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RESEARCH PAPER

The endangered spirlin (*Alburnoides bipunctatus*) as an indicator of biotic integrity

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Abstract. Previous research has demonstrated the value of the spirlin (*Alburnoides bipunctatus*), a small freshwater fish, as an indicator species for monitoring the ecological health of European rivers. In this study, we examine the temporal occurrence of spirlin in a lowland section of the River Drzewiczka, Poland, at the centre of the European distribution of the species. Using a mixed modelling approach, we show a significant interaction of freshwater fish species richness and time period on the probability of occurrence of spirlin across multiple microhabitats, with a strong positive relationship between the probability of occurrence and species richness at the end of the 20th Century, but an erosion in this relationship two decades later. This change in the predicted relationship of spirlin occurrence with freshwater fish species richness corresponds with a temporal decline in the water level of the River Drzewiczka. We discuss these findings in the context of the spirlin as an indicator species of the biotic integrity of European river environments.

Key words: Bernoulli model, conservation, fish, hydroecology, indicator species

Introduction

Freshwater habitats, including rivers, lakes, wetlands, and streams, face numerous threats that jeopardise their ecological integrity, biodiversity, and the ecosystem services they provide to human and natural systems. These threats arise from multiple sources, including the introduction of man-made pollutants, habitat destruction and modification, resource overexploitation, land use, and the introduction of alien species, as well as natural processes, such as long-term cycles in precipitation and temperature (Reid et al. 2019, Przybylski et al. 2020).

Indicator species are valuable tools for assessing the biotic integrity of ecosystems. They reflect the health of their environment because they are sensitive to specific ecological changes. By monitoring their presence and abundance, it is possible to readily infer the overall condition of a given habitat. Thus, indicator species can potentially provide signals of ecological degradation, enabling timely conservation interventions (Fausch et al. 1990, Schiemer 2000, Lasne et al. 2007).

The spirlin *Alburnoides bipunctatus* (Bloch, 1972) is a freshwater fish species that has potential as an indicator of environmental integrity due to its wide distribution across Europe and parts of Central Asia, though recent decades have seen a significant decline in the distribution and abundance of this riverine cyprinid (Marszał et al. 2018). Despite its threatened status, the spirlin has not been subject to commercial exploitation, stocking, or any specific management measures and is typically associated with fluvial habitats that have experienced minimal disturbance (Copp et al. 2010).

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Spirlin populations are typically found in the middle course of unpolluted and unmodified streams and rivers, within the barbel and grayling zones, characterised by a significant gradient, well-oxygenated water, gravel or rocky substrates, and limited aquatic vegetation (Treer et al. 2006, Jakovljević et al. 2023). The species is considered vulnerable or endangered in several European countries, including Poland, Slovakia, the Czech Republic, Austria, and Hungary (Schiemer et al. 2004, Kováč et al. 2006, Erős 2007, Jurajda et al. 2007, Witkowski et al. 2009), highlighting the need for conservation efforts (Marszał et al. 2018). The recent decline of the spirlin is primarily attributed to anthropogenic influences, such as pollutants leading to eutrophication, siltation of spawning sites, and river flow alteration through engineering projects (Kruk 2007). Other threats to the spirlin include the impacts of invasive fish species and the introduction of salmonids (Aarts et al. 2004).

The goal of this study was to model the presence of spirlin in the River Drzewiczka to test the hypothesis of Marszał & Smith (2024) that it can serve as an indicator species for the monitoring and maintenance of the ecological health of river habitats across its range in Poland. We predicted that spirlin would be associated with habitat variables, including freshwater fish species diversity, that indicated high ecological integrity, supporting its proposed status as an indicator species.

Material and Methods

The River Drzewiczka is an 81.3 km long tributary of the River Pilica (River Vistula basin) with a total catchment area of 1,089.9 km^2. The study was carried out on a 210 m long lowland section (third-order river stretch with a bed slope of 0.765 ‰) located in the town of Drzewica (51°27′1.09′′ N, 20°29′12.10′′ E), approximately 2 km downstream from Lake Drzewieckie, an artificial reservoir that was dammed between 1932-1936. Throughout the study period, discharge varied from 2.1 to 12.0 m^3 s^{-1} (Dukowska et al. 2007). Fish sampling was undertaken in autumn, winter, spring, and summer from 1998 to 1999 and from 2019 to 2020 in every month (except for December 1998 and June 1999), yielding a total sample size of 90 fish surveys for the first sampling period from 1998 to 1999 and 108 surveys for the period from 2019 to 2020. Each month, sampling occurred at the same nine sites, each selected to capture the full range of available riverine microhabitats (Table S1). Eight sites were located along the left river bank, and the ninth site was in the middle part of the river bed. Fish were sampled by electrofishing during ten excursions for a total of 90-point abundance samples (adapted from Persat & Copp 1989), which were collected in a downstream-to-upstream direction during daytime (10:00-17:00) using an AC electroshocker (IUP-12, Radet, Poznań, Poland) with an effective range of approximately 1.5 m^2. At each sampling point, all stunned fish were collected in a dipnet, identified, measured for total length (TL), and returned to the water unharmed. In addition, at each sampling point, the following environmental parameters were measured: water depth (cm), water velocity (cm s^{-1}, taken 10 cm above the substrate), and proportion of substrate covered by gravel (%). An unpaired t-test was performed to compare the mean proportion of

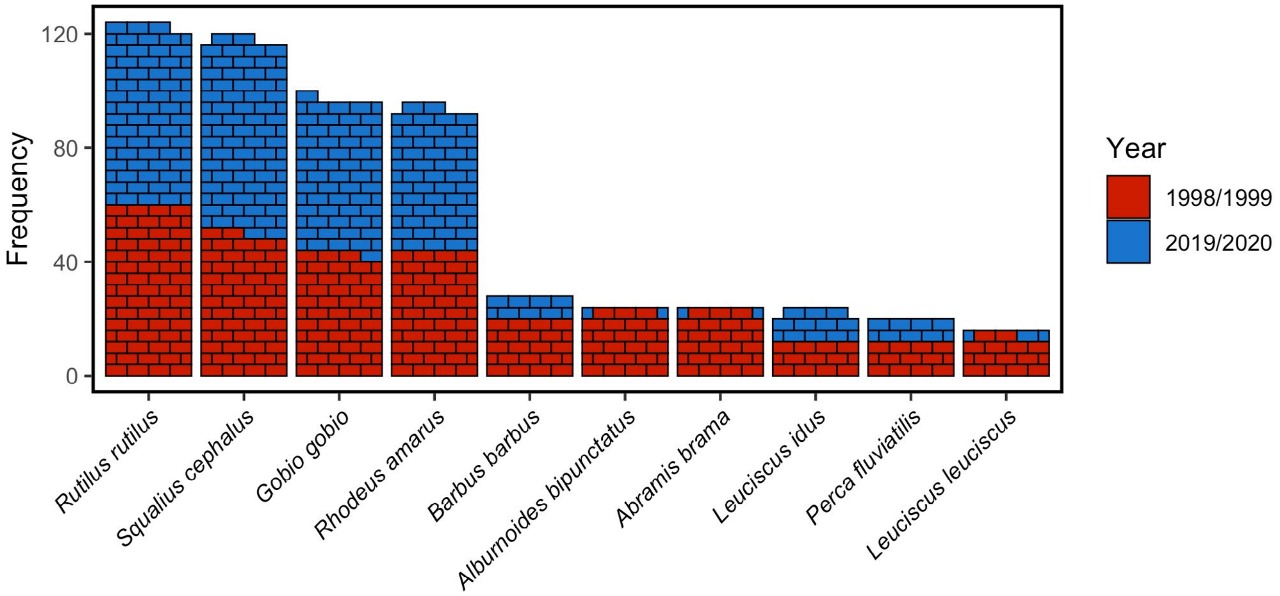

Fig. 1. Frequency plot of the ten most common fish species captured in the River Drzewiczka in two sampling periods.

Table 1. Parameters of the final Bernoulli GLMM fitted to data on the presence of spirlin in the River Drzewiczka showing log-odds estimate and 95% confidence intervals (95% CI). σ^2 is the mean random effect variance for the model; $\tau_{00\ habitat}$ is the model between-subject variance, indicating how much different levels of the random term 'habitat' differ from each other; $\tau_{00\ season}$ is the model between-subject variance for 'season'; ICC is the intra-class correlation coefficient, which is a measure of the degree of correlation within groups; $N_{habitat}$ indicates the number of levels in the random effect 'habitat' and N_{season} indicates the number of levels in the random effect 'season'.

Coefficient	Log-Odds	95% CI	*P*-value
Fixed Effects			
Intercept$_{(98/99)}$	–2.10	–5.16-0.96	0.178
Year$_{(19/20)}$	–4.38	–6.69-–2.06	< 0.001
Fish species diversity	0.53	0.14-0.92	0.008
Water velocity	0.67	–2.29-3.62	0.658
Water depth	–0.04	–0.09-0.02	0.177
Random Effects			
σ^2	3.29		
$\tau_{00\ habitat}$	1.69		
$\tau_{00\ season}$	1.45		
ICC	0.49		
$N_{habitat}$	9		
N_{season}	4		
Observations	198		
Marginal R^2/Conditional R^2	0.453/0.720		

gravel substrate, a critical spawning substrate for spirlin, between the two sampling periods.

The presence and absence of spirlin in samples was modelled using a Bernoulli GLMM with logit link function, with the presence of spirlin as the response variable, freshwater fish species diversity (measured as species richness), year of sampling, water velocity and depth as fixed terms, and microhabitat type and season (autumn, winter, spring, summer) as random terms. An interaction between species diversity and the year of sampling was also included in the model as a fixed term to examine whether the predicted relationship between the presence of spirlin and species diversity changed between sampling periods. The model was implemented with the *glmmTMB* package (ver. 1.1.9) (Brooks et al. 2017) in R version 4.4.0 (R Development Core Team 2024). Data exploration was conducted following a six-step protocol (Smith & Warren 2023), and residual diagnostics were conducted using the *DHARMa* package (Hartig 2022). Model selection was not undertaken, with the exception of dropping non-significant interaction terms.

Hydrological and meteorological data were obtained from the archives of the Polish Institute of Meteorology and Water Management (Instytut Meteorologii i Gospodarki Wodnej; https://meteo.imgw.pl). A nonparametric test for a monotonic trend in time series data for the maximum air temperature and water level at the sampling site was conducted using Kendall's trend test with the *EnvStats* package (ver. 2.8.1) (Millard & Kowarik 2023).

Results

A total of 3,371 fish and lampreys were caught in both sampling periods in 1998-1999 and 2019-2020 belonging to 16 species: common bleak *Alburnus alburnus*, spirlin, stone loach *Barbatula barbatula*, white bream *Blicca bjoerkna*, spined loach *Cobitis taenia*, northern pike *Esox lucius*, Ukrainian brook lamprey *Eudontomyzon mariae*, three-spined stickleback *Gasterosteus aculeatus*, gudgeon *Gobio gobio*, ide *Leuciscus idus*, common dace *Leuciscus leuciscus*, weatherfish *Misgurnus fossilis*, European perch *Perca fluviatilis*, European bitterling *Rhodeus amarus*, roach *Rutilus rutilus* and chub *Squalius cephalus* (Fig. 1). Spirlin were captured in all except two microhabitats (Table S2). Invasive species of goby (*Neogobius fluviatilis*, *Babka gymnotrachelus*) and topmouth gudgeon (*Pseudorasbora parva*) are present in the River Pilica, of which the River Drzewiczka is a tributary, though none were encountered in surveys.

A preliminary data exploration showed that habitat substrate was collinear with water velocity and

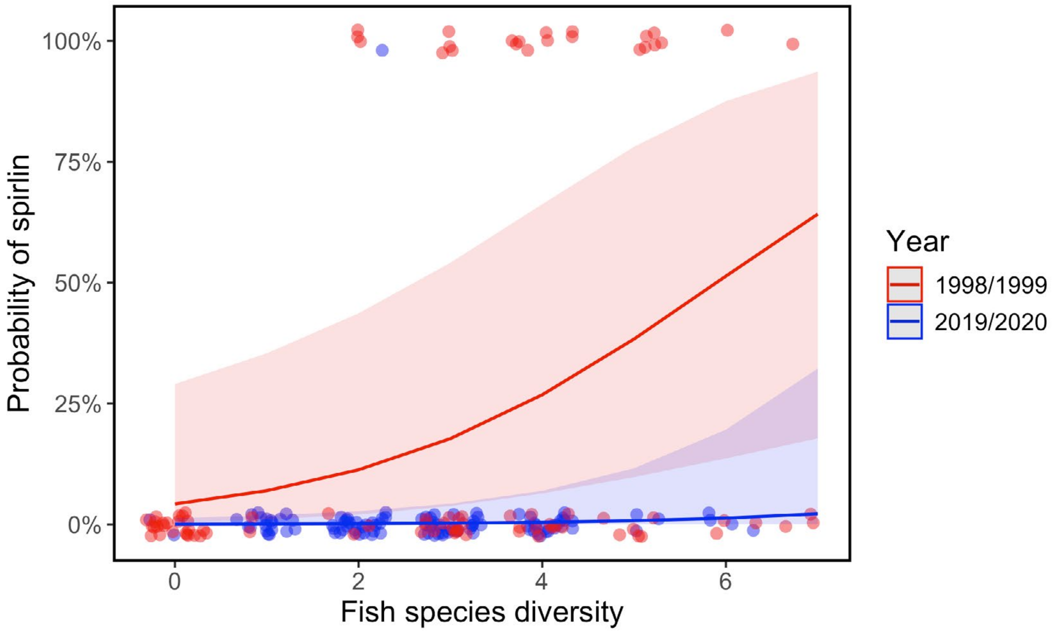

Fig. 2. Mean fitted probability of the occurrence of spirlin as a function of fish species diversity in the River Drzewiczka in two sampling periods modelled with a Bernoulli GLMM. Points are observed data. Shaded areas are 95% confidence intervals.

microhabitat, so it was subsequently dropped from the analysis to avoid variance inflation. The proportion of gravel substrate at sampling sites differed significantly between sampling years (t-test, t_{196} = 4.91, $P < 0.001$); the mean proportion in 1998-1999 was 16.0% (SE = 0.61) and 10.4% (SE = 0.90) in 2019-2020.

In the Bernoulli GLMM, the interaction term between species diversity and the year of sampling included in the initial model did not improve model fit and was subsequently dropped from the analysis. The final fitted model showed a positive association of spirlin with fish species richness ($P = 0.008$) and a significant effect of the period of sampling ($P < 0.001$), with a substantially greater probability of spirlin in 1998-1999 (215 specimens) than in 2019-2020 (two specimens) (Fig. 2, Table 1, Table S2). Water velocity showed a positive relationship with the occurrence of spirlin ($P = 0.658$) and water depth a negative relationship ($P = 0.177$), though the slope of neither relationship was statistically significant (Fig. 3A, B, Table 1). Model residual diagnostics showed no departure from normality ($P = 0.661$), no overdispersion ($P = 0.888$) and no outliers ($P = 0.999$). Plots of standardised residuals were used against model predictions, and each of the variables in the model showed no significant patterns. Fixed effects in the model explained approximately 45% of the variance in the presence of spirlin among samples, while random effects explained approximately 27% of the variance (Table 1).

There was a non-significant positive trend in maximum air temperature at the sampling site between 1997 and 2020 (Kendall's trend test, t = 0.051, $Z = 1.29$, $P = 0.196$, Fig. 4A) and a significant negative trend in water level (t = –0.292, $Z = -7.38$, $P < 0.001$, Fig. 4B).

Discussion

In this study, we used a fine (microhabitat) spatial scale to evaluate the response of a spirlin population to specific stresses by comparing its abundance over a two-decade period. As lotic ecosystems can be viewed as mosaics of habitat patches, sampling habitats that individually vary in structure and function can more efficiently capture heterogeneity than the transect approach, which emphasises the dominant habitat (Hitchman et al. 2018). In a previous larger-scale study, the presence of dams above a sampling reach, as well as reduced water depth, were identified as stressors potentially responsible for a substantial decrease in the abundance of the spirlin population and also showed a positive association between the number of other fish taxa and the presence of spirlin (Marszał & Smith 2024). In the present study, we similarly demonstrated a significant association of fish species diversity as a predictor of the presence of spirlin, as well as a significant temporal decline in their probability of occurrence (Fig. 2, Table 1). We failed to detect a significant effect of water velocity or depth on the presence of spirlin, at least after

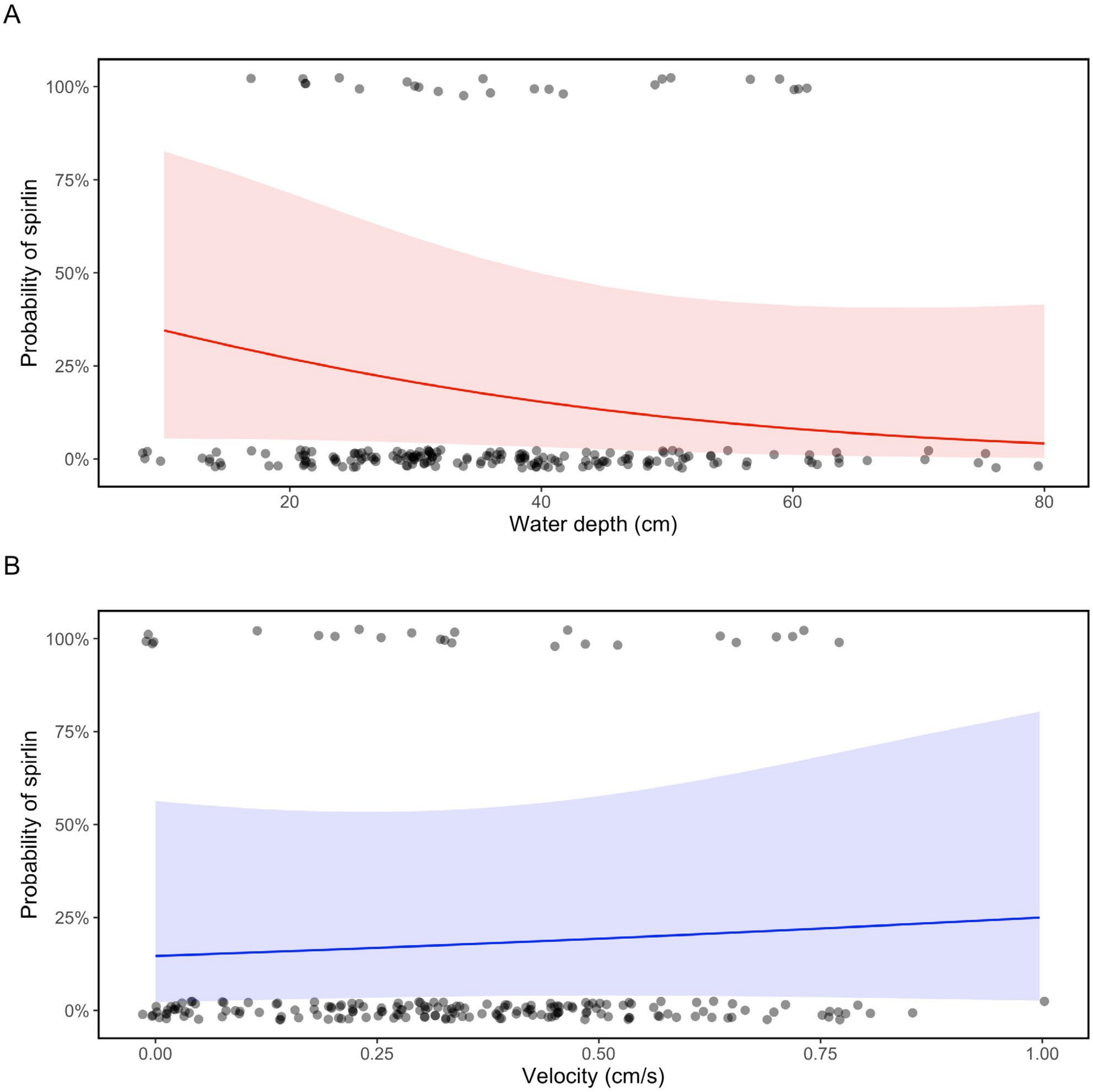

Fig. 3. Mean fitted probability of the occurrence of spirlin as a function of: A – water depth, B – water velocity in the River Drzewiczka modelled with a Bernoulli GLMM. Points are observed data. Shaded areas are 95% confidence intervals.

controlling for microhabitat and season effects in the data (Table 1).

The pooled proportion of spirlin in the local fish community between the two sampling periods declined precipitously from 11% to 0.1%. Changes that occurred to the river during this period included a phased dredging of the Drzewiczka dam between 2002-2013. During this time, the dam reservoir was emptied and dredged, with the River Drzewiczka returning to its natural discharge level for several years before the reservoir was refilled. This disruption to flow was a potential contributor to the substantial decline of spirlin over the course of the study.

Schiemer (2000) underlined the early life history phase of rheophilic fishes as critical due to their narrow requirements regarding current velocity, temperature, and food availability. In the surveyed section of the River Drzewiczka, there have been no apparent changes in riverine inshore structures that could result in the loss of nursery habitats. On the other hand, we found, on average, a highly significant decline in the proportion of the gravel fraction of the substrate, which would indicate less availability of spawning habitats. This type of change most likely occurs as a result of fluctuating water levels, which causes a change in the position of microhabitats in the riverbed (Schiemer 2000). Notably, there was a significant

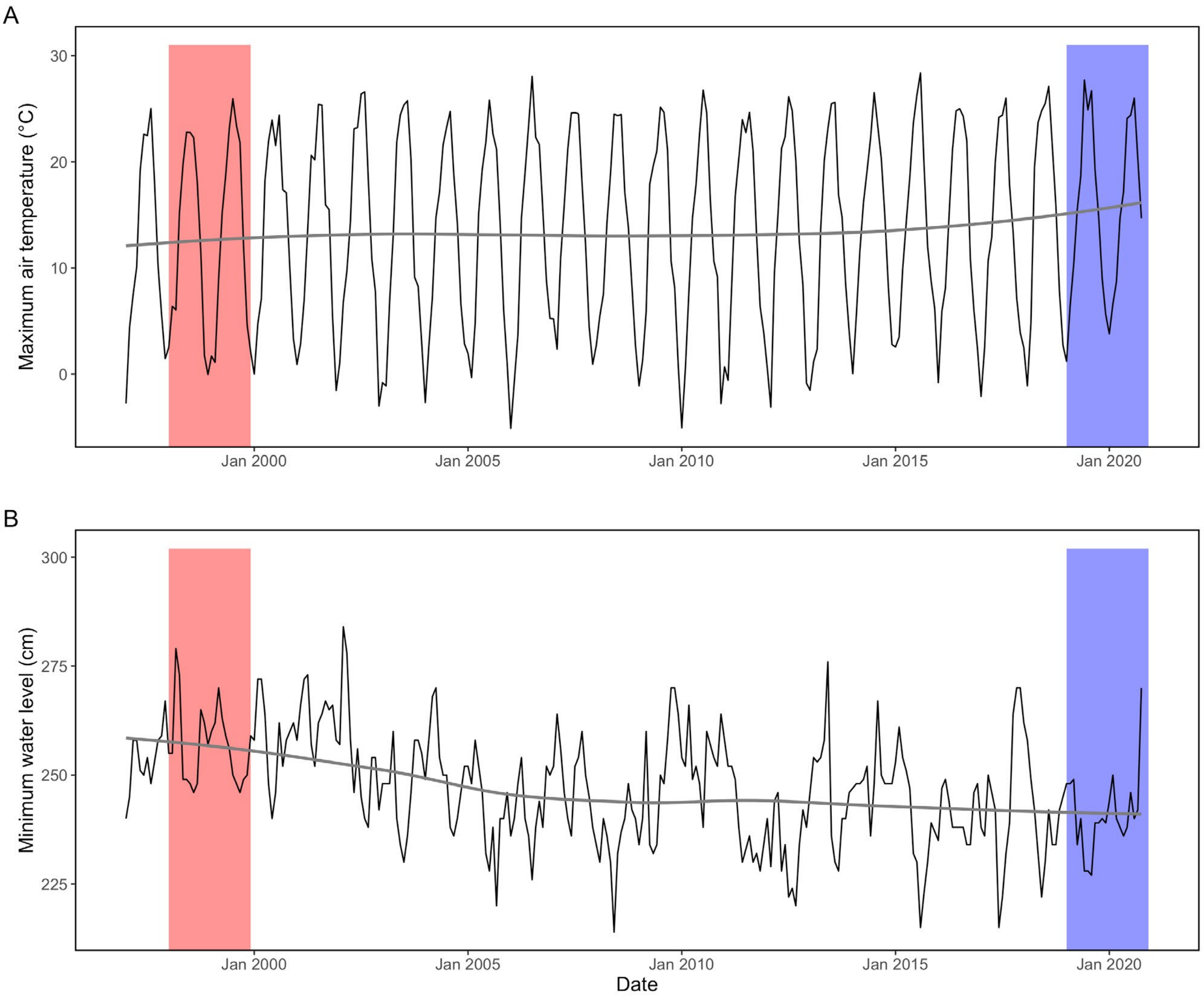

Fig. 4. Temporal trend of: A – mean air temperature (°C), B – water level (cm) at the sampling site on the River Drzewiczka between 1997 and 2020. Red shaded areas indicate the sampling period in 1998-1999 and blue shaded areas in 2019-2020.

decline in water level over the study period (Fig. 4B), which may have resulted in the exposure of otherwise suitable spawning substrate, possibly arising from the dredging of the Drzewiczka dam. Jellyman et al. (2013) highlighted the importance of bed movement due to flow disturbance. Bed-movement measures appeared to better predict spatial and temporal variability in fish biomass and community structure than hydrological measures such as flow variability. Minor barriers, including low-head dams, weirs, culverts, bridges, and road crossings, are frequently linked to local alterations in river physical morphology. These modifications mainly result in the homogenisation of microhabitat features, such as current velocity, water depth, and substrate composition (Santucci et al. 2005, Alexandre & Almeida 2010).

Many freshwater ecosystems are under severe pressure from interacting threats, resulting in their alteration and a loss of aquatic biodiversity (Craig et al. 2016). Research has been focused on conserving these habitats from progressive degradation and creating reliable protocols for managers and relevant authorities to aid in habitat management. To this end, increasingly sophisticated tools for assessing the existing state of freshwaters are being proposed, and the search for the causes that most negatively affect their functioning is an ongoing challenge. Among the many threats identified for flowing waters (Reid et al. 2019), the most frequently highlighted aspect is damming as a leading cause of fluvial habitat degradation (e.g. Dudgeon et al. 2006, Liermann et al. 2012, Birnie-Gauvin et al. 2017). River engineering schemes that disrupt natural flow and thermal regimes and result in habitat fragmentation can also entail additional consequences, such as decreased river-floodplain connectivity, modified aquatic productivity and limited fish access to spawning and nursery habitats (Aarts et al. 2004, Poff & Zimmerman 2010, Fuller et al. 2015). The last decade has also seen increased research concerning the adverse effects of climate change on river ecosystems, especially in the context that many systems are already exposed to numerous human-induced pressures (Craig et al. 2016, Reid et al. 2019). Freshwater ecosystems can be considered integrators of local catchment and regional processes, highly sensitive to the net effects of multiple stressors (Jackson et al. 2015, Reid et al. 2019). An increase in temperatures globally inevitably results in elevated water temperatures but also altered discharge rates, water levels and interactions between these and other stressors. Climate change has been predicted to place at risk around 50% of freshwater fish species worldwide and may intensify several emerging concerns, including the spread of invasive species, pathogens, eutrophication, and increased salinity levels (Darwall & Freyhof 2015).

Fish community structure (i.e. individual species patterns, flow preference guilds, species richness) is primarily determined by environmental variables like slope, temperature and depth, and on a local scale by damming and pollution (Matthews 1998, Pont et al. 2006, Lasne et al. 2007). The biological consequences of altered flow regimes encompass the ongoing substitution of sensitive taxa with species that possess the resilience to tolerate changed conditions or species with adaptations necessary to thrive in reduced flow conditions (Santucci et al. 2005). The substitution of rheophils by eurytopic fish species has already been observed in modified Polish rivers (Penczak & Kruk 2000, Záhorská et al. 2024).

The utility of rheophilic spirlin as an indicator species of the health of river systems was previously examined by analysing predictors of its distribution in a large-scale spatiotemporal study (Marszał & Smith 2024). It was shown that river obstacles affected fish community structure and were associated with the absence of spirlin (Marszał & Smith 2024). Similarly, the negative impact of damming on the young-of-the-year fish community structure was observed by Musil et al. (2012) and manifested itself in the loss of rheophilic species. This effect is typically evident in migratory species initially, which tend to go extinct (e.g. Aarts et al. 2004, Costa et al. 2021). As the negative impact of obstacles increases with their increasing number and decreasing distance between them (Musil et al. 2012), the survival of small-bodied, more stationary rheophils depends on the existence of sufficiently long sections of river between obstacles. Having all freshwater life stages linked to the lotic environment, rheophilic fish species are particularly useful indicators of riverine environment health. Many integrity indices (e.g. Index of Biotic Integrity, IBI: Karr 1981) use the domination of functional guilds in fish communities, considering the link between fish species and specific habitat requirements (Oberdorff et al. 2002, Pont et al. 2006). Spirlin expresses limited migratory behaviour (Aarts et al. 2004), and the species can complete its life cycle within river sections comprising spawning and nursery habitats, similar to unspecialised eurytopic species. For this reason, it is an effective bioindicator in rivers that are already altered by dams since it can indicate further degradation of the river environment in rivers from which large-bodied diadromous and potamodromous species have already been eliminated. In the present study, flow disruption during the dredging of the Drzewiczka dam appears to have resulted in a catastrophic decline in the spirlin population, likely indicating wider ecological damage.

An additional advantage of choosing a specific indicator taxon is that it is useful when only qualitative presence-absence data are available for fish species, and it can also be easily applied with semi-quantitative data (such as relative abundance). In addition, this approach is conceptually simple, requiring no complex theory (Fausch et al. 1990). The main disadvantage of using this method is that it does not accurately assess a species' sensitivity to a particular source of degradation. This sensitivity can fluctuate due to additional influencing factors, and the species may not effectively reflect the relative severity of the degradation experienced (Fausch et al. 1990).

The protection, preservation, restoration, and management of riverine ecosystems requires a comprehensive understanding of the ecological requirements of key indicator species. This understanding is crucial for accurately assessing the effects of habitat modifications at a local scale (Jungwirth et al. 2000). In the case of spirlin, we have information from a large-scale and long-term study (Marszał & Smith 2024) that its presence is adversely influenced by flow disturbance and that its presence is more likely in reaches where there is a high fish species richness, which is itself an indicator of habitat quality. When we have historical data on the distribution of the spirlin, its disappearance or a significant decline in its populations should be a warning for prompt action to determine which environmental factors should be improved and serving as an indication of how river restoration efforts should proceed. Due to the various pressures affecting freshwater ecosystems, it is crucial that conservation tools and mitigation strategies address all co-occurring stressors to preserve these environments effectively (Reid et al. 2019).

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Author Contributions

M. Przybylski conceived the manuscript idea, M. Przybylski and L. Marszał designed the sampling plan, M. Przybylski analysed the data, and M. Przybylski and L. Marszał wrote the manuscript. All applicable international, national and institutional guidelines for the care and use of animals were followed.

Data Availability Statement

The data and RScript supporting this study's findings are available in the FigShare Digital Repository: https://doi.org/10.6084/m9.figshare.27210690.v1.

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Supplementary online material

Table S1. Main hydro morphological and substrate composition variables of the studied microhabitats (H1-H9). Sampling periods: I – 1998-1999, II – 2019-2020. Mean values ± SD (min-max). Mean values of depth and velocity were calculated from data collected each sampling month on two sampling occasions (I – 10 months, II – 12 months).

Table S2. Number of spirlin individuals caught during the survey.

(https://www.ivb.cz/wp-content/uploads/JVB-vol.-73-2024-Marszal-L.-PrzybylskiM.-Table-S1-S2.pdf)