

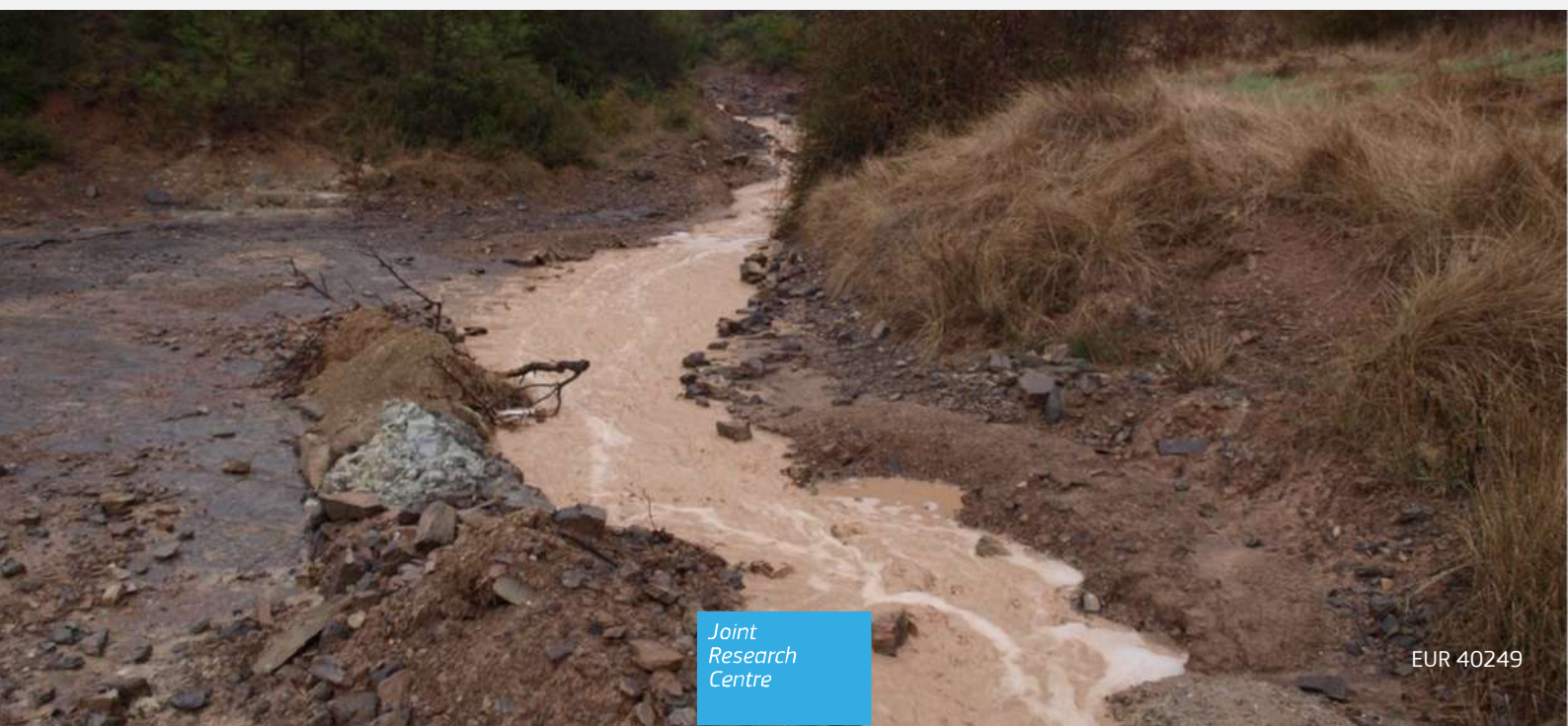


Preparing European freshwaters for a saltier future

Cañedo-Argüelles, M., Beklioğlu, M., Crabot, J., Cunillera-Montcusí, D., Free, G., Hernández-Carrasco, D., Kelly, M.G., Moyano Salcedo A.J., Schäfer, R.B., Poikane, S.



2025



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JRC141037

EUR 40249

PDF ISBN 978-92-68-25279-6 ISSN 1831-9424 doi:10.2760/7705629 KJ-01-25-157-EN-N

Luxembourg: Publications Office of the European Union, 2025

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How to cite this report: European Commission, Joint Research Centre, Cañedo-Argüelles, M., Beklioğlu, M., Crabot, J., Cunillera-Montcusí, D., Free, G., Hernandez- Carrasco, D., Kelly, M.G., Moyano Salcedo, A.J., Schäfer, R.B. and Poikane, S., *Preparing European freshwaters for a saltier future*, Publications Office of the European Union, Luxembourg, 2025, <https://data.europa.eu/doi/10.2760/7705629>, JRC141037.

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Abstract

Humans are altering the quantity and composition of salts in freshwater ecosystems worldwide through a wide variety of activities (e.g. agriculture, resource extraction, transportation, urbanization). This phenomenon is known as freshwater salinization and it leads to drastic reduction in freshwater biodiversity, alteration of ecosystem functioning, socioeconomic damage and risks to human health. In spite of the potential ecological and socioeconomic impacts, freshwater salinization is still poorly managed. For example, most legislation and regulations that target salinity have been designed to ensure water quality for irrigation and human consumption, but only a few consider biodiversity and ecosystem functioning. In Europe, salinization is a direct pressure defined in Annex V of the Water Framework Directive. This means that member states have to: (1) set salinity thresholds for high and good status classes; (2) evaluate salinity conditions and report this to the WFD-WISE reporting systems. However, this has been done by roughly a half of the countries. Fifteen countries have standards for parameters that relate to the salinization of rivers, while 9 countries have set standards for lakes. Different metrics (conductivity and/or chlorides) and different measures (annual average, seasonal average, 90th percentile, maximum concentrating) are used, rendering comparison difficult. Moreover, the rationale behind these thresholds is not always present or accompanied by evidence that shows their effectiveness for protecting freshwater organisms. This report aims to address the overlooked issue of increasing salinization in European water bodies, which poses a threat to biodiversity, ecosystem integrity and food and water security. Using a European-wide dataset that includes information on salinity, salinization drivers, and aquatic macroinvertebrate communities (selected as a potential Biological Quality Element for monitoring salinization's impact) we explore ecologically relevant salinity criteria and identify potential salinization indicators. We also assess the potential interactions between salinization and climate change in freshwater ecosystems focusing on the Oder River and Turkish lakes as two case studies. In this report policy-makers and water managers can find a model-based extent of river salinization at the EU-scale, invertebrate-based metrics to monitor freshwater salinization, salinization criteria that can protect freshwater biodiversity and information on the potential interaction of salinization and climate change in rivers and lakes. There is an important take-home message from this report: we need to develop standards for salinization (i.e. increase in salinity) rather than for salinity per se. This is because salinity has both natural and anthropogenic causes, but the effect that they have on biota is very different. Also, the changes in relative ion concentrations could be even more important than the differences in total salinity. This is because different ions have different toxicities. Thus, ion-specific standards should be developed taking into account natural ion mixtures and the ecological impacts derived from their alteration. Although this report constitutes a major step forward into the effective regulation of freshwater salinization in Europe, it needs to be used with caution. For example, we provide a salinization threshold that should potentially protect freshwater biodiversity and suggest that around 22% of the European rivers and streams could be threatened by salinization. Since this threshold has been calculated at the EU scale, we warn against its direct application. Developing river-basin specific or national standards following the guidelines offered by this report will probably result in more reliable standards that more accurately account for the ecological context. The data and technical expertise to develop such standards is readily available. Therefore, we urge EU member states to take action and we hope that this report can guide their efforts.

Acknowledgements

We thank all governmental authorities that provided data. We thank Mathieu Floury for providing processed data for France, and Heikki Mykrä and Jukka Aroviita for providing processed data for Finland. We thank Alfredo Corrochano (TRAGSATEC) and Juan Alánde Rodríguez (Dirección General del Agua, Ministerio para la Transición Ecológica y el Reto Demográfico, Gobierno de España) for providing processed data for Spain. David Cunillera-Montcusí received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101062388. This work was supported by the i-LINK 2022 funding scheme (CSIC, Spanish National Research Council). Research on Turkish lakes and establishment of mesocosm set up were funded by TUBITAK program BIDEB 2232 International Fellowship for Outstanding Researchers (project 118C250) and running the mesocosms systems for two experiments were funded by EU-H2020, INFRAIA project AQUACOSM-Plus (no 871081).

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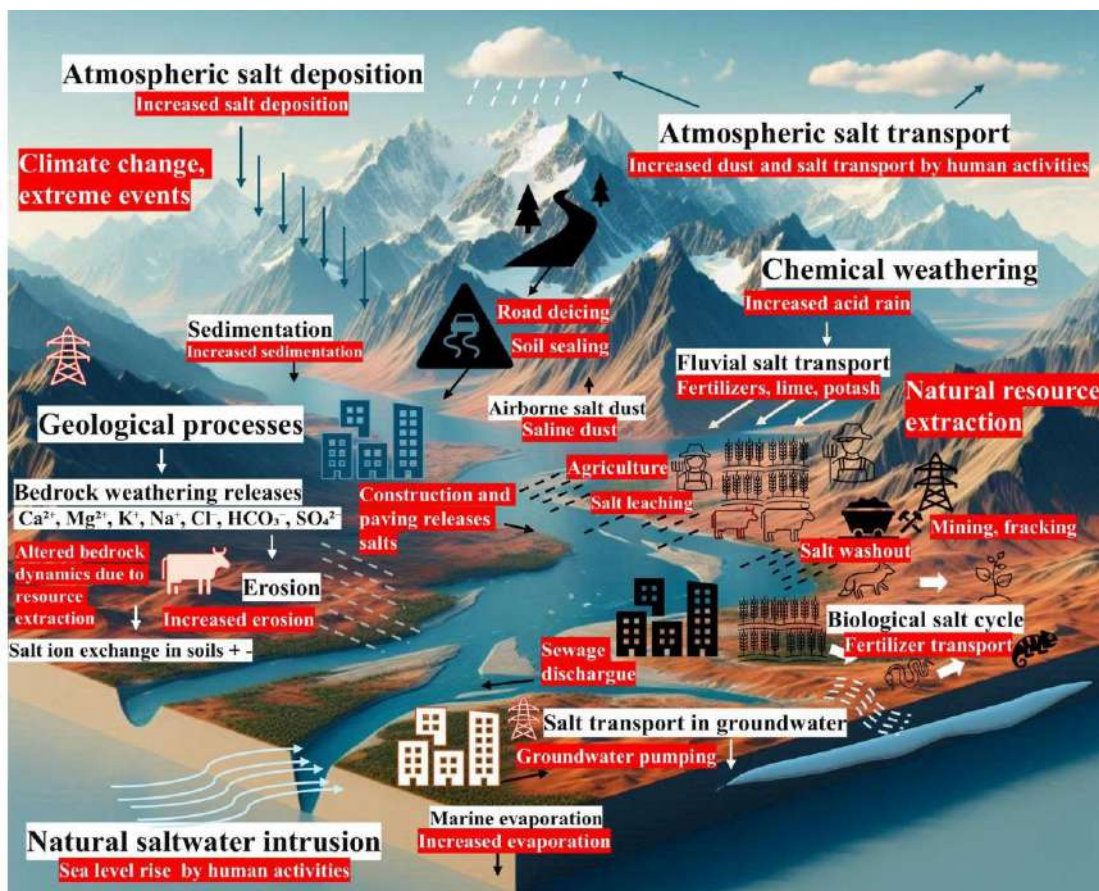
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1. Introduction

1.1 Freshwater salinization: definition and causes

All inland water bodies, including lakes, rivers, streams and wetlands, contain dissolved ions (i.e. salts). This is mainly because (1) rainwater carries ions resulting from cloud dynamics and microphysical processes (Mouli et al. 2005; Keresztesi et al. 2020), (2) surface runoff and groundwater dissolve ions contained in soils and rocks (Kaushal et al. 2023), and (3) seawater enters inland water bodies (Ibañez et al. 1997; Carreira et al. 2014). Climatic and hydrogeological forces also shape the final concentration of dissolved ions in inland water bodies. For example, droughts result in increased salinity due to a reduction in the dilution capacity of surface waters (Jones & van Vliet, 2018). Inland water bodies around the world can therefore present very different ion composition and concentrations depending on their distance to the sea, the geology of their catchments and their climate (Meybeck, 2003a). For instance, in the central Amazon the sodium (Na^+) concentration of pristine rivers and streams ranges between 10 and 100 $\mu\text{mol/L}$, whereas in Japan it is one order of magnitude higher, ranging between 100 and 1000 $\mu\text{mol/L}$ (Meybeck, 1994). All these natural processes that simultaneously modulate the salinity of inland water bodies form part of the “natural salt cycle” (Kaushal et al. 2023). However, since the beginning of the industrial revolution, the concentrations of dissolved ions in inland water bodies have been profoundly altered by human activities at a global scale (Meybeck, 2003a, 2003b), leading to the “anthropogenic salt cycle” (Figure 1).

Figure 1. Main sources, causes, transport, dynamics, and storage of natural salinity (in white) and salinization (in red).



Source: Created by Álvaro Moyano based on Kaushal et al., 2023.

The drivers of the anthropogenic salt cycle are diverse and differ among regions, also leading to different chemical cocktails (Kaushal et al. 2024). The most important are:

1. Agriculture: Irrigation of agricultural fields is one of the main global drivers of freshwater salinization through several mechanisms (Thorslund et al., 2021) including the use of saline water for irrigation, saltwater intrusion caused by excessive pumping, and the displacement of freshwater by saline water (Kaushal et al. 2024). Additionally, salts can move downward through the unsaturated zone (i.e., the layer of soil above the phreatic zone), dissolve from minerals in the soil, and become concentrated as plants absorb water and release it through evapotranspiration (Kaushal et al. 2024). For example, an annual irrigation of 1000 mm with irrigation water with salt content as low as 300 mg/l adds up to 300 kg of salts per hectare, depending on leaching (Fienen & Arshad, 2016). Furthermore, excessive use of both surface and ground waters for irrigation can lead to an accumulation of salts in lakes, ponds and rivers through evaporation as the water balance becomes negative, which results in the salinization of these originally freshwater habitats. In addition to irrigation water, the application of fertilizers contributes to freshwater salinization because they contain salts. For example, fertilizers constituted the main sources of sulphates into a Mediterranean aquifer (Elmeknassi et al., 2022).
2. Resource extraction: Mining has been responsible for some of the most notable river salinization cases in Europe. For instance, potash mining led to the severe salinization of the Llobregat river in Spain and the Werra river in Germany. During the extraction of potash, the mining waste (mainly composed by NaCl) was stockpiled and continuously dissolved by rainwater and humidity, thereby reaching nearby rivers and streams through surface runoff and groundwater infiltration. As a result, a small stream flowing into the Llobregat River registered an electrical conductivity up to 132 mS cm^{-1} (Ladreda et al. 2017), which is nearly 3 times higher than that of seawater. In the Werra river, the water authorities found maximum chloride concentrations of 30 g/L, which is nearly 2 times higher than that of seawater (Bäthe & Coring, 2011). Coal mining has also been shown to greatly increase the concentration of ions (especially sulfate) in surrounding water bodies because it exposes rocks to weathering (Palmer et al., 2010; Halabowski et al. 2020). Gas and oil extraction are also important drivers of salinization because, after hydraulic fracturing, the pressure barriers are removed and the water used in the extraction emerges to the surface. Once the well begins to produce gas, this water is referred to as "produced water". Produced waters are a mixture of injected fluids and water that was originally present in the target or surrounding formations (formation water), and they present extremely high salinity levels (Vidic et al. 2013). In some cases, produced waters are directly discharged into rivers and streams. For example, in the Peruvian Amazon (a hotspot of freshwater biodiversity) thousands of liters of produced water with a maximum chloride concentration of 193 g/L were disposed daily into rivers and streams (Yusta-García et al. 2017), significantly increasing the Na^+ and Cl^- export of the whole Amazon River (Moquet et al. 2014). In other regions, produced waters are treated and/or reinjected, but they can still contribute to salinization because treatments do not effectively recover the original salt concentration and there are still many accidental spills or direct disposal into the environment (Folkerts et al. 2020).
3. Transportation: Salts are widely used as de-icing agents around the world to safeguard driving safety. In Europe, the total annual usage of road salt for de-icing varies significantly depending on winter conditions, ranging from approximately 5 to 17.5 million tons (Gaudé,

2021). In Spain, 101,995 tons of NaCl were applied to roads in the winter 2021-2022 (Spanish Government, 2024). These salts are then washed into surrounding water bodies, which are naturally oligohaline, through snowmelt and precipitation (Szklairek et al. 2022). This results in great salinity increases, exceeding the 2000‰ in relation to baseline levels (Kaushal et al., 2005). Roads can also contribute to salinization because they contain base cations and carbonates that are chemically weathered (Kaushal et al., 2017).

4. Wastewaters: Urban wastewaters have high salt concentrations that come from multiple products used in our daily life, such as dishwashers and laundry powder or fabric softeners (Tjandraatmadja et al., 2010), in addition to the salt that we consume in our diet. Industrial wastewaters, such as those produced by the textile and pulp and meat industries, can also present high salinity levels (Cañedo-Argüelles et al., 2013). Although the water quality of industrial and urban effluents depends on the characteristics of the wastewater treatment plants, they are usually saline because conventional treatments are not effective for salt-removal (Salcedo et al. 2024a).

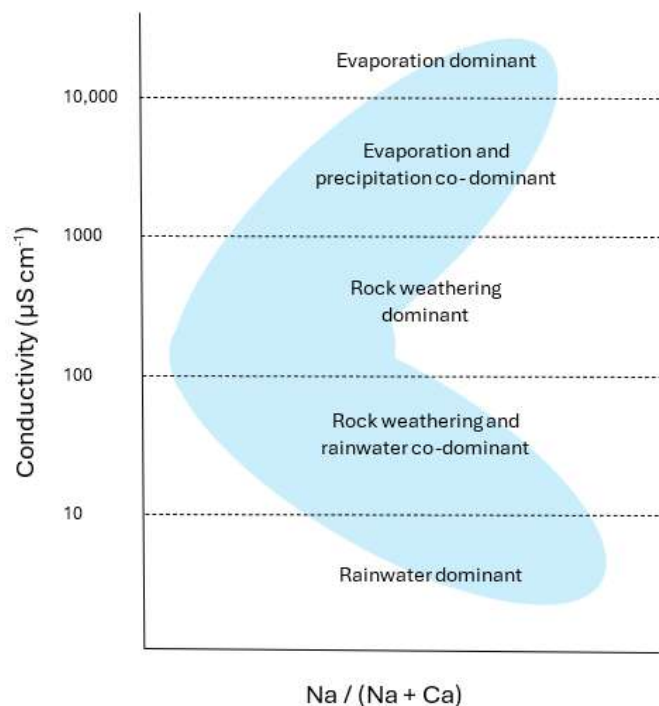
Since all these human activities generate many other chemicals, salinization interacts with them leading to chemical cocktails (Kaushal et al. 2019). Pesticides, for instance, are typically organic chemicals but can exist in various formulations and combinations (Weisner et al., 2021), which may contribute to the chemical mix found in salinized agricultural freshwater around the world (Beketov et al., 2013). Freshwater ecosystems impacted by road runoff can also contain other potentially toxic substances, such as metals (Rose & Shea, 2007) and hydrocarbons (Hwang et al., 2019), which originate from waste products such as road dust, exhaust emissions, catalytic converters, lubrication fluids, brake linings, and tire and road wear. Finally, the chemical mixture in coal-mine effluents is marked by highly acidic waters (acid mine drainage) that reduce the pH of the surrounding water bodies and release metals (Acharya & Kharel, 2020). These are just some examples of chemical cocktails associated with freshwater salinization, but there are many more, and we still lack a proper characterization for most of them.

1.2. The geochemical context

Four principal mechanisms determine the ionic strength and chemical composition of surface water: precipitation inputs, rock and soil weathering, seawater intrusion and evaporation/crystallisation, the latter principally in saline lakes and oceans. Gibbs (1970) developed a model to visualise the relative importance of these three mechanisms in determining the chemistry of a surface water body by plotting total dissolved solids against the ratio of Na^+ to the sum of Na^+ and Ca^{2+} (Figure 2). For example, a stream in a siliceous catchment close to the sea will have a low Ca^{2+} concentration relative to that of Na^+ resulting in a high ratio, while in a limestone catchment the concentration of Ca^{2+} will be high resulting in a low ratio. Evaporation will increase overall ionic strength to the point where Ca^{2+} will precipitate, leading to a higher proportion of Na^+ . Although the Gibbs model has been criticised (Eilers et al., 1992), it is a useful means for classifying natural waters as well as for tracing alterations to the base geochemistry over time. The Gibbs diagram forms a “v”-shaped relationship, where the upper arm represents “salinization”, as understood in this report, and the lower arm represents “alkalinization”, which is an acknowledged pressure in soft water lakes (Arts, 2002), albeit beyond the scope of this report. This definition of “salinization” reflects both ionic strength and composition although the most widely-used definitions and measures (see below) just measure strength. We also stress that sites may move in both directions along these axes, and it is possible that a naturally saline water body will become more dilute due to human activities (a problem in the soda pans and a risk for Lake Neusiedl in Eastern Austria, Krachler et al. 2009), some saline streams in Spain (Gutiérrez-Cánovas et al.,

2012) or transitional waters cut off from the sea (e.g. Lake Mandra in Bulgaria, Michev & Stoyneva 2007). Again, this is beyond our scope, but readers need to be aware that such situations do exist. Whilst there have been other attempts to describe salinity and salinization in terms of particular ion ratios (e.g. Zieman & Schulz, 2011), these are often context-specific and less suitable for a global overview. Such an approach could also be used to compare a reference background value and trace deviation away from this resulting from anthropogenic pressure. However, the Gibbs model has been criticised (Eilers et al., 1992) because it fails to adequately describe very dilute lakes or lakes with very high marine inputs. The model also assumes Ca^{2+} weathering to be indicative of mineral weathering and Na^+ to be indicative of precipitation inputs. This may not be the case when Na^+ is an important weathering product relative to Ca^{2+} or if there is minimal marine influence in precipitation. Whilst Gibb (1970) focussed on cations, emphasised the importance of anions, defining salinized waters as those where total salinity : chloride < 4 (or conductivity : chloride < 6).

Figure 2. Relationship between total dissolved solids and $\text{Na}^+ / \text{Na}^+ + \text{Ca}^{2+}$.



Source: Gibbs, 1970.

1.3. Consequences for ecosystems and human welfare

Freshwater organisms need to maintain an internal osmotic pressure relative to that of the water in their habitats (rivers, lakes, wetlands, etc.). If the salinity of the water becomes higher than the internal salinity, they have to use energy to expel ions and retain water through osmoregulation (Griffith, 2017). This has important consequences for the organisms, since this energy cannot be spent in basic functions such as growth and reproduction (Hassell et al., 2006; Hintz & Relyea, 2017). Therefore, salinization acts as a stressor, triggering oxidative responses (Cañedo-Argüelles et al., 2016a) and activating genes related to osmotic and chemical stress in all tissues (Escobar-Sierra et al., 2024). Overall, sub-lethal effects (i.e., negative effects that do not directly imply the death of the organism) on organisms' physiology can lead to reduced fitness and compromise the survivorship of the individuals and the viability of their populations. If salinity concentrations reach critical points, the osmoregulation mechanisms collapse and the organism dies. Although physiological mechanisms might be more complex than what we have described (Kefford, 2019),

field studies unequivocally report a drastic decline in the abundance and richness of freshwater organisms along salinity gradients. For example, Pinder et al. (2005) found that the freshwater species richness started to drastically decline when a salinity threshold of 2.6 g/L was reached in the wetlands of the Australian Wheatbelt region. Concordantly, data from field works as well as mesocosms experimental systems show that physical and chemical properties as well as biota might respond to salinization in a non-linear fashion with certain salinity thresholds (Jeppesen et al., 2020; Vidal et al., 2021; Hintz et al., 2022).

As stenohaline freshwater species disappear, euryhaline species (including invasive species) are able to colonize salinized habitats, leading to novel communities (Kefford et al., 2016). This can have implications for ecosystem functioning. For example, most of the aquatic invertebrates responsible for leaf litter decomposition in streams are salt sensitive; thereby salinization reduces decomposition and the availability of organic matter within the stream (Canhoto et al., 2021). Studies performed in lakes in north-west China showed drastic reductions in biodiversity, food chain length, and average trophic position, indicating loss of ecosystem functioning with increasing salinity (Vidal et al., 2021, Jeppesen et al., 2020). Even a small change in salinity may have drastic effects on the structure and functions of aquatic ecosystems if critical salinity levels are surpassed. For example, the salinization of lakes can boost algal production through the elimination of large zooplankton that feed on them (Hintz et al., 2022). Such thresholds could depend on the abiotic context and therefore change with climate. However, the ecological mechanisms behind salinity thresholds are largely unknown and need to be explored with a wide geographic coverage as well as with gradient design mesocosm experiments including different local biotas.

Increases in the salinity of freshwater can limit its use for human activities (Figure 3). For example, a salinity higher than 1 g/L can have an impact on the quality of drinking water, irrigation water, and water for industries that require intermediate water quality. Therefore, freshwater salinization can jeopardize water and food security (van Vliet et al., 2017). Although the economic costs of freshwater salinization have rarely been quantified, available estimates suggest that they can be very high. For example, the annual economic cost of anthropogenic salinization in the Colorado River Basin (USA) from agricultural practices is estimated at \$300 million (Kucera, 2019). This includes approximately \$176 million worth of damage to crops, and \$81 million worth of damage to households each year. In the Murray-Darling Basin (Australia) the total annual impact costs of dryland and urban salinity to key stakeholders were estimated at \$304 million per year (Wilson, 2004). Similarly, due to freshwater salinization, the city of Barcelona now relies on desalination or membrane technologies to meet drinking water quality demands, whose installation cost surpassed €130 million and which maintenance cost is around €1.2 million per year (Honey-Roses, 2012).

Even more worrying than the economic costs of freshwater salinization are its potential effects on human health. In some cases, health effects are directly caused by salt exposure. In the coast of Bangladesh, wells are affected by seawater intrusion, leading to a high NaCl daily intake by the local population through drinking water. This NaCl intake has been related to pre-eclampsia in pregnant women (Khan et al., 2014), causing miscarriage (Hossain et al., 2024). In other cases, the health effects are indirectly related to salinization and therefore more difficult to anticipate and detect. For instance, in Flint (Michigan, USA), salts corroded water infrastructure and contributed to increased lead concentrations in drinking water, which had serious deleterious effects on the population, especially children (Kaushal, 2016). In Spain, rivers impacted by potash mining exhibited elevated levels of bromomethanes (Gorostiza & Sauri, 2017), compounds that have been associated with an increased risk of cancer in humans (Min & Min, 2016). In addition to the risks posed to drinking water quality, freshwater salinization can impact human health through various pathways, including wind-borne dust causing respiratory issues, increased virus transmission by

fostering mosquito populations, and mental health challenges stemming from environmental degradation (Jardine et al., 2007). For example, in the Aral Sea basin as well as Lake Urmia, salt dust inhalation has been related with incidences of infant mortality, typhoid, hepatitis, respiratory illnesses and esophageal cancers (Weinthal, 2002).

Figure 3. Overview of the effect of salinity on water uses. Green = conditions will support the use; yellow = some effects may be observed; red = likely to be detrimental to use. EC = electric conductivity, TDS = total dissolved solids, Cl⁻ = chloride concentration. Based on data from WHO (2022) [drinking water quality], Ayers and Westcot (1985) [irrigation], DWAF (1996) [industrial water use], ANZECC (2000) [water for livestock].

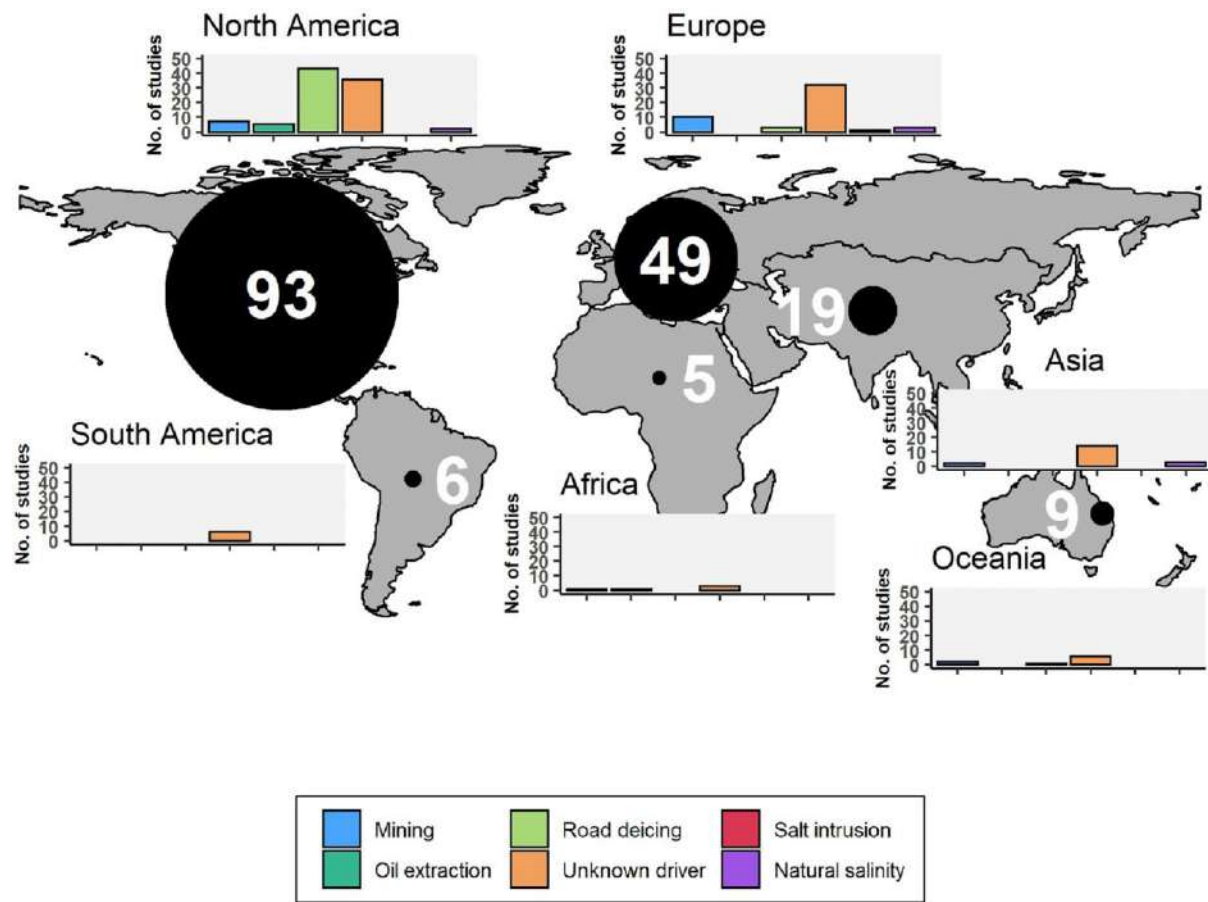
TDS (mg/l)	Drinking water quality	Water quality for irrigation	Water quality for livestock (dairy cattle)	Industries that require intermediate quality water (e.g., food and beverage industry)
200	Good palatability TDS < 600 mg/L	No restriction on use TDS < 450 mg/L EC < 700 µS/cm	No adverse effects TDS < 2500 mg/L	No effect of product quality TDS < 200 mg/L; EC < 300 µS/cm
500 600				Slight to moderate impairment to product quality TDS 200-800 mg/L EC 300-1200 µS/cm Cl ⁻ 40-200 mg/L
800		Fair palatability TDS 600-1000 mg/L		Slight - moderate restriction on use TDS 450-2000 mg/L EC 700-3000 µS/cm
1000	Significantly unpalatable Distinctly salty taste TDS > 1000 mg/L Cl ⁻ > 250 mg/L	Severe restriction on use TDS > 2000 mg/L EC > 3000 µS/cm	Satisfactory, short-term effects TDS 2500-4000 mg/L	
1500				Decline in animal health / production TDS 4000-7000 mg/L Unacceptable TDS > 7000 mg/L
2000				
2500				
4000				
7000				

Source: Kelly et al., 2024.

1.4. Management and regulation

Historically, freshwater salinization has been perceived by scientists, water managers and society at large as a phenomenon that only occurs in arid areas. Moreover, some agents involved in the salinization of freshwater systems (e.g. mining companies) have denied responsibility for environmental damage, arguing that salts are a natural component of freshwater ecosystems (Gorostiza & Sauri, 2019). While the global extent of freshwater salinization remains uncertain, available data shows that it is a pervasive environmental pressure worldwide, affecting a wide range of ecosystems (from high mountain streams to estuaries). For instance, Kaushal et al. (2018) estimated that 37% of the drainage area in the contiguous USA had been impacted by salinization, with statistically significant increases observed at 39% of sites for specific conductance, 34% for sodium, 29% for calcium, 33% for magnesium, and 36% for potassium levels. Similarly, Estévez et al. (2019) reported that approximately 27% of streams in Spain exhibited some degree of salinization. Additionally, Le et al. (2019) identified statistically significant upward trends in electrical conductivity (EC) for nearly 80% of streams in Germany. It is important to recognize that there is a strong geographical bias in freshwater salinization research, probably associated with the main scientific interests and geographical location of the research groups that are leading the investigations (Figure 4).

Figure 4. Global representation of studies on salinisation from 2017 to 2021 considering the causes of salinity changes in the studied systems (bar plot colours), including mining, road de-icing, salt intrusion, and oil extraction (e.g., fracking).



Trends in Ecology & Evolution

Source: Cunillera-Montcusí et al., 2022.

In spite of the potential ecological and socioeconomic impacts, freshwater salinization is still poorly managed worldwide (Cañedo-Argüelles et al. 2016b). For example, most legislation and regulations that target salinity have been designed to ensure water quality for irrigation and human consumption, but only a few consider biodiversity and ecosystem functioning. Salinization is a direct pressure defined in Annex V of the Water Framework Directive (WFD; EU, 2000). The criteria (set out in Annex V of the WFD) are collectively termed “ecological status”, with “good ecological status” (GES: defined as no more than a slight change from that expected under unimpaired conditions) being the threshold for sustainable water resources. The criteria are subdivided into:

- Biological quality elements;
- Physico-chemical supporting elements;
- Hydromorphological supporting elements.

“Salinity” is included as one of the physico-chemical supporting elements, both for inland, transitional and coastal water bodies. This means that member states have to: (1) set salinity thresholds for high and good status classes; (2) evaluate salinity conditions and report this to the WFD-WISE reporting systems. However, this has been done by roughly a half of the countries (Table1).

Table 1. Overview of salinity thresholds and salinity conditions reported for the WFD implementation.

	Salinity thresholds		Salinity condition (2 nd RBMP)	
	Rivers	Lakes	Rivers	Lakes
Austria	yes	yes		
Belgium	yes		yes	yes
Bulgaria	yes		yes	yes
Croatia				
Cyprus	yes		yes	yes (only 3 wb)
Czech Republic	yes	yes	yes	yes (only 2 wb)
Denmark				
Estonia				
Finland				
France			yes	
Germany	yes		yes	
Greece			yes	
Hungary	yes	yes	yes	yes
Iceland	yes	yes		
Ireland				
Italy			yes (only 1 wb)	
Latvia				
Lithuania				
Luxembourg	yes		yes	
Malta			yes (only 3 wb)	yes (only 2 wb)
Netherlands	yes	yes	yes	yes
Norway			yes (only 4 wb)	yes
Poland	yes	yes	yes	yes
Portugal	yes	yes	yes	yes
Romania	yes		yes	yes (only 1 wb)
Slovakia	yes		yes	
Slovenia				
Spain	yes	yes	yes	yes
Sweden				

Source: Water Framework Directive 2nd River basin management plans.

Fifteen countries have standards for parameters that relate to the salinization of rivers, while 9 countries have set standards for lakes (Table 2). Different metrics (conductivity and/or chlorides) and different measures (annual average, seasonal average, 90th percentile, maximum concentrating) are used, rendering comparison difficult. Moreover, the rationale behind these thresholds is not always present or accompanied by evidence that shows their effectiveness for protecting freshwater organisms. In general, most of the thresholds fall in the range of 450-1000 µS/cm for conductivity and 50-200 mg/L for chloride concentrations.

Several river and lake types have extremely high conductivity thresholds; however, a more detailed look shows that these high values belong to specific water body types linked to either saline water sources (Poland, Spain, and the Netherlands) or the karst origin of lakes (Spain).

Table 2. Summary table of conductivity ($\mu\text{S}/\text{cm}$) and chloride thresholds (mg/L) for rivers and lakes in Europe used to implement the Water Framework Directive. AA – annual average, SA – seasonal average, MAC – Maximum allowable concentration, P90 – 90th percentile.

Maximum allowable concentration, 150 – 500 percentage.					
		Rivers		Lakes	
	Metrics	Conductivity µS/cm	Chloride mg/L	Conductivity µS/cm	Chloride mg/L
Single threshold or Range of type-specific thresholds (median value)					
Austria	AA	-	150	1010 ¹	60 ¹ and 150
	MAC	-	600	-	-
Belgium	AA	800 (one river type)	150 (all river types)	-	-
	P90	600-1000	120-200	-	-
Bulgaria	AA	750-900 (750)	-	-	-
Czech Republic	AA	-	150 65 (drinking water treatment)	150 65 (drinking water treatment)	-
Cyprus	AA	750	-	-	-
Germany	AA	-	200	-	-
Hungary	AA	600 - 1200 (1000)	20-60 (50)	20 - 1500 (70)	-
Iceland	AA	112-264	-	104-220	-
Luxembourg	AA	-	200	-	-
Netherlands	SA	-	40-300 (150)	-	40 – 200 (200)
Poland	AA	300 - 2300 (620)	-	100 - 600 (600)	-
Portugal	AA	165-1100 (740)	70	100-1000 (530)	-
Romania	P90	1500	-	-	-
Slovakia	P95	50-70	-	-	-
Spain	AA	300 - 350 (300) 20-3500 ²	50-500 ²	350 20-700 ²	-
	MAC	300 - 700 (500) 100 - 2200 ²	-	600 - 3600 ³ (600)	-

¹ Threshold for Lake Neusiedl - a unique soda lake, represents a minimum value for conductivity and chlorides

² Specific thresholds set for several types

³ Highest type-specific values for two karstic calcareous lake types

Source: Salinity thresholds reported for the WFD implementation for 2nd River Basin Management Plans.

For example:

- In Poland, two river types have conductivity thresholds 2300 $\mu\text{S}/\text{cm}$. However, these types are described as “Estuarine river / brooks under the influence of salty waters”, so high salinity levels are natural (in fact, these river types should be under “transitional waters”). The next highest conductivity threshold - 850 $\mu\text{S}/\text{cm}$ – is allocated to very large lowland rivers.

- In Hungary, the highest conductivity threshold 1200 $\mu\text{S}/\text{cm}$ is for river type 7 - very large calcareous rivers (catchment > 10.000 km²).
- In Spain, an extremely high value 60,000 $\mu\text{S}/\text{cm}$ is allocated for one type, however this water body type is “salt marshes” so clearly belongs to transitional waters. Other high values are for “high mountain rivers” (probably specific geology), this type has also the highest chloride thresholds of 300 mg/L. For lakes, two Spanish lake types L-T10 and LOT12 - have extremely high conductivity values up to 3600 $\mu\text{S}/\text{cm}$, however these lake types are karst calcareous, so with naturally high mineralization levels.

In alignment with the WFD, the United Nations Sustainable Development Goal 6 (SDG 6.3.2) aims “to ensure availability and sustainable management of water and sanitation for all”, and includes surface water salinity as a parameter to be monitored, but no salinity threshold is provided to differentiate between acceptable and unacceptable water quality (Kelly et al., 2024). Globally, the few existing salinity regulations that aim at protecting freshwater life (Schuler et al., 2019) have some important limitations: 1) They are mostly based on laboratory-toxicity assays and therefore fail to protect whole biological communities and ecosystems (Hintz et al., 2022); 2) They refer to total salinity or chloride concentrations, neglecting that different ions could have different toxicities and each of them should be regulated (Cañedo-Argüelles et al., 2016b); 3) They are often recommendations, not legally-enforced (Schuler et al., 2019). Beyond regulatory mechanisms, management actions that effectively mitigate freshwater salinization are generally lacking. For example, the salt waste generated from potash mines in Catalonia was collected and transported into the Mediterranean Sea through a 120 km long pipe. This resulted in severe environmental degradation because: 1) the collection system was flawed and salts leached into surrounding streams and groundwater systems (Ladrera et al., 2017); 2) the pipe was corroded by the salts and there were periodical leaks (Gorostiza et al., 2022); 3) the marine and estuarine ecosystem can be impacted by brines (Schulz & Canedo-Argüelles, 2019). Also, although some technical solutions that could reduce freshwater salinization exist (e.g. alternative de-icers, backfilling, drip-irrigation, reverse-osmosis) they are rarely implemented due to a combination of factors, such as economic costs and the lack of environmental awareness and political will (Cañedo-Argüelles, 2020).

2. Aims and scope

This report aims to address the overlooked issue of increasing salinization in European water bodies, which poses a threat to biodiversity, ecosystem integrity and food and water security. Using a European-wide dataset that includes information on salinity, salinization drivers, and aquatic macroinvertebrate communities (selected as a potential Biological Quality Element for monitoring salinization's impact) we explore ecologically relevant salinity criteria and identify potential salinization indicators. We also assess the potential interactions between salinization and climate change in freshwater ecosystems focusing on the Oder River and Turkish lakes as two case studies. The specific objectives of this report are to:

- 1) Develop a salinization metric based on the deviation of observed electrical conductivity (EC, used as a proxy to salinity) from expected background values (section 4: “Estimating freshwater salinization”).
- 2) Assess the changes in the Ecological Quality Ratio (coming from the EIONET database) along the salinization gradient to estimate the risk of failing to achieve environmental targets (i.e. good ecological status) (section 5 “Assessing the risk of failing to achieve good ecological status due to salinization”).
- 3) Analyse the change in the relative richness of sensitive taxa and the response of ecological traits along a salinization gradient (section 6: “Setting salinity thresholds”).
- 4) Setting the most probable salinity thresholds combining the results of analysis (section 6: “Setting salinity thresholds”).
- 5) Exploring the potential interactions between salinization and climate change in freshwater ecosystems and how this might affect ecological quality (section 7: “The interactions between salinization and climate change”).
- 6) Evaluating the current salinity standards for their capacity to protect inland waters from salinization (section 8: “Discussion”).
- 7) Developing the methodology for setting the salinity thresholds for implementing European policies and SDG 6.3.2, including their adaptation to climate change (section 8: “Discussion”).

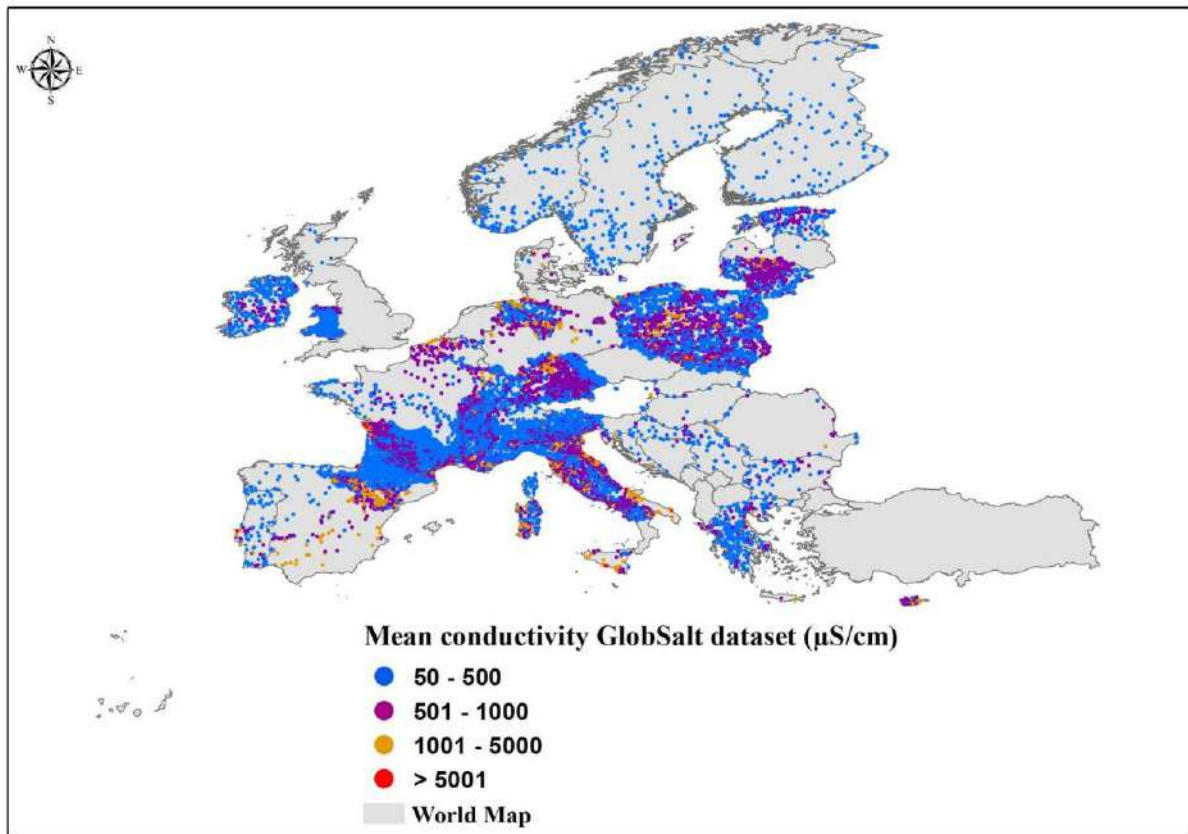
3. Methods

3.1 Study sites

3.1.1 Salinity data

GlobSalt is a global dataset that consolidates water quality data with a focus on riverine salinity parameters. GlobSalt combines records of electrical conductivity (EC), major ions, organic matter and nutrient concentrations from 62 public sources (e.g. Waterbase), providing harmonized time series data on salinity variations (Figures 5 and 6). The dataset covers the years 1980–2024 and includes information for 35 European countries (Table 3), with the most extensive coverage in Germany, France and Switzerland.

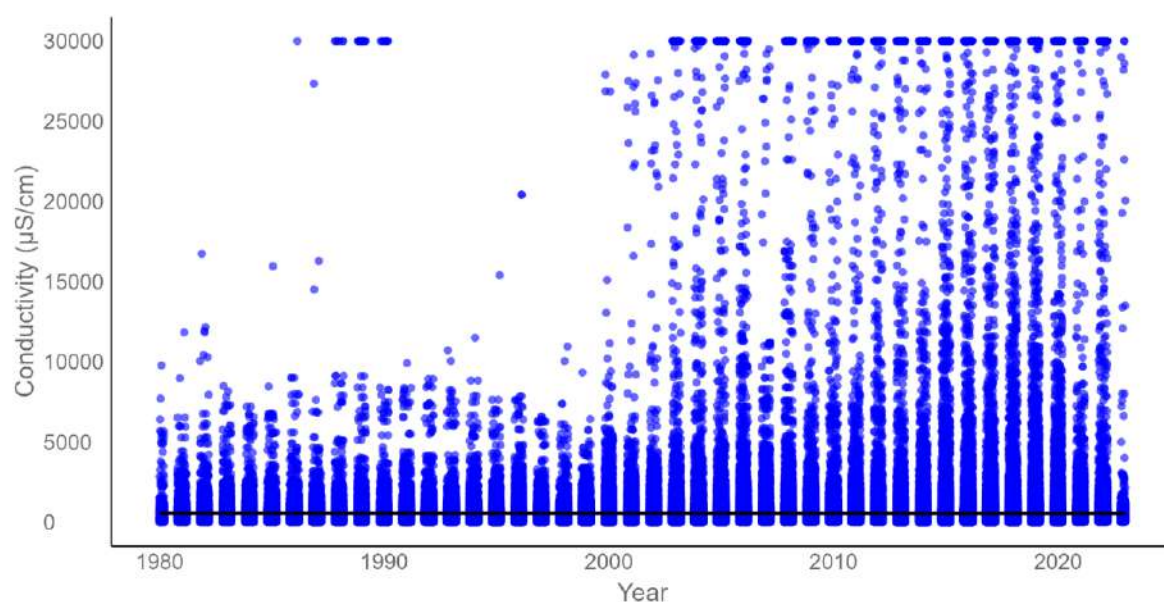
Figure 5. European distribution of measured mean electrical conductivity (EC) and station density. The global map in the panel shows the measured mean EC values per river in each country, based on observations included in the GlobSalt database, over the entire data period (1980–2023). Blue, purple, orange, and red dots represent low, moderate, moderately high, and high EC, respectively.



Source: GlobSalt database.

It is important to notice that the amount of data available and the EC range covered by that data varies temporally, with a greater number of sites and a better representation of the EC gradient after the year 2000 (Figure 5). This is most likely related to the implementation of the European Framework Directive, which led to a significant increase in water quality monitoring efforts across all member states (Hering et al. 2010a).

Figure 6. Temporal dispersion plot of electrical conductivity (EC) for Europe. EC observations (blue dots) from the GlobSalt database span the entire data period (1980-2023).



Source: GlobSalt database.

The geographical bias is also important, with the number of sites and observations strongly differing from country to country (Table 3). Overall, Northern Europe is better represented than Southern Europe, and Eastern Europe is better represented than Western Europe.

Table 3. Distribution of mean conductivity (EC) data relative to area across European countries in GlobSalt.

Country	Number of Sites	Number of Data	Area (km ²)	Nº Data/km ²	Median Conductivity (EC) (µS/cm)
Austria	16	1,474	83,871	0.0176	370
Belarus	1	60	207,600	0.0003	424
Belgium	63	5,361	30,689	0.1747	822
Bosnia and Herzegovina	14	1,002	51,197	0.0196	400
Bulgaria	74	3,849	110,879	0.0347	414
Croatia	51	4,057	56,594	0.0717	388
Cyprus	37	1,480	9,251	0.16	737
Czech Republic	2	489	78,867	0.0062	522
Denmark	11	18	42,930	0.0004	425
Estonia	263	4,659	45,227	0.103	445
Finland	106	5,400	338,145	0.016	13
France	6,642	370,873	643,801	0.5761	326
Germany	4,048	281,889	357,022	0.7895	565
Greece	360	2,008	131,957	0.0152	346
Hungary	16	2,844	93,028	0.0306	386
Ireland	190	25,535	70,273	0.3634	240
Italy	2,654	29,074	301,340	0.0965	488
Latvia	15	648	64,589	0.01	493

Country	Number of Sites	Number of Data	Area (km²)	N° Data/km²	Median Conductivity (EC) (uS/cm)
Lithuania	463	9,150	65,300	0.1401	521
Luxembourg	2	58	2,586	0.0224	797
Moldova	6	597	33,846	0.0176	540
Netherlands	5	261	41,543	0.0063	489
Norway	182	15,584	323,802	0.0481	30
Poland	3,293	43,144	312,685	0.138	479
Portugal	87	3,886	92,090	0.0422	479
Romania	27	4,699	238,391	0.0197	391
Serbia	83	7,288	77,474	0.0941	411
Slovakia	9	1,287	49,037	0.0262	423
Slovenia	3	556	20,273	0.0274	314
Spain	1,440	82,162	505,370	0.1626	558
Sweden	948	26,583	450,295	0.059	10
Switzerland	126	11,118	41,290	0.2693	425
UK	898	23,020	243,610	0.0945	198

Source: GlobSalt database.

3.1.2 Other environmental data

Data from other environmental datasets were compiled, including RiverATLAS (Linke et al., 2019), CHELSA Bioclim (Karger et al., 2017), FutureStreams (Bosmans et al., 2022), GIRES (Messenger et al., 2021) and LUCAS LUC (Hoffmann et al., 2023). This encompasses a wide range of natural (e.g. elevation, soil properties, bioclimate) and human-related variables (e.g. land cover, human footprint). Except for RiverATLAS and GIRES, the spatial resolution differed between datasets and we aggregated all environmental variables at the scale of the river reaches of the RiverATLAS network at a resolution of 15 arcsecond. If one river reach was located across several raster cells of CHELSA, FutureStreams or LUCAS LUC, we averaged the value across cells for the associated variables. In addition, we approximated the area of major lithological classes in the catchments upstream of each river reach. For this purpose, we used the GLIM lithological classes (Hartmann & Moosdorf, 2012) as reported at the smallest spatial scale available in the BasinATLAS dataset (the 12th hierarchical level of BasinATLAS, i.e. basins of approximately 150km² on average; Linke et al., 2019). BasinATLAS provides the main lithological class of each basin, so for each river reach, and for each lithological class, we summed the area of all upstream basins presenting the associated lithological class.

3.1.3 Biological data

In this report we focus on aquatic macroinvertebrates as one of the most widely used biological quality elements in Europe due to their diversity, fast and strong response to anthropogenic stress and ubiquity. However, it is important to keep in mind that other aquatic organisms hold a great potential to monitor freshwater salinization too (e.g. diatoms; Ziemann, 1997). Benthic macroinvertebrate samples from 23 countries across Europe were gathered. These samples stem from national monitoring programs for 8 countries (Austria, France, Germany, Greece, Poland, Slovakia, Spain, Switzerland) and from a previous data compilation from Haase et al. (2023) for sites of all other countries. To minimize possible biases related to differences in sampling effort, only the samples collected during or after the year 2000 were kept from the original data, as most sampling campaigns after 2000 have been carried out according to standard protocols following the implementation of the WFD. We only considered the presence/absence of taxa to avoid biases related

to the different existing protocols to estimate abundances. The taxonomic resolution of some datasets reached genus and species levels but thousands of samples were identified only at the family level, especially in the Mediterranean area. In order to keep the maximum number of sampling sites, we worked at the family level and aggregated all samples that were available at finer resolutions. The taxonomic homogenization (e.g. avoid synonyms among taxa in the final dataset) was carried out with the biomonitoR package (Laini et al., 2022) and the final taxonomic list was checked by experts. Additionally, we collected information on the Ecological Quality Ratio (EQR) based on benthic invertebrates from the Waterbase database (EEA, 2024).

The biological sampling points were snapped to the RiverATLAS network in order to combine biological and environmental data at the scale of the river reach. If several sampling points occurred on the same RiverATLAS river reach, samples were pooled. As the RiverATLAS network does not include small headwaters, this step discarded many sampling points (22%). In addition, as the salinity sampling points were generally not overlapping with the biological sampling points, salinity data were aggregated and averaged at the spatial scale of 8th-level basins from BasinATLAS. This choice was the result of a trade-off between having a large overlap between biological and salinity data, and avoiding gathering salinity across too many sites with contrasted environmental conditions. This means that a basin was characterized by an average conductivity value, but potentially multiple biological samples and other environmental variables, because they were characterized at the reach scale.

Table 4. Characteristics of the datasets of biological samplings. *Dirección General del Agua, Ministerio para la Transición Ecológica y el Reto Demográfico, Gobierno de España. **Plan PIMA adapta. Servicio técnico para la vigilancia de las estaciones de referencia y obtención de la línea base para evaluar las alteraciones debidas al cambio climático y a la contaminación atmosférica. Subdirección de protección de las aguas y gestión de riesgos. MITERD

Country	Source	Main taxonomic resolution	Nb sites	First sampling year	Last sampling year	Average nb sampling years/ site
Austria	Water Information System Austria	genus	175	2010	2020	2
Belgium	Haase et al. 2023	genus	42	2000	2020	7
Bulgaria	Haase et al. 2023	family	6	2000	2019	6
Cyprus	Haase et al. 2023	family	2	2006	2019	8
Denmark	Haase et al. 2023	species	38	2000	2019	18
Estonia	Haase et al. 2023	species	10	2003	2019	8
Finland	Finland Stream Monitoring	species	105	2001	2022	2
France	Naiades	genus	760	2005	2020	13
Germany	German national monitoring	species	5149	2005	2013	2
Greece	Hellenic Centre for Marine Research	family	386	2012	2023	4
Hungary	Haase et al. 2023	species	13	2001	2017	11

Country	Source	Main taxonomic resolution	Nb sites	First sampling year	Last sampling year	Average nb sampling years/ site
Ireland	Haase et al. 2023	genus	10	2003	2019	17
Italy	Fornaroli et al. 2020	family	798	2007	2018	2
Latvia	Haase et al. 2023	species	3	2000	2015	15
Luxembourg	Haase et al. 2023	genus	4	2007	2017	10
Netherlands	Haase et al. 2023	species	10	2000	2019	12
Norway	Haase et al. 2023	genus	60	2000	2020	11
Poland	Główny Inspektorat Ochrony Środowiska	family	1533	2000	2019	1
Portugal	Haase et al. 2023	genus	2	2000	2019	20
Slovakia	Slovakian Water Research Institute	species	34	2003	2008	2
Spain	NABIA*	family	994	2010	2022	4
Spain	REFCON**	genus	16	2020	2022	2
Spain	Haase et al. 2023	genus	54	2000	2019	14
Sweden	Haase et al. 2023	species	25	2000	2019	14
Switzerland	MIDAT - Info Fauna (CSCF)	species	375	2010	2022	2
Switzerland	Haase et al. 2023	species	1	2000	2018	7
United Kingdom	Haase et al. 2023	family	11	2000	2019	9

Data source: The authors' analysis.

3.1.4 Oder River case study

Data on in situ parameters were obtained from the Brandenburg State Office for the Environment (LfU) at the Oder River at site Hoenwutzen (DE) (Site OD_0070 (Brandenburg)) (<https://apw.brandenburg.de>). Data from the period between 2010 and 2023 were considered due to data completeness, as water level was available from this date onwards. Climatic data for the Oder catchment were obtained from ERA5, the fifth-generation ECMWF reanalysis for the global climate and weather (data on single levels) and all ERA5 data documentation is available online. These included: 2m air temperature, potential evaporation, runoff, surface runoff, surface solar radiation downwards, total cloud cover, and total precipitation. Derived data included the standardized precipitation evapotranspiration index (SPEI), standardized precipitation index (SPI), climatic water balance and potential evapotranspiration and were calculated using the r package SPEI (Beguería & Vicente-Serrano, 2023). SPI and SPEI from 1981 to 2010 were used as the reference period. Sulphate is a known useful indicator of mine drainage contamination (Gray, 1996), but also occurs naturally from marine influence. The concentration of non-marine sulphate

was calculated to adjust for the proportion based on its ratio with Cl^- in seawater. This is not an appropriate adjustment when there are industrial sources of Cl^- but it can indicate relative changes in loss to the environment from industrial sources for example.

3.1.4 Turkish lakes case studies

3.1.4.1 Study areas for assessing changes in areas and salinity, fish and waterfowls of lakes

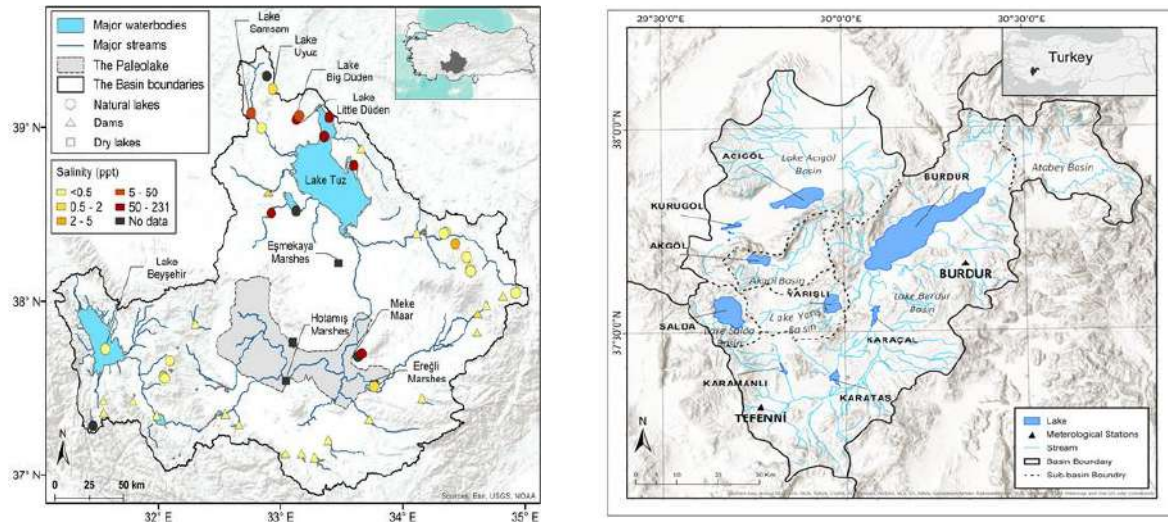
Our study areas include both Konya and Burdur Closed Basin (KCB, BCB, respectively) in the central Anatolia of Türkiye. KCB (49805 km²) is located at a mean elevation of 900–1050 m in the south-Central Anatolian Plateau (Figure 7). The KCB holds many lakes, including the iconic saline Lake Tuz and the largest Turkish freshwater lake, Lake Beyşehir (also the largest freshwater lake in the entire Mediterranean region). The KCB has a diverse climate with a Mediterranean climate in the southwest basin, a cold-dry steppe climate in the northern basin and a desert climate in the central Karapınar region. The geology of the basin is dominated by marl and limestone. Long-term annual mean precipitation in the basin was 340 mm during 1970–2020. Around 56% of the KCB area was used for arable farming across 27×10^3 km², of which 8×10^3 km² were irrigated (Republic of Türkiye Ministry of Agriculture and Forestry, 2018). Although a semiarid climate prevails in most of the KCB, with 70% of the precipitation occurring outside the crop-growing period, cultivation of water thirsty crops has been widespread in the KCB farmlands (Berke et al. 2014).

Burdur Closed Basin (BCB, area 629 km²) is a mountainous and located in the southwest of Türkiye, has a mean elevation of 1200 m (Figure 7). The BCB includes several types of aquatic ecosystems which support high biodiversity, including one Ramsar site, six Important Bird Areas, and a considerable richness of native and endemic fish species. The basin includes six large natural lakes: Lakes Acıgöl, Akgöl, Burdur, Salda, Yarıklı, and Karataş (Figure 7). Lake Akgöl has already dried out. Lakes Salda (max. depth: 184 m) and Burdur (max. depth: 110 m) are the second- and third-deepest lakes in Türkiye, respectively. The long-term monthly mean temperature of the basin is 13.2 °C, and the long-term total annual precipitation is 413 mm. The Sentinel-2 MSI and Landsat data on the long-term (>40 years) were assessed for the changes in the lake surface area and salinity of 3 lakes located in the Konya closed Basin (Lakes Düden, Little Düden, and Uyuz). These lakes cover a wide salinity range (48.4, 65.3, and 1.2 ppt of measured salinity, respectively) though they share similar meteorological and climatic conditions due to their spatial proximity. For salinity inference shortwave infrared bands (2.2 µm of Landsat 8, 2.205 µm of Landsat 5, and 2.194 µm of Sentinel data), near infrared (NIR) bands (0.865 µm of Landsat 8, 0.835 µm of Landsat 5), and a red-edge band (0.78 µm) of Sentinel data were used to create the salinity index $(\text{SWIR-NIR})/(\text{SWIR} + \text{NIR})$ (see Yılmaz et al., 2020 for the details). The salinity index was calibrated using ground truth data (using a YSI ProDSS Multiparameter Water Quality Meter, Yellow Springs, OH, USA) obtained from 14 lakes in the region in June 2020 having a salinity between 0.5 and 230 ppt. For 5 other lakes Located in Burdur Closed Basins (Lakes Acıgöl, Burdur, Karataş, Salda and Yarıklı) changes in surface area were also estimated using the same approach (see Çolak et al., 2022).

Data on agricultural land area, crop patterns, and their biomass production (i.e., crops, vegetables, fruits) for both KCB and BCB for 1980–2019 were obtained from the database of the Turkish Statistical Institute (TUIK 2020). In addition, remotely sensed land-use data, from the Coordination of Information on the Environment Land Cover (CORINE) (2006 and 2018), were obtained from Copernicus Land Monitoring Service. Monthly mean air temperature and annual precipitation data were obtained from the Turkish State Meteorological Service for the period 1970–2020 for estimating evapotranspiration. The surface water and mean monthly discharge data in the basin

were obtained from the State Hydraulic Works of Turkey (DSI). The data on the changes in fish and waterfowl species and distributions were also collected from various reports and publications to assess the impact of lake surface area and salinity changes. For the details of all the methodologies please see Yılmaz et al. (2021) and Çolak et al. (2022).

Figure 7. Konya (left) and Burdur (right) closed basins, sub-basins, lakes, and locations of meteorological stations. Salinity of lakes and reservoirs is shown for KCB based on YSI multiprobe.



Source: Yılmaz et al., 2021 and Çolak et al., 2022.

3.1.4.2 Synchronized Mesocosms Experiments

We established similar experimental mesocosm facilities in two different climate zones, which include cold-dry semi arid Ankara, and hot Mediterranean Mersin in Türkiye, specifically designed to simulate the effects of salinization and climate change on shallow lake ecosystems (Özkan et al., 2023). A total of 24 mesocosms were established at each location, allowing for a 3×2 factorial experimental design with four replicates per treatment. Each mesocosm had a diameter of 2 m, 1.8 m high, and had a volume of 5 m^3 . They were made of high-density polyethylene (HDPE) due to the material's resistance to high salinities and UV exposure, Please see Özkan et al. (2023) for the details of the set ups. These two facilities were used for two different experiments that included (1) a salinity gradient experiment consisting of 16 salinity levels (range: 0–50 g/L); and (2) a heat wave experiment where two different temperature regimes (no heatwave and $+6^\circ\text{C}$ for two weeks) were crossed with two salinity levels (4 and 40 g/L), with four replicates in each treatment. The experiments lasted 8 and 2 months, respectively, and the experimental mesocosms were monitored frequently (Özkan et al. 2023).

The first experiment aimed to elucidate the threshold response of shallow lakes to increasing salinity, using a salinity gradient of 16 different salinity levels (0, 2, 4, 6, 8, 10, 12, 14, 17, 20, 23, 26, 30, 35, 40, and 50 g/L salt concentrations) between the 9th of September 2021 and the 13th May 2022. The selected salinity range reflected the observed salinities in the central Anatolian lakes sampled. The short-term response to increasing salinity by raising the salinity daily by 1/30 of the target concentration with salt additions (salinization phase) lasted for a month (Özkan et al. 2023). Then the experiment was conducted for 5 and 2 months of stable and desalinization periods, respectively.

3.2 Data analysis

3.2.1 Freshwater salinization

We created a salinization variable by first calculating a theoretical conductivity under reference conditions and then comparing the observed conductivity to this theoretical value. To do so, we modeled the conductivity on a subset of reference (i.e. least impacted) sites with a random forest using the 'ranger' package (Wright & Ziegler 2017, Wright et al., 2023). Reference sites were selected using the 2009 Human Footprint Index (HFI; Venter et al. 2016) and land cover as proxies for anthropogenic pressure. We set a maximal threshold of HFI at 100, and a threshold of impacted land covers (crops and urban areas) at 10%, which resulted in a subset of 11% of sites from the complete dataset. Different thresholds were tested based on the European reference condition concept (Pardo et al. 2012), and these ones were retained as a trade-off between keeping enough sites in the model and being conservative enough to least impacted sites. A set of 37 environmental variables were selected for their ecological relevance and their low collinearities (correlation threshold set at 0.7; Dormann et al., 2013) and were used as predictors of the conductivity on the reference sites. They encompass lithology, soil properties, bioclimatic variables, water temperature, discharge and more (see Appendix 1 for the complete list of variables retained). The random forest was computed using 1000 trees, with a number of 7 variables to possibly split in each node, as advised for the total number of predictors we had, and a minimal node size of 5 sites, which is the default for regression trees. The quality of the random forest model was assessed with the R^2 , providing the explained variance computed on the out-of-bag data. The model, providing an expected relationship between environmental variables under near-pristine conditions and conductivity, was then used to predict a theoretical reference value of conductivity across all sites. The difference between the observed conductivity and the theoretical conductivity predicted by the model is the salinization variable. When the predicted value was lower than the observed value (which happened in 2.5 % of the cases), we assumed no salinization occurring at that site and we set the salinization value to 0.

3.2.2 Salinization impacts on ecological quality rations (EQR)

Ecological Quality Ratio (EQR) values were combined with salinization indices to directly relate ecological quality with aquatic systems salinization. Additionally, due to the complex nature of the relationship between EQR with other environmental variables we carried out other analysis considering the main descriptor related to human footprint index (HFI), river area in total watershed upstream of reach point (Area), percentage cover of non-irrigated crops (Agr1), percentage cover of irrigated crops (Agr2), percentage cover of urban land cover (Urban), elevation averaged in total watershed upstream of reach point (Elev), and main lithological classes (Geol). For both relationships we used generalized linear mixed models considering site identity as a random factor and a beta family distribution due to the proportional nature of EQR values (ranging between 0 and 1), using the glmmTMB package (Magnusson et al. 2020). When EQR values were above 1, higher quality with respect to reference status, we set those values to 1. Salinization was log-transformed to ensure a normal distribution. Lithological classes were considered as a factor variable. Finally, we assess correlation between variables to identify highly correlated relationships before analysing the main relationships. We performed these analyses to quantify the overarching relationships between EQR and salinization individually and consider the other potential drivers of EQRs. However, we were also interested in the cross-effects between all these variables and for that we used structural equation modelling (SEM) considering linear mixed models (piecewiseSEM package; Lefcheck, 2016). Within this modelling schema we considered the previously described metrics in

three mixed models following a nested structure where EQR can be explained by all described factors as follows:

Table 5. Model structure used within the structural equation modelling to identify crossed correlations between selected parameters an EQR.

Response	Explicative	Relationship interpretation
EQR	Salinization; HFI; Agr1; Agr2; Urban; Elev; Geol	EQR relation with all identified major drivers of ecological quality.
Salinization index	HFI; Agr1; Agr2; Urban; Elev; Geol	Relation of salinization index with the major drivers that drive its values at the catchment scale.
HFI	Agr1; Agr2; Urban; Elev; Geol	Human footprint index relationship with main site descriptors linked to both human activity and natural features.

Source: The authors` analysis.

This nested structure ensures the identification of significant indirect relations between the considered variables. For example, agricultural surface may not have a direct relationship with EQR values but it may increase salinization that in turn, will impact EQR values. We retained only significant interactions to identify significant models.

3.2.3 Taxa responses to salinization: spatial turnover

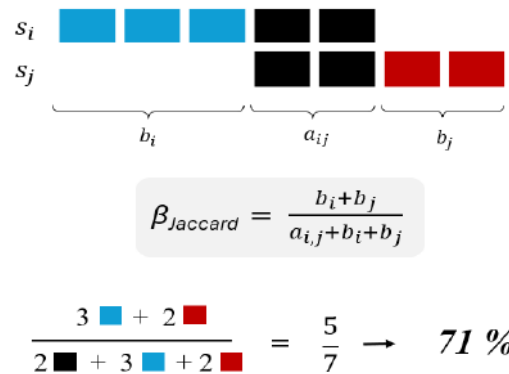
We assessed taxa-specific and trait-mediated responses to salinization with Joint Species Distribution Models (JSDM; Warton et al., 2015; Clark et al., 2017; Ovaskainen, et al., 2017). JSDMs are multivariate extensions of single Species Distribution Models (SDM) that incorporate dependencies among taxa, which can lead to more accurate predictions. We fitted JSDMs using a Generalized Latent Variable Modeling approach (GLLVM; Niku et al., 2019) to account for residual correlation between taxa and potential heterogeneities among datasets. Trait-environment relationships were obtained through the so-called fourth corner approach (Brown et al., 2014). We modelled expected occurrence rates with a binomial distribution and a logit link, which is a common choice for binary data, and assumed linear responses to environmental variables in the link scale.

In addition to salinization, we included background salinity (see section [3.2.1 Freshwater salinization](#)), bioclimatic variables (average precipitation and temperature), river order, and a human footprint index to account for other potential stressors. We also included country in the latent variable structure to account for potential spatial patterns in community composition. Although country is a political rather than purely geographical feature, it allowed us to account for potential differences between datasets, which in most cases have been collected at the country level. We fitted two models: (1) a model without traits to assess the response of individual taxa and (2) a model with traits to investigate how different trait modalities mediate the response to salinization through the fourth corner approach. The one hundred most common families across Europe were included in the former model, and all families with more than 50 occurrences were included in the latter.

The fitted taxa-specific model was used to assess community level changes in response to salinization. For this task, we computed the expected community composition along the salinization gradient for each country, setting other environmental factors to national averages and accounting for residual country-level variability in species composition. We then computed the Jaccard dissimilarity index (Podani & Schmera; 2011; Legendre & De Cáceres, 2013) to compare the expected

community composition without salinization, to that expected at different degrees of salinization (Figure 8).

Figure 8. Jaccard dissimilarity index (β_{Jaccard}) as the proportional change in community composition. The Jaccard index takes the number of unshared taxa between two communities (b_i, b_j) as a proportion of the total species in both communities (a_{ij}, b_i, b_j).



Source: The authors' analysis.

Additionally, we used TITAN analysis to identify taxa that were strongly responding to the salinization gradient and found threshold values according to the aggregated response of the taxa (Baker & King, 2010). TITAN is a blend of univariate regression trees and Indicator Species Analysis (Dufrêne & Legendre; 1997). Like a regression tree, TITAN considers all possible values that split salinization into two groups. The group of low values (z-) represents species that respond negatively to increases in salinization. The group of high values (z+) includes species that respond positively to increases in salinization gradient. For each division of salinization, TITAN performs an Indicator Species Analysis (ISA). This analysis determines, for each species, whether it is more strongly associated with the z- group or the z+ group, along with the corresponding Indicator Value (IndVal). After evaluating all possible values, each species is assigned to the group where it achieved the highest IndVal. Subsequently, the values of salinization are permuted, and the IndVal is recalculated. These permutations help standardize the observed IndVals into z-scores. The value of salinization that created the strongest division into two groups was referred to as the salinization threshold. Essentially, this is the point along the salinization gradient where the taxon is most clearly differentiated into two groups, making it a highly reliable indicator for one of the groups. To conduct this analysis we used the titan function in the R package TITAN2 (Baker et al., 2023).

3.2.4 Taxa responses to salinization: temporal turnover

Using a subset of sites from which conductivity and biological data had been monitored along time (i.e. sites with more than 5 years of monitoring), we conducted an analysis of community trajectories (De Cáceres et al., 2019; Sturbois et al., 2021a) to assess the relationship between the temporal change in community composition (i.e. trajectory lengths based on Jaccard dissimilarity) and the change in conductivity (i.e. conductivity difference between surveys). Trajectory analysis represents an optimal way to represent the relative changes in community composition over time (Sturbois et al., 2021a; 2023). This approximation has recently been formalized with a set of metrics that are suitable to quantify how communities change throughout time. In this report, we have used Trajectory length (De Cáceres et al., 2019) in order to quantify the compositional change between consecutive surveys. We used Jaccard dissimilarity index to quantify compositional differences to have a proportional measure of community differences (Podani & Schmera; 2011; Legendre & De Cáceres,

2013). We then used the package *ecotraj* (De Cáceres & Sturbois, 2023) to calculate trajectory lengths and the *vegan* package to obtain Jaccard dissimilarity indices (Oksanen et al., 2013). Once we obtained all trajectory lengths and conductivity differences between consecutive surveys, we analyzed their relationship considering trajectory length as response variable (i.e. the proportional change in communities between surveys) and conductivity difference (i.e. the change in conductivity between surveys) and the human footprint index as explicative variables. We related them using a generalized linear mixed model considering site identity as a random factor and a beta family distribution due to the proportional nature of trajectories calculated with Jaccard dissimilarities. We tested this relationship to assess the effect that a change in conductivity may have in the community composition while including the effect of human activities in explaining this relationship. Finally, we estimated breaking points with regression models considering segmented relationships (Muggeo, 2024). With this, we aimed to identify salinization thresholds (measured as conductivity difference between consecutive surveys) at which the community dynamics become altered (i.e. change in community trajectory). We considered a simpler linear model, considering community change as the response variable and conductivity difference as the explicative variable to identify an overarching breaking point in their relationship.

3.2.5 Taxa tolerance to salinity: toxicity data

Acute toxicity data for freshwater organisms and sodium chloride was extracted from the Standartox database (Scharmüller et al., 2020). The Standartox database is an aggregated and polished version of the US Environmental Protection Agency ECOTOX database (Olker et al, 2022), with additional taxonomic and habitat information. Standartox continuously incorporated the ever-growing number of test results in an automated process workflow that ultimately leads to a harmonized ecotoxicity data collection and provides methods to derive single aggregated ecotoxicity values for a specific chemical-organism test combination. Standartox makes use of the publicly available and quarterly updated ECOTOX database and restricts the data to commonly used endpoints in ecotoxicology, such as half maximal effective concentrations (EC50) or no-observed-adverse-effect concentrations (NOEC), leading to about 600,000 ecotoxicological test results, including about 8000 chemicals, tested on about 10,000 taxa in the current version. We then used this data to fit Sensitivity Distributions (SSD) using the *r* function `ssd_fit_diists` in the *R* package *ssdtools* (Thorley et al., 2024).

3.2.6 Long-term trends in salinization: Oder river study case

For the Oder river, in order to quantify the degree to which parameters were temporally aligned we carried out a Time Alignment Measurement (TAM) during the period 2010 to 2023 (Folgado et al. 2018). TAM values range from 0, indicating perfect temporal alignment to 3, indicating completely out of temporal alignment. It is essentially a similarity index in the temporal domain and can be useful here to compare what parameters tend to be more aligned. Prior to calculation, each variable was standardized. An ARIMAX model was fit to the combined residual and seasonal components of the conductivity timeseries to see if climatic parameters could explain additional variation in the time series of conductivity during the period 2010 to 2023 using the *r* package *forecast* (Hyndman & Khandakar, 2008).

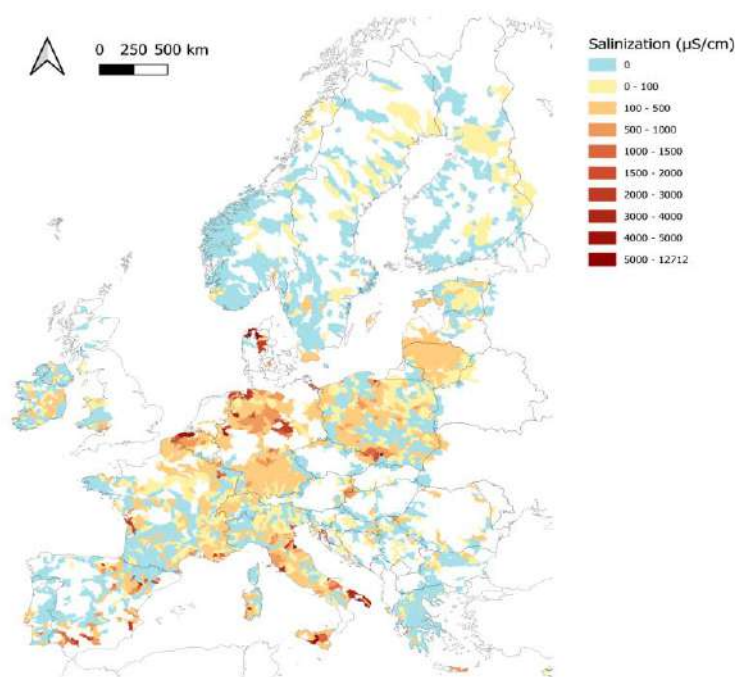
3.2.7 Synchronized mesocosms experiments

All the statistical analyses were performed in the programming language R (version 4.3.0). For the first experiment, generalized additive models (GAM) were generated, assuming a Gaussian distribution of the data, to estimate the relationships between the response variables and salinity using the package *mgcv* in R (Wood et al., 2011). For the second experiment, because of the presence of some non-normal or non-homogeneous data, the effects of the factors which include place, salinity, heat wave, and phases of the experiment on the response variables were tested with a four-way analysis of variance with aligned rank transformation (ART-ANOVA) using the package *ARTool* in R (Kay et al., 2021).

4. Estimating freshwater salinization

According to our model 57% of the European river network could be salinized to some extent (Figure 9). The salinized river reaches are scattered throughout Europe, showing that salinization is not restricted to arid landscapes. However, it should be noted that many of the salinized reaches are close to the sea. Although seawater intrusion is one of the main drivers of freshwater salinization (e.g. 58% of the 82 coastal hydrogeological units in Spain have shown some evidence of seawater intrusion; Estrela et al. 1996), it is unlikely to be captured by our model because it does not include the distance to the sea. Thus, some of the areas that have been identified as salinized (e.g. Northern Denmark) could indeed be naturally saline. Also, some of the naturally saline streams (e.g. some streams in Sicily; Gutiérrez-Cánovas et al., 2019a) have been identified as salinized by our model, most likely due to the difficulties in predicting EC at the high end of the gradient. However, our model was able to capture salinization driven by key anthropogenic drivers such as agriculture or mining. For example, the results for Spain align with previous models available for some of the catchments (Estévez et al., 2019; Thorslund et al., 2021).

Figure 9. Salinization of European rivers calculated as difference between the observed conductivity and the theoretical conductivity predicted by Random Forest models (see section 3.2.1 for further details).

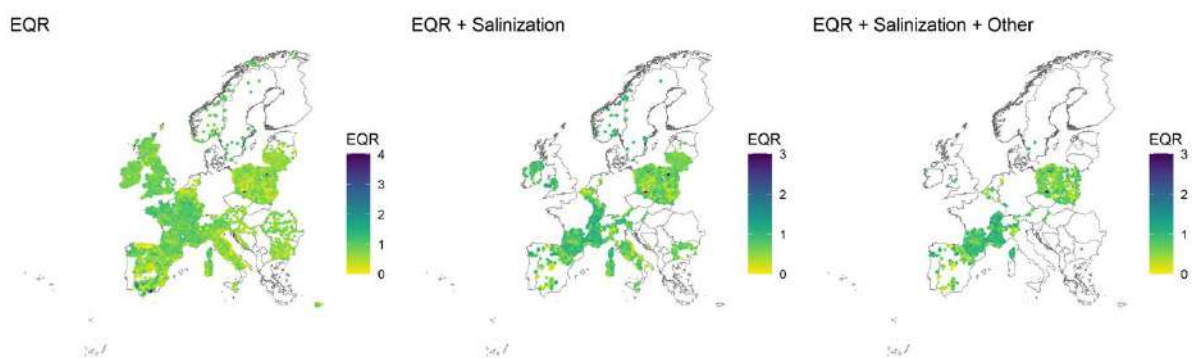


Source: The authors' analysis.

5. Assessing the risk of failing to achieve good ecological status due to salinization

Even though we had a good coverage of EQR values across European countries (8139 entries), when combined with the salinization index we lost 47% of the values (3895 entries) and when further combined with other relevant data informing about co-occurring stressors this loss was even greater, and only 19% of EQR values were retained (1573 entries). This loss of data means that interpretation of current results focuses on a smaller geographic area mostly encompassed by the Ebro basin in Spain, southern France and Poland (Figure 10). It also highlights the current need to expand monitoring networks to collect relevant data (EC, ion concentrations) that can be used to assess salinization.

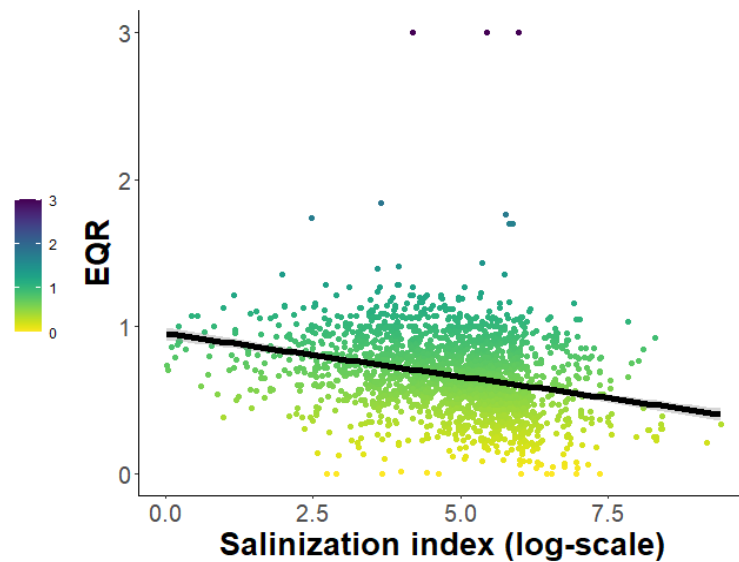
Figure 10. Coverage of the different datasets employed in this section and the loss of information as we incorporated new datasets. Left: all EQR data. Center: data retained after combining EQR with salinization. Right: data retained after combining EQR with salinization and with other relevant drivers.



Source: The authors' analysis.

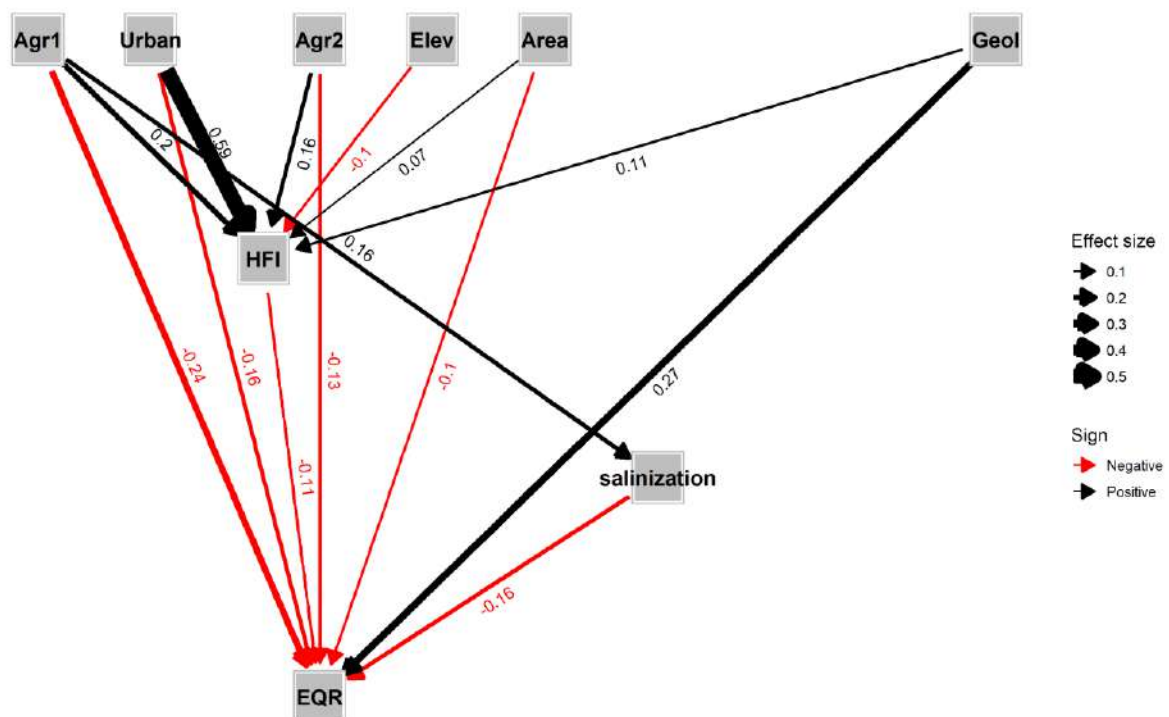
We found a negative but weak ($R^2 = 0.13$) relationship between the invertebrate Ecological Quality Ratio (EQR) and salinization (Figure 11), suggesting that the biotic indices currently used to assess the ecological status of European rivers according to the WFD do not strongly respond to salinization. When we included other predictors, the strength of our model increased ($R^2 = 0.44$) and we could identify that other major drivers were affecting EQR. For example, we found a significant negative effect comparable to that of salinization with HFI, non-irrigated and irrigated agricultural surface (Agr1 and Agr2) and urban land cover. We did not find strong correlations among the used predictors, with urban land cover and human footprint index being most strongly correlated ($R^2 = 0.57$). Overall, our results indicate that EQR is responding to multiple anthropogenic drivers and is not suitable for measuring the ecological effects of salinization. This was expected, since current biological indices have not been designed to capture salinization, but rather on general environmental degradation and other stressors such as nutrient pollution (Birk et al. 2012). When assessing cross correlations between salinization, EQR and other relevant drivers using structural equations modelling (Figure 12) the direct effect of other parameters such as agricultural land cover (total negative effect of 0.47 on EQR), urban land cover and HFI (total negative effect of 0.27) on EQR were clearly greater than the effect of salinization (total negative effect of 0.16). Additionally, agriculture, especially non-irrigated, had a strong role in explaining salinization (0.26 of Agr1 over 0.11 of Agr2). Thus, the direct effect of salinization weakens when considering the crossed effects among the considered variables. The three models (Table 5) had an R^2 of 0.28, 0.05 and 0.41 respectively. The explanatory power in relation to salinization was the smallest (second model with a 0.05). Thus, salinization played a significant role in explaining EQR, although weaker in comparison to other parameters.

Figure 11. Relationship between EQR and salinization. The linear regression line is shown in black. EQR values are coloured following a yellow-purple gradient as in previous maps. Note that for the analysis EQR values greater than 1 have been transformed to 1 to ensure the proportional nature of the EQR index. This transformation did not affect the overall relationship and we obtained similar results when ignoring it.



Source: The authors` analysis.

Figure 12. Results of the path analysis considering the three mixed linear models from table 5. Only significant positive (in black) and negative (in red) paths are plotted. Arrow size defines the relevance of each path (effect size).



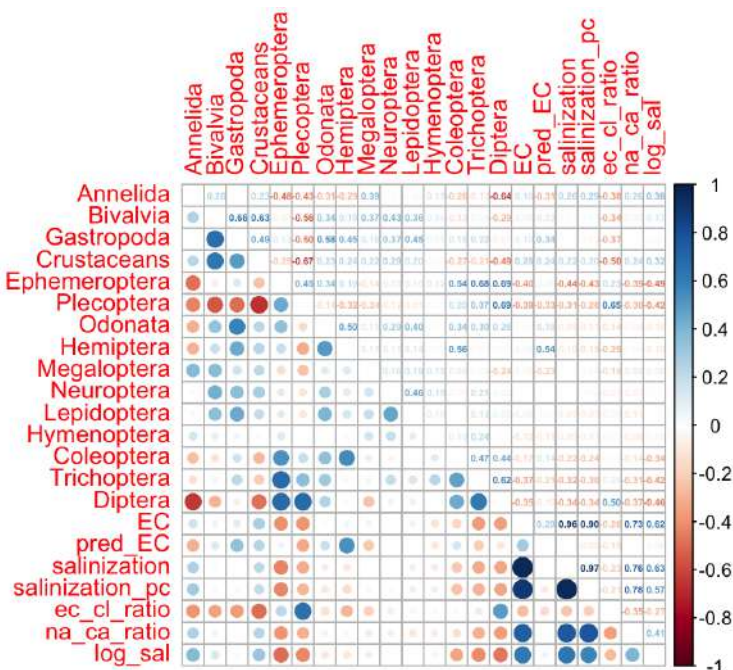
Source: The authors` analysis.

6. Setting salinity thresholds

6.1 Invertebrate orders as potential salinization indicators

In this section we explore how the richness of different orders of aquatic invertebrates respond to salinization, thereby determining community structure. Taxa were grouped by order because: 1) we only had access to presence/absence data, so we could not assess changes in relative abundance and we assessed changes in relative richness instead (i.e. number of families belonging to certain orders vs. total number of families); 2) different studies have shown that some orders such as Ephemeroptera, Plecoptera and Trichoptera (EPT) are sensitive to salinization (Kefford et al., 2011; Cañedo-Argüelles et al., 2013), suggesting that there could be a strong phylogenetic signal in relation to salt stress (Carew et al. 2011). In addition to the salinization metric calculated as the deviation from background salinity (see section 4), we tested different ion ratios that have been suggested to serve as a proxy to salinization because they inform about natural and anthropogenic drivers of salinity (Ziemann, 1997). Since ion concentrations were only available for a limited number of sites, we first analyzed a sub-group of sites ($n = 271$) for which both conductivity and ion concentrations were available. According to the Spearman correlation analysis (Figure 13) Ephemeroptera and Plecoptera show a moderate negative response to salinization, whereas Crustacea show a moderate positive response. Plecoptera and Crustacea also responded strongly to the ratio between chloride and EC. Taking all of this into account we decided to assess the ratio between the relative richness of Plecoptera + Ephemeroptera vs. the relative richness of Crustacea as a potential metric to monitor freshwater salinization, measured both as a deviation of observed from expected EC and as the ratio between chloride and EC.

Figure 13. Spearman correlations between the richness of the aquatic macroinvertebrate orders, electrical conductivity (EC), predicted conductivity (pred-EC, see section 4), salinization (absolute value, see section 4), percentage of salinization (see section 4), electrical conductivity vs. chloride ratio (ec_cl_ratio), sodium vs. calcium ratio (na_ca_ratio) and the logarithm of electrical conductivity (log_sal).

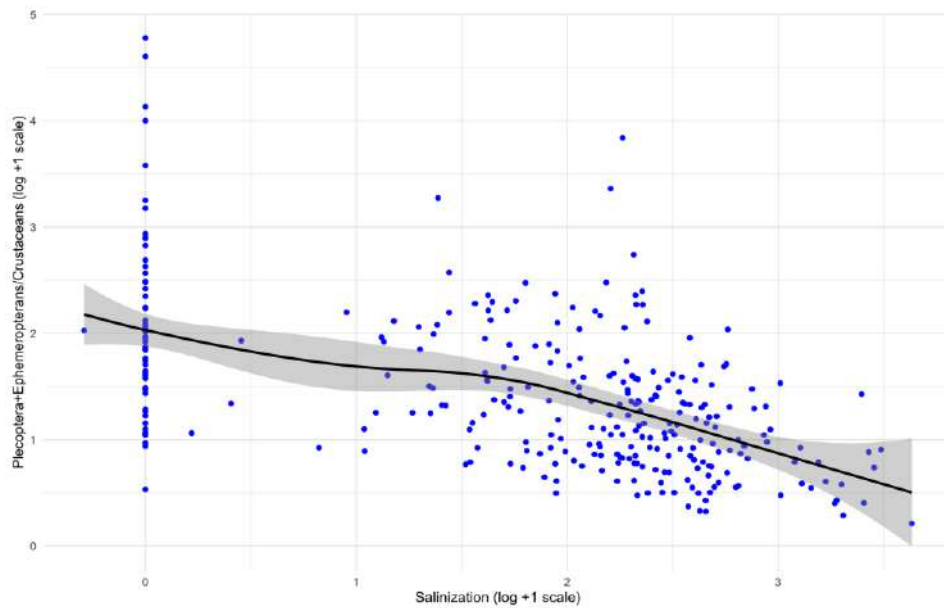


Source: The authors' analysis.

We found that the ratio of Plecoptera + Ephemeroptera vs. Crustacea strongly declined along the salinization gradient ($R^2 = 0.26$; Figure 14) and it responded even more strongly to the EC vs. chloride

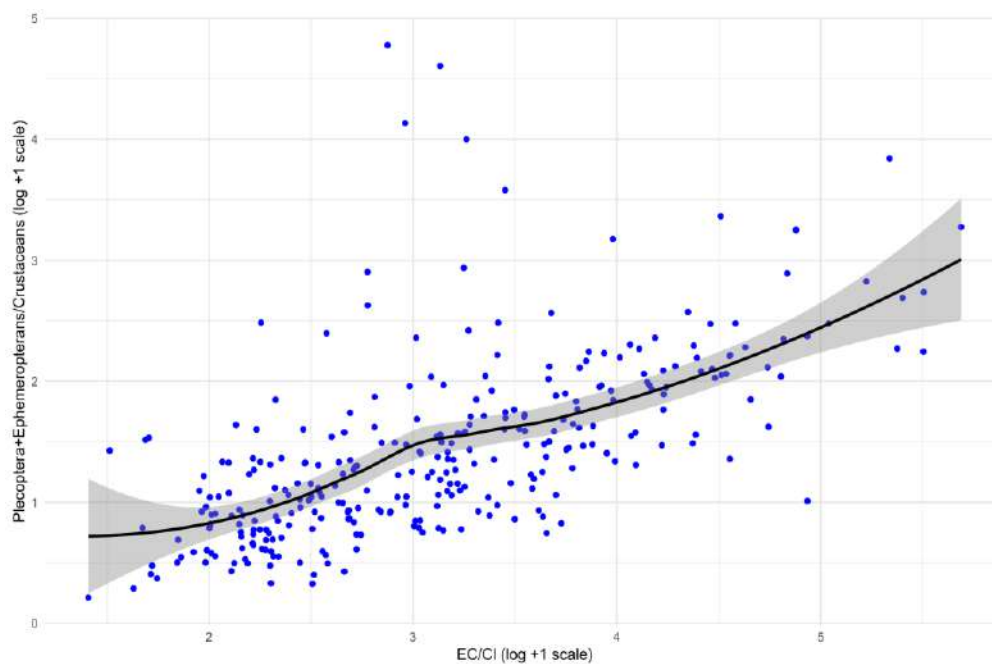
ratio ($R^2 = 0.39$; Figure 15). This suggests that: 1) the EC vs. chloride ratio could be a very good approximation to salinization and might capture biological effects; 2) the ratio of Plecoptera + Ephemeroptera vs. Crustacea (EP/C) could be explored as a potential metric to monitor freshwater salinization.

Figure 14. Ratio of (Plecoptera + Ephemeroptera) : Crustacea richness (log x + 1 scale) vs. salinization (log x + 1 scale).



Source: The authors` analysis.

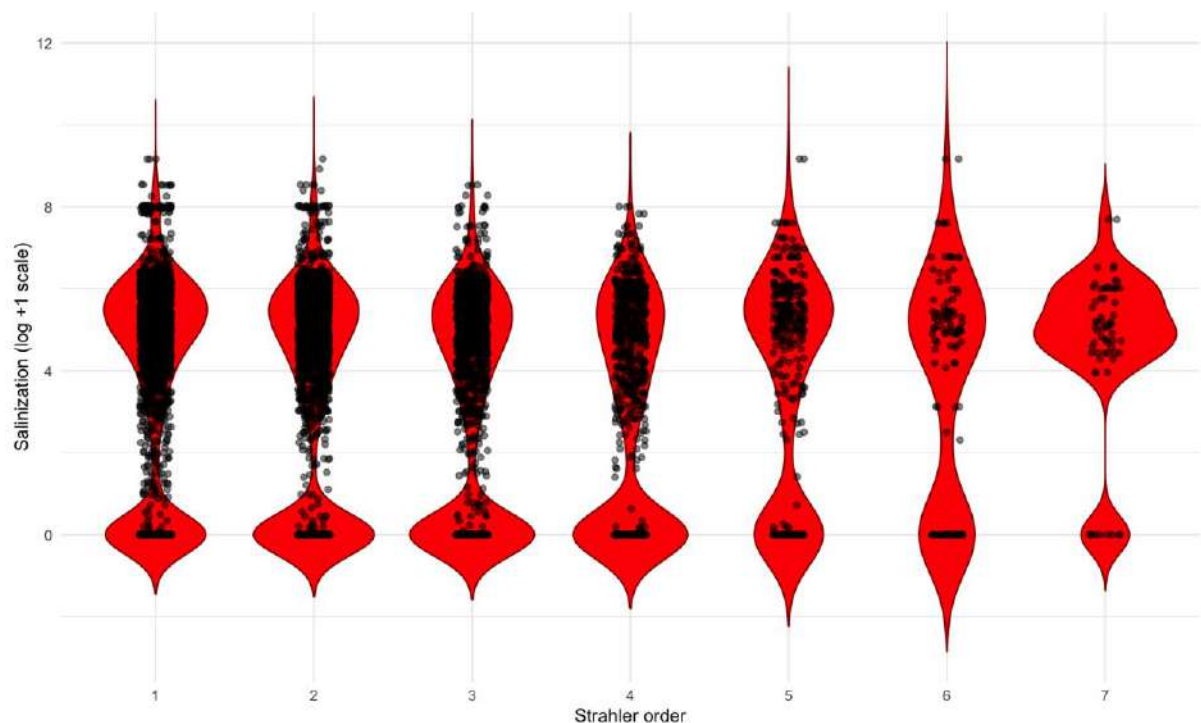
Figure 15. Ratio of (Plecoptera + Ephemeroptera) : Crustacea richness (log x + 1 scale) vs. Electrical conductivity/Chloride concentration (EC/Cl, log x+1 scale).



Source: The authors` analysis.

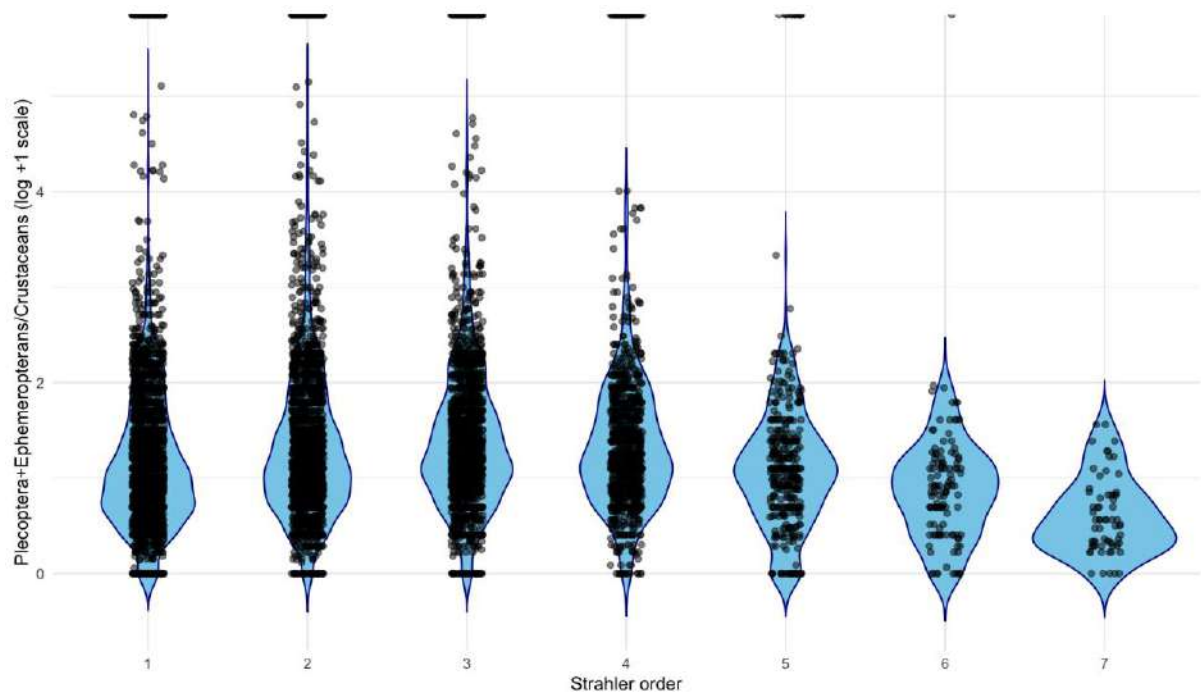
Regarding the use of EP/C as salinization indicators, it is important to keep in mind that the relative richness and abundance of these macroinvertebrate taxa varies naturally in streams and rivers dependent on habitat and environmental conditions, which are also influenced by river size (Vannote et al., 1980; Doretto et al., 2020). As we move from low order to high order sections, the river widens and increases in size, shifting from heterotrophy (i.e. allochthonous organic matter inputs from riparian vegetation dominate) to autotrophy (i.e. instream algal production dominates). This leads to a replacement of shredders and scrapers (which include most EP) by collectors (such as crustaceans). Given this, we performed an exploration of the relationships between EP/C and EC for the different river orders. We found that although salinization occurs across river orders it is more common in high orders (Figure 16). This is most likely due to the fact that they include large estuaries with strong tidal influence, and our model is not robust enough to clearly differentiate between anthropogenic and natural salinity in areas influenced by seawater (see section 4). Although there was a broad range of E+P/C values across river orders, the tendency decreased strongly, with the highest E+P/C values found at low river orders (Figure 17). The E+P/C values of river order 4 were more balanced, with most observations falling within the intermediate range of values. Concordantly, the relationship between EP/C and salinization was strongest for river order 4, with a strong negative relationship and little dispersion (Figure 18). Thus, we decided to focus on river order 4 sites to find a salinization threshold based on the changes in the relative richness of EP vs. C (Figure 19).

Figure 16. Violin plot showing the distribution of river salinization (log x + 1 scale) values across the different Strahler orders' (Strahler, 1957).



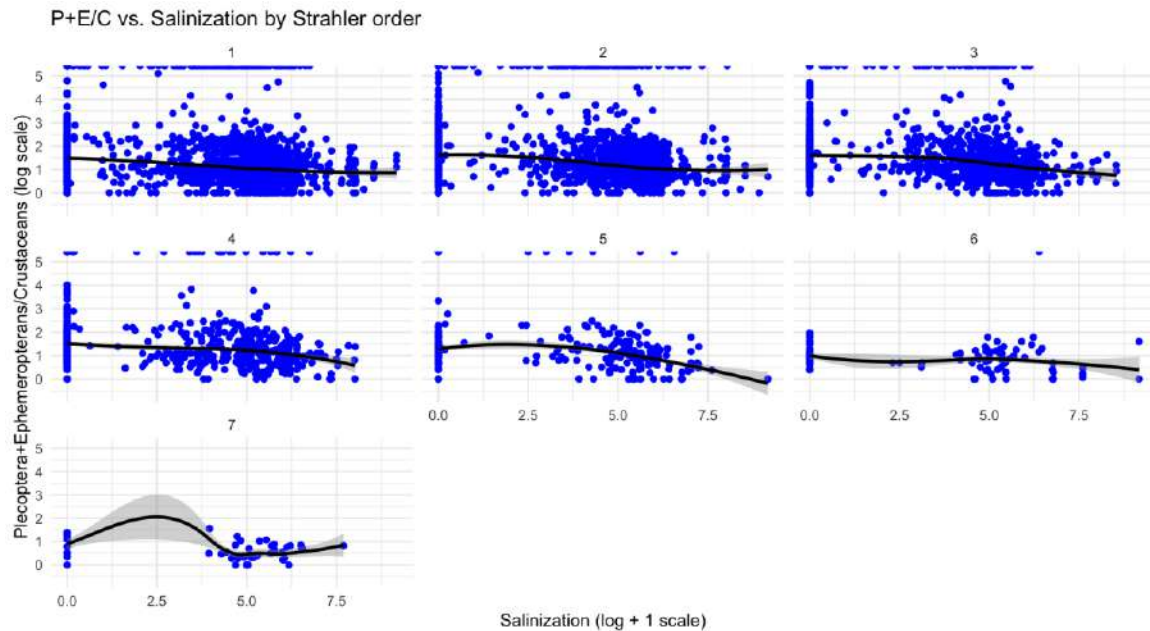
Source: The authors` analysis.

Figure 17. Violin plot showing the distribution of (Plecoptera + Ephemeroptera) : Crustacea richness (log x +1 scale) across the different Strahler orders' (Strahler, 1957).



Source: The authors` analysis.

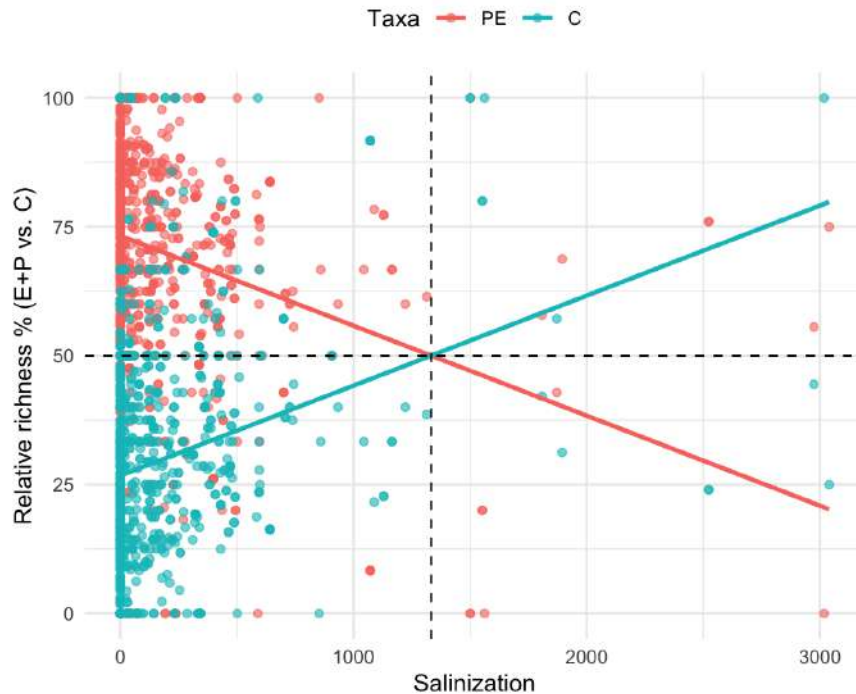
Figure 18. Linear regressions between the (Plecoptera + Ephemeroptera) : Crustacea richness (log x +1 scale) and salinization (log x +1 scale) across the different Strahler orders' (Strahler, 1957).



Source: The authors` analysis.

We finally fit a linear regression for EP vs. salinization and another one for C vs. salinization (Figure 19), and computed the intersection point between both (corresponding to 1333 $\mu\text{S}/\text{cm}$) as a potential threshold, as this has been used in previous studies to define the limit between the good and the moderate ecological status categories (Kelly et al., 2008; Cañedo et al., 2013).

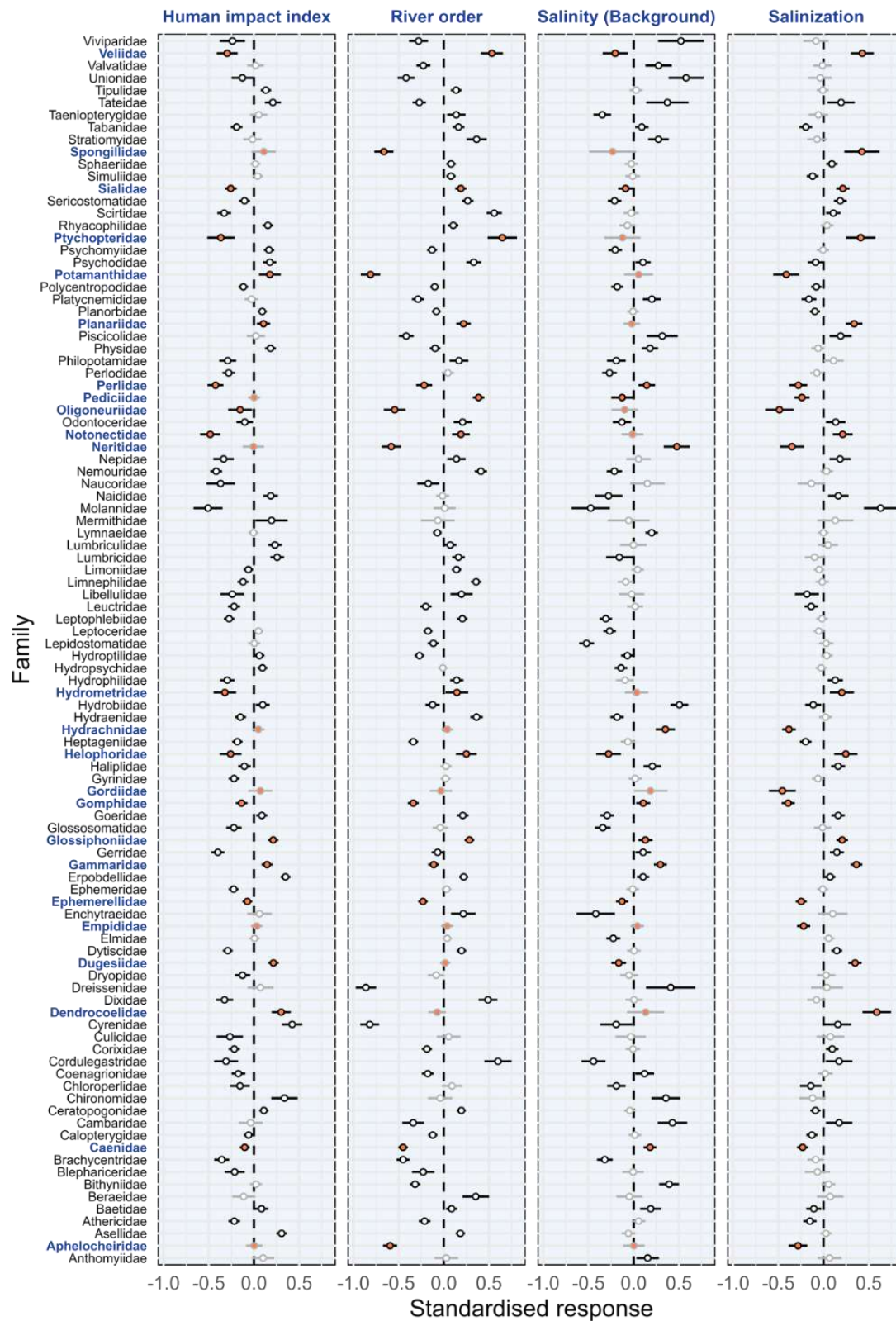
Figure 19. Relative richness of Plecoptera + Ephemeroptera (PE; red dots) and Crustacea (C; blue dots) along salinization (log x +1 scale). The fitted lines of the linear regression are plotted and its intersection calculated (corresponding to a salinization of 1333 $\mu\text{S}/\text{cm}$. Analysis carried out for river order 4 (Strahler, 1957).



Source: The authors' analysis.

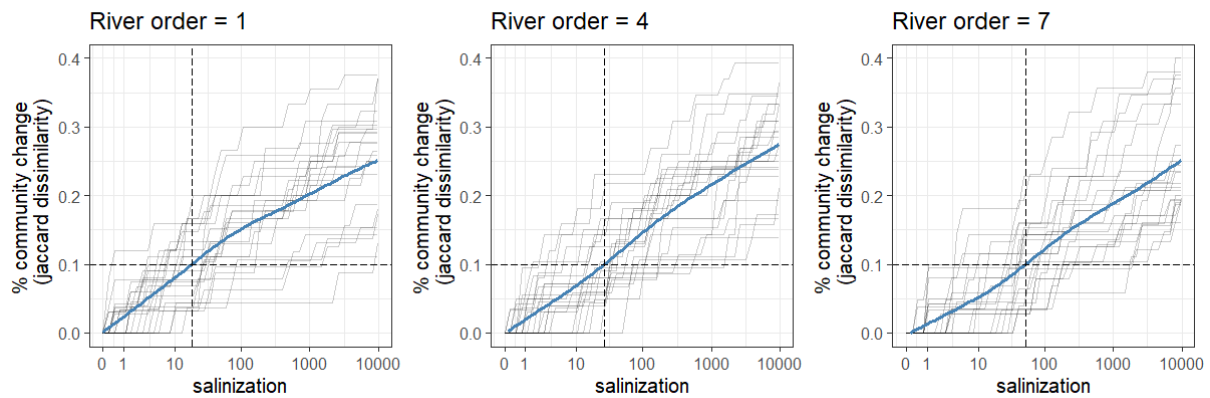
There are more factors to consider and carefully assess before using EP/C as an indicator of freshwater salinization. For example, bio-geographical and evolutionary factors might limit the distribution of many species belonging to these groups (Grigoropoulou et al. 2023; García-Girón et al. 2024). This can therefore affect the local richness of each of these orders, as is shown by our dataset, with some regions of Europe being clearly richer than others depending on the group considered (Figure 22). This could be interpreted in the context of the normative definitions for moderate status for river macroinvertebrates in the WFD which specifically refer to sensitive and insensitive taxa: “the ratio of disturbance-sensitive taxa to insensitive taxa, and the level of diversity, are substantially lower than the type-specific level and significantly lower than for good status” (EU, 2000).

Figure 20. Standardised responses of the one-hundred most common families. Positive values indicate increasing occurrence probability along the gradient, whereas negative values indicate decreasing occurrence.



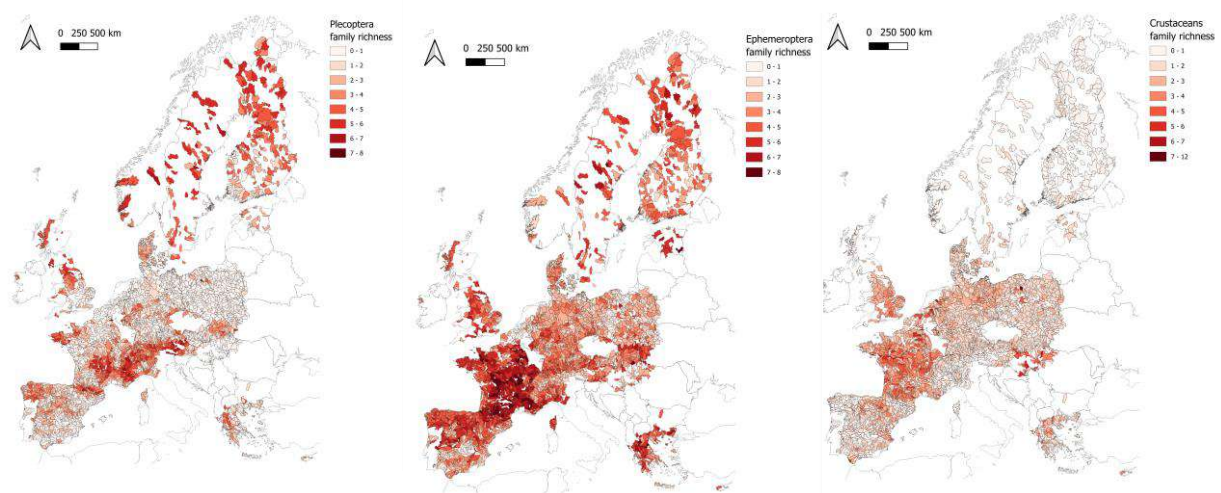
Source: The authors' analysis.

Figure 21. Community change along a salinization gradient. Gray thin lines indicate expected composition change with increasing salinization for different countries, whereas blue thick lines indicate the average value across countries. Dashed lines indicate the degree of salinization at which a 10% of the community change is expected, which corresponds to 18.6, 27.2 and 53.7 ($\mu\text{S}/\text{cm}$) for rivers of order one, four and seven, respectively (Strahler, 1957). The mean value is 33.2 ($\mu\text{S}/\text{cm}$). García-Girón; et al. 2024). This can therefore affect the local richness of each of these orders, as is shown by our dataset, with some regions of Europe being clearly richer than others depending on the group considered (Figure 22).



Source: The authors` analysis.

Figure 22. Family richness of Plecoptera (left), Ephemeroptera (center) and Crustacea (right) for each catchment included in our database (see section 3.1.3 for further details).

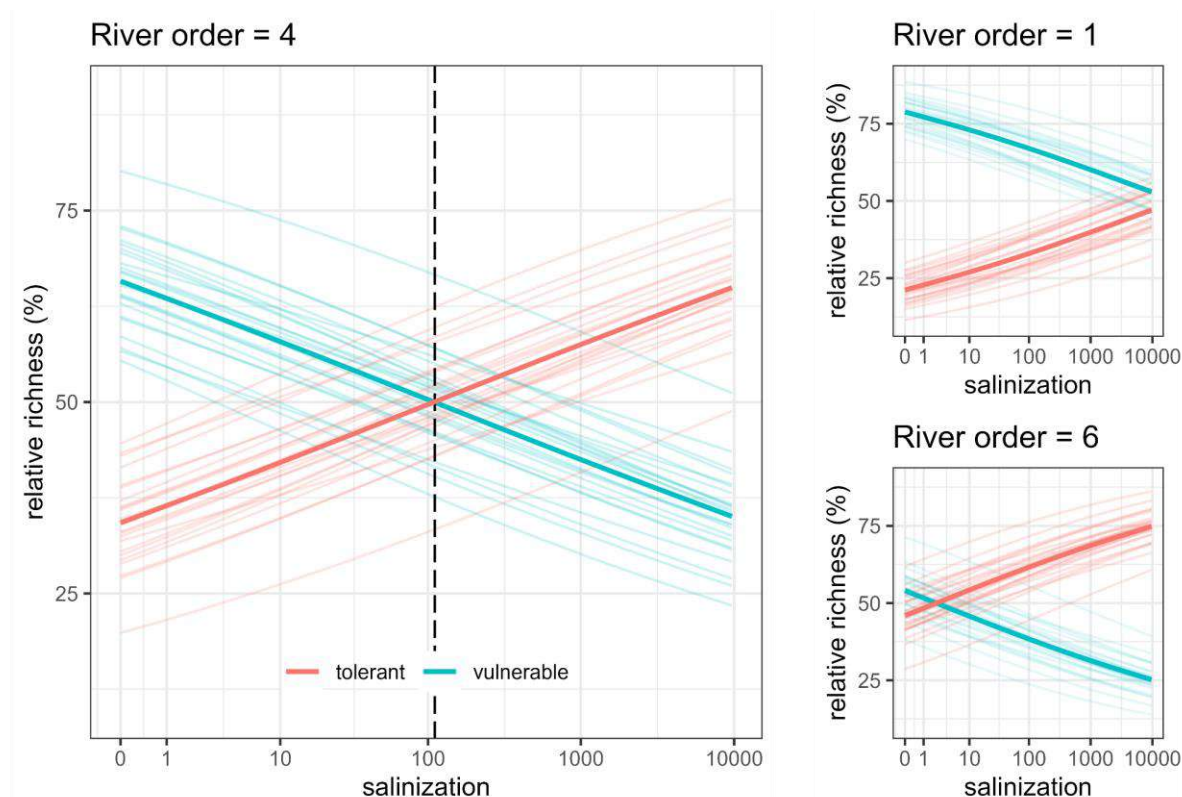


Source: The authors` analysis.

6.2 Taxa specific responses and community turnover

According to Joint Species Distribution Models, 24 taxa strongly responded to salinization (Figure 20). In many cases (e.g. Notonectidae, Perlidae) they responded both to salinization and general environmental degradation (measured as the Human Impact Index). Also some of them (e.g. Oligoneuriidae, Ptychopteridae, Veliidae) were strongly associated with different river orders. Although further analyses disentangling the effect of multiple stressors and the differences between river types are needed before strong conclusions can be drawn from our analyses, we explored the changes in the relative richness of tolerant (i.e. strong positive response to salinization) vs. sensitive (i.e. strong negative response to salinization) taxa along the salinization gradient. We found that a salinization between 18.6 and 53.7 $\mu\text{S}/\text{cm}$ was enough to cause a 10% replacement in community composition (i.e. taxa turnover) depending on the river order, and the response was consistent across countries (Figure 21). Since the relative proportion of sensitive and tolerant taxa was most balanced at order 4 river reaches, which aligned with results at the order level, we focused on them to find a potential salinization threshold for taxa turnover. Following the same procedure as in the previous section, we fit a linear regression for tolerant taxa vs. salinization and another one for sensitive taxa vs. salinization (Figure 23), and computed the intersection point between both (corresponding to 110 $\mu\text{S}/\text{cm}$) as a potential threshold for acceptable salinization (Cañedo et al., 2013; Kelly et al. 2024b).

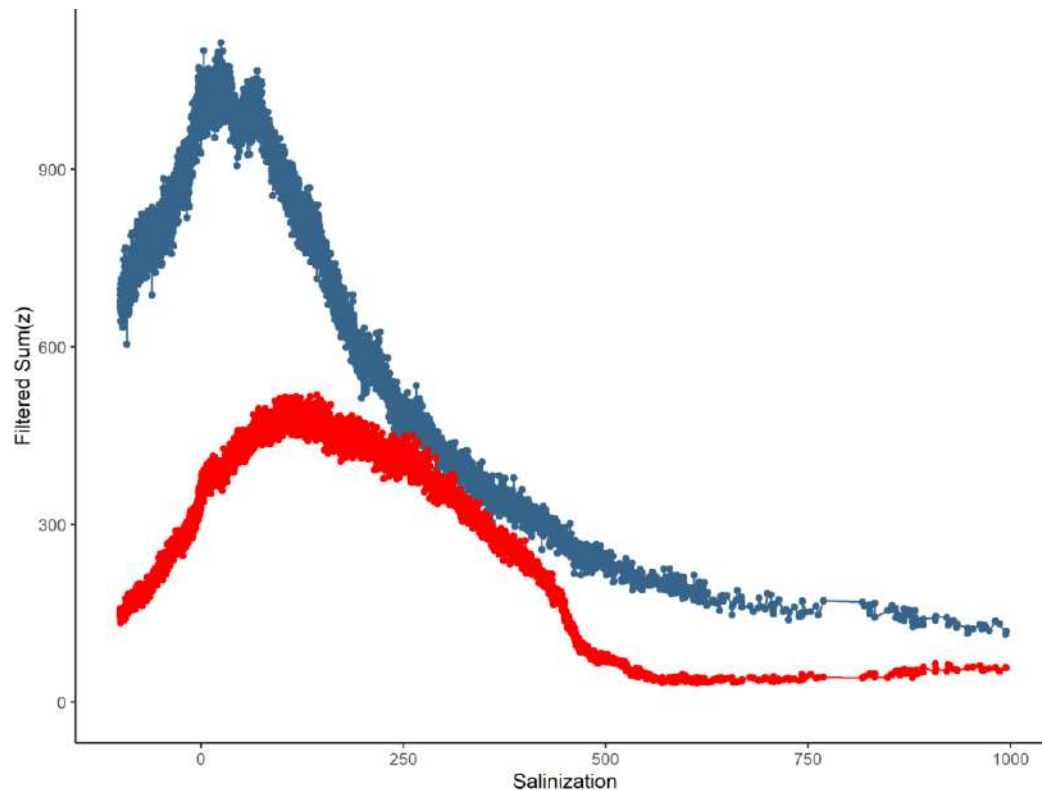
Figure 23. Relative richness of tolerant and vulnerable taxa along the salinization gradient. Panels correspond to different river orders (Strahler, 1957). Thin lines indicate the expected trend for different countries.



Source: The authors' analysis.

According to TITAN, there was a peak response of sensitive taxa (i.e. z^- , see section 3.2.4) at a salinization of 25 $\mu\text{S}/\text{cm}$ and a peak response of tolerant taxa (i.e. z^+ , see section 3.2.4) at a salinization of 143 $\mu\text{S}/\text{cm}$ (Figure 24). The taxa that responded more strongly to salinization (Appendix 3) were: Perlodidae; Heptageniidae, Leuctridae, Empididae, Blepharicedidae and Philopotamidae (negative response); and Naididae; Tateidae, Gammaridae (positive response). This aligned with the results from the Joint Species Distribution Models (Figure 20).

Figure 24. Filtered z-scores (y-axis) along the salinization gradient (x-axis) according to TITAN analysis. Taxa are classified as z+ (positive response to salinization, shown in red) or z- (negative response to salinization, shown in blue). The z-score represents the magnitude of a taxon's response to the salinization gradient. The filtered z-score is calculated after excluding noisy taxa that do not show a statistically significant or consistent response (based on permutations and confidence intervals). By "filtering out" these taxa, TITAN focuses only on those taxa that demonstrate robust responses. The filtered z-score is used to summarize the strength of responses across all retained taxa. Peaks in the filtered z-scores correspond to potential ecological thresholds where the community structure shifts significantly. Negative salinization scores correspond to sites where the predicted EC was higher than the observed EC (see section 3.2.1).

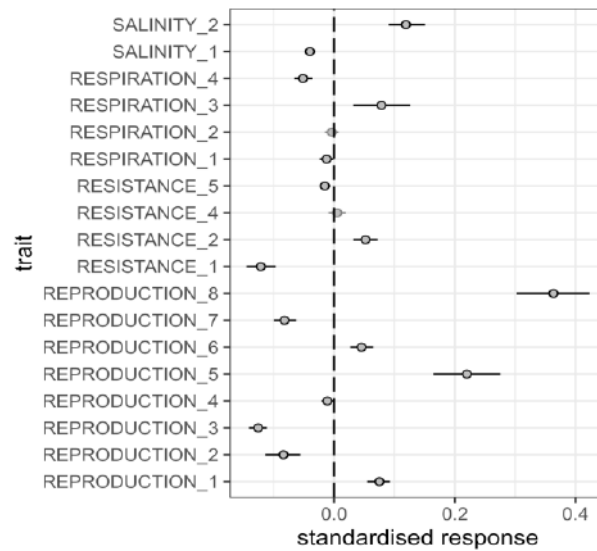


Source: The authors' analysis.

6.3 Ecological traits

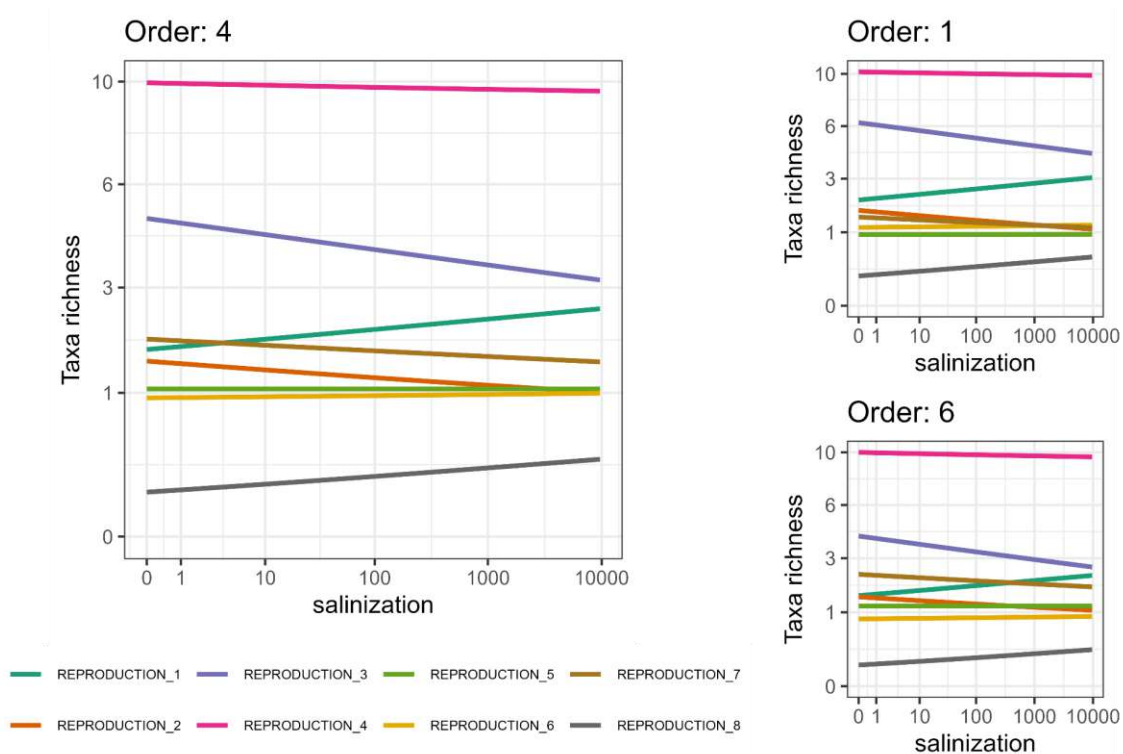
We found that different traits responded to salinization, potentially serving as biological indicators. The traits positively responding to salinization were: brackishwater preference, plastron respiration, resistance to drought through cocoons, asexual reproduction, ovoviviparity and reproduction through free clutches and clutches left in vegetation. The traits negatively responding to salinization were: freshwater preference, spiracle respiration, resistance to drought through egg laying or statoblasts, reproduction through terrestrial clutches and reproduction through egg-laying. The taxa having traits that show a positive response could be better equipped to cope with salinization than those having traits with a negative response. Therefore, the change in the relative proportion of "positive" and "negative" traits could be used to monitor freshwater salinization (Figure 26).

Figure 25. Estimated effect of salinization on the occurrence of different traits. Positive values indicate increasing occurrence (i.e., positive slope) whereas negative values indicate decreasing occurrence along the salinization gradient. Error bars indicate 95% confidence intervals. Salinity: 1 = freshwater preference; 2 = brackishwater preference. Respiration: 1 = tegument; 2 = gill; 3 = plastron; 4 = spiracle; 5 = hydrostatic vesicle. Resistance: 1 = eggs, statoblasts; 2 = cocoons; 4 = diapause or dormancy; 5 = none. Reproduction: 1 = ovoviviparity; 2 = isolated eggs, free; 3 = isolated eggs, cemented; 4 = clutches cemented; 5 = clutches free; 6 = clutches in vegetation; 7 = clutches terrestrial; 8 = asexual reproduction.



Source: The authors' analysis.

Figure 26. Change in the expected number of taxa (Taxa richness) showing different reproduction modes along the salinization gradient for different river orders (Strahler, 1957).

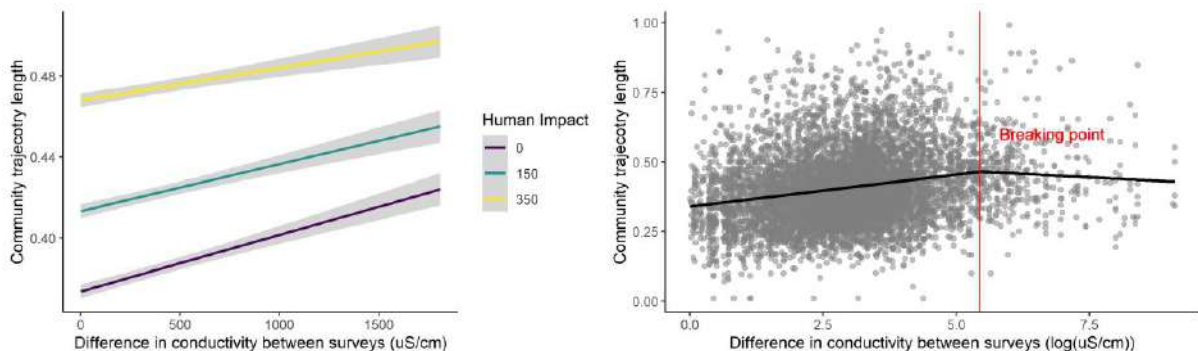


6.4 Community trajectories over time

We found a significant relationship between community trajectories (i.e. proportional change in composition with between two consecutive surveys) and conductivity difference (i.e. the difference in conductivity between the two surveys). This significant relationship indicates that a greater change in conductivity is associated with a greater dissimilarity between macroinvertebrate communities (Figure 27). Thus, for every increase in conductivity (in log-scale), communities became 4% more dissimilar in their composition. Human footprint index modulated this relationship by increasing its intercept, with higher human footprint implying greater change in community composition (Figure 27). There was no interaction between conductivity difference and human footprint index. Nonetheless, this relationship was markedly influenced by the basin identity, with a conditional R^2 of 0.75 in comparison to the 0.06 from the explicative variables. This marked difference was rather expected due to variability in sampled years, biogeographical context, and sampling protocols.

Interestingly, when analyzing the relationship between community change and conductivity differences through regression models with segmented relationships, we could identify a breaking point. The relationship between the two metrics was positive (estimated slope of 0.02) until a conductivity difference of 230 $\mu\text{S}/\text{cm}$ was reached (5.45 in log scale; Figure 27). Then, the relationship became slightly negative (estimated slope of -0.009). This implied that when conductivity between two surveys increased more than 230 $\mu\text{S}/\text{cm}$, the expected corresponding increase in dissimilarity between communities was no longer following a positive trend. Thus, above 230 $\mu\text{S}/\text{cm}$, community change stagnates and remains similar regardless of the difference in conductivity.

Figure 27. Relationship between community trajectory length (relative change in community similarity) and difference in conductivity values between consecutive surveys ($\mu\text{S}/\text{cm}$). Left panel summarises the positive relationship between these two variables and how the human impact (i.e. Human Footprint Index) modulates this response. Right panel indicates the breaking point detected in this relationship at 230 $\mu\text{S}/\text{cm}$. This breaking point marks the change in relationship from positive (i.e. a greater change in conductivity implies greater dissimilarity in communities) to flat.



Source: The authors` analysis.

6.5 Ecotoxicological data

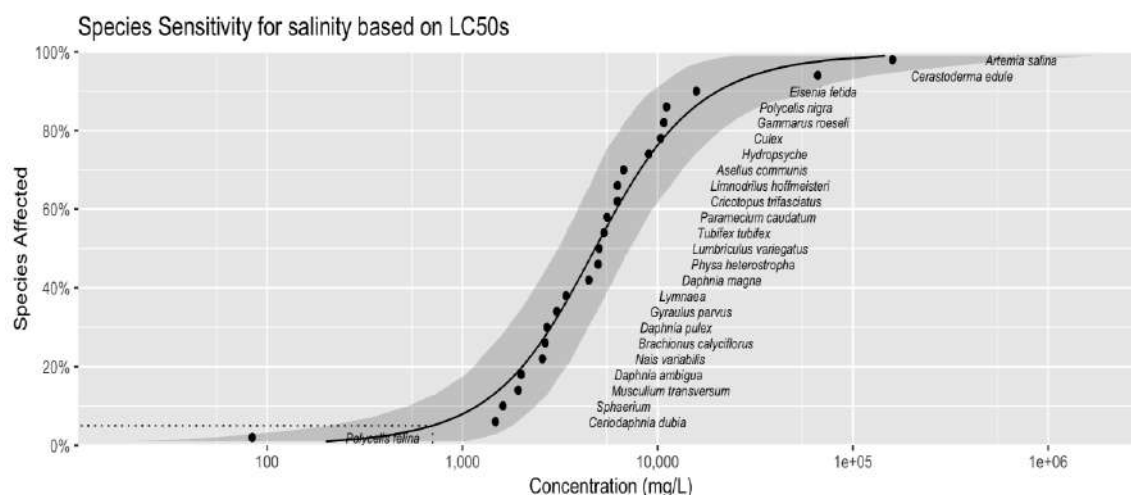
The analysis of mortality tests revealed high salinity tolerance for aquatic invertebrates (Figure 28), with 95% of the taxa analyzed having a lethal concentration 50 (i.e. the salt concentration needed to kill 50% of the individuals of a given species) higher than 704 mg/L. This suggests that any salinity below that would be acutely toxic for less than 5% of the tested taxa. However, these

values need to be interpreted with caution and the value of 704 mg/L should by no means be interpreted as a salt concentration for inland aquatic ecosystems in Europe that protects 95% of taxa. This is because:

1) Most of the taxa included in our analyses are salt-tolerant according to previous studies and may not be representative of the taxa in real-world ecosystems. For example, decapods, amphipods and isopods have a marine origin (Väinölä et al., 2008; Wilson, 2008; Anger, 2016, Ah Yong & Huang, 2020) and, therefore, many of them are known to tolerate wide salinity ranges and thrive in estuarine environments (Wilson & Fleeger, 2013). Other taxa such as Coenagrionidae and Physidae are known to tolerate high salinity according to field studies and laboratory assays (Kefford & Nuggeoda, 2005; Kefford et al., 2006; Cañedo-Argüelles & Rieradevall, 2009). Finally, some of the insects included in the Standartox database, such as Chironomidae, Culicidae and Hydropsychidae are generally regarded as pollution tolerant taxa that can also tolerate salt pollution (Bonada et al., 2004; Berger et al., 2017). We encourage future salt toxicity tests to focus on sensitive taxa such as Ephemeroptera and Plecoptera (Kefford et al., 2011).

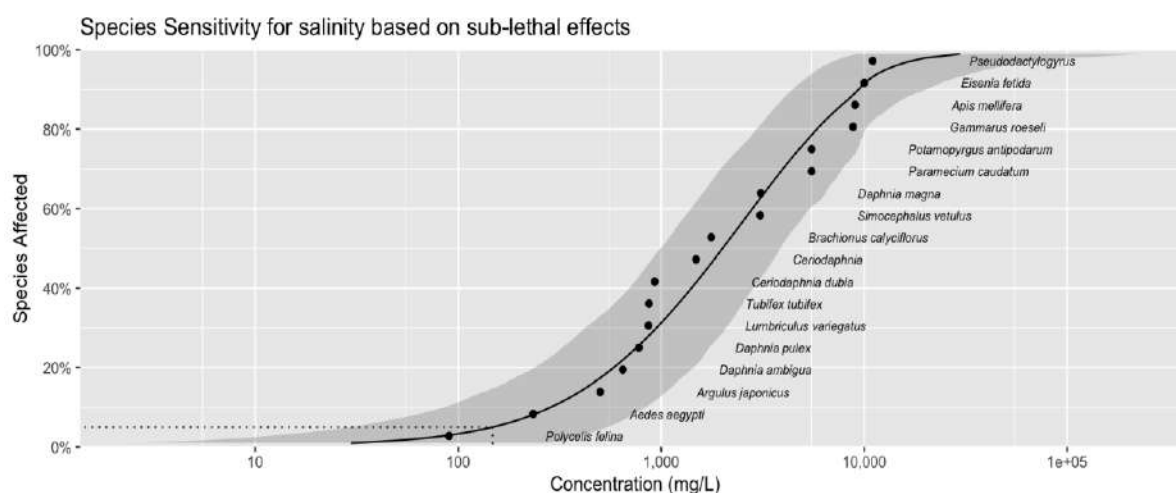
2) Lethal concentrations are informing about the maximum salinity that an organism can withstand for a relatively short period of time (e.g. test duration typically between 24 and 96 h), and they can serve as a proxy to the maximum salinity at which the taxa can be found in nature (Kefford & Nuggeoda, 2004). However, salinization causes stress at considerably lower levels (Escobar et al., 2024) that has an impact on important physiological functions such as reproduction or feeding (Hassell et al., 2006; Hintz & Relyea, 2017), thereby altering ecological interactions and determining community structure (Cañedo-Argüelles et al., 2014, 2016a; Salcedo et al., 2024b). Also, the LC50 already represents an effect measure where only 50% of a population below the threshold would be protected against acute effects. A more suitable measure if the aim was to protect species would be the LC10 or similar, but related data are lacking. Moreover, sub-lethal endpoints represent a more conservative alternative. In our case, sublethal endpoints suggested that 148 mg/L was a “safe” salt concentration, protecting 95% of the analyzed taxa (Figure 29).

Figure 28. Mean and standard deviation of the lethal concentration 50 (LC50) of the taxa included in the Standartox database that are present in Europe. The dashed line indicates the salinity (704 mg/L) beyond which 95% of the taxa experience significant mortality. This 704 mg/L salinity approximately corresponds to an EC of 1260 μ S/cm (US Geological Survey, 2019).



Source: The authors' analysis.

Figure 29. Mean and standard deviation of the concentration that has a significant sub-lethal effect on the taxa included in the Standartox database that are present in Europe. The dashed line indicates the salinity (148 mg/L) beyond which 95% of the taxa experience significant sublethal effects. This 148 mg/L salinity approximately corresponds to an EC of 266 $\mu\text{S}/\text{cm}$ (US Geological Survey, 2019).



Source: The authors' analysis.

6.6 Setting salinization thresholds

In this report we compile multiple lines of evidence (Table 6) to find a salinization threshold (th.) beyond which the aquatic macroinvertebrate communities included in our analyses are strongly impacted. Since aquatic macroinvertebrates are one of the Biological Quality Elements included in the WFD (EU, 2000) and they have often been used as a proxy for the general ecological status of river ecosystems, this threshold could be safeguarding the good ecological status. However, the thresholds produced by different approaches are all very different, spanning three orders of magnitude and there are many limitations to be considered (see discussion section) so we advise EU member states to develop their own salinization criteria using this report as a guide. Also, it is very important to keep in mind that we are proposing a salinization threshold, i.e. the increase in EC from reference (i.e. background) values. So, before our threshold can be used, it is necessary to calculate salinization. If possible, the modelling approach that we have developed (see section 4) should be followed. Alternatively, if chloride concentrations are available, the ratio between EC and chloride seems to be a good alternative to measure salinization (Ziemann, 1997).

All of these measures need to be evaluated in relation to the normative definitions of “good status” in the WFD, which refers to a “slight change” in structure and function of the type-specific community, and should also consider the likelihood of interference from other stressors. A change in EP/C, for example, could arise due to changes in oxygen supply and food types arising from organic pollution, and we also need to make a judgement about how the negative positive responses observed in the TITAN analysis relate to the normative definitions. Could one of these be indicating a threshold other than the good/moderate, in which case, is the negative response “high status” and the positive response “good status”, or do these represent “good” and “moderate” status thresholds? Similarly, is there a case for giving greater weight to analyses based on field data over that derived from laboratory tests, as the former will more closely reflect the interactions amongst taxa that will occur, or to sub-lethal effects over lethal effects? If we deprecate EP/C (likely interactions with other stressors), LC50s (significant ecological change will occur likely before

lethality) and the negative response of indicator taxa (assumed to reflect “high”, rather than “good” status), then the median salinization threshold drops from 186 $\mu\text{S}/\text{cm}$ to 143 $\mu\text{S}/\text{cm}$. If we assume osmotic homeostasis in the absence of salinization, then this reflects the extent to which the ionic strength can be increased (or decreased) before the community starts to show effects concordant with the normative definition for good status given in the WFD. Overall, it is important to note that this report is based on EU-level data, with a relatively coarse spatial resolution and not on river typologies defined according to the natural drivers of ecology. Therefore, this threshold is not ready to be adopted as a safe threshold in all EU member countries. For example, in regions where rivers are oligohaline (e.g. $\text{EC} < 50 \mu\text{S}/\text{cm}$) an EC increase of 186 $\mu\text{S}/\text{cm}$ represents more than a threefold increase in conductivity compared to that to which the organisms are habituated and is likely to have a negative impact on freshwater biota.

Table 6. Salinization thresholds derived from different endpoints assessed in this report and resulting in a median value (186 $\mu\text{S}/\text{cm}$) that can be cautiously interpreted as a potential salinization limit to protect European rivers.

Metric	Salinization threshold ($\mu\text{S}/\text{cm}$)	Description
EP/C	1333	Intersection between the relative richness of Plecoptera + Ephemeroptera vs. Crustacea along the salinization gradient.
LC50s	1260	EC above which 95% of the taxa experience significant mortality according to LC50s reported in the Standartox database.
Sublethal endpoints	266	EC above which 95% of the taxa experience significant stress according to sub-lethal endpoints reported in the Standartox database.
Taxa turnover	33	Salinization at which a 10% of the community is replaced.
Taxa richness	110	Relative richness of tolerant and vulnerable taxa along the salinization gradient.
Indicator taxa	25	Salinization value at which the taxa that responded negatively to salinization was most clearly differentiated into two groups.
Indicator taxa	143	Salinization value at which the taxa that responded positively to salinization was most clearly differentiated into two groups.
Community trajectories	230	Community trajectories (i.e. proportional change in composition between two consecutive surveys) vs. conductivity difference (i.e. the difference in conductivity between the two surveys).
Median	186	Median salinization threshold

Source: The authors' analysis.

Although the proposed threshold could be useful to establish the limit between the good and moderate boundaries, there are other class boundaries to be considered within the WFD: High/Good, Moderate/Poor and Poor/Bad (EU, 2000). To establish these categories the first step would be to

select a metric (it could be a single metric or a multi-metric index) that responds to salinization. In this regard, the metrics that we present in this report can be helpful, but they should be explored at regional or national scales and then inter-calibrated at the EU level. Once a suitable metric is selected, a reference value should be established for different typologies (to be defined, see discussion section). This can be done by modelling the metric at reference/least impaired condition using meaningful predictors (e.g. climate, hydrology, geology). The model can be then used to obtain expected (i.e. reference) values for all water bodies and compare them with observed values, quantifying the biological response to the stressor (i.e. salinization). This procedure is similar to the one followed in this report to calculate the salinization metric (see section 4). The difference between expected and observed values can be scaled to a 0-1 range and used as an Ecological Quality Ratio (EQR; Solimni et al., 2008). The EQR can be then divided into the WFD categories with reference to the normative definitions in the WFD and the boundary setting procedure documented in guidance document 14 (EC, 2000; EC, 2011; Kelly et al., 2023). The final step would be to translate these ecological categories into salinization thresholds. At the moment, the recommended technique is to use binary logistic regression to compute a confusion matrix and determine how many sites will be misclassified (e.g. “good” status according to the EQR, but unacceptable salinization values) with any chosen boundary value (Kelly et al., 2022a). This process has already been implemented into a free Shiny app through a collaborative framework involving the Member States, EFTA countries, and other stakeholders including the European Commission (Varbiro et al., 2018).

7. The interaction between salinization and climate change

7.1. Climate change and salinity in the Oder river

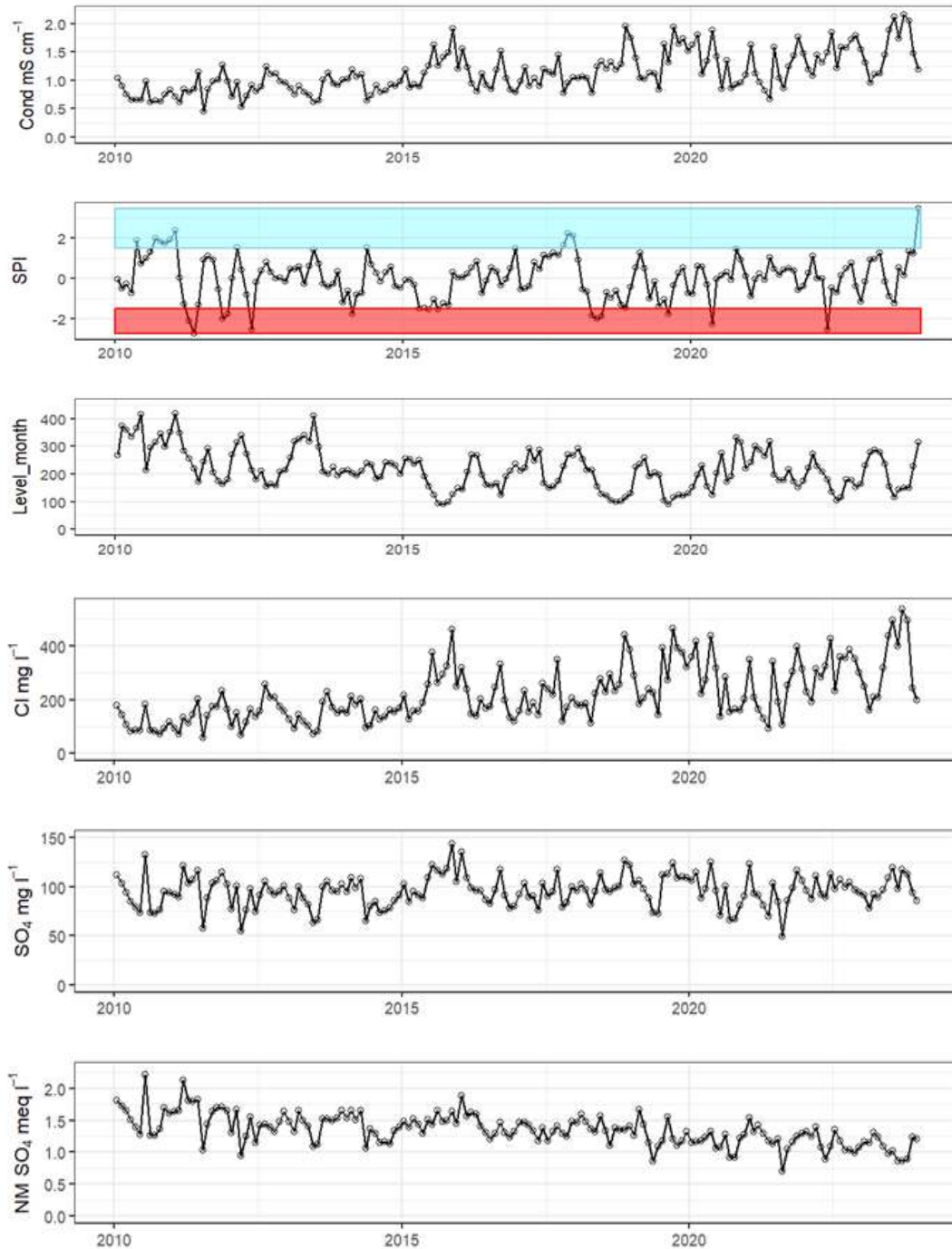
During August 2022 massive fish kills were noted on the Oder river and eventually resulted in the death of approximately 1000 tonnes of fish and other aquatic organisms and had an ecological impact along 500 km of the river. Their deaths were caused by a substantial toxic algal bloom that happened at this time. The causal species was identified as *Prymnesium parvum*, a species adapted to brackish salinities. A key factor that enabled the proliferation of this species was the high salinity of the Oder river during this time, due, at least partly, to discharges of industrial wastewater with a high salt content (e.g. from mining activities). Other potential factors were the drought and the resulting low water levels reducing dilution and flow and also hydromorphological modifications to the river. High nutrient concentrations, especially phosphorus and nitrogen were also key in promoting the algal blooms. Although it was clearly a multifactorial event difficult to anticipate, the increased incidence of droughts and alteration of hydrology suggest that this kind of events will be promoted by climate change and become more frequent in the future.

Conductivity over the period ranged from 0.45 to 2.6 mS/cm (Figure 30) and a Theil-Sen trend analysis indicated a significant upward trend with a monthly increase of 0.40% (see Appendix 4). A similar upward trend was noted for chloride but not for SO_4^{2-} . In contrast, a significant downward trend was found for calculated non-marine SO_4^{2-} (-0.23%). Drought periods, as indicated by more negative SPI values tended to be followed by an increase in conductivity once the SPI started to increase with precipitation, this can be seen more clearly in 2015, 2018, 2019 and 2022. The percentage decrease in river level measured in situ and the runoff for the catchment estimated

from ERA5 data were both minus 0.26, indicating significant declines over the period. A significant decline in the SPEI was noted over time but not the SPI.

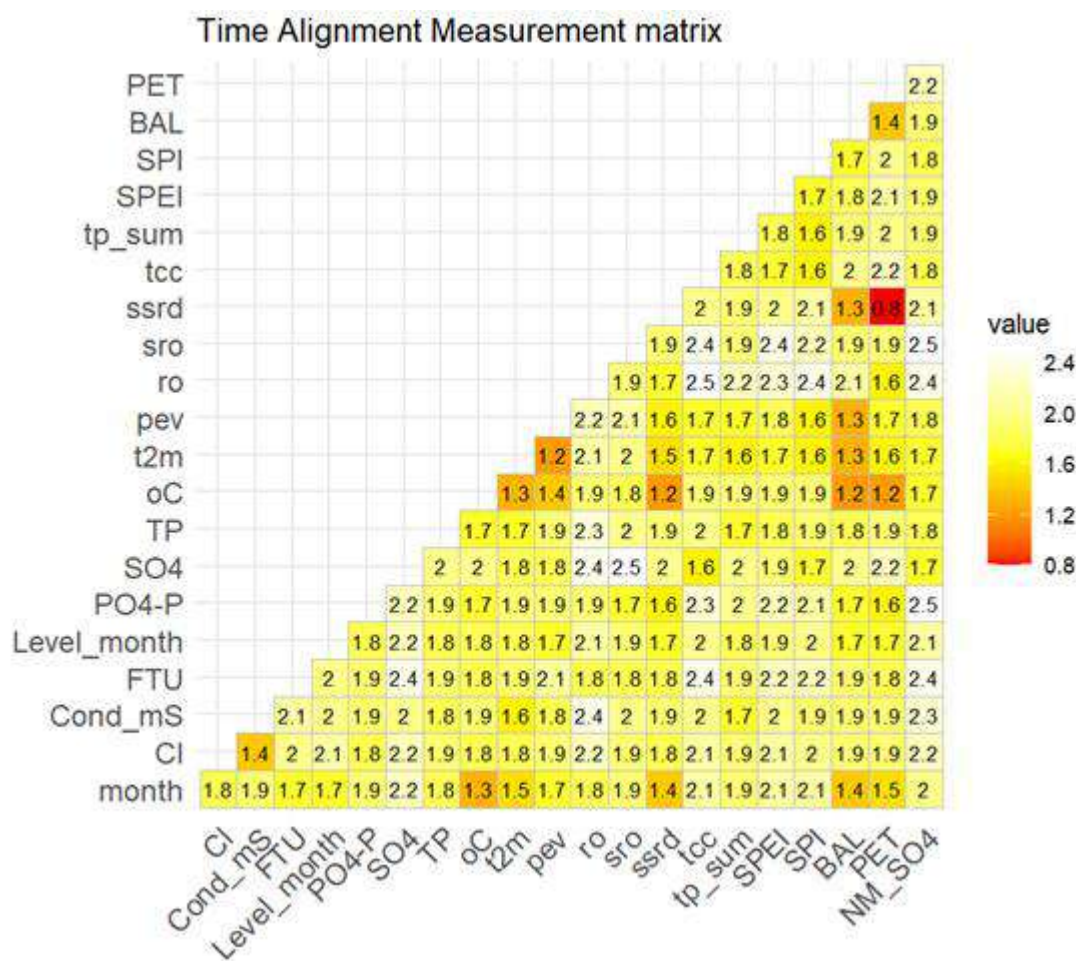
In order to examine the temporal interrelationships we carried out a TAM analysis (Figure 31). Conductivity was in closest synchrony with climatic parameters such as air temperature (t2m), total precipitation (tp_sum) and indicators of drought (SPI, SPEI, BAL and PET) while being most similar to Cl (TAM = 1.4) which would be expected. Some parameters were more asynchronous such as ro (runoff) with Cl and SO_4^{2-} both having a TAM value of 2.4.

Figure 30. Monthly time series (2010 to 2023) for the river Oder for conductivity, SPI (blue >1.5, red <-1.5 highlight wet and dry conditions, respectively), river level, Cl^- , SO_4^{2-} and non-marine SO_4^{2-} .



Source: The authors' analysis.

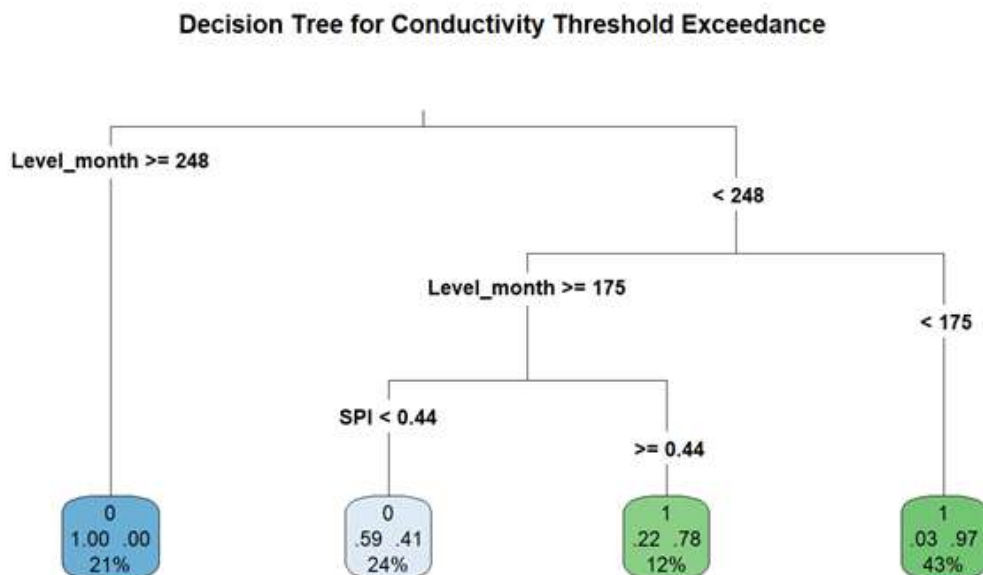
Figure 31. TAM (Time Alignment Measurement) values for pairwise analysis performed on data from 2010 to 2023. The TAM represents an index of similarity in the temporal domain. A TAM of 0, indicates perfect temporal alignment while 3 indicates completely out of alignment. See Appendix 4 for abbreviations



Source: The authors` analysis.

The seasonal and residual components were analysed together in an AIRMAX model to understand which environmental parameters were important in explaining the variation in conductivity. Environmental variables that had a significant positive z score included total cloud cover (4.08) and river level with a 3 month lag (2.26). Parameters with a significant negative z score included the river level of the current month (-4.13), SPI (-2.48) and total cloud cover with a 3 month lag (-3.23). Variables that were significant in the ARIMAX model were used to create a decision tree to predict when conductivity would exceed 1.2 mS/cm using data from the most recent 6-year period (2018 to 2023) (Figure 32). Although 1.5 mS/cm is used more commonly as a threshold to expect *Prymnesium* blooms, in the Oder river the range between 1 and 1.5 mS/cm corresponded to a transition stage from absence to initial presence of the species (Hartman, 2021; IOŚ PIB, 2023). The decision tree indicated that 100% of river levels above 248 cm did not exceed the conductivity threshold while when levels were below 175 cm then 97% did. SPI was of limited use in distinguishing whether the threshold was exceeded at intermediate river levels.

Figure 32. Decision tree for conductivity (mS/cm) exceedance of 1.2 mS/cm for the Oder river between 2018 and 2023.



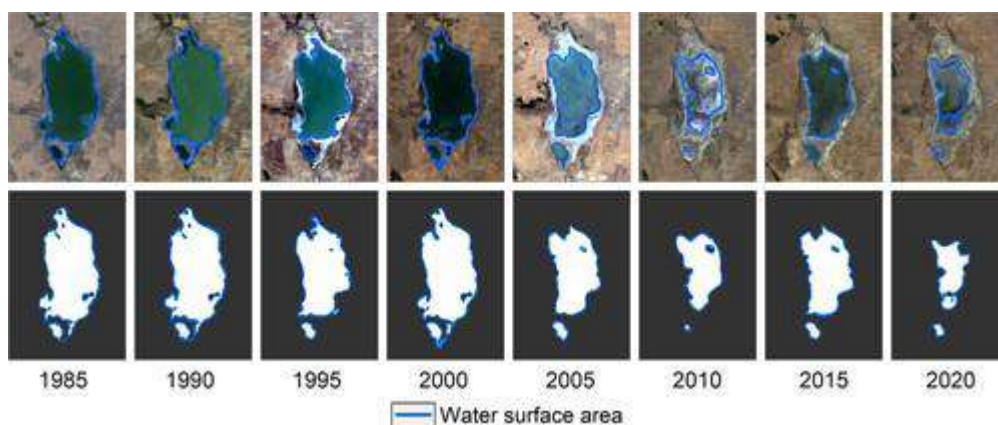
Source: The authors` analysis.

7.2. Impact of Land-use and climate change on surface area, salinity and biota in the Turkish lakes

7.2.1 Change in lake surface area and salinity

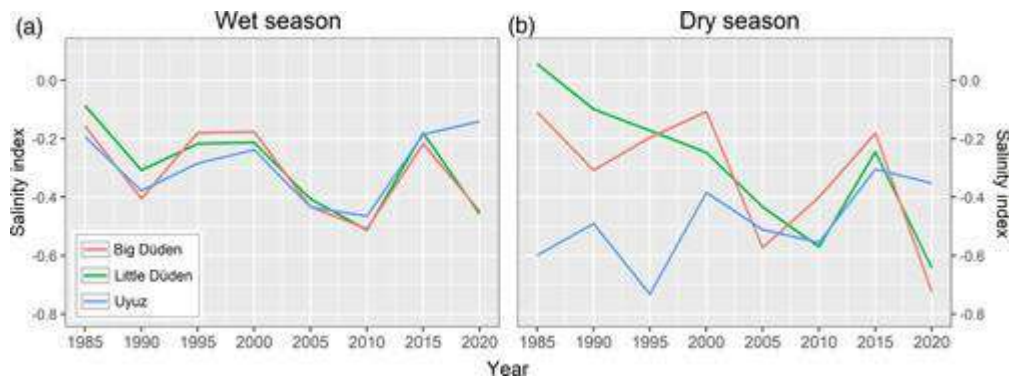
About 50% of the surface area of the 3 lakes studied were lost during the last 35 years and the decrease in surface area was more prominent during the dry season (Figure 33). Based on the remote-sensing salinity index, during the observed period, the salinity of the 3 lakes strongly increased (lower the index value higher the salinity) especially during the dry periods (Figure 34).

Figure 33. Landsat true-colour RGB images (upper pane) and black and white images marking the surface area (lower panel) of Lakes Big Düden and Little Düden at 5 years intervals from 1985 to 2020.



Source: Yilmaz et al., 2020.

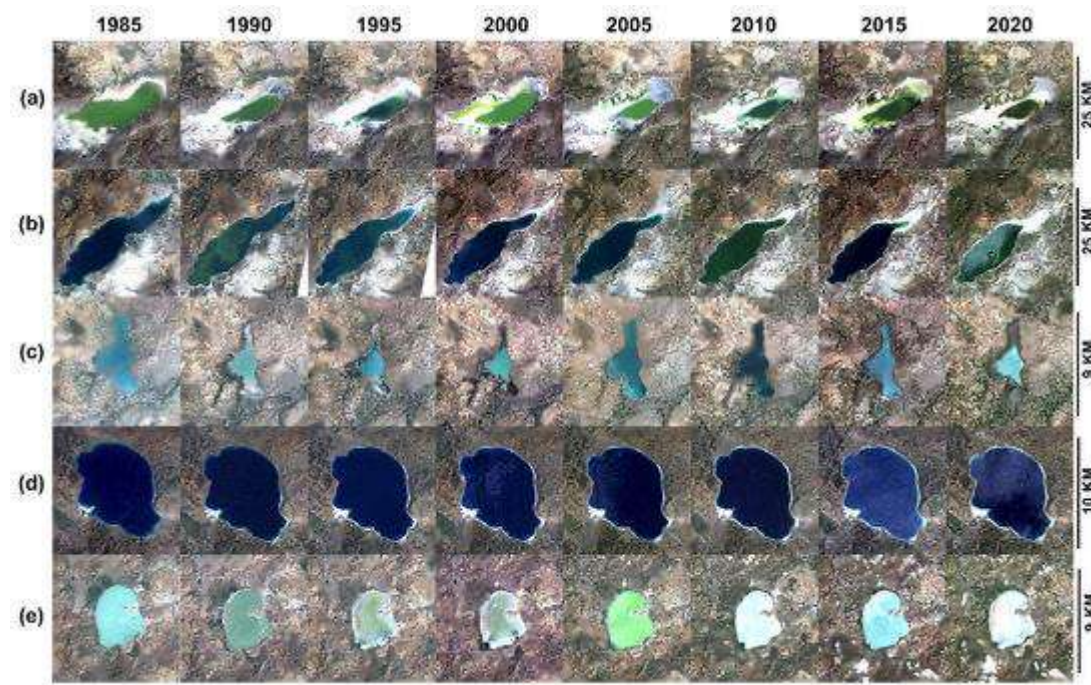
Figure 34. Changes in salinity of Lakes Düden, Little Düden and Uyuz m 1985 to 2020 at 5-year intervals in (a) the wet season and (b) the dry season.



Source: Yılmaz et al., 2020.

Same as in KCB, the lakes in BCB had major surface areas lost. The surface areas of Lakes Acıgöl, Akgöl, Burdur, Karataşı, and Yarışlı decreased drastically, whereas this was not the case in deep Lake Salda (Figure 35). A major decrease in the surface area of Lake Acıgöl occurred after 2005 in the wet season. Lake Akgöl was completely dry from 1988 to 2009 during the dry season.

Figure 35. Change in the surface areas of the lakes located in BCB (a) Acıgöl, (b) Burdur (c) Karataşı, (d) Salda, (e) Yarışlı for wet periods from 1985 to 2020.



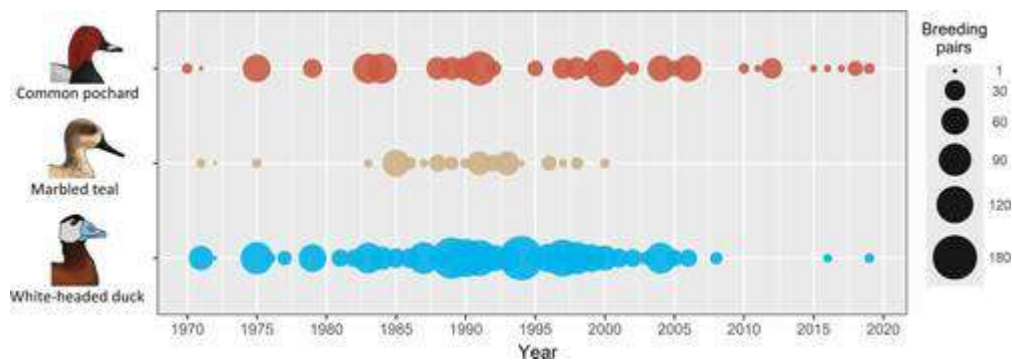
Source: Çolak et al., 2022.

Major reduction in lakes' surface areas and increase in salinity (the latter quantified only in KCB) coincided with the major increase in water use in irrigated crop farming in both basins. In KCB, the crop production increased substantially after the year 2000, coinciding with increased use of water for irrigation. In 2019, the dominant crops were sugar beet and maize, accounting for 28% and 18% of the total production, respectively (Yılmaz et al., 2020). Although sugar beet is the second most water intensive crop, its production in the KCB largely increased with the establishment of new infrastructure for processing sugar beet (e.g. sugar factories). In 2019, 43.6% of the land in

the BCB was cultivated (Çolak et. al., 2022). The main products in 2019 were maize, tomatoes, alfalfa, sugar beets, which constituted 34, 11.6, 9.4 and 9% of the total production, respectively. The establishment of a new fodder-crop factory and the distribution of government-supported maize-gathering machines led to an increase in maize and alfalfa production after 2004 that they mainly served the purpose of feeding an increasing number of livestock (Çolak et. al., 2022).

The changes in lake area and salinity in the KCB were associated with major changes in the waterbird communities of 3 of the globally threatened waterbird species, used here as an example. Common pochard had a small population of breeding birds but large migrating and wintering populations in the KCB. However, its breeding population had declined by 95% to only 6 pairs in 2019. Marbled teal used to breed at several localities in the basin, all of which have either been totally lost or severely degraded, The species has not been observed in the KCB since 2000 and not in Turkey since 2015. White-headed duck populations in the KCB have suffered major losses over the last few decades (Figure 36) (Yılmaz et al., 2020, Özcengil et al., accepted).

Figure 36. Changes in the sizes of the breeding populations of the 3 globally threatened waterbird species: common pochard, marbled teal, and white-headed duck from 1970 to 2020 in the KCB.



Source: Yılmaz et al., 2020.

Closed basins can be an important indicator of the regional climate conditions and the local hydrological cycles through the expansion and reduction of lakes' surface areas as well as of salinization. A drastic loss of lake surface area and salinisation have occurred during the past 40 years in the KCB and BCBs, in part due to largely a result of water abstraction and landscape regulation conducted to support an increasing agricultural production (Yılmaz et al., 2020, Çolak et al., 2022). In both KCB and BCB, a key factor driving the water loss in the lakes is the crop-farming irrigation which has increased notably since 2000 and 2004, respectively, leading to intensive water abstraction from the lakes and their inflows, as well as from groundwater (rate of reduction in groundwater was up to 1 m/yr), to meet the increased demand for irrigation owing to intensive crop farming of mainly water thirsty crops (e.g. sugar beet, maize). The consequences of this are the salinization of lakes with a strong effect on their ecosystem structure, functioning and biodiversity (see section 7.2.2), as well as the loss of many habitats (e.g. large shallow lakes, wetlands).

Three globally threatened waterbird species face extinction in the KCB, and 18 of the 62 previous breeding species have already been lost. The BCB is home to 106 bird species with either probable or confirmed breeding records and 55 more with possible breeding records. Of these, five species are included in the IUCN red-list as globally threatened (Çolak et al., 2022). The six Important Bird Areas (IBA) in the BCB have had worsening scores and conditions. Over the last 50 years, the wintering waterbird population size has shown a significant negative trend from more than 360,000 waterbirds in the late 1960 (the largest record ever in Türkiye) to only 26,000 for the period of 2015–2020 (Çolak et al., 2022).

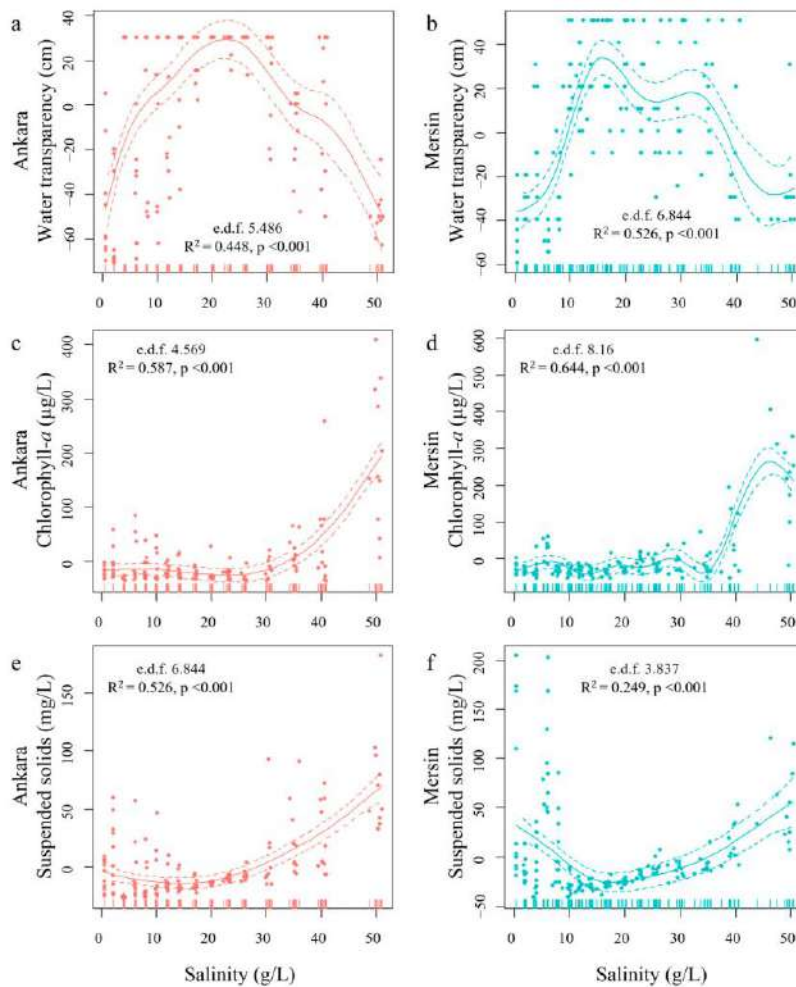
Both closed basins are famous for fish species richness and especially for endemic fish species. There are 38 and 24 fish species in KCB and BCB, respectively, with 74% and 54% being endemic, respectively (Yılmaz et al., 2020 and Çolak et al., 2022). Of the endemic species in the KCB, 61% are considered threatened or near threatened by the IUCN and already one species is extinct coinciding with habitat loss or salinization. Also, once widespread endemic fish species are now restricted to small refuges (Yılmaz et al., 2020). Of the 13 endemic fish species, 7 are categorized as threatened: 2 as critically endangered and 5 as endangered (Çolak et al., 2022, see references therein).

7.2.2 Synchronized Mesocosm Experiments

7.2.2.1 Experiment 1: Salinity Gradient experiment

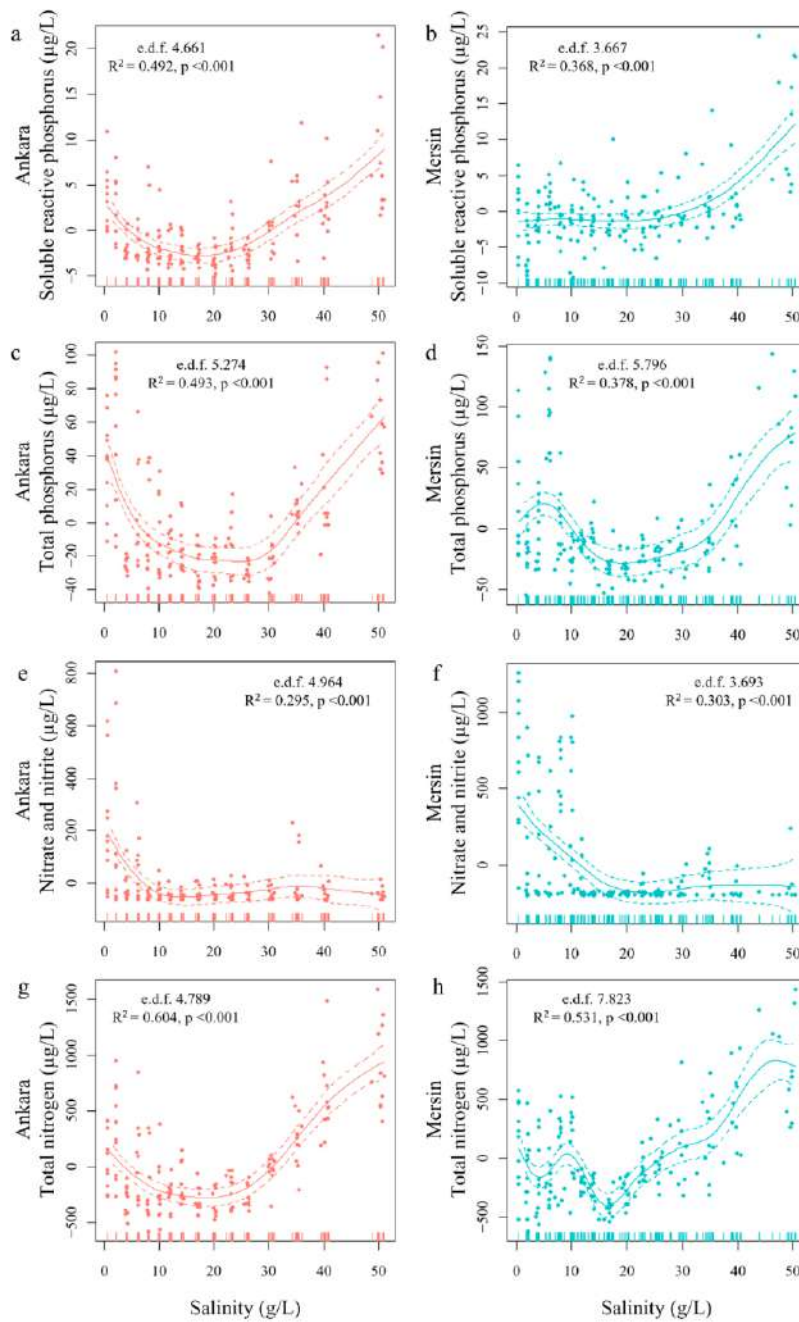
To further explore the potential impact of salinization on ecosystem structure, functioning and biodiversity, we conducted a salinity gradient experiment with 16 different salinity levels (see section 3.1.4.2). The selected salinity range reflected the observed salinities in the central Anatolian lakes sampled. All water transparency and nutrient concentration variables showed a significant non-linear relationship with the salinity gradient during the stable phase (generalized additive models with $p < 0.001$) in the Mersin and Ankara mesocosms (Figures 37 and 38).

Figure 37. Temporal changes in salinity (a,b) and average water temperatures (c,d) in the 16 different treatments of the gradient experiment in the Ankara and Mersin mesocosm facilities, respectively.



Source: Özkan et al., 2023.

Figure 38. Response variable values estimated through generalized additive models (GAM) for soluble reactive phosphorus (a,b), total phosphorus (c,d), nitrite and nitrate (e,f), and total nitrogen (g,h) in Ankara and Mersin, respectively, and their response to the salinity gradient in the stable phase of the experiment.



Source: Özkan et al., 2023.

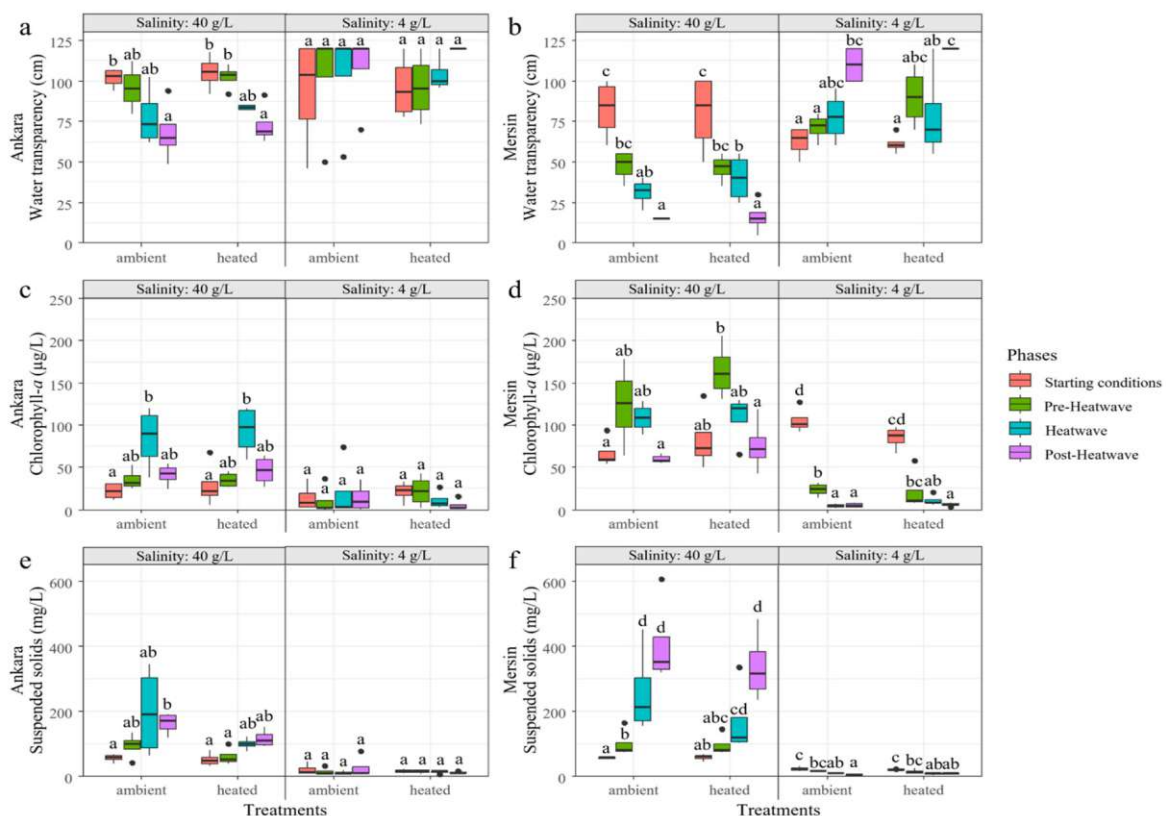
The higher turbidity at lower salinities was likely due to fish activity, while at higher salinities, it was likely due to both high phytoplankton growth and inorganic turbidity triggered by high salinities (Figures 37 and 38). The effect of high salinities on the flocculation dynamics of suspended particulate matter through physicochemical processes in the water column has been widely observed in brackish waters (Droppo, 2021). Even though the same amount of N and P were added to all mesocosms every week, the treatments showed different nutrient concentrations relative to the salinity treatments (Figure 38). TP, SRP and TN concentrations showed a U-shaped response to the salinity gradient in both experimental facilities, with high concentrations in the freshwater and low salinity mesocosms (0–12 g/L of salts), decreasing concentrations in the intermediate salinity

mesocosms, followed by an increase in the high salinity mesocosms (35–50 g/L salts). The concentrations of $\text{NO}_3 + \text{NO}_2$ decreased markedly after the first phase of the experiment at both sites (Figure 38) (Özkan et al., 2023).

7.2.2.2 Experiment 2: Heat wave and Salinization experiment

The second synchronized factorial design mesocosm experiment tested the impact of a heatwave (6 °C) crossed with relatively low (4 g/L) and high salinities (40 g/L) in both mesocosm facilities. The experiments included 3 phases: pre-heat wave, during heat wave and post heat wave. Throughout the experiment the desired temperature and the salt concentrations were maintained (see Özkan et al., 2023 for details).

Figure 39. Variation of water transparency (a,b), chlorophyll-a (c,d), and suspended solids (e,f) in the treatments with 4 and 40 g/L salinity crossed with ambient temperatures and heatwave treatments at the start of the experiment, at the beginning (Pre-Heatwave), during (Heatwave), and after (Post-Heatwave) the heatwave period in Ankara and Mersin mesocosm facilities.



Source: Özkan et al., 2023.

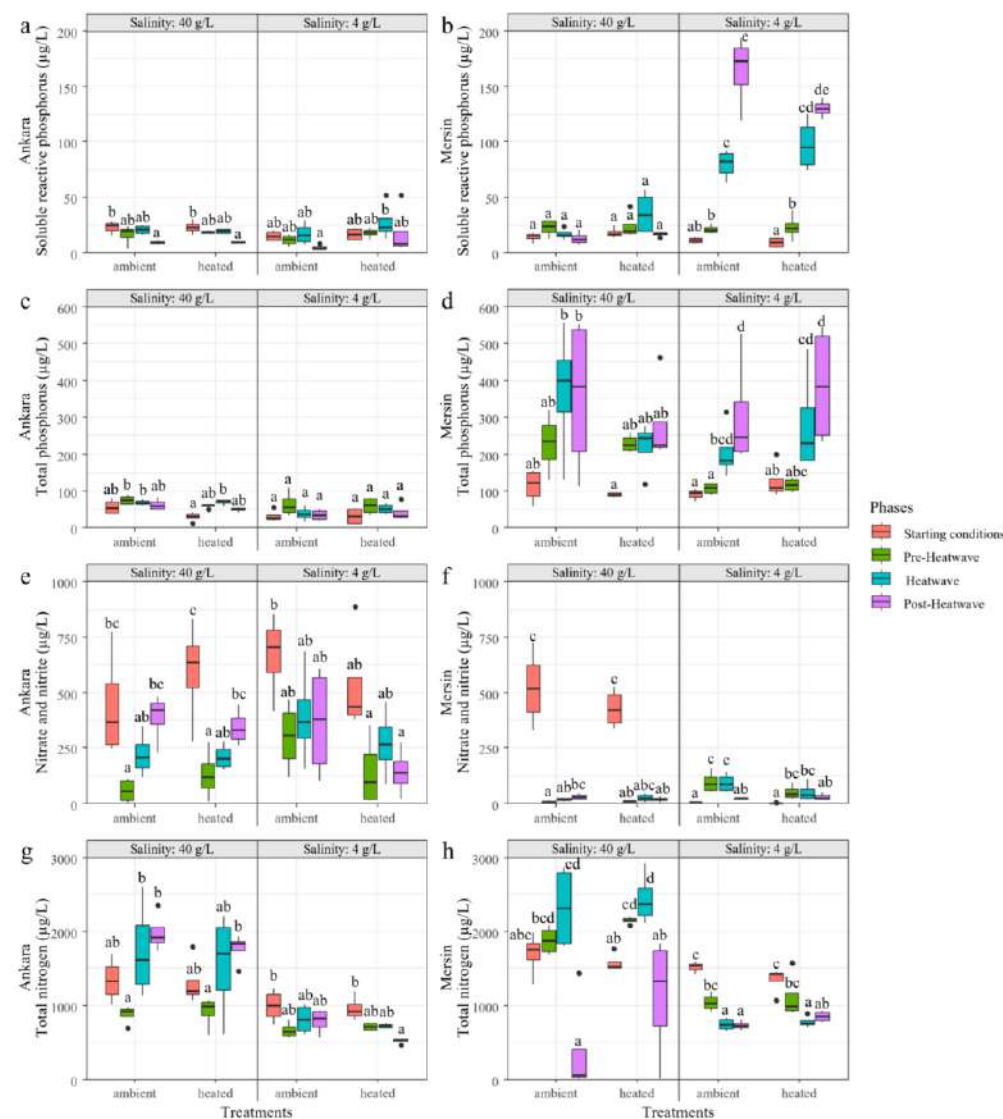
Overall, mesocosms with high salinity (40 g/L) were more turbid, with higher concentrations of chl-a, SS, TP, and TN. Both the salinity gradient and the factorial design heatwave-salinity experiments demonstrated a strong effect of salinity on ecosystem structure and functioning, which was modulated by the natural climate differences between the two facilities. The warmer Mersin facility experiences eutrophic conditions, which were worsened at high salinities. In Ankara, eutrophic conditions were only observed at high salinity (40 g/L).

The salinity gradient experiment (Figure 39) revealed a strong effect of salinity on primary producers (i.e., chl-a) and water transparency, which was modulated by temperature and fish. At high salinities water was highly turbid due to phytoplankton growth and SS induced turbidity, whereas at low salinities the top-down effect of fish (inducing turbidity) prevailed. At intermediate salinities the water clarity was the highest probably owing to the high top-down control of

zooplankton on phytoplankton biomass in the absence of fish feeding induced turbidity. However, further research on how phytoplankton, zooplankton and fish interact along salinity gradients is needed. The U-shaped response of nutrients (TN and TP) to the salinity gradient in both experimental facilities is partly in line with previous research from semi-arid regions showing that salinization enhances nitrogen retention (Jiang et al., 2023).

The experiment on the effects of the heatwave crossed with two different salinities (Figure 40) showed that the effect of climate was very pronounced when comparing the semi-dry central Anatolia and the Mediterranean climates. However, no significant effect of the two-week heatwave was observed on water transparency and nutrient concentrations.

Figure 40. Variation of soluble reactive phosphorus (a,b), total phosphorus (c,d), nitrite and nitrate (e,f), and total nitrogen (g,h) in the treatments with 4 and 40 g/L salinity crossed with ambient temperatures and heatwave treatments at the start of the experiment, at the beginning (Pre-Heatwave), during (Heatwave), and after (Post-Heatwave) the heatwave period in Ankara and Mersin mesocosm facilities.



Source: Özkan et al. 2023.

8. Discussion

This report provides valuable information for the regulation of freshwater salinization in Europe, aiming at preserving aquatic biodiversity and ecosystem functioning while safeguarding food and water security. This is not only necessary to comply with keystone policies such as the WFD or the United Nation's SDGs, but also urgent due the lack of consistent evidence-based salinity standards across Europe (Kelly et al. 2024). In this report policy-makers and water managers can find an estimation of the extent of river salinization at the EU-scale, invertebrate-based metrics to monitor freshwater salinization, salinization criteria that can protect freshwater biodiversity and information on the potential interaction of salinization and climate change in rivers and lakes. However, we warn against the direct use of this information at the country level for various reasons that are explained in the following paragraphs. Here we present an ambitious attempt to assess freshwater salinization at the European-scale, but working at a large scale with relatively coarse spatial resolution is not ideal for setting meaningful standards at the regional scale. Adapting the methods presented in this report to the biogeographical, climatic and even socio-political context of each EU member country is highly recommended. Fortunately, salinity has been routinely reported by water quality monitoring programs and most of the information that we have used in this report is publicly available at the European scale. Thus, data availability should not be a constraint to take action and develop ecologically meaningful salinity standards in all European countries.

The first limitation that we need to acknowledge is that we did not define river typologies. This is important because salinity can naturally vary between regions due to differences in climate, geology and proximity to the ocean, among other causes (Kaushal et al., 2023). Thus, we can find very different salinity-types (e.g. calcareous mountain streams vs. arid-land saline streams) that not only differ in their salt concentration, but also in their ion composition (e.g. CaCO_3 vs. NaCl) (Meybeck, 2003a). Although developing salinity-typologies was out of the scope of this report, it seems both necessary and accomplishable. Ecoregions could be a good starting point for defining these typologies, although the concordance between ecoregions and biological communities has recently been questioned for European rivers (Jupke et al., 2022). For example, Griffith (2014) estimated reference values for specific conductivity and major ions in the ecoregions of the US and found distinct patterns mainly associated with underlying geology, precipitation and evaporation. In the case of Europe, typologies have already been defined for surface water bodies according to the WFD, however the definition criteria vary widely among countries (Moss, 2008; Jupke et al., 2022). In this regard, existing attempts to inter-calibrate the different typologies and produce broad river types (Solheim et al., 2019) could be explored within the context of freshwater salinization, even though they would need to be adapted to better capture biological communities (Jupke et al., 2022). Alternatively, our salinization model goes a step forward from typology-based reference, to location-specific predictions. However, it would need further validation and it could benefit from including more explanatory variables (e.g. related with seawater influence or naturally saline water bodies). Combining both approaches, salinization could be modelled for different typologies, which could yield stronger results given that some key explanatory variables (e.g. lithological classes) are categorical.

Developing typologies might also be useful for setting reference conditions, not only for biological communities, but also for background salinity levels and ion mixtures. There is an important take-home message from this report: we need to develop standards for salinization (i.e. increase in salinity) rather than for salinity per se. This is because salinity has both natural and anthropogenic causes, but the effect that they have on biota is very different. While natural salinity allows for

adaptation to take place, salinization is a more disruptive and fast process that usually leads to impoverished communities and promotes biological invasions (Gutiérrez-Cánovas et al., 2019b; Kefford et al., 2016). Also, the relative increase in salinity is much more important than its absolute value. For example, a moderate increase in salinity can have a very strong impact on the aquatic organisms of mountain streams, which have evolved under very low salinities, whereas it might have a negligible impact on those inhabiting estuarine environments (Niedrist et al., 2021). Finally, the changes in relative ion concentrations could be even more important than the differences in total salinity. This is because different ions have different toxicities. For example, different studies have shown that K^+ is more toxic than other ions for aquatic fauna, whereas Ca^{2+} might even ameliorate the toxicity of other ions and metals (Schulz & Canedo-Argüelles, 2019). Also, Ziemann (1967) found that the alteration of $K^+ : Ca^{2+}$ ratios could determine the distribution of euryhaline diatom species. Thus, ion-specific standards should be developed taking into account natural ion mixtures and the ecological impacts derived from their alteration (Cañedo-Argüelles et al., 2016).

Another limitation of our approach is that we did not integrate the interactive effects of salinization with other stressors. Salinization often co-occurs with other stressors, e.g. salinization and pesticide pollution in agricultural fields or salinization and metal pollution in mining areas (Kaushal et al., 2024). However, we still have very little information about the interactive effects of salinization and multiple stressors on aquatic biodiversity (Velasco et al., 2019), but it is clear that they should be accounted for as much as possible. This is a challenge that applies to all stressors (e.g. nutrient pollution) when establishing the ecological status within the WFD, and probably requires a harmonised solution at the EU-level (Posthuma et al., 2019a). In our approach, the effect of other stressors was incorporated through a general human impact metric (the Human Footprint, HF), which incorporates 1) built environments, 2) population density, 3) electric infrastructure, 4) crop lands, 5) pasture lands, 6) roads, 7) railways, and 8) navigable waterways (Venter et al., 2016). The HF was incorporated into our models to estimate salinization, and when assessing the response of individual taxa to salinization. However, this approach is not fully capturing interactive effects and other approaches such as improved component-based methods (Posthuma et al., 2019b) and effect-based methods (Brack et al., 2019) should be explored. Within the context of multiple stressors salinization might play a critical role. For example, when Heß et al. (2024) used WFD monitoring data from Saxony (Germany) to investigate the importance of 85 environmental variables (including 34 micropollutants) for 18 benthic invertebrate metrics at 108 sites, salinity (measured as EC) ranked as the most important.

Accounting for multiple stressors is necessary to isolate the effect of freshwater salinization and to demonstrate causality. To this end, ecotoxicological data are helpful and, despite their limitations (e.g. lack of realism because they fail to capture the complexity of ecosystems), they can complement field data analyses. In this report we derived species sensitivity distributions from acute and sub-lethal toxicity data for sodium chloride compiled in the US EPA Ecotox database (Olker et al., 2022). Classical thresholds in chemical risk assessment are the concentrations corresponding to 5% affected species (Posthuma et al., 2002). This yielded to values in the range of hundreds mg/L NaCl, corresponding to a few hundreds to thousand $\mu S/cm$ in terms of EC. These values are certainly lower than the background salinity in some least impacted surface waters (Le et al., 2019). Thus, to derive ecological threshold needs to account for the natural background ion concentrations and very likely the ecoregion and stream type. Notwithstanding, the toxicity data provide information on effect thresholds from a precautionary perspective that are in the range of observed changes in species richness and community turnover of macroinvertebrates along salinity gradients in Germany (Le et al., 2021) and South-East Australia (Simão et al., 2021). Although toxicity data are informative, field studies should always complement laboratory assays since they can better capture the complexity

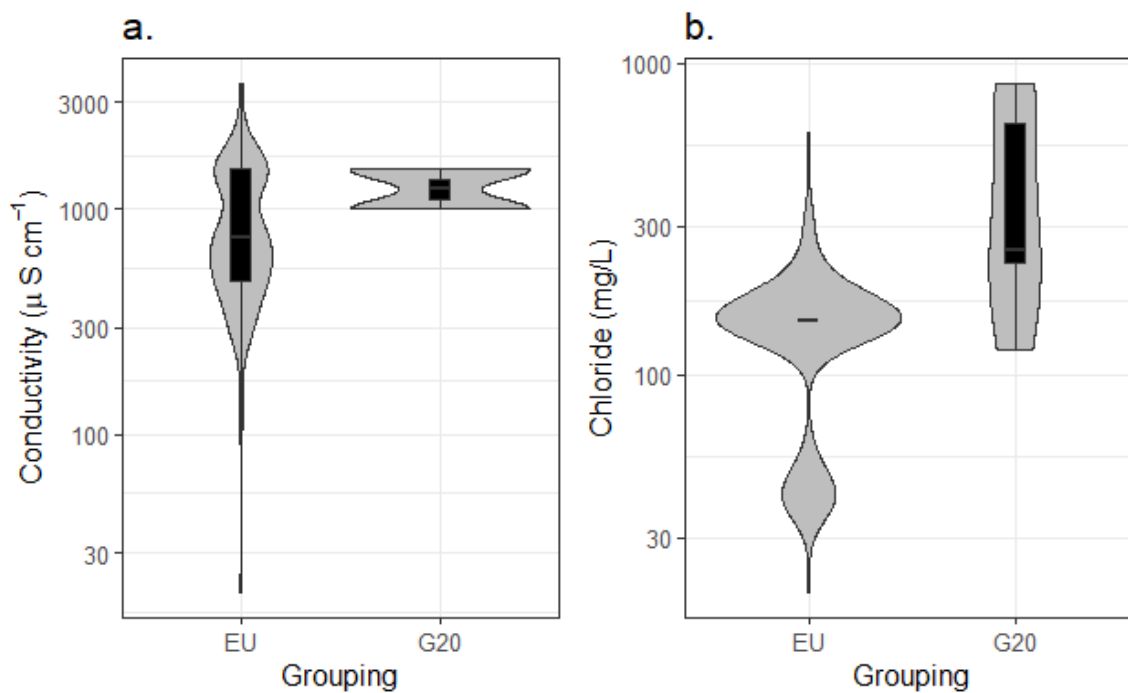
of ecosystems (Kefford et al., 2023). Laboratory experiments with single species ignore additional stressors that may strongly exacerbate the effects, thereby decreasing the threshold for protecting 95% of taxa (Liess et al., 2016). Also, mesocosm studies can be a powerful tool to explore biological responses to salinization under controlled experimental conditions (Stewart et al., 2003). For example, the synchronised gradient salinization experiment carried out in two sites in Türkiye mimicking shallow lake ecosystems, highlighted that the response of ecosystem structure to a wide range of salinity is non-linear (it follows a threshold response) and that salinization can affect bottom-up and top-down processes.

Overall, we found that different Ephemeroptera and Plecoptera were sensitive to salinization, whereas Crustacea tended to be tolerant. This is a broad generalization that needs to be taken with caution (e.g. there are some Gammaridae species that are found in oligohaline habitats such as springs; Barquín & Death, 2004), and can differ between species and between regions, but it aligns with previous studies. For example, Le et al. (2021) found that with higher salinization (measured as the EC change from background levels) were dominated by the amphipods *Gammarus tigrinus* and *Chelicorophium curvispinum*, whereas ephemeroptera *Epeorus assimilis* and the plecopterans *Protonemura* sp. and *Leuctra* sp. were indicators of low salinization. Similarly, many experimental and field studies have reported a much higher salt sensitivity of Ephemeroptera and Plecoptera than Crustacea (Cañedo-Argüelles et al., 2013; Schulz & Canedo-Argüelles, 2019; DWA-Themenband, 2024). Overall, although our results offer some guidance for the development of invertebrate-based salinization metrics, we recommend the selection of indicator taxa and the development of metrics to be conducted at the regional level. This is because there might be differences in salt sensitivity (even among populations of the same species) arising from ecological properties that vary between taxa, such as the individual plasticity of organisms (Schröder et al., 2015), historical salinity exposure of species (Sala et al., 2016), and regional differences including the occurrence of stressors and biotic competitors (Velasco et al., 2019). Future advances in the development of biological indicators of salinization can benefit from regional efforts that have shown promising results in Australia (Chessman, 2023), Germany (Ziemann & Schulz, 2011; Feld et al. 2023) and the UK (Pickwell et al., 2022). However, the salt sensitivity of the taxa defined in these indices tends to rely on expert criteria, and common guidelines and inter-calibration exercises are still lacking. In addition, ecological traits can be explored as an alternative for developing metrics that can be used at the European level. Another possibility is to use ecological traits, which do not depend on the taxonomic identity of the taxa and therefore can be used at the European scale. In our case we found that respiration and reproduction could potentially serve to monitor ecological responses to salinization. For example, plastron respiration was benefited over spiracle respiration in salinized rivers. This makes sense since plastron respiration minimizes the entry of water into the body (Buchwalter et al., 2002), thereby minimizing the exposure to salinization. Also, reproduction through egg clutches left within the water seems to be a good strategy to cope with salinization, which makes sense because these clutches are laid in a gelatinous matrix that provides protection from desiccation (Williams, 1996; Bonada & Bogan, 2024) and, most likely, salinization. Similarly, the internal development of eggs (i.e. ovoviviparity) seemed to protect against salinization. Our results partly agree with previous studies (Szöcs et al., 2014; Gutiérrez-Cánovas et al., 2019b), but information on the trait-sensitivity to salinization is still scarce (Walker et al., 2023). Overall, we have shown that the development of salinization indices at the EU-level is necessary because current EQRs are weakly related to salinization. Also, if we take our proposed salinization threshold 186 $\mu\text{S}/\text{cm}$ and look at the ecological quality (i.e. EQR) of those that exceed it, 48% of them are in Good or High ecological status. This suggests that current indices are not capturing ecological degradation associated with salinization.

A final limitation that needs to be acknowledged is that we did not differentiate between acute and chronic toxicity, although in our case toxicity data were most related to the former and field data to the latter (thus, we combined information from both for the derivation of the salinization threshold). Different studies have shown that pulse (i.e. acute) and press (i.e. chronic) salt releases can have different ecological effects (Marshall & Bailey, 2004; Cañedo-Argüelles et al., 2014; Cocherio et al., 2017; Vendrell-Puigmitja et al., 2022; Canhoto et al., 2023). Moreover, some salinization drivers are more likely to cause acute toxicity (e.g. road salt application, leading to salt spikes during snowmelt), whereas others (e.g. wastewater treatment plants) are most likely to result in chronic toxicity. Incorporating this into the definition of salinization is also advised, although it was out of the scope of the present study.

Evaluating the current salinity standards for their capacity to protect inland waters from salinization based on our results is challenging because they are all referred to salinity instead of salinization, thereby making our results difficult to compare. If we refer to current European salinity standards, they are higher than our proposed threshold of 186 $\mu\text{S}/\text{cm}$ (Tables 1 & 2, Figure 41). However, since we are referring to salinization, our threshold should be summed to background EC to get a comparable estimation. If we take all the river reaches that were not salinized (i.e. observed EC - predicted EC ≤ 0) according to our model, their mean EC was $272 \pm 91 \mu\text{S}/\text{cm}$. Thereby, our threshold would be around 458 $\mu\text{S}/\text{cm}$. This is a very rough estimation that would need to be re-calculated for each river type rather than taking a European-wide estimate. However, even if considering this threshold as “safe”, most of the current EU salinity standards fall above it (Tables 1 & 2, Figure 41). Thus, overall, our conclusion is that current EU salinity standards do not protect freshwater biodiversity from salinization. If we take other standards derived from technical reports and scientific papers we reach the same conclusion. For example, Halle & Müller (2013), found that a mean Cl^- concentration of around 25 mg/L ($\approx 41 \mu\text{S}/\text{cm}$) marked a shifting point for invertebrate community composition, with most taxa being negatively affected once concentrations reached 50 mg/L ($\approx 82 \mu\text{S}/\text{cm}$). Also, a chloride threshold of 200 mg/L ($\approx 330 \mu\text{S}/\text{cm}$) was set for German flowing waters but Halle et al. (2017) suggested that it should be revised and lowered according to river typologies. However, these findings are still debated within the German Association for Water, Wastewater and Waste (DWA) working group GB-5.4 (salt pollution of watercourses) and require further research (DWA-Themenband, 2024). Finally, an internationally coordination mesocosm experiment including locations in Canada, Europe and the US found that at 73% of the study sites, Cl^- concentrations that caused a $\geq 50\%$ reduction in cladoceran abundance were lower than the lowest Cl^- thresholds established in Canada (120 mg $\text{Cl}^-/\text{L} \approx 198 \mu\text{S}/\text{cm}$) and the United States (230 mg $\text{Cl}^-/\text{L} \approx 330 \mu\text{S}/\text{cm}$). The situation is even more worrying for other G20 countries, where EC standards are above 1000 $\mu\text{S}/\text{cm}$ (Figure 41), or in many other countries of the world (including EU member states) where salinity standards do not even exist (Kelly et al., 2024). Developing such standards would help us to get an estimation of how many freshwater bodies within Europe are threatened by salinization. In this regard, if we use our 186 $\mu\text{S}/\text{cm}$, a total length of 139891 km of the river network analyzed in this report (Appendix 5) can be considered as threatened by salinization (i.e. salinization is above safe levels for aquatic biodiversity), which represents 22% of the total river network in Europe.

Figure 41. Comparison of a) conductivity and b) chloride standards in EU (rivers) and other G20 countries. EU data from Kelly et al. (2022b); G20 data collated for this study. Note log scale. Low conductivity standards in the EU protect against “alkalinisation” in soft water streams (mostly in Spain).

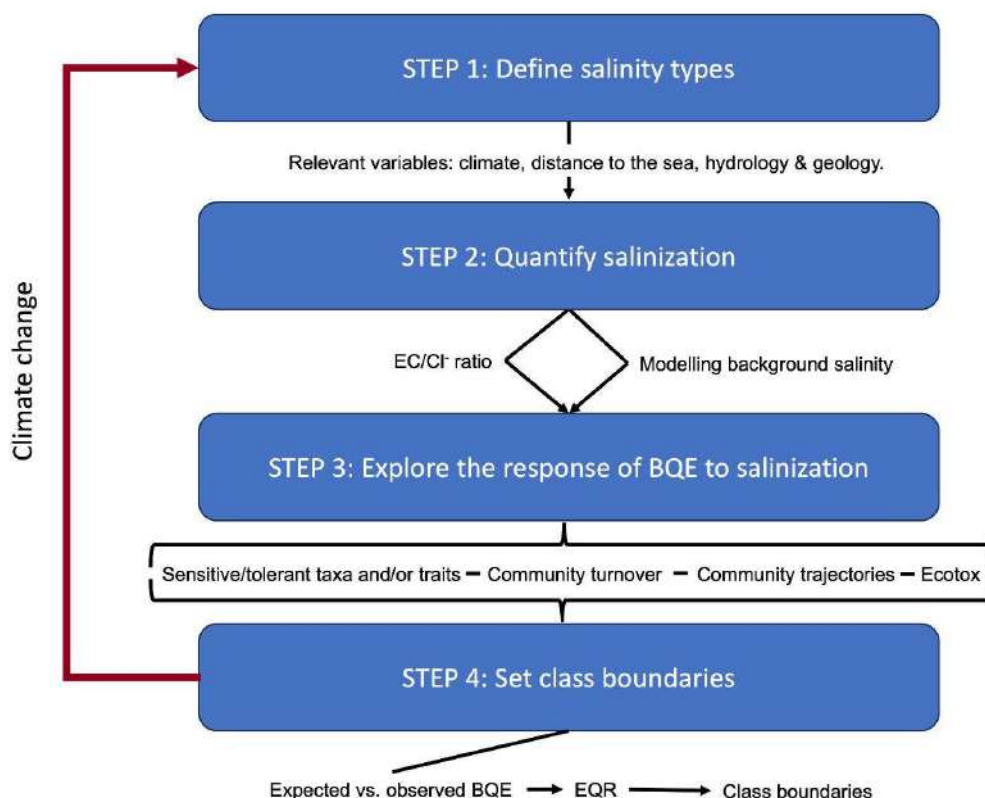


Source: Kelly et al., 2024..

In spite of its potential limitations, this report offers some technical guidelines for setting salinity standards that we hope will be useful (Figure 42). The first step, as we argued in the second paragraph of this section, is to establish typologies according to the main natural drivers of salinity (climate, distance to the sea, geology and hydrology). Once these typologies are established their ecological relevance could be explored by assessing their congruence with biological communities of aquatic organisms (Jupke et al., 2022), and they could be adjusted accordingly. After that (step 2), background salinity conditions should be defined for each type (see section 4). Ideally, these background salinity conditions should not only include total salinity, but also the concentration of major ions: K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} . If sufficient data of reference (i.e. least disturbed sites) conditions is available for each type, it can be used to model background salinity for all the sites under consideration (e.g. a whole catchment or the entire river network of a country). Then salinization can be calculated as the differences between the expected salinity under minimal human disturbance and the observed salinity. If the models are not robust enough (e.g., because of lack of data) but chloride concentrations are available, the ratio between salinity and chloride concentration can be a reliable estimation of salinization (Ziemann, 1997). Once the salinization metric has been calculated, it can be used to explore the response of relevant BQEs such as diatoms, macrophytes, zooplankton, benthic invertebrates and fish (Step 3). In this report we have explored different methods that complement each other. Having multiple lines of evidence (from laboratory assays to mesocosm and field studies) can lead to stronger and more reliable results to inform the final standards. Thus, we advocate for using as many methodological approaches as possible and to avoid relying solely on expert criteria. Finally (Step 5), the biological metrics selected as indicators of salinization can be modelled using only sites with little to no salinization (i.e. reference sites) so the expected metric values corresponding to un-impacted biological communities can be obtained. Then we can calculate the deviation of the metric at each site (observed - expected) and build the EQR. This EQR can be used to set class boundaries (i.e.

High/Good, Good/Moderate, Moderate/Poor, Poor/Bad) following the WFD criteria. These categories can be further validated using confusion matrices (Phillips et al., 2024).

Figure 42. Methodological flowchart to set salinity criteria. Step 1: see discussion section. Step 2: see section 4. Step 3: see section 6. Step 4: see section 6. Section 7 provides some information on how climate change might modulate salinity.



Source: The authors' analysis.

When designing salinity standards, we should anticipate potential climate change effects, which can modify reference conditions and lead to shifting baselines (Moe et al., 2014; Polazzo et al., 2024). For example, climate change will bring a decrease in surface runoff (Döll & Schmied, 2012) and an increase in evapotranspiration (Dai et al., 2018) in many regions of the world, thereby reducing water level in surface water bodies and decreasing their salt dilution capacity (Jeppesen et al., 2015). The river Oder case study showed the importance of river level in influencing the conductivity in the river, with drier periods leading to higher conductivity levels. The SPEI rather than the SPI had a significant decline indicating that evapotranspiration may be more important rather than rainfall levels in controlling streamflow as found in this region for the Rhine river (Teuling et al., 2019). However, despite conductivity and Cl^- increasing, the SO_4^{2-} did not increase and calculated NM-SO_4 was actually declining, this may be related to variability in industrial discharges or to the lower susceptibility of Cl^- to chemical precipitation or to the lower runoff reducing the dissolution of sulfide bearing minerals (Mittelstädt et al., 2023). Important climatic factors influencing conductivity in time series analysis were cloud cover, river level and SPI with antecedent conditions shown to be important by inclusion of lagged variables (SPI itself is a 3-month lagged index). Drought periods, as indicated by more negative SPI values tended to be followed by an increase in conductivity once the SPI started to increase with precipitation. This corresponds to one of the anticipated impacts of climate change where drought periods can lead to a concentration of metals and salts from mining waste, which

become released with the first rainfall event in a pulse with implications for freshwater life (Nordstrom, 2009). The decision tree was useful in identifying water level at which to expect an increase in salinity. These findings can help management prepare for the arrival of conditions conducive to bloom development but must be incorporated with active discharge monitoring and management to achieve water quality objectives.

The experience from Turkish lakes also serves to illustrate the potential of salinization to interact with temperature. This interaction can be expected in many lakes around Europe (especially in the Mediterranean). As evapotranspiration increases the lake water levels drop and salinity increases. According to mesocosm experiments, increasing temperature can affect the response of lake ecosystems to salinization. We found a strong response in productivity (i.e., chl-a) and water transparency to salinization that was modulated by temperature. Also, the experiment carried out in the same facilities on the effects of heatwaves crossed with two different salinities (low and high) showed that the climatic differences between the semi-dry central Anatolia and the hot Mediterranean region determined the ecosystem response to salinization.

In addition to increased salinization due to climate change, it is important to account for potential physiological effects of climate change on aquatic organisms that might increase their salt sensitivity. For example, Orr & Buchwalter (2022) showed that increasing temperature generally increased ion transport rates and reduced the survival of aquatic insects subjected to salinization. Overall, we recommend salinity standards to be revised and adapted according to climate change. One option would be to track the effects of climate change on least impacted sites through long-term monitoring networks (Hering et al. 2010b; Peñas et al., 2023). Then, if changes in physico-chemical characteristics and/or biological communities are detected, reference conditions should be re-defined and the whole methodological flowchart should be followed to re-calculate salinity standards (Figure 42). Alternatively, climate change scenarios can be used to model future salinity (Le et al., 2019; Olson, 2019) and species distributions (Domisch et al., 2013), thereby enabling the calculation of new salinity standards adapted to climate change. However, climate change is forming a more pervasive threat to the WFD assessment procedures than anticipated, altering reference conditions may only be a temporary solution to what are likely to be successive changes in community structure and new approaches to retro-fit climate change into the WFD are needed (O'Briain, 2019; Free et al., 2024).

9. Conclusions

Current salinity standards in the European Union are not protecting freshwater ecosystems for several reasons: 1) they do not assess salinization (i.e. the deviation of salinity from background levels due to human activities); 2) they do not consider the different toxicity of different ions; 3) salinity limits are mostly relying on expert criteria or on laboratory toxicity assays that lack realism; 4) the established salinity limits are above the acceptable limits to protect aquatic diversity; 5) they do not account for climate-change effects on salinity. Within this context, this report offers valuable information and tools to address these regulation flaws and create ecologically meaningful salinity standards. These salinity standards are needed to prepare European freshwaters for a saltier future driven by climate change (Le et al., 2019; Olson, 2019) and an increase in resource extraction and water demand (Cañedo-Argüelles et al., 2013). This is urgent because salinization might be fuelling the global freshwater biodiversity crisis (Harrison et al., 2018; Haase et al., 2023) and because it can compromise food and water security (van Vliet et al., 2017), or even pose risks to human health (Cañedo-Argüelles, 2020). Although this report constitutes a major step forward into the effective regulation of freshwater salinization in Europe, it needs to be used with caution. For example, we provide a salinization threshold that should potentially protect freshwater biodiversity and suggest that around 22% of the European rivers and streams could be threatened by salinization. Since this threshold has been calculated at the EU scale, we warn against its direct application. Developing regional or national standards following the guidelines offered by this report will probably result in more reliable standards that more accurately account for the ecological context. The data and technical expertise to develop such standards is readily available. Therefore, we urge EU member states to take action and we believe that this report can guide their efforts.

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Annexes

Annex 1: Complete list of environmental variables considered and those used in the random forest model.

Included in the model	Category	Short_name	Name	Unit	Projection available for future	Resolution	Source	Original source	Citation original source
yes	Climate	scd	Snow cover days	count	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio1	mean annual air temperature	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio2	mean diurnal air temperature range	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio3	isothermality	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
yes	Climate	bio4	temperature seasonality	°C/100	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio5	mean daily maximum air temperature of the warmest month	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio6	mean daily minimum air temperature of the coldest month	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio7	annual range of air temperature	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio8	mean daily mean air temperatures of the wettest quarter	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
yes	Climate	bio9	mean daily mean air temperatures of the driest quarter	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio10	mean daily mean air temperatures of the warmest quarter	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio11	mean daily mean air temperatures of the coldest quarter	°C	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
yes	Climate	bio12	annual precipitation amount	kg m ⁻² year ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio13	precipitation amount of the wettest month	kg m ⁻² month ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio14	precipitation amount of the driest month	kg m ⁻² month ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022

Included in the model	Category	Short_name	Name	Unit	Projection available for future	Resolution	Source	Original source	Citation original source
yes	Climate	bio15	precipitation seasonality	kg m ⁻²	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio16	mean monthly precipitation amount of the wettest quarter	kg m ⁻² month ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio17	mean monthly precipitation amount of the driest quarter	kg m ⁻² month ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio18	mean monthly precipitation amount of the warmest quarter	kg m ⁻² month ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Climate	bio19	mean monthly precipitation amount of the coldest quarter	kg m ⁻² month ⁻¹	Yes	30 arcsecond	CHELSA Bioclim	-	Karger et al. 2017 Brun et al. 2022
no	Hydrology	Q-mean	Annual mean streamflow	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-min	Minimum weekly flow	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-max	Maximum weekly flow	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-wm	Mean flow of the wettest month	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-dm	Mean flow of the driest month	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-hm	Mean flow of the hottest month	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-cm	Mean flow of the coldest month	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-wq	Mean flow of the wettest quarter	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	Q-dq	Mean flow of the driest quarter	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-hq	Mean flow of the hottest quarter	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-cq	Mean flow of the coldest quarter	m ³ .s ⁻¹	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	Q-zfw	Number of zero flow weeks		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-range	Annual streamflow range		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	Q-si	Streamflow seasonality index $\sigma(Q)/Q\text{-mean}$		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022

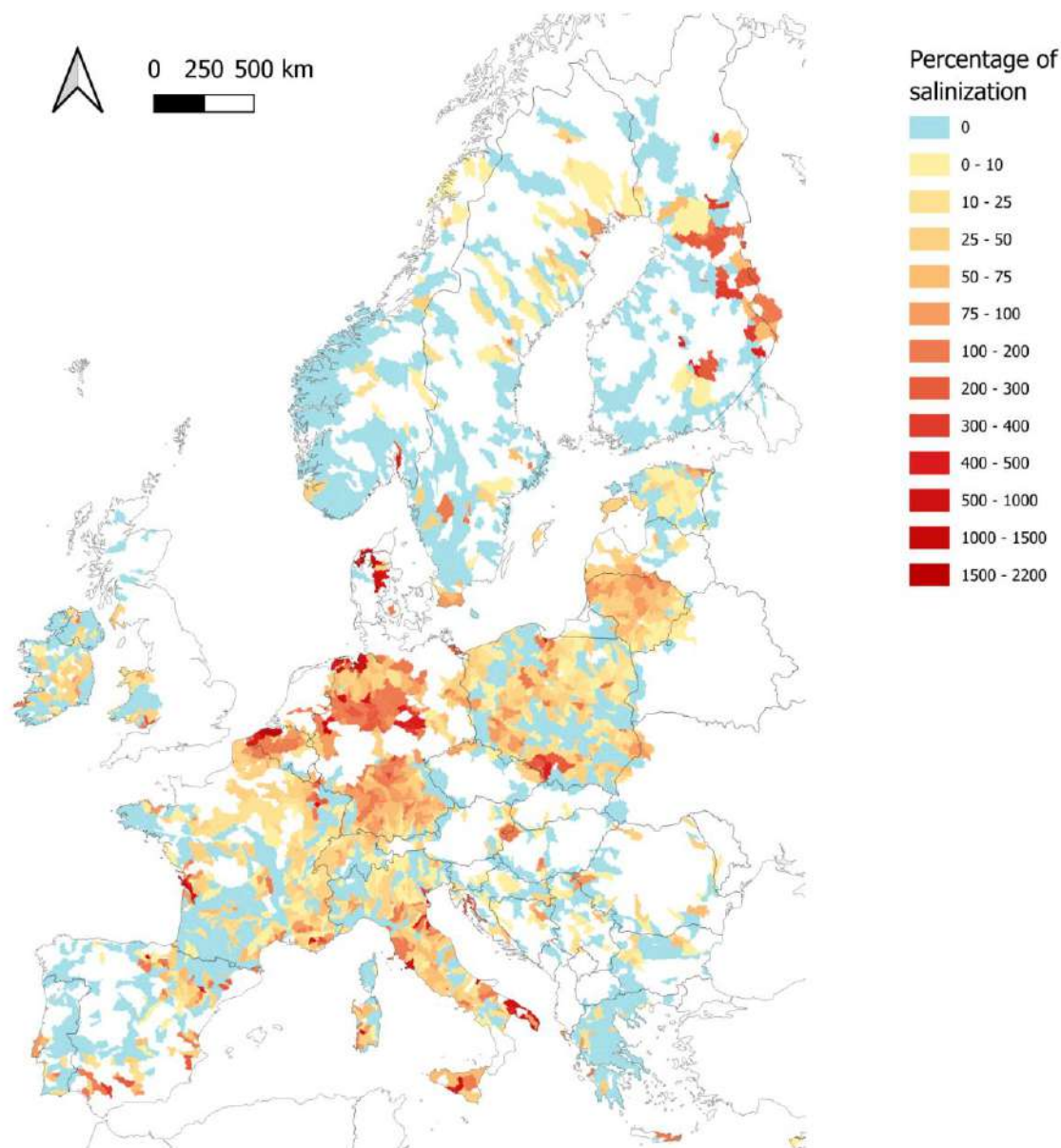
Included in the model	Category	Short_name	Name	Unit	Projection available for future	Resolution	Source	Original source	Citation original source
no	Hydrology	Q-wmin	Week of minimum weekly flow		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	Q-wmax	Week of maximum weekly flow		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-mean	Annual mean water temperature	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	WT-min	Minimum weekly water temperature	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-max	Maximum weekly water temperature	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	WT-wm	Mean water temperature of the wettest month	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-dm	Mean water temperature of the driest month	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-hm	Mean water temperature of the hottest month	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-cm	Mean water temperature of the coldest month	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-wq	Mean water temperature of the wettest quarter	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-dq	Mean water temperature of the driest quarter	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-hq	Mean water temperature of the hottest quarter	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-cq	Mean water temperature of the coldest quarter	°C	Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	WT-ztw	Number of weeks with WT =< 0.5 °C		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
yes	Hydrology	WT-range	Annual water temperature range		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-si	Water temperature seasonality index $\sigma(WT)/WT-mean$		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-wmin	Week of minimum water temperature		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022
no	Hydrology	WT-wmax	Week of maximum water temperature		Yes	5 arcminute	FutureStreams	-	Wanders et al. 2022 Bosmans et al. 2022

Included in the model	Category	Short_name	Name	Unit	Projection available for future	Resolution	Source	Original source	Citation original source
no	Hydrology	predprob1	Predicted probability that river reach ceases to flow for at least one day per year on average somewhere along its length		No	15 arcsecond	GIRES	-	Messenger et al. 2021
yes	Hydrology	predprob30	Predicted probability that river reach ceases to flow for at least 30 days per year on average somewhere along its length		No	15 arcsecond	GIRES	-	Messenger et al. 2021
no	Hydrology	predcat1	Predicted flow intermittence class (based on predprob1)		No	15 arcsecond	GIRES	-	Messenger et al. 2021
no	Hydrology	predcat30	Predicted flow intermittence class (based on predprob30)		No	15 arcsecond	GIRES	-	Messenger et al. 2021
yes	Landcover	impacted_lc	Cover percentage of: crops (irrigated or non-irrigated) and urban areas	%	Yes	5 arcminute	LUCAS LUC	-	Hoffmann et al. 2022
yes	Landcover	natural_lc	Cover percentage of: forest (deciduous trees and temperate broadleaf evergreen trees), shrubs (coniferous and deciduous), C3 grass	%	Yes	5 arcminute	LUCAS LUC	-	Hoffmann et al. 2022
yes	Anthropogenic	pop_ct_csu	Population Count in reach catchment	count (thousands)	No	15 arcsecond	RiverATLAS	GPW v4	CIESIN 2016
no	Anthropogenic	pop_ct_usu	Population Count in total watershed upstream of reach pour point	count (thousands)	No	15 arcsecond	RiverATLAS	GPW v4	CIESIN 2016
no	Anthropogenic	ppd_pk_cav	Population Density in reach catchment	people. km ⁻²	No	15 arcsecond	RiverATLAS	GPW v4	CIESIN 2016
no	Anthropogenic	ppd_pk_uav	Population Density in total watershed upstream of reach pour point	people. km ⁻²	No	15 arcsecond	RiverATLAS	GPW v4	CIESIN 2016
yes	Anthropogenic	hft_ix_c09	Human Footprint in reach catchment in 2009	index value (x100)	No	15 arcsecond	RiverATLAS	Human Footprint v2	Venter et al. 2016
no	Anthropogenic	hft_ix_u09	Human Footprint in total watershed upstream of reach pour point in 2009	index value (x100)	No	15 arcsecond	RiverATLAS	Human Footprint v2	Venter et al. 2016
no	Climate	ari_ix_cav	Global Aridity Index - average in reach catchment	index value (x100)	No	15 arcsecond	RiverATLAS	Global Aridity Index	Zomer et al. 2008
yes	Climate	ari_ix_uav	Global Aridity Index - average in total watershed upstream of reach pour point	index value (x100)	No	15 arcsecond	RiverATLAS	Global Aridity Index	Zomer et al. 2008
no	Climate	cmi_ix_cav	Climate Moisture Index - average in reach catchment	index value (x100)	No	15 arcsecond	RiverATLAS	WorldClim & Global-PET	Hijmans et al. 2005
no	Climate	cmi_ix_uav	Climate Moisture Index - average in total watershed upstream of reach pour point	index value (x100)	No	15 arcsecond	RiverATLAS	WorldClim & Global-PET	Hijmans et al. 2005
no	Climate	snw_pc_cyr	Snow Cover Extent - annual average in reach catchment	%	No	15 arcsecond	RiverATLAS	MODIS/Aqua	Hall & Riggs 2016
no	Climate	snw_pc_uyr	Snow Cover Extent - annual average in total watershed upstream of reach pour point	%	No	15 arcsecond	RiverATLAS	MODIS/Aqua	Hall & Riggs 2016

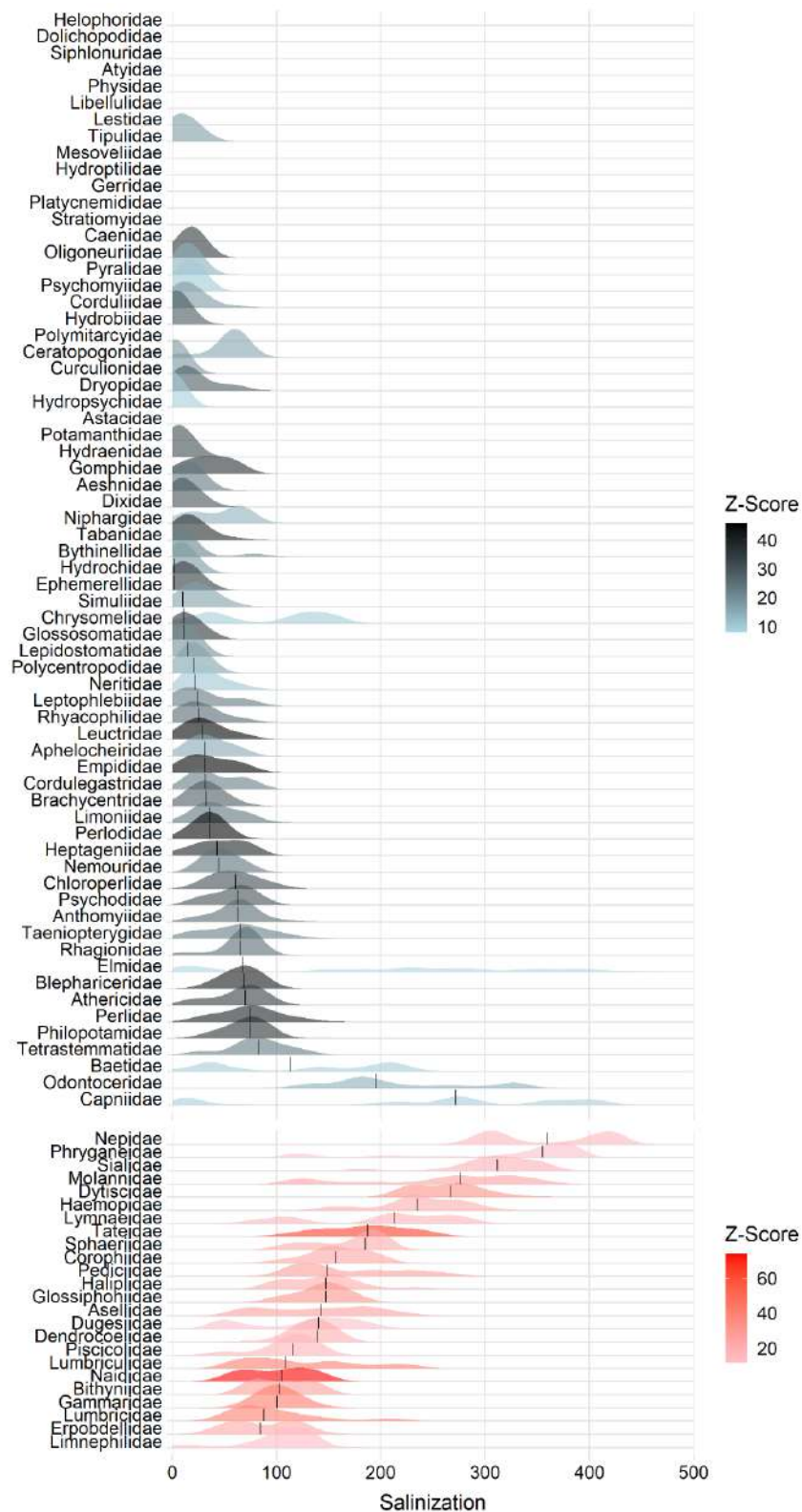
Included in the model	Category	Short_name	Name	Unit	Projection available for future	Resolution	Source	Original source	Citation original source
no	Climate	pet_mm_cyr	Potential Evapotranspiration - annual average in reach catchment	mm	No	15 arcsecond	RiverATLAS	Global-PET	Zomer et al. 2008
no	Climate	pet_mm_uyr	Potential Evapotranspiration - annual average in total watershed upstream of reach pour point	mm	No	15 arcsecond	RiverATLAS	Global-PET	Zomer et al. 2008
yes	Climate	aet_mm_cyr	Actual Evapotranspiration - annual average in reach catchment	mm	No	15 arcsecond	RiverATLAS	Global Soil-Water Balance	Trabucco & Zomer 2010
no	Climate	aet_mm_uyr	Actual Evapotranspiration - annual average in total watershed upstream of reach pour point	mm	No	15 arcsecond	RiverATLAS	Global Soil-Water Balance	Trabucco & Zomer 2010
yes	Hydrology	ria_ha_rsu	River Area along reach segment	ha	No	15 arcsecond	RiverATLAS	HydroSHEDS & WaterGAP	Lehner & Grill 2013
no	Hydrology	ria_ha_usu	River Area in total watershed upstream of reach pour point	ha	No	15 arcsecond	RiverATLAS	HydroSHEDS & WaterGAP	Lehner & Grill 2013
no	Hydrology	riv_tc_rsu	River Volume along reach segment	thousand m ³	No	15 arcsecond	RiverATLAS	HydroSHEDS & WaterGAP	Lehner & Grill 2013
yes	Hydrology	riv_tc_usu	River Volume in total watershed upstream of reach pour point	thousand m ³	No	15 arcsecond	RiverATLAS	HydroSHEDS & WaterGAP	Lehner & Grill 2013
yes	Hydrology	gwt_cm_cav	Groundwater Table Depth	cm	No	15 arcsecond	RiverATLAS	Global Groundwater Map	Fan et al. 2013
no	Landcover	gla_pc_cse	Glacier Extent - spatial extent in reach catchment	%	No	15 arcsecond	RiverATLAS	GLIMS	GLIMS & NSIDC 2012
yes	Landcover	gla_pc_use	Glacier Extent - spatial extent in total watershed upstream of reach pour point	%	No	15 arcsecond	RiverATLAS	GLIMS	GLIMS & NSIDC 2012
no	Physiography	ele_mt_cav	Elevation - average in reach catchment	m a.s.l.	No	15 arcsecond	RiverATLAS	EarthEnv-DEM90	Robinson et al. 2014
yes	Physiography	ele_mt_cmn	Elevation - min in reach catchment	m a.s.l.	No	15 arcsecond	RiverATLAS	EarthEnv-DEM90	Robinson et al. 2014
no	Physiography	ele_mt_cmx	Elevation - max in reach catchment	m a.s.l.	No	15 arcsecond	RiverATLAS	EarthEnv-DEM90	Robinson et al. 2014
no	Physiography	ele_mt_uav	Elevation - average in total watershed upstream of reach pour point	m a.s.l.	No	15 arcsecond	RiverATLAS	EarthEnv-DEM90	Robinson et al. 2014
no	Physiography	slp_dg_cav	Terrain Slope in reach catchment	degrees (x10)	No	15 arcsecond	RiverATLAS	EarthEnv-DEM90	Robinson et al. 2014
no	Physiography	slp_dg_uav	Terrain Slope in total watershed upstream of reach pour point	degrees (x10)	No	15 arcsecond	RiverATLAS	EarthEnv-DEM90	Robinson et al. 2014
no	Soils & Geology	cly_pc_cav	Clay Fraction in Soil in reach catchment	%	No	15 arcsecond	RiverATLAS	SoilGrids1km	Hengl et al. 2014
no	Soils & Geology	cly_pc_uav	Clay Fraction in Soil in total watershed upstream of reach pour point	%	No	15 arcsecond	RiverATLAS	SoilGrids1km	Hengl et al. 2014
yes	Soils & Geology	slt_pc_cav	Silt Fraction in Soil in reach catchment	%	No	15 arcsecond	RiverATLAS	SoilGrids1km	Hengl et al. 2014

Included in the model	Category	Short_name	Name	Unit	Projection available for future	Resolution	Source	Original source	Citation original source
no	Soils & Geology	slt_pc_uav	Silt Fraction in Soil in total watershed upstream of reach pour point	%	No	15 arcsecond	RiverATLAS	SoilGrids1km	Hengl et al. 2014
no	Soils & Geology	snd_pc_cav	Sand Fraction in Soil in reach catchment	%	No	15 arcsecond	RiverATLAS	SoilGrids1km	Hengl et al. 2014
yes	Soils & Geology	snd_pc_uav	Sand Fraction in Soil in total watershed upstream of reach pour point	%	No	15 arcsecond	RiverATLAS	SoilGrids1km	Hengl et al. 2014
yes	Soils & Geology	ero_kh_cav	Soil Erosion in reach catchment	kg.ha ⁻¹ .year ⁻¹	No	15 arcsecond	RiverATLAS	GloSEM v1.2	Borrelli et al. 2017
no	Soils & Geology	ero_kh_uav	Soil Erosion in total watershed upstream of reach pour point	kg.ha ⁻¹ .year ⁻¹	No	15 arcsecond	RiverATLAS	GloSEM v1.2	Borrelli et al. 2017
yes	Soils & Geology	x---	Lithological Classes (sum of upstream catchment areas for each class)	km ²	No	15 arcsecond	BasinATLAS	GLiM	Hartmann & Moosdorf 2012

Annex 2: Percentage of salinization calculated as (observed EC-predicted EC)/predicted EC.



Annex 3: TITAN z+ (positive response to salinization, shown in red) and z- (negative response to salinization, shown in blue) scores, representing the magnitude of a taxon's response to the salinization gradient. Negative salinization scores correspond to sites where the predicted EC was higher than the observed EC (see section 3.2.1).

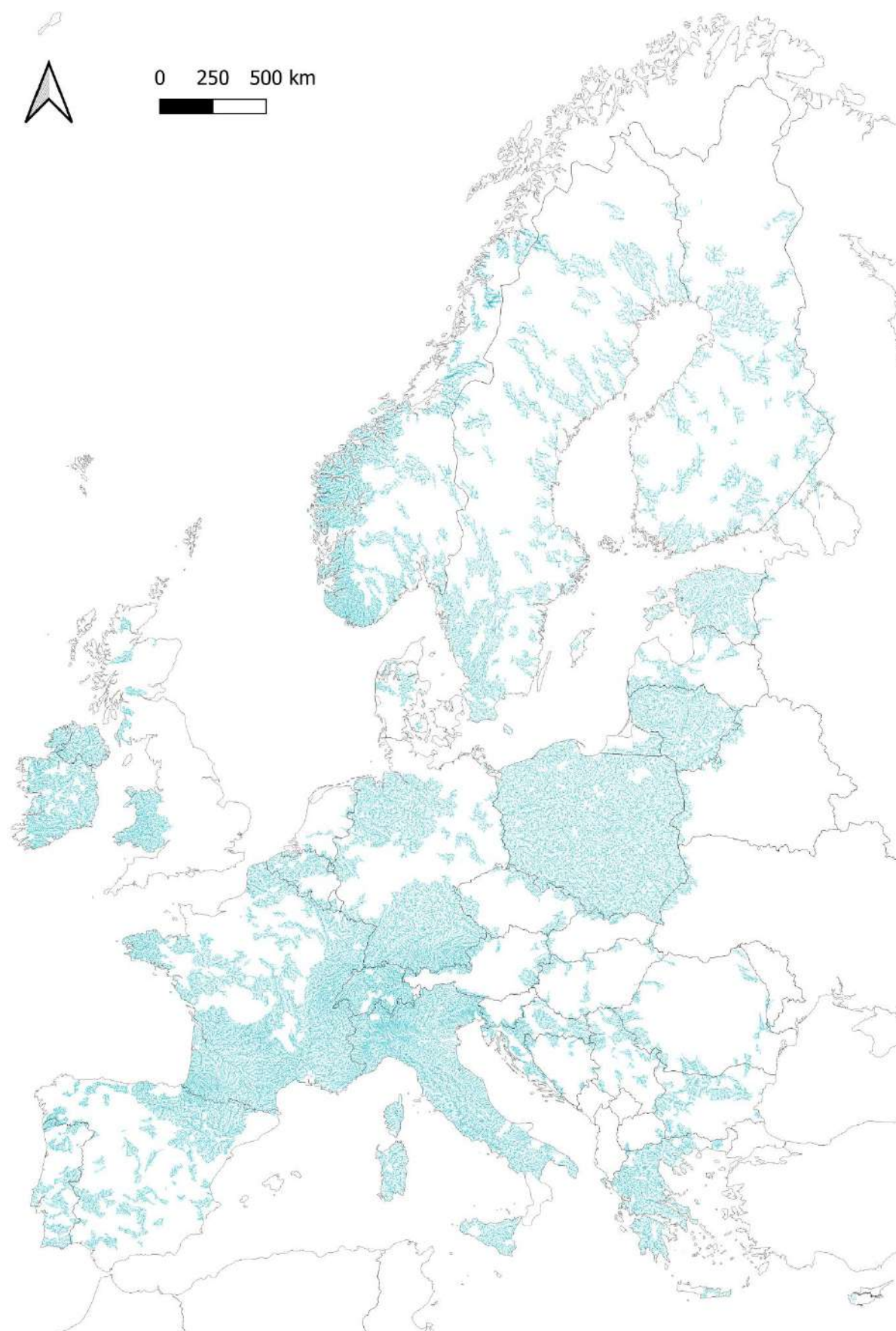


Annex 4: Summary statistics and results of Theil-Sen trend analysis for river Oder for 2010 to 2023.

Parameter	Abbreviation	unit	Slope	Slope %	p.value	Mean	Median	SD	Min	Max
Conductivity	Cond_mS	mS cm ⁻¹	0.004	0.39	≤ 0.001	1.14	1.06	0.36	0.45	2.16
Chloride	Cl	mg l ⁻¹	1.252	0.58	≤ 0.001	217.35	192.50	103.43	56.30	538.00
Sulphate	SO4	mg l ⁻¹	0.038	0.04	0.104	95.53	95.95	15.92	49.00	144.00
Non marine sulphate	NM_SO4	meq l ⁻¹	-0.003	-0.23	≤ 0.001	1.35	1.33	0.24	0.71	2.22
Turbidity	FTU	FTU	-0.019	-0.12	0.053	15.67	13.33	8.84	3.80	53.00
River level	Level_month	cm	-0.561	-0.26	≤ 0.001	216.57	210.50	72.11	91.00	419.00
Phosphate	PO4.P	mg l ⁻¹	0.0001	0.20	0.035	0.03	0.03	0.02	0.00	0.08
Total Phosphorus	TP	mg l ⁻¹	0.000	-0.06	0.150	0.12	0.11	0.04	0.04	0.32
Water Temperature	oC	°C	0.015	0.11	0.154	13.37	12.76	7.41	0.60	27.30
2m air temperature	t2m	Kelvin (K)	0.012	0.00	0.331	282.78	282.56	7.23	266.86	295.23
Potential evaporation	pev	meters (m)	0.000	-0.03	0.646	-0.00213	-0.00176	0.00170	-0.00614	-0.00012
Runoff	ro	meters (m)	0.000	-0.26	0.001	0.00021	0.00018	0.00015	0.00005	0.00094
Surface runoff	sro	meters (m)	0.000	-0.06	0.528	0.00003	0.00002	0.00002	0.00000	0.00016
Surface solar radiation downwards	ssrd	J/m ²	-16	0.00	0.990	10923963	10706818	6694441	1584388	23407063
Total cloud cover	tcc	Proportion (0 to 1)	0.000	0.00	0.888	0.66	0.66	0.12	0.34	0.91
Total precipitation	tp_sum	meters (m)	0.000	0.05	0.425	0.06	0.05	0.03	0.00	0.17

Standardized Precipitation Evapotranspiration Index	SPEI	Index	-0.006	-0.74	≤ 0.001	-0.82	-0.88	0.88	-2.97	1.73
Standardized Precipitation Index	SPI	Index	0.000	-0.93	0.977	0.01	0.01	1.06	-2.70	3.44
Climatic Water Balance	BAL	mm	-0.042	-0.07	0.376	-59.09	-47.14	49.53	-157.24	0.08
Potential evapotranspiration	PET	mm	0.043	0.07	0.386	59.16	47.21	49.54	0.00	157.28

Annex 5: river network considered in the salinization model.



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