (mg L^{-1})	11.14	11.29	1.38	2.53
N_2 (sat %)	109.78	103.50	0.64	0.74
Na^+ (mg L^{-1})	14.31	1.15	10.73	0.90
N-NH_4 (mg L^{-1})	0.40	0.06	0.45	0.06
N-NO_3 (mg L^{-1})	0.82	0.89	0.48	0.43

O_2 (mg L^{-1})	102.77	100.70	4.63	16.94
pH (mg L^{-1})	6.69	7.52	3.16	0.22
PO_4^{3-} (mg L^{-1})	0.07	0.06	0.03	0.05
SO_4^{2-} (mg L^{-1})	39.89	11.95	22.78	4.58
Temp (°C)	0.89	14.68	0.46	0.88

Table S3 – Average values of intragravel (nest) water quality parameters during winter 2022 and spring 2023. Values are pooled from all three investigated systems.

Parameter	Winter 2022 Average	Spring 2023 Average	Winter 2022 SD	Spring 2023 SD
Ar (µM)	16.36	16.11	0.37	1.06
Ca^{2+} (mg L^{-1})	76.84	76.67	12.46	17.97
CH_4 (mg L^{-1})	799.16	3638.47	1201.26	8447.08
Cl^- (mg L^{-1})	47.08	13.37	37.64	11.48
F^- (mg L^{-1})	0.45	0.11	0.13	0.03
HCO_3^- (mg L^{-1})	185.75	248.45	30.77	43.55
K^+ (mg L^{-1})	2.81	1.56	1.10	0.72
Mg^{2+} (mg L^{-1})	11.83	10.03	0.90	1.96
N_2 (sat %)	109.68	106.04	0.99	4.25
Na^+ (mg L^{-1})	16.62	0.88	13.27	0.59
N-NH_4 (mg L^{-1})	0.31	0.12	0.38	0.13
N-NO_3 (mg L^{-1})	1.09	0.91	0.82	0.50
O_2 (mg L^{-1})	81.94	68.53	46.58	27.58
pH (mg L^{-1})	5.92	7.45	3.35	0.64
PO_4^{3-} (mg L^{-1})	0.07	0.08	0.06	0.04
SO_4^{2-} (mg L^{-1})	46.20	9.79	23.20	3.64
Temp (°C)	1.06	14.87	0.30	0.77

Klaipėdos universiteto leidykla

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SURFACE-GROUNDWATER INTERACTIONS, HYDROLOGICAL REGIME AND LAND USE: IMPACTS ON STREAM FUNCTIONING FOR SALMONID SPAWNING AND INCUBATION PHASES
Doctoral dissertation

PAVIRŠINIO IR GRUNTINIO VANDENS SĄVEIKOS, HIDROLOGINIO REŽIMO IR ŽEMĖNAUDOS POVEIKIS UPĖS FUNKCIONAVIMUI LAŠIŠINIŲ ŽUVŲ NERŠTO IR INKUBACIJOS FAZĖSE
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