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Patterns and drivers of native, non-native, and at-risk freshwater fish richness in Canada

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1 **Patterns and drivers of native, non-native, and at-risk freshwater fish richness in Canada**

2 M.U. Mohamed Anas*† and Nicholas E. Mandrak

3 Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail,
4 Toronto, ON M1C 1A4 Canada

5 *Corresponding author. Email: anas.usoof@gmail.com

6 †Present address: Dept. of Biological Sceinces, University of Alberta, Edmonton AB T6G 2E9
7 Canada

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Abstract

Understanding the relative roles of the historical, environmental, and anthropogenic processes underlying spatial biodiversity patterns is crucial to predict the impacts of global environmental changes. We quantified the relative roles of these factors in influencing species richness of total, native, non-native, and at-risk freshwater fishes in 985 tertiary watersheds across Canada, while accounting for correlations among descriptors and spatial autocorrelation. Our findings indicate differences in factors influencing richness patterns among species categories. Environmental factors related to energy availability and historical factors related to post-glacial recolonization both played roles in shaping spatial variation in native species richness. In contrast, variation in non-native species richness was largely related to human activities increasing propagule pressure and habitat disturbance, which were greater for foreign species (i.e. not native to Canada) than for translocated native species. Anthropogenic processes and environmental conditions were both important determinants of at-risk species richness. Our study emphasizes the importance of an integrated approach that simultaneously considers natural and anthropogenic processes to better predict the fish biodiversity change at the landscape scale.

Keywords: freshwater fishes, species richness, threatened species, invasive species, climatic factors

42 Introduction

43 Understanding the processes underlying the spatial variation in biodiversity is a central
44 pursuit in macroecology (Ricklefs 2004). Historical, environmental, and anthropogenic processes
45 are known to influence the taxonomic diversity at broad spatial scales (Kerr et al. 2007; Heino
46 2011). Broad-scale environmental constraints, such as environmental energy availability and
47 habitat availability/heterogeneity, may lead to niche differentiation among species, facilitating
48 species turnover and speciation (Hawkins et al. 2003; Field et al. 2009; Heino 2011). In addition,
49 historical factors, such as Quaternary climate oscillations, glaciations and subsequent
50 recolonization, and headwater captures, can influence current biodiversity patterns (Reyjol et al.
51 2007; Griffiths 2010; Heino 2011; Leprieur et al. 2011; Oberdorff et al. 2011). Anthropogenic
52 pressures may impact biodiversity patterns through habitat destruction, overexploitation, and
53 non-native species introductions (McKinney and Lockwood 1999; Mack et al. 2000; McKinney
54 2002). An improved understanding of the relative influences of these processes on biodiversity
55 patterns is crucial for predicting the impacts of global environmental changes, which can help
56 effectively direct conservation resources to areas and aspects most vulnerable (Kerr et al. 2007).

57 Unambiguously estimating the relative roles of different processes underlying
58 biodiversity patterns at broad scales is challenging due to collinearity among explanatory
59 variables and/or spatial dependencies among observations. For example, the distribution of
60 human populations is often influenced by favorable environmental conditions associated with
61 climatic factors, such as suitability for agriculture and other economic activities, land cover, and
62 access to natural resources (Gaston 2005; Luck 2007). Meanwhile, collinearity may exist
63 between historical and present climates (Ricklefs 2004; Field et al. 2009; Griffiths et al. 2014). In
64 addition, spatial autocorrelation (i.e. lack of independences between observations) is pervasive in

species distribution data with broad geographic extents (Diniz-Filho and Bini 2005). This can be the result of spatially contagious biotic processes (such as dispersal, population growth, differential mortality, and species interactions), underlying exogenous processes (e.g. environmental, historical, anthropogenic) that are themselves spatially structured, and/or simply from data-collection methods (e.g. richness data often derived from overlapping species ranges and when ranges are larger than the size of sampling unit, adjacent units likely have similar species composition/richness) (Diniz-Filho and Bini 2005; Griffith and Peres-Neto 2006). Presence of spatial autocorrelation violates the assumption of stochastic independence among observations in most classical statistical models and, thus, may affect the statistical inference due to inflated type I error rate (Diniz-Filho and Bini 2005; Griffith and Peres-Neto 2006; Peres-Neto and Legendre 2010). Therefore, the roles of historical, environmental, and anthropogenic factors in influencing biodiversity should be simultaneously assessed in an integrated context, while accounting for their multicollinearity and spatial autocorrelation (Marini et al. 2009; Walther et al. 2009; Pyšek et al. 2010; Brucet et al. 2013).

Conservation planning is often focused on overall species diversity, assuming that it is an appropriate proxy for other dimensions of biodiversity (Orme et al. 2005; Howard et al. 2020). However, native, non-native, and at-risk species diversity may not be spatially congruent as they are likely regulated by different mechanisms (Orme et al. 2005). For example, distributions of native species may be at least partially constrained by historical processes (Heino 2011; Leprieur et al. 2011; Oberdorff et al. 2011) in addition to environmental and anthropogenic processes (McKinney 2002; Hawkins et al. 2003). In contrast, historical factors are unlikely to constrain the distributions of non-native species (Stohlgren et al. 2006; Leprieur et al. 2009), rather anthropogenic introduction pathways (Lockwood et al. 2005; Leprieur et al. 2008; Dawson

et al. 2017) and environmental conditions influencing the post-introduction establishment success (Stohlgren et al. 2006; Blanchet et al. 2009; Carboni et al. 2010; Gallardo et al. 2015) should play important roles. Of non-native species, establishment of foreign species (i.e. those introduced from other countries) may be more constrained by environmental conditions compared to translocated species (i.e. native to the country, but introduced beyond their historical distribution) (Bobeldyk et al. 2015; Rahel and Smith 2018; Anas and Mandrak 2021). The relative importance of different introduction pathways (e.g. stocking for hunting, sport fishing and biocontrol, release/escape from the pet and live food trade) may also vary between the two non-native species categories (Strecker et al. 2011; Bobeldyk et al. 2015; Carpio et al. 2017). Meanwhile, spatial variation in the number of at-risk species (i.e. species vulnerable to extinction) can be associated with both environmental conditions that predispose species to rarity and anthropogenic pressures responsible for reducing their natural populations to threatened levels (Kerr and Currie 1995; Davies et al. 2006; Howard et al. 2020). Therefore, identifying where high densities of native, non-native, and at-risk species occur, and which processes regulate their distributions, can help conservation practitioners to develop strategies targeting the different aspects of biodiversity impacts (Marini et al. 2009; Howard et al. 2019).

Biodiversity in freshwater ecosystems is more endangered by human-induced environmental changes at a global scale than terrestrial or marine systems as a result of spatial co-occurrence of disproportionately high species richness (primarily fish species) and multiple human pressures (Dudgeon et al. 2006; Chu et al. 2014; Reid et al. 2019). Yet, from a conservation perspective, freshwater fish biodiversity is poorly studied relative to terrestrial biodiversity (Reid et al. 2019). Macroecological studies of freshwater fish richness have detected divergent patterns for the relative effects of environmental, historical, and anthropogenic factors

(Stohlgren et al. 2006; Leprieur et al. 2008b; Blanchet et al. 2009; Pyšek et al. 2010; Brucet et al. 2013; Dawson et al. 2017; Anas and Mandrak 2021), which is likely a function of geographic location, spatial extent, and spatial resolution. Such effects have been rarely investigated simultaneously for total, native, non-native, and at-risk freshwater fish diversity in a given geographic region.

In this study, we simultaneously analyzed current species richness of total, native, non-native (overall, foreign, translocated), and at-risk freshwater fishes in 985 watersheds across Canada in relation to contemporary environmental and anthropogenic gradients, and historical factors. Specifically, we quantified the overall relative importance of historical, environmental, and anthropogenic factors in explaining the richness variation in each species category and estimated the relative contributions of individual explanatory factors in each case while accounting for covariation among explanatory variables and spatial autocorrelation. We hypothesized that historical and environmental factors would be more important for native species richness, and anthropogenic factors would be more important drivers of at-risk and non-native species richness. Further, we predicted that the importance of environmental factors and associations with individual anthropogenic factors would vary between foreign and translocated non-native richness.

Materials and Methods

Data sources

Current species richness of total, native, non-native, and at-risk freshwater fishes in 991 tertiary watersheds (i.e. third level of the hierarchically nested watershed boundaries delineated by the Water Survey of Canada 1977) across Canada were estimated using a dataset of freshwater fish species distributions in Canada compiled from multiple sources (See online

Supplementary Materials for a detailed description of sources and data compilation approach). This dataset contained current distribution data for 192 native species, of which 19 were considered translocated species in some parts of their range, and 11 foreign species with established populations (i.e. populations that show evidence of successful reproduction and overwinter survival). Several foreign species with highly localized distributions in Canada (e.g. Mosquitofish [*Gambusia affinis*] in Banff Hot Springs) were not included in the present analysis. We combined established foreign species and translocated native species to estimate overall non-native species richness in each watershed. In the case of native species with translocated occurrences, only the translocated occurrences were used to estimate non-native richness, while only native occurrences (i.e. within native range) were used to estimate native richness. To estimate at-risk richness, we included all “designatable units” of freshwater fishes in the official list of Canadian wildlife species at risk (COSEWIC 2019) as the at-risk species occurrences. Designatable units are either an entire taxonomic species or any population/group of populations within a taxon if it/they: (i) are recognized as subspecies or varieties; or, (ii) if they are “discrete” from other members of the same taxon, and if that discreteness is of some evolutionary significance to the particular taxon (Taylor et al. 2013; COSEWIC 2018). In total, 95 designatable units for 57 taxonomic species were used to estimate at-risk species richness.

Data for historical, environmental, and anthropogenic variables were extracted or calculated (summary statistics per watershed) from several national, continental, and global databases and digital maps (Table S1, Supplementary Information). Recent sources, available at an adequate spatial resolution for the present study, were used to extract contemporary environmental and anthropogenic data. Given the practical difficulty in including all variables in the data analysis, we performed Principal Component Analysis (PCA) separately on standardized

(scaled to unit variance) climatic and human population density-related variables to reduce sets of highly correlated variables to a smaller number of composite variables (Fig. S1, Table S2 and S3, Supplementary Information). PCA minimizes the redundancy among correlated variables by evaluating the correlation structure within the original data matrix and represents a significant portion of its variance as new orthogonal, composite variables (i.e. principal components) (Pearson 1901), which were used as variables in further statistical analysis. The final set of environmental variables included in the data analysis were: a composite variable representing a temperature and summer precipitation gradient (Clim_PC1); a composite variable representing a aridity and winter precipitation gradient (Clim_PC2) (Fig. S1 and Table S2, Supplementary Information); mean elevation (Elev); mean terrain roughness index (TRI; proxy for habitat heterogeneity); and, watershed area (Area) (Table 1). Historical variables included :distance from watershed centroid to the nearest major Wisconsinan refugium, i.e. Atlantic, Mississippian, Missourian, Pacific and Beringian (NRDist; Fig. 1); distance from watershed centroid to nearest glacial waterbody (GLDist; Fig. 1); distance from the watershed centroid to closest ice edge at the glacial maximum, ca. 18,000 ybp (IEDist) as a proxy for proximity to minor refugia that occurred along the edges of Laurentide and Cordilleran ice sheets and/or in coastal areas (Ross 2013; Wilson and Mandrak 2021); duration uncovered by ice (ice cover <50% of watershed area) since the glacial maximum, ca. 18,000 ybp (IFDur); and, duration covered by glacial waterbodies (waterbody extent >25% of watershed area) since ca. 18,000 ybp (GWDur). Anthropogenic factors included: a composite variable representing urbanization gradient (Urban_PC1; derived from PCA of population density, dwelling density; % human settlement area,% impervious surface and average nighttime light; Fig. S1 and Table S3, Supplementary Information), crop and animal farm density (CAFD); cultivated land density (CLD); pastureland density (PLD); density

of manufacturing facilities (ManuD); density of facilities for utilities (UtilD); density of mining facilities (MiniD); road density (RD); and, large dam (height >15m and/or reservoir volume >3 million m³) density (DamD). Six watersheds representing the Laurentian Great Lakes, Lake St. Clair, Detroit River and Lake Winnipeg were excluded from the data analysis as watershed-based estimates for environmental and anthropogenic variables could not be assigned to them, resulting in 985 watersheds used in the analysis.

Data analysis

Our data analysis consisted of three main components. First, richness by tertiary watershed was mapped separately for each species category and relationships between richness of total, native, non-native (overall, foreign, translocated), and at-risk fishes were evaluated using Spearman's rank correlation test. Second, we quantified the overall relative importance of historical, environmental, and anthropogenic variable groups in explaining the variation in the richness of the different species categories using two complementary approaches i.e., variance partitioning and the Lindeman et al. (1980) method of hierarchical partitioning (henceforth LMG). Third, we estimated the independent relative contributions of individual explanatory variables for explaining the richness variation of different species categories using LMG method.

Overall relative importance of explanatory variable groups in explaining species richness

We performed variance partitioning based on multiple linear regression to estimate the proportions of variation in richness of different species categories explained by historical,

environmental, and anthropogenic variable groups, jointly and independently. To account for spatial autocorrelation, we employed a spatial eigenvector-based method together with variance partitioning (Griffith and Peres-Neto 2006; Peres-Neto and Legendre 2010). Briefly, this method extracts eigenvectors of a connectivity matrix among survey units (known as graph-based Moran's eigenvector maps; gb-MEM) describing all possible mutually orthogonal map patterns of the study region, which are then used as additional predictors of the response variables (Diniz-Filho and Bini 2005; Griffith and Peres-Neto 2006). For detailed procedure for extracting and selecting optimum subset of gb-MEM variables, see Bauman et al. (2018) and Anas and Mandrak (2021). Details of the optimum subsets for each species category are provided in the Supplementary Information (Table S4). Prior to the analyses, all explanatory variables, except principal components (Clim_PC1, Clim_PC2, Urban_PC1) and spatial variables, were either $\log_{10}(x + 0.01)$ or $\log_{10}$ -transformed to improve linear model fits. The results were expressed in terms of adjusted R^2 (adj. R^2), which is an unbiased estimate of explained variation as it accounts for number of explanatory variables and sample size (Peres-Neto et al. 2006). The overall relative importance of each explanatory variable group was determined based on its proportional unique contributions, i.e. variation not shared with other explanatory variable groups (Peres-Neto et al. 2006; Peres-Neto and Legendre 2010). We repeated this procedure for each response variable, i.e. total, native, at-risk, and overall and different categories of non-native species richness.

We performed LMG hierarchical variance partitioning (Lindeman et al. 1980) based on multiple linear regression as an additional analysis to quantify the relative importance of the historical, environmental, anthropogenic, and spatial (gb-MEM variables generated above) variable groups for explaining richness variation in the different species categories. The LMG

method decomposes R^2 into independent (i.e. corrected for the shared contribution due to inter-set correlations, Fig. S2, Supplementary Information) contributions attributable to each explanatory variable group based on averaging over all orderings of explanatory variables in a multiple regression setting (Lindeman et al. 1980; Grömping 2006). An important difference between this approach and variance partitioning with respect to estimating overall relative importance of explanatory variable groups should be noted - variance partitioning is an exploratory approach based on addition and subtraction of R^2 computed from different models, which provides an indication of relative importance of explanatory variable groups by partitioning out the shared variation resulting from inter-set correlations, whereas LMG analysis addresses the effect of among-regressor correlations through a computational approach. We calculated the 95% bootstrap percentile confidence interval (using 500 bootstrap samples) for the estimated independent contribution of each explanatory variable group.

Relative importance of individual explanatory variables in explaining species richness

To estimate the independent relative contributions of individual historical, environmental, and anthropogenic factors for explaining the variation in richness of different species categories, we performed multiple linear regression combined with hierarchical variance partitioning based on the LMG method (Lindeman et al. 1980; Grömping 2006). This analysis was not aimed at developing the ‘best predictive model’, but rather ranking the explanatory variables based on their independent (i.e. corrected for the shared contribution with other correlated explanatory variables, Fig. S2) explanatory power, which was estimated by decomposing R^2 into independent contributions of each explanatory variable as described above. We used an eigenvector-based spatial-filtering approach (Griffith and Peres-Neto 2006) that has been shown to be effective for controlling the effect of spatial autocorrelation in regression analysis of species richness data

(Diniz-Filho and Bini 2005). The procedure for generating spatial filters (gb-MEM) was similar to that used in the variance partitioning with few exceptions (Bauman et al. 2018; Anas and Mandrak 2021). The selected gb-MEM subset (Table S4, Supplementary Information) was incorporated into the regression model with environmental, historical, and anthropogenic variables (either $\log_{10} [x + 0.01]$ or $\log_{10}$ -transformed as indicated above) to correctly estimate the effect (statistical significance, form of relationship, and relative importance) of each explanatory variable unbiased by spatial autocorrelation in residuals (Diniz-Filho and Bini 2005; Griffith and Peres-Neto 2006). The LMG analysis allows independent relative importance values to be aggregated by pre-defined groups of explanatory variables, making our analysis with a large number of explanatory variables computationally feasible (Grömping 2006). For example, when calculating the independent relative importance of individual historical variables, other variables in the model were grouped as environmental, anthropogenic, and spatial factors. The 95% bootstrap percentile confidence intervals for the estimated independent contribution of each explanatory variable group was calculated using 500 bootstrap samples. This procedure was repeated for each response variable, i.e. total, native, at-risk, overall and different categories of non-native species richness.

All statistical analyses were performed using the following packages within R statistical software version 3.6.0 (R Core Team 2019)); 'tmap' (Tennekes 2018) ver. 3.3-1 for mapping; 'vegan' (Oksanen et al. 2019) ver. 2.5-5 for PCA and variance partitioning; 'adespatial' (Dray et al. 2020) ver. 0.3-8 and 'spdep' (Bivand et al. 2013) ver. 1.1-7 for gb-MEM variable generation and subset selection; 'relaimpo' (Grömping 2006) ver. 2.2-3 for hierarchical partitioning; and, 'ggplot2' (Wickham 2016) ver. 3.3.3 for plotting.

270 Results

271 There is considerable non-random variation in total (1-133 species), native (1-111), non-
272 native (0-21), and at-risk (0-14) freshwater fish richness across the 991 watersheds (Fig. 2). In
273 general, total richness exhibits an inverse latitudinal gradient with the southern parts of Quebec,
274 Ontario, and Manitoba being particularly speciose. Native richness is highly correlated and
275 spatially congruent with total richness (Table 2). Native richness is largely spatially congruent
276 with non-native and at-risk species, resulting in high correlations among species categories
277 (Table 2). In addition, a cluster of watersheds with high numbers of non-native and at-risk
278 species is apparent in southern British Columbia (Fig. 2). The distribution of non-native species
279 is largely limited to southern parts of the country, of which translocated species are more
280 widespread than foreign species (Fig. S3, Supplementary Information). At-risk species are not
281 found in Arctic regions (Fig. 2).

282 Historical, environmental, anthropogenic, and spatial factors collectively explained most
283 of the variation in total, native, non-native, and at-risk species richness (93%, 93%, 87%, and
284 86% of total variation, respectively based on adj. R^2 ; Fig. 3 and Fig. 4). For all species
285 categories, variance partitioning revealed that most of the richness variation explained by each
286 explanatory variable group was shared by other variable groups. This resulted in minor unique
287 fractions (0.1% - 1% of total variation) explained by all explanatory variable groups (relative to
288 total explained variation) except for spatial factors (27% – 36% of total variation), indicating the
289 major role of spatial factors in explaining richness variation of three species categories and the
290 difficulty in determining the hierarchical importance of remaining explanatory variable groups.
291 However, all unique fractions were statistically significant ($p < 0.05$), except for historical factors
292 in explaining at-risk species richness (Fig. 3). The results of LMG relative independent

importance analysis further supported the predominant relative importance of spatial factors in all cases (Fig. 4). The relative independent importance of environmental, historical, and anthropogenic variable groups was similar (16%, 11%, and 15% of total variation, respectively) for total and native richness. Although the historical variable group was ranked as less important relative to environmental and anthropogenic factors, bootstrap confidence intervals used for ranking can be somewhat liberal (Grömping 2006). On the other hand, the relative independent importance of anthropogenic factors in explaining variation in overall non-native richness was greater (16% of total variation) than environmental and historical variable groups (10% and 8% of total variation, respectively). However, when analyzed separately based on geographic origin (i.e. foreign vs. translocated), this pattern only held for foreign non-native richness (Fig. 5 and Fig. S4, Supplementary Information). The historical factors had a higher relative contribution for explaining translocated non-native richness (10% of total variation) compared to foreign non-native richness (3% of total variation).. In contrast, both anthropogenic and environmental variable groups were more important (12% and 17% of total variation, respectively) than historical factors (2% of total variation) for at-risk species richness (Fig. 4).

The LMG results indicated the relative independent importance of individual environmental factors were largely similar among four species categories (Fig. 6). Clim_PC1 (composite variable representing temperature and summer precipitation gradient) was the major independent contributor in explaining the richness variation in all species categories (6-10% of total variation). The form of relationship of explanatory variables (positive or negative; based on the sign of regression coefficients) was largely consistent among the species categories. Further, most of the individual explanatory variables were statistically significant ($p < 0.05$) for total, native, and at-risk richness. Interestingly, despite the predominant independent contributions of

316 Clim_PC1 and Clim_PC2 (composite variable representing aridity and winter precipitation
317 gradient) for explaining non-native richness, they were not statistically significant ($p > 0.05$).
318 This can be indicative of indirect influence of these variables on non-native richness through
319 their influence on certain other predictors (Grömping 2007). While Clim_PC1 was considerably
320 more important than Clim_PC2 for explaining foreign non-native richness (6% and 2% of total
321 variation, respectively), they were both equally important (each explaining 4% of total variation)
322 for explaining translocated non-native richness (Fig. 7).

323 Similarities were apparent among the four species categories with respect to independent
324 relative importance of, and nature of relationship with, historical variables (Fig. 6). The historical
325 variables with the greatest independent relative importance indicated that richness of all species
326 categories decreases with increasing distance to the nearest refugium (NRDist, 5% of total
327 variation in each case, $p < 0.05$) and nearest glacial waterbody (GLDist, 2.5-4% total variation,
328 $p < 0.05$ for total and native richness) and, conversely, is higher in watersheds covered by glacial
329 waterbodies for longer periods of time (GWDur, 3-4% total variation, $p < 0.05$). On the other
330 hand, IEDist (0.5-2% total variation) and IFDur (0.2-0.5% total variation) were least important
331 for all species categories.

332 Precise ranking of individual anthropogenic factors based on relative importance was
333 difficult given the overlapping bootstrap confidence intervals in most cases (Fig. 6). Urban_PC1
334 (composite variable representing an urbanization gradient), CAFD, CLD, PLD, and RD were
335 among the variables with higher relative contributions in explaining total (4%, 6%, 5%, 4%, and
336 3% of total variation, respectively), native (4%, 5%, 4%, 4%, and 3% of total variation,
337 respectively), and non-native richness (5%, 3%, 6%, 4%, and 5% of total variation, respectively).
338 Urban_PC1 was most important in explaining foreign non-native richness (8% of total variation),

whereas its importance (2% of total variation) was marginal relative to other anthropogenic variables for translocated non-native richness (Fig. 7). For at-risk species richness, Urban_PC1, CAFD, ManuD, and UtilD had higher relative importance (5%, 4%, 4% and 4% of total variation, respectively). Urban_PC1, CAFD, and UtilD had significant ($p < 0.05$) positive associations with all species richness categories, but the nature of relationship and statistical significance of other explanatory variables were not consistent among species categories.

Discussion

Our analysis indicated that, despite moderate correlations and spatial congruence among native, non-native, and at-risk species richness across Canada, they were differentially influenced by historical, environmental, and anthropogenic processes. Environmental variables likely related to thermal energy (Evans et al. 2005a) and habitat space availability (McAllister et al. 1986; Oberdorff et al. 1995), and historical factors related to postglacial recolonization (Tedesco et al. 2005; Griffiths et al. 2014) likely play important roles in shaping spatial variation in native richness. Observed associations of anthropogenic variables with native richness are likely indicative of covariation of both human activities and native diversity with natural resource availability and sampling bias (Luck 2007). The results for total richness were largely similar to those for native richness, which is not surprising given the predominant contribution of native species to the total species pool. In contrast, variation in non-native species richness is largely related to the human stressors likely facilitating the establishment of invaders through increased propagule pressure and habitat disturbance (Gallardo et al. 2015; Cabra-Rivas et al. 2016). Among non-native species, the distribution of foreign species was strongly influenced by anthropogenic factors related to urbanization compared to translocated species. Both environmental conditions predisposing species to rarity (such as energy availability) and

anthropogenic processes are likely important in determining the at-risk species richness in a watershed (Kerr and Currie 1995; Davies et al. 2006; Howard et al. 2020).

Relative roles of historical, environmental, and anthropogenic factors in influencing the richness of native, non-native, and at-risk species

Among environmental factors, climatic constraints are likely more influential than habitat availability/heterogeneity/isolation in shaping spatial variation in total, native, non-native, and at-risk species richness at the watershed level. Increase in river discharge with precipitation can result in increased habitat space available for fish species, leading to a positive relationship between precipitation-related variables and species richness (McAllister et al. 1986; Oberdorff et al. 1995; Xenopoulos and Lodge 2006). Previous broad-scale studies also found positive associations between climatic correlates of environmental energy availability (i.e. temperature and precipitation) and freshwater fish species richness (McAllister et al. 1986; Stohlgren et al. 2006; Oberdorff et al. 2011; Griffiths et al. 2014). Energy availability may influence species richness through a range of mechanisms, such as constraints on species' population sizes, niche breadth and position, food-web structure and complexity, and physiological tolerances (see Evans et al. 2005 for a review). However, quantifying the relative importance of such mechanisms for the observed pattern is beyond the scope of present study. Many fish species in southern Canada are at the northern edge of their range and, given their limited geographic distribution within the country, they are more likely to be assessed as at risk by COSEWIC (Staton and Mandrak 2006; Glass et al. 2017). Our findings indicate that range limits of such species are largely determined by the thermal constraints. Energy-related mechanisms may also be linked to the occurrence of other naturally rare species not at the edge of their range and, thus, are vulnerable to imperilment (Rohde 1992; Hawkins et al. 2003; Howard et al. 2019). Positive

relationships between climatic factors and non-native richness can be an indirect result of covariation of human activities (promoting introduction effort of non-native species and habitat disturbance) with favorable climatic conditions (Evans et al. 2005b; Marini et al. 2012). However, our analysis indicates that climatic factors are still important after accounting for the potentially confounding effects of human factors. Therefore, it is likely that climatic factors play a minor, but important, role in the post-introduction establishment success and range expansion of non-native species (Chown et al. 2005; Evans et al. 2005a; Gallardo et al. 2015).

Our findings indicate that historical factors played an important role in influencing the variation in native fish richness, contrary to previous studies that concluded they were of marginal importance (Kerr and Currie 1999; Oberdorff et al. 2011; Tisseuil et al. 2013; Griffiths et al. 2014; Anas and Mandrak 2021; but see Tedesco et al. 2005). It is not surprising that historical factors play an important role in Canada because almost all of the country was glaciated during the late Pleistocene, which left a pronounced imprint on contemporary fish species distributions (Wilson and Mandrak 2021; Mandrak et al. 2022). In contrast, such glaciation effects may have been overshadowed in other continental or global studies, which included both glaciated and unglaciated areas during the Pleistocene (Oberdorff et al. 1995; Anas and Mandrak 2021). The postglacial colonization of formerly glaciated watersheds by native fishes was likely more constrained by the availability (proximity and duration) of dispersal pathways than by length of time deglaciated and proximity to ice edge. The major refugia considered in the present study represent remnants of pre-glaciation habitats where extinctions may have occurred during the Pleistocene (Matthews 1998; Griffiths 2010; Oberdorff et al. 2011). Dispersal of vagile species from these species pools into formerly glaciated areas depended on drainage connections as dispersal corridors from refugia, which became sparse as

408 distance from refugia increased (Mandrak 1995; Oberdorff et al. 1997; Griffiths 2015; Mandrak
409 et al. 2022). Associations of native richness with distance to nearest glacial waterbody and
410 duration covered by glacial waterbodies corroborate the role of glacial waterbodies as dispersal
411 corridors from refugial areas into newly deglaciated regions for fishes (Bailey and Smith 1981;
412 Crossman and McAllister 1986; Lindsey and McPhail 1986; Mandrak and Crossman 1992;
413 Mandrak 1995; Matthews 1998; Mandrak et al. 2022). However, length of time since
414 deglaciation and distance to closest ice edge were not good descriptors of species richness, as
415 watersheds deglaciated for long periods of time or close to the ice edge were not more accessible
416 for colonization unless they were near, or covered by, glacial waterbodies or near major refugia
417 along the ice edge.

418 Our findings indicate that anthropogenic factors are of greater importance than
419 environmental factors in influencing non-native richness in watersheds. While the anthropogenic
420 variables considered in our analysis are not proximate determinants of fish invasions, they are
421 related to causes that determine the outcome of non-native species introductions, including
422 pathways of introduction, propagule pressure, and intensity of habitat disturbance (Lockwood et
423 al. 2005; Marini et al. 2009; Pyšek et al. 2010). Opposing viewpoints exist on the relative
424 importance of such anthropogenic-based processes versus post-introduction environmental
425 filtering in determining the invasion success (Lockwood et al. 2005; Marini et al. 2009). Our
426 results are consistent with several broad-scale studies of freshwater fish invasions that support
427 predominant role of anthropogenic-based processes (Stohlgren et al. 2006; Leprieur et al. 2008a;
428 Blanchet et al. 2009; Gallardo et al. 2015; Dawson et al. 2017; Anas and Mandrak 2021).
429 Anthropogenic factors, especially urbanization, are more important for foreign species than for
430 translocated species, likely due to higher frequency and intensity of unauthorized foreign fish

releases from aquaria, aquaculture and live fish markets, and sportfish introductions by public (Rixon et al. 2005; Mandrak and Cudmore 2010; Bobeldyk et al. 2015; Rahel and Smith 2018) in heavily populated areas (Copp et al. 2010; Strecker et al. 2011; Lockwood et al. 2019). Conversely, translocations of native fish species are primarily related to recreational fisheries (stocking of sport and forage fishes, bait releases) via both authorized and unauthorized means (Rixon et al. 2005; Rahel and Smith 2018), of which authorized introductions did not particularly target highly populated regions (McKinney 2001; Rahel and Smith 2018). The stronger correlation of translocated richness with historical variables compared to foreign richness may be an artefact caused by authorized translocations of species into areas closer to their native ranges to ensure climate matching (Lapointe et al. 2016).

Relationships among environmental factors, human activities and biodiversity

Overall, relationships among environmental factors, anthropogenic factors, and species richness identified in our study (Fig. S5, Supplementary Information) presents a concerning picture for freshwater fish biodiversity in Canada. Not surprisingly, the richness of native species and rare species vulnerable to imperilment are positively related to favourable environmental conditions. Favourable environmental conditions that support native species are also preferred by humans, leading to a positive correlation between human stressors and native/at-risk species richness (Chown et al. 2003; Luck 2007; Chu et al. 2014). As human activities can promote the introduction and establishment of non-native species (Lockwood et al. 2005; Marini et al. 2009; Pyšek et al. 2010), more non-native species are accumulated in the very watersheds with high native and at-risk species richness. Consequently, impacts of invasive species and other human stressors (e.g. habitat degradation and fragmentation, overexploitation) can be greater in areas

453 with higher densities of native and at-risk species (McKinney 2002; Chu et al. 2003, 2014;
454 Dextrase and Mandrak 2006).

455 *Caveats*

456 When interpreting the results of our analysis, some caveats should be noted. First, our
457 analysis may be confounded by uneven sampling effort for native, at-risk, and non-native species
458 (Barbosa et al. 2013; Gallardo et al. 2015). The known distributions of many species in Canada
459 are likely not accurately delineated due to poor survey coverage in northern regions (Chu et al.
460 2003). Second, there may be a temporal mismatch between some of the anthropogenic data we
461 used, which are snapshots in time, and the species richness data, which are measured across a
462 longer time span. Our analysis is based on the assumption that anthropogenic data are
463 representative of the long-term spatial variability of human stressors across the study extent.
464 Further, time lags in species responses to anthropogenic processes may confound accurate
465 detection of relationships between human stressors and species diversity (Forester and Machlist
466 1996; Davies et al. 2006). Third, our correlative approach is vulnerable to identifying spurious or
467 artefactual relationships (e.g. correlation between historical factors and non-native richness due
468 to translocation of species closer to their native ranges). Fourth, insights into causality are limited
469 by the plausible factors evaluated in the present study. The spatially structured variation in the
470 richness patterns that remained unaccounted for by the explanatory variables may be, in part, a
471 result of effects of unmeasured factors (e.g. across-watershed connectivity influencing the
472 dispersal, nutrient availability influencing the productivity/energy availability, fishing pressure
473 influencing the population sizes) (Field et al. 2009; Peres-Neto and Legendre 2010; Griffiths
474 2017). More precise explanatory variables are necessary to identify anthropogenic pressures that
475 are the most proximate causes of threats to fish biodiversity. Fifth, the coarse spatial resolution

of our study prevented us from evaluating the role of biotic interactions, which is an important determinant of invasion at finer grain sizes (Levine and D'Antonio 1999). Finally, although we focused only on historical variables related to recent Pleistocene glaciation given their particular interest in the Canadian context, longer-term evolutionary processes may also impact richness patterns (Field et al. 2009; Griffiths et al. 2014). However, glaciations and subsequent dispersal have erased the evolutionary signals in richness patterns of vagile groups, such as freshwater fishes in North America (Kerr and Currie 1999).

Conservation implications

Improved understanding of the relationships among historical, environmental, anthropogenic, and spatial factors, and biodiversity patterns can inform spatial prioritization of conservation efforts under current and future environmental conditions (Chown et al. 2003; Pyšek et al. 2010; Howard et al. 2020). With the increased recognition of the importance of combining area-based conservation approaches with species-by-species approaches, the identification of regions with high extinction risk is critical (Chu et al. 2014; Howard et al. 2019; Muir et al. 2021). Some regions harbour high densities of range-restricted native species due to natural factors (e.g. environmental and historical) and consequently, biodiversity in such regions is inherently more vulnerable than in others to impacts of threatening processes (Howard et al. 2020; Muir et al. 2021). Based on our findings for at-risk richness, we can infer that the geographical distribution of inherent vulnerability of watersheds to biodiversity loss is influenced by climatic factors rather than by glacial history. Understanding the drivers of inherent vulnerability of watersheds to threats can aid decisions on prioritizing conservation actions (Orme et al. 2005; Davies et al. 2006; Howard et al. 2020). For example, watersheds with low human pressures in warmer regions (that have higher inherent vulnerability due to high

densities of naturally range-restricted species) could be prioritized for proactive conservation efforts (e.g. protected areas), whereas remediation measures may be more appropriate in colder regions (Arlidge et al. 2018). In addition, our results indicate that native diversity should not be assumed as an appropriate surrogate for at-risk species richness to guide conservation planning (Prendergast et al. 1993; Orme et al. 2005), because drivers of richness vary between the two species categories. Range expansions of native (Mandrak 1989; Chu et al. 2005) and invasive (Sharma et al. 2007; Rahel and Olden 2008) fish species in North America in response to increase in water temperature with climate change have been predicted. Our findings show that climatic factors are important drivers of non-native species richness, yet they are not solely sufficient to predict future invasions. Rather, anthropogenic (e.g. land-use and socio-economic variables) and climate data should be used together to make more accurate, spatially explicit predictions of invasion likelihood, which can inform geographically targeted strategies to prevent or manage fish invasions (Pyšek et al. 2010; Gallardo et al. 2015; Anas and Mandrak 2021).

Conclusions

Our study indicates that the hierarchical importance of environmental, historical and anthropogenic processes on richness patterns vary among native, non-native, and at-risk species categories. The prominent historical signal in native richness pattern is not surprising given that Canada was almost completely glaciated during the Pleistocene Epoch. At least some of the environmental processes that regulate native richness (e.g. those related to energy availability) likely also influence non-native richness, yet are superseded by anthropogenic processes. The predisposing environmental conditions are as important as anthropogenic processes in determining where high densities of at-risk fish species occur, which suggests that some regions

may be inherently more vulnerable than others to the impacts of threatening anthropogenic processes. Overall, our study highlights the importance of an integrated approach that simultaneously considers natural and anthropogenic processes to better understand the risks of biodiversity change at the landscape scale.

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Author contributions

M.A and N. M. conceived the study. N. M. compiled species distribution data. M. A. compiled environmental, historical and anthropogenic data, performed statistical analyses and wrote the manuscript with a significant contribution from N. M.

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542

543 **Data Accessibility:** The data associated with this paper are available through Dryad Digital

544 Repository: <https://doi.org/10.5061/dryad.sn02v6x3n>

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546 **References**

547 Anas, M.U.M., and Mandrak, N.E. 2021. Drivers of native and non-native freshwater fish

548 richness across North America: Disentangling the roles of environmental, historical and

549 anthropogenic factors. *Glob. Ecol. Biogeogr.* **30**: 1232–1244. doi:10.1111/geb.13298.

550 Arlidge, W.N.S., Bull, J.W., Addison, P.F.E., Burgass, M.J., Gianuca, D., Gorham, T.M., Jacob,

551 C.D.S., Shumway, N., Sinclair, S.P., Watson, J.E.M., Wilcox, C., and Milner-Gulland, E.J.

552 2018. A global mitigation hierarchy for nature conservation. *Bioscience* **68**(5): 336–347.

553 doi:10.1093/biosci/biy029.

554 Bailey, R.M., and Smith, G.R. 1981. Origin and geography of the fish fauna of the Laurentian

555 Great Lakes Basin. *Can. J. Fish. Aquat. Sci.* **38**: 1539–1561.

556 Barbosa, A.M., Pautasso, M., and Figueiredo, D. 2013. Species-people correlations and the need

557 to account for survey effort in biodiversity analyses. *Divers. Distrib.* **19**(9): 1188–1197.

558 doi:10.1111/ddi.12106.

559 Bauman, D., Drouet, T., Fortin, M.J., and Dray, S. 2018. Optimizing the choice of a spatial

560 weighting matrix in eigenvector-based methods. *Ecology* **99**(10): 2159–2166.

561 doi:10.1002/ecy.2469.

562 Blanchet, S., Leprieur, F., Beauchard, O., Staes, J., Oberdorff, T., and Brosse, S. 2009. Broad-

- scale determinants of non-native fish species richness are context-dependent. *Proc. R. Soc. B Biol. Sci.* **276**(1666): 2385–2394. doi:10.1098/rspb.2009.0156.
- Bobeldyk, A.M., Ru, J., and Lamberti, G.A. 2015. Freshwater hotspots of biological invasion are a function of species – pathway interactions. *Hydrobiologia* **746**: 363–373. doi:10.1007/s10750-014-2009-z.
- Brucet, S., Pédrón, S., Mehner, T., Lauridsen, T.L., Argillier, C., Winfield, I.J., Volta, P., Emmrich, M., Hesthagen, T., Holmgren, K., Benejam, L., Kelly, F., Krause, T., Palm, A., Rask, M., and Jeppesen, E. 2013. Fish diversity in European lakes: Geographical factors dominate over anthropogenic pressures. *Freshw. Biol.* **58**(9): 1779–1793. doi:10.1111/fwb.12167.
- Cabra-Rivas, I., Saldaña, A., Castro-Díez, P., and Gallien, L. 2016. A multi-scale approach to identify invasion drivers and invaders' future dynamics. *Biol. Invasions* **18**(2): 411–426. doi:10.1007/s10530-015-1015-z.
- Carpio, A.J., Jose, G., Jose, B., Tortosa, F.S., Vicente, J., Hillstrom, L., and Delibes-Mateos, M. 2017. Hunting as a source of alien species: a European review. *Biol.* **19**: 1197–1211. doi:10.1007/s10530-016-1313-0.
- Chown, S.L., Hull, B., and Gaston, K.J. 2005. Human impacts, energy availability and invasion across Southern Ocean Islands. *Glob. Ecol. Biogeogr.* **14**(6): 521–528. doi:10.1111/j.1466-822x.2005.00173.x.
- Chown, S.L., Van Rensburg, B.J., Gaston, K.J., Rodrigues, A.S.L., and Van Jaarsveld, A.S. 2003. Energy, species richness, and human population size: Conservation implications at a national scale. *Ecol. Appl.* **13**(5): 1233–1241. doi:10.1890/02-5105.

- 585 Chu, C., Mandrak, N.E., and Minns, C.K. 2005. Potential impacts of climate change on the
586 distributions of several common and rare freshwater fishes in Canada. *Divers. Distrib.* **11**:
587 299–310. doi:10.1111/j.1366-9516.2005.00153.x.
- 588 Chu, C., Minns, C.K., Lester, N.P., and Mandrak, N.E. 2014. An updated assessment of human
589 activities, The environment, And freshwater fish biodiversity in Canada. *Can. J. Fish.*
590 *Aquat. Sci.* **72**(1): 135–148. doi:10.1139/cjfas-2013-0609.
- 591 Chu, C., Minns, C.K., and Mandrak, N.E. 2003. Comparative regional assessment of factors
592 impacting freshwater fish biodiversity in Canada. *Can. J. Fish. Aquat. Sci.* **60**(5): 624–634.
593 doi:10.1139/F03-048.
- 594 Copp, G.H., Vilizzi, L., and Gozlan, R.E. 2010. The demography of introduction pathways,
595 propagule pressure and occurrences of non-native freshwater fish in England. *Aquat.*
596 *Conserv. Mar. Freshw. Ecosyst.* **20**(5): 595–601. doi:10.1002/aqc.1129.
- 597 COSEWIC. 2018. Operations and procedures manual. Ottawa, Ontario. Committee on the Status
598 of Endangered Wildlife in Canada, Environment Canada, Canadian Wildlife Service.
599 Ottawa, Ontario. ix + 359 pp.
- 600 COSEWIC. 2019. Canadian Wildlife Species at Risk. Committee on the Status of Endangered
601 Wildlife in Canada. Available from [https://www.canada.ca/en/environment-climate-](https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html)
602 [change/services/species-risk-public-registry.html](https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html) [accessed 15 March 2020].
- 603 Crossman, E.J., and McAllister, D.E. 1986. Zoogeography of freshwater fishes of the Hudson
604 Bay drainage, Ungava Bay and the Arctic Archipelago. *In* *Zoogeography of North*
605 *American Freshwater Fishes. Edited by C.H. Hocutt and E.O. Wiley.* John Wiley & Sons,
606 New York. pp. 53–104.

- 607 Davies, R.G., Orme, C.D.L., Olson, V., Thomas, G.H., Ross, S.G., Ding, T.S., Rasmussen, P.C.,
608 Stattersfield, A.J., Bennett, P.M., Blackburn, T.M., Owens, I.P.F., and Gaston, K.J. 2006.
609 Human impacts and the global distribution of extinction risk. *Proc. R. Soc. B Biol. Sci.*
610 **273**(1598): 2127–2133. doi:10.1098/rspb.2006.3551.
- 611 Dawson, W., Moser, D., Van Kleunen, M., Kreft, H., Pergl, J., Pyšek, P., Weigelt, P., Winter,
612 M., Lenzner, B., Blackburn, T.M., Dyer, E.E., Cassey, P., Scrivens, S.L., Economo, E.P.,
613 Guénard, B., Capinha, C., Seebens, H., García-Díaz, P., Nentwig, W., García-Berthou, E.,
614 Casal, C., Mandrak, N.E., Fuller, P., Meyer, C., and Essl, F. 2017. Global hotspots and
615 correlates of alien species richness across taxonomic groups. *Nat. Ecol. Evol.* **1**(JUNE): 1–
616 7. doi:10.1038/s41559-017-0186.
- 617 Dextrase, A.J., and Mandrak, N.E. 2006. Impacts of alien invasive species on freshwater fauna at
618 risk in Canada. *Biol. Invasions* **8**: 13–24. doi:10.1007/s10530-005-0232-2.
- 619 Diniz-Filho, J.A.F., and Bini, L.M. 2005. Modelling geographical patterns in species richness
620 using eigenvector-based spatial filters. *Glob. Ecol. Biogeogr.* **14**(2): 177–185.
621 doi:10.1111/j.1466-822X.2005.00147.x.
- 622 Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque,
623 G., Legendre, P., Madi, N., and Wagner, H.H. 2020. *adespatial*: multivariate multiscale
624 spatial analysis. . R package version 0.3-8. Available from [https://cran.r-](https://cran.r-project.org/package=adespatial)
625 [project.org/package=adespatial](https://cran.r-project.org/package=adespatial).
- 626 Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C.,
627 Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., and Sullivan, C.A. 2006.
628 Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biol. Rev.*

- 629 Camb. Philos. Soc. **81**(2): 163–182. doi:10.1017/S1464793105006950.
- 630 Evans, K.L., Warren, P.H., and Gaston, K.J. 2005a. Species-energy relationships at the
631 macroecological scale: A review of the mechanisms. Biol. Rev. Camb. Philos. Soc. **80**(1):
632 1–25. doi:10.1017/S1464793104006517.
- 633 Evans, K.L., Warren, P.H., and Gaston, K.J. 2005b. Does energy availability influence classical
634 patterns of spatial variation in exotic species richness? Glob. Ecol. Biogeogr. **14**(1): 57–65.
635 doi:10.1111/j.1466-822X.2004.00134.x.
- 636 Field, R., Hawkins, B.A., Cornell, H. V., Currie, D.J., Diniz-Filho, J.A.F., Guégan, J.F.,
637 Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., and Turner,
638 J.R.G. 2009. Spatial species-richness gradients across scales: A meta-analysis. J. Biogeogr.
639 **36**(1): 132–147. doi:10.1111/j.1365-2699.2008.01963.x.
- 640 Forester, D.J., and Machlist, G.E. 1996. Modeling Human Factors That Affect the Loss of
641 Biodiversity. Conserv. Biol. **10**(4): 1253–1263. doi:10.1046/j.1523-1739.1996.10041253.x.
- 642 Gallardo, B., Zieritz, A., and Aldridge, D.C. 2015. The importance of the human footprint in
643 shaping the global distribution of terrestrial , freshwater and marine invaders. PLoS One **10**:
644 e0125801. doi:10.1371/journal.pone.0125801.
- 645 Gaston, K.J. 2005. Biodiversity and extinction: Species and people. Prog. Phys. Geogr. **29**(2):
646 239–247. doi:10.1191/0309133305pp445pr.
- 647 Glass, W.R., Corkum, L.D., and Mandrak, N.E. 2017. Living on the edge: Traits of freshwater
648 fish species at risk in Canada. Aquat. Conserv. Mar. Freshw. Ecosyst. **27**(5): 938–945.
649 doi:10.1002/aqc.2781.
- 650 Griffith, D.A., and Peres-Neto, P.R. 2006. Spatial Modeling in Ecology : The Flexibility of

- 651 Eigenfunction Spatial Analyses. *Ecology* **87**(10): 2603–2613.
- 652 Griffiths, D. 2010. Pattern and process in the distribution of North American freshwater fish.
653 *Biol. J. Linn. Soc.* **100**(1): 46–61. doi:10.1111/j.1095-8312.2010.01404.x.
- 654 Griffiths, D. 2015. Connectivity and vagility determine spatial richness gradients and
655 diversification of freshwater fish in North America and Europe. *Biol. J. Linn. Soc.* **116**(4):
656 773–786. doi:10.1111/bij.12638.
- 657 Griffiths, D. 2017. Connectivity and vagility determine beta diversity and nestedness in North
658 American and European freshwater fish. *J. Biogeogr.* **44**(8): 1723–1733.
659 doi:10.1111/jbi.12964.
- 660 Griffiths, D., Mcgonigle, C., and Quinn, R. 2014. Climate and species richness patterns of
661 freshwater fish in North America and Europe. *J. Biogeogr.* **41**(3): 452–463.
662 doi:10.1111/jbi.12216.
- 663 Grömping, U. 2006. Relative importance for linear regression in R: the package relaimpo. *J. Stat.*
664 *Softw.* **17**(1): 1–27.
- 665 Hawkins, B.A., Field, R., Cornell, H. V, Currie, D.J., Guégan, J.-F., Kaufman, D.M., Kerr, J.T.,
666 Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E., and Turner, J.R.G. 2003.
667 Energy, water, and broadscale geographic patterns of species richness. *Ecology* **84**: 3105–
668 3117. doi: 10.1890/03-8006.
- 669 Heino, J. 2011. A macroecological perspective of diversity patterns in the freshwater realm.
670 *Freshw. Biol.* **56**(9): 1703–1722. doi:10.1111/j.1365-2427.2011.02610.x.
- 671 Howard, C., Flather, C.H., and Stephens, P.A. 2019. What drives at-risk species richness?
672 Environmental factors are more influential than anthropogenic factors or biological traits.

- 673 Conserv. Lett. **12**(2): 1–9. doi:10.1111/conl.12624.
- 674 Howard, C., Flather, C.H., and Stephens, P.A. 2020. A global assessment of the drivers of
675 threatened terrestrial species richness. Nat. Commun. **11**(1): 1–10. Springer US.
676 doi:10.1038/s41467-020-14771-6.
- 677 Kerr, J.T., and Currie, D.J. 1995. Effects of human activity on global extinction risk. Conserv.
678 Biol. **9**(6): 1528–1538. doi:10.1046/j.1523-1739.1995.09061528.x.
- 679 Kerr, J.T., and Currie, D.J. 1999. The relative importance of evolutionary and environmental
680 controls on broad-scale patterns of species richness in North America. Ecoscience **6**: 329–
681 337. doi:10.1080/11956860.1999.11682546.
- 682 Kerr, J.T., Kharouba, H.M., and Currie, D.J. 2007. The macroecological contribution to global
683 change solutions. Science (80-.). **316**(5831): 1581–1584. doi:10.1126/science.1133267.
- 684 Lapointe, N.W.R., Fuller, P.L., Neilson, M., Murphy, B.R., and Angermeier, P.L. 2016.
685 Pathways of fish invasions in the Mid-Atlantic region of the United States. Manag. Biol.
686 Invasions **7**(3): 221–233.
- 687 Leprieur, F., Beauchard, O., Blanchet, S., Oberdorff, T., and Brosse, S. 2008a. Fish invasions in
688 the world's river systems: When natural processes are blurred by human activities. PLoS
689 Biol. **6**(2): 0404–0410. doi:10.1371/journal.pbio.0060028.
- 690 Leprieur, F., Beauchard, O., Hugueny, B., Grenouillet, G., and Brosse, S. 2008b. Null model of
691 biotic homogenization: A test with the European freshwater fish fauna. Divers. Distrib. **14**:
692 291–300. doi:10.1111/j.1472-4642.2007.00409.x.
- 693 Leprieur, F., Olden, J.D., and Lek, S. 2009. Contrasting patterns and mechanisms of spatial
694 turnover for native and exotic freshwater fish in Europe. J. Biogeogr. **36**: 1899–1912.

- doi:10.1111/j.1365-2699.2009.02107.x.
- Leprieur, F., Tedesco, P.A., Hugueny, B., Beauchard, O., Dürr, H.H., Brosse, S., and Oberdorff, T. 2011. Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. *Ecol. Lett.* **14**(4): 325–334. doi:10.1111/j.1461-0248.2011.01589.x.
- Levine, J.M., and D’Antonio, C.M. 1999. Elton revisited: A review of evidence linking diversity and invasibility. *Oikos* **87**(1): 15. doi:10.2307/3546992.
- Lindeman, R.H., Merenda, P.F., and Gold, R.Z. 1980. Introduction to Bivariate and Multivariate Analysis. Scott Foresman & Co., Glenview, Ill.
- Lindsey, C.C., and McPhail, J.D. 1986. Zoogeography of fishes of the Yukon and Mackenzie basins. *In* Zoogeography of North American Freshwater Fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, New York. pp. 639–674.
- Lockwood, J.L., Cassey, P., and Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. *Trends Ecol. Evol.* **20**(5): 223–228. doi:10.1016/j.tree.2005.02.004.
- Lockwood, J.L., Welbourne, D.J., Romagosa, C.M., Cassey, P., Mandrak, N.E., Strecker, A., Leung, B., Stringham, O.C., Udell, B., Episcopo-sturgeon, D.J., Tlusty, M.F., Sinclair, J., Springborn, M.R., Pienaar, E.F., Rhyne, A.L., and Keller, R. 2019. When pets become pests : The role of the exotic pet trade in producing invasive vertebrate animals. : 323–330. doi:10.1002/fee.2059.
- Luck, G.W. 2007. A review of the relationships between human population density and biodiversity. *Biol. Rev.* **82**(4): 607–645. doi:10.1111/j.1469-185X.2007.00028.x.

- 717 Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., and Bazzaz, F.A. 2000.
718 Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol. Appl.*
719 **89**(4): 341–343. doi:10.1890/0012-9623(2008)89[341:iie]2.0.co;2.
- 720 Mandrak, N.E. 1989. Potential invasion of the Great Lakes by fish species associated with
721 climatic warming. *J. Great Lakes Res.* **15**: 306–316.
- 722 Mandrak, N.E. 1995. Biogeographic patterns of fish species richness in Ontario lakes in relation
723 to historical and environmental factors. *Can. J. Fish. Aquat. Sci.* **52**(7): 1462–1474.
724 doi:10.1139/f95-141.
- 725 Mandrak, N.E., and Crossman, E.J. 1992. Postglacial dispersal of freshwater fishes into Ontario.
726 *Can. J. Zool.* **70**(11): 2247–2259. doi:10.1139/z92-302.
- 727 Mandrak, N.E., and Cudmore, B. 2010. The fall of native fishes and the rise of non-native fishes
728 in the great Lakes basin. *Aquat. Ecosyst. Heal. Manag.* **13**(3): 255–268.
729 doi:10.1080/14634988.2010.507150.
- 730 Mandrak, N.E., Curry, A., Dumont, P., Reist, J., Taylor, E., and Watkinson, D. 2022.
731 Zoogeography of freshwater fishes in Canada. *In* *Freshwater fishes and fisheries in Canada.*
732 *Edited by* S. Cooke, C.T. Hasler, and N.E. Mandrak. American Fisheries Society,
733 Baltimore, MD. (accepted)
- 734 Marini, L., Battisti, A., Bona, E., Federici, G., Martini, F., Pautasso, M., and Hulme, P.E. 2012.
735 Alien and native plant life-forms respond differently to human and climate pressures. *Glob.*
736 *Ecol. Biogeogr.* **21**(5): 534–544. doi:10.1111/j.1466-8238.2011.00702.x.
- 737 Marini, L., Gaston, K.J., Prosser, F., and Hulme, P.E. 2009. Contrasting response of native and
738 alien plant species richness to environmental energy and human impact along alpine

- elevation gradients. *Glob. Ecol. Biogeogr.* **18**(6): 652–661. doi:10.1111/j.1466-8238.2009.00484.x.
- Matthews, W.J. 1998. *Patterns in Freshwater Fish Ecology*. Chapman & Hall, New York.
- McAllister, D.E., Platania, S.P., Schueler, F.W., Baldwin, M.E., and Lee, D.S. 1986. Ichthyofaunal patterns on a geographic grid. *In The Zoogeography of North American Freshwater Fishes. Edited by C.H. Hocutt and E.O. Wiley*. John Wiley & Sons, Toronto, ON. pp. 17–51.
- McKinney, M., and Lockwood, J. 1999. Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends Ecol. Evol.* **14**(11): 450–453. Available from <http://www.ncbi.nlm.nih.gov/pubmed/10511724>.
- McKinney, M.L. 2001. Effects of human population, area, and time on non-native plant and fish diversity in the United States. *Biol. Conserv.* **100**(2): 243–252. doi:10.1016/S0006-3207(01)00027-1.
- McKinney, M.L. 2002. Do human activities raise species richness? Contrasting patterns in United States plants and fishes. *Glob. Ecol. Biogeogr.* **11**(4): 343–348. doi:10.1046/j.1466-822X.2002.00293.x.
- Muir, G., Lawrence, E.R., Grant, J.W.A., and Fraser, D.J. 2021. Assessing biodiversity hotspots below the species-level in Canada using designatable units. *Glob. Ecol. Conserv.* **26**. Elsevier Ltd. doi:10.1016/j.gecco.2021.e01506.
- Oberdorff, T., Guégan, J., Hugueny, B., Oberdorff, T., Guegan, J., and Hugueny, B. 1995. Global scale patterns of fish species richness in rivers. *Ecography.* **18**(4): 345–352. Available from <http://www.jstor.org/stable/3682818>.

- 761 Oberdorff, T., Hugueny, B., and Guégan, J.F. 1997. Is there an influence of historical events on
762 contemporary fish species richness in rivers? Comparisons between Western Europe and
763 North America. *J. Biogeogr.* **24**(4): 461–467. doi:10.1111/j.1365-2699.1997.00113.x.
- 764 Oberdorff, T., Tedesco, P.A., Hugueny, B., Leprieur, F., Beauchard, O., Brosse, S., and Dürr,
765 H.H. 2011. Global and regional patterns in riverine fish species richness: A review. *Int. J.*
766 *Ecol.* **2011**. doi:10.1155/2011/967631.
- 767 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson,
768 G.L., Solymos, P., Henry, M., Stevens, H., Szöcs, E., and Wagner, H. 2019. vegan:
769 community ecology package. R package version 2.53-5. Available from [http://cran.r-](http://cran.r-project.org/package=vegan)
770 [project.org/package=vegan](http://cran.r-project.org/package=vegan).
- 771 Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V.A., Webster, A.J.,
772 Ding, T.S., Rasmussen, P.C., Ridgely, R.S., Stattersfield, A.J., Bennett, P.M., Blackburn,
773 T.M., Gaston, K.J., and Owens, I.P.F. 2005. Global hotspots of species richness are not
774 congruent with endemism or threat. *Nature* **436**(7053): 1016–1019.
775 doi:10.1038/nature03850.
- 776 Pearson, K. 1901. On lines and planes of closest fit to systems of points in space. *Philos. Mag.* **2**:
777 559–572.
- 778 Peres-Neto, P.R., and Legendre, P. 2010. Estimating and controlling for spatial structure in the
779 study of ecological communities. *Glob. Ecol. Biogeogr.* **19**(2): 174–184.
780 doi:10.1111/j.1466-8238.2009.00506.x.
- 781 Peres-Neto, P.R., Legendre, P., Dray, S., and Borcard, D. 2006. Variation partitioning of species
782 data matrices: estimation and comparison of fractions. *Ecology* **87**(10): 2614–25. Available

- 783 from <http://www.ncbi.nlm.nih.gov/pubmed/17089669>.
- 784 Prendergast, J.R., Quinn, R.M., Lawton, J.H., Eversham, B.C., and Gibbons, D.W. 1993. Rare
785 species, the coincidence of diversity hotspots and conservation strategies. *Nature*
786 **365**(6444): 335–337. doi:10.1038/365335a0.
- 787 Pyšek, P., Jarošík, V., Hulme, P.E., Kühn, I., Wild, J., Arianoutsou, M., Bacher, S., Chiron, F.,
788 Didžiulis, V., Essl, F., Genovesi, P., Gherardi, F., Hejda, M., Kark, S., Lambdon, P.W.,
789 Desprez-Loustau, M.L., Nentwig, W., Pergl, J., Poboljšaj, K., Rabitsch, W., Roques, A.,
790 Roy, D.B., Shirley, S., Solarz, W., Vilà, M., and Winter, M. 2010. Disentangling the role of
791 environmental and human pressures on biological invasions across Europe. *Proc. Natl.*
792 *Acad. Sci. U. S. A.* **107**(27): 12157–12162. doi:10.1073/pnas.1002314107.
- 793 R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for
794 Statistical Computing, Vienna, Austria. Available from <http://www.r-project.org/>.
- 795 Rahel, F.J., and Olden, J.D. 2008. Assessing the effects of climate change on aquatic invasive
796 species. *Conserv. Biol.* **22**(3): 521–533. doi:10.1111/j.1523-1739.2008.00950.x.
- 797 Rahel, F.J., and Smith, M.A. 2018. Pathways of unauthorized fish introductions and types of
798 management responses. *Hydrobiologia* **817**: 41–56.
- 799 Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A.,
800 MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K.,
801 Vermaire, J.C., Dudgeon, D., and Cooke, S.J. 2019. Emerging threats and persistent
802 conservation challenges for freshwater biodiversity. *Biol. Rev.* **94**(3): 849–873.
803 doi:10.1111/brv.12480.
- 804 Reyjol, Y., Hugueny, B., Pont, D., Bianco, P.G., Beier, U., Caiola, N., Casals, F., Cowx, I.,

- 805 Economou, A., Ferreira, T., Haidvogel, G., Noble, R., de Sostoa, A., Vigneron, T., and
806 Virbickas, T. 2007. Patterns in species richness and endemism of European freshwater fish.
807 Glob. Ecol. Biogeogr. **16**(1): 65–75. doi:10.1111/j.1466-8238.2006.00264.x.
- 808 Ricklefs, R.E. 2004. A comprehensive framework for global patterns in biodiversity. Ecol. Lett.
809 **7**(1): 1–15. doi:10.1046/j.1461-0248.2003.00554.x.
- 810 Rixon, C.A.M., Duggan, I.C., Bergeron, N.M.N., Ricciardi, A., and Macisaac, H.J. 2005.
811 Invasion risks posed by the aquarium trade and live fish markets on the Laurentian Great
812 Lakes. Biodivers. Conserv. **14**(6): 1365–1381. doi:10.1007/s10531-004-9663-9.
- 813 Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause.
814 Oikos **65**(3): 514–527.
- 815 Ross, S.T. 2013. Ecology of North American freshwater fishes. University of California Press,
816 Berkeley, CA.
- 817 Sharma, S., Jackson, D.A., Minns, C.K., and Shuter, B.J. 2007. Will northern fish populations be
818 in hot water because of climate change? Glob. Chang. Biol. **13**(10): 2052–2064.
819 doi:10.1111/j.1365-2486.2007.01426.x.
- 820 Staton, S.K., and Mandrak, N.E. 2006. Focusing Conservation Efforts for Freshwater
821 Biodiversity. In Proceedings of the Parks Research Forum of Ontario Annual Meeting.
822 Edited by G.N. et Al. University of Waterloo, Waterloo, ON. pp. 197–204.
- 823 Stohlgren, T.J., Barnett, D., Flather, C., Fuller, P., Peterjohn, B., Kartesz, J., and Master, L.L.
824 2006. Species richness and patterns of invasion in plants, birds, and fishes in the United
825 States. Biol. Invasions **8**(3): 427–447. doi:10.1007/s10530-005-6422-0.
- 826 Strecker, A.L., Campbell, P.M., Olden, J.D., Strecker, A.L., Campbell, P.M., The, J.D.O., and

- 827 Campbell, P.M. 2011. The aquarium trade as an invasion pathway in the Pacific Northwest.
828 Fisheries **36**: 74–85. doi:10.1577/03632415.2011.10389070.
- 829 Taylor, E.B., Darveau, C.A., and Schulte, P.M. 2013. Setting conservation priorities in a
830 widespread species: Phylogeographic and physiological variation in the lake chub, *Couesius*
831 *plumbeus* (pisces: Cyprinidae). Diversity **5**(2): 149–165. doi:10.3390/d5020149.
- 832 Tedesco, P.A., Oberdorff, T., Lasso, C.A., Zapata, M., and Hugueny, B. 2005. Evidence of
833 history in explaining diversity patterns in tropical riverine fish. J. Biogeogr. **32**(11): 1899–
834 1907. doi:10.1111/j.1365-2699.2005.01345.x.
- 835 Tennekes, M.. 2018. tmap: Thematic Maps in R. J. Stat. Softw. **84**: 1–39.
836 doi:10.18637/jss.v084.i06.
- 837 Tisseuil, C., Cornu, J.F., Beauchard, O., Brosse, S., Darwall, W., Holland, R., Hugueny, B.,
838 Tedesco, P.A., and Oberdorff, T. 2013. Global diversity patterns and cross-taxa
839 convergence in freshwater systems. J. Anim. Ecol. **82**(2): 365–376. doi:10.1111/1365-
840 2656.12018.
- 841 Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S.,
842 Botta-Dukát, Z., Bugmann, H., Czúcz, B., Dauber, J., Hickler, T., Jarošík, V., Kenis, M.,
843 Klotz, S., Minchin, D., Moora, M., Nentwig, W., Ott, J., Panov, V.E., Reineking, B.,
844 Robinet, C., Semenchenco, V., Solarz, W., Thuiller, W., Vilà, M., Vohland, K., and Settele,
845 J. 2009. Alien species in a warmer world: risks and opportunities. Trends Ecol. Evol.
846 **24**(12): 686–693. doi:10.1016/j.tree.2009.06.008.
- 847 Water Survey of Canada. 1977. Reference index hydrometric map supplement. Inland Water
848 Directorate, Water Survey of Canada, Environment Canada, Ottawa.

- 849 Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.
850 Available from <http://ggplot2.org>.
- 851 Wilson, C.C., and Mandrak, N.E. 2021. Paleoecology. *In* The Lake Charr *Salvelinus namaycush*:
852 Biology, ecology, distribution, and management. Fish and Fisheries Series 39. *Edited by*
853 A.M. Muir, C.C. Krueger, M.J. Hansen, and S.C. Riley. Springer Nature. pp. 41–68.
- 854 Xenopoulos, M.A., and Lodge, D.M. 2006. Going with the flow: Using species-discharge
855 relationships to forecast losses in fish biodiversity. *Ecology* **87**(8): 1907–1914.
856 doi:10.1890/0012-9658(2006)87[1907:GWTFUS]2.0.CO;2.

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Figure captions

Fig. 1. Maximum extent of Wisconsinan ice sheet, ca. 18,000 YBP, and of glacial waterbodies, 14,000 YBP – present (based on deglaciation map sequence in Dyke et al. 2003). Length of time (in thousands of years) that areas were covered by glacial waterbodies represented by the gradient of gray shading; The five major Wisconsinan refugia for freshwater fishes are labelled based on Crossman and McAllister (1986). The Map is in Canada Lambert Conformal Conic projection. Adapted by permission from Springer Nature Customer Service Centre GmbH: Wilson and Mandrak (2021), Copyright © 2021 Springer Nature Switzerland AG.

Fig. 2. Total, native, non-native, and at-risk freshwater fish richness in tertiary watersheds across Canada. At-risk richness was estimated based on the “designatable units” identified in the COSEWIC (2019). Species richness of Great Lakes are from Roth et al. (2012). Watershed boundaries (grey lines) are based on third level of the Water Survey of Canada (1977) hierarchical watershed classification. Maps are in Canada Lambert Conformal Conic projection. [Colour online.]

Fig. 3. Results of variance partitioning, showing the contributions environmental, historical, anthropogenic, and spatial explanatory variable groups for explaining richness variation of native, non-native, and at-risk freshwater fish species richness in 985 watersheds of Canada. Each ellipse represents the total variation in richness explained by each variable group. Overlapping sections represent shared variations attributable to two or more variable groups, while non-overlapping sections represent variations uniquely attributable to each variable group.

881 Fractions are expressed as percentages of total variation (based on adj. R^2); * $p < 0.05$. [Colour
882 online.]

883

884 Fig. 4. Relative independent importance of environmental, historical, anthropogenic and spatial
885 predictor variable groups in explaining native, non-native, and at-risk freshwater fish species
886 richness in 985 watersheds of Canada based on LMG metric. The relative independent
887 importance of each predictor variable group is expressed as the percentage of total variation
888 (based on R^2) in each case; Vertical bars represents 95% bootstrap confidence intervals; Letters
889 indicate the ranks covered by bootstrap confidence intervals for each species category.

890

891 Fig. 5. Relative independent importance of environmental, historical, anthropogenic and spatial
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897

898 Fig. 6. Relative independent importance of individual environmental, historical and
899 anthropogenic factors in explaining native, non-native, and at-risk freshwater fish species
900 richness in 985 watersheds of Canada based on LMG metric. The relative independent
901 importance of each predictor is expressed as the percentage of total variation (based on R^2) in
902 each case; Horizontal bars represents 95% bootstrap confidence intervals; * variables with

903 statistically significant ($p < 0.05$) regression coefficient; [+] / [-] represents direction of the
904 relationship (based on sign of the regression coefficient); For variable codes, see Table 1.

905

906 Fig. 7. Relative independent importance of individual environmental, historical and
907 anthropogenic factors in explaining foreign and translocated non-native freshwater fish species
908 richness in 985 watersheds of Canada based on LMG metric. The relative independent
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910 each case; Horizontal bars represents 95% bootstrap confidence intervals; * variables with
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912 relationship (based on sign of the regression coefficient); For variable codes, see Table 1.

Table 1. Environmental, historical, anthropogenic, and spatial variables used to explain the variation in native, non-native, and at-risk freshwater fish richness of 985 watersheds in Canada.

Abbreviation	Description
<i>Environmental variables</i>	
Clim_PC1	PCA (performed on bioclimatic variables) Axis 1 scores representing temperature and summer/total precipitation related variables i.e. Mean annual temperature (°C), mean temperature during warmest quarter (MTWQ; °C), mean temperature during coldest quarter (MTCQ; °C), continentality (MTWQ – MTCQ; °C), annual precipitation (mm), precipitation seasonality, summer (June to August) precipitation (mm), annual potential evapotranspiration (mm year ⁻¹), frost free period (days) and degree-days above 5°C; The data are averages for the years 1970-2000.
Clim_PC2	PCA (performed on bioclimatic variables) Axis 2 scores representing aridity and winter precipitation related variables i.e. precipitation as snow (mm), winter (Dec to Feb) precipitation (mm), evapotranspiration seasonality (mm month ⁻¹), Thornthwaite's aridity index, Hargreave's climatic moisture index and mean annual solar radiation (MJ m ⁻² d ⁻¹); The data are averages for the years 1970-2000.
Elev	Mean elevation (m)
TRI	Mean Terrain Roughness Index (proxy for habitat heterogeneity)

Area Watershed area (km²)

Historical variables

NRDist Distance from watershed centroid to approximate centroid of nearest major refuge i.e. Atlantic, Mississippian, Missourian, Pacific and Beringian (km)

GLDist Distance from watershed centroid to nearest glacial waterbody (km)

IEDist distance from the watershed centroid to closest ice edge at the glacial maximum i.e. ca. 18,000 ybp (km)

IFDur Duration uncovered by ice (ice cover <50% of watershed area) since the glacial maximum i.e. ca. 18,000 ybp (1000 years)

GWDur Duration covered by glacial waterbodies (waterbody extent >25% of watershed area) since ca. 18,000 ybp (1000 years)

*Anthropogenic
variables*

Urban_PC1 Axis 1 scores of PCA performed on human population density related variables i.e. population density (individuals per km²), dwelling density (no. of dwellings per km²), % human settlement area (percentage area of watershed), % impervious surface (percentage area of watershed) and average nighttime light (average per watershed; represent human infrastructure in rural and suburban areas that are not well represented in % human settlement area data); The data are for the years 2010-2016.

CAFD	Crop and animal farm density (count per 1000 km ² of watershed area); The data are for the year 2016.
CLD	Cropland density (km ² per 1000 km ² of watershed area); The data are for the year 2000.
PLD	Pastureland density (km ² per 1000 km ² of watershed area); The data are for the year 2000.
ManuD	Density of manufacturing facilities (count per 1000 km ² of watershed area); The data are for the year 2008.
UtilD	Density of facilities for utilities (count per 1000 km ² of watershed area); The data are for the year 2008.
MiniD	Density of mining facilities (count per 1000 km ² of watershed area); The data are for the year 2008.
RD	Road density (km per 1000 km ² of watershed area); The data are for the years 1980-2010.
DamD	Large (height >15m and/or reservoir volume >3 million m ³) dam density (count per 1000 km ² of watershed area); The data are for the year 2019.

Spatial factors

gb-MEM	Graph-based Moran’s eigenvector maps
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Table 2. Spearman rank correlation coefficients among richness of freshwater fish species categories in 985 watersheds of Canada.

Variable	Total richness	Native richness	Overall non-native richness	Foreign non-native richness	Translocated non-native richness
Native richness	0.99*				
Overall non- native richness	0.64*	0.57*			
Foreign non- native richness	0.48*	0.43*	0.78*		
Translocated non-native richness	0.65*	0.59*	0.96*	0.63*	
At-risk species richness	0.47*	0.46*	0.44*	0.40*	0.40*

Note: * $p < 0.0001$

Fig. 1. Maximum extent of Wisconsin ice sheet, ca. 18,000 YBP, and of glacial waterbodies, 14,000 YBP – present (based on deglaciation map sequence in Dyke et al. 2003). Length of time (in thousands of years) that areas were covered by glacial waterbodies represented by the gradient of gray shading; The five major Wisconsin refugia for freshwater fishes are labelled based on Crossman and McAllister (1986). The Map is in Canada Lambert Conformal Conic projection. Adapted by permission from Springer Nature Customer Service Centre GmbH: Wilson and Mandrak (2021), Copyright © 2021 Springer Nature Switzerland AG.

243x187mm (150 x 150 DPI)

Fig. 2. Total, native, non-native, and at-risk freshwater fish richness in tertiary watersheds across Canada. At-risk richness was estimated based on the “designatable units” identified in the COSEWIC (2019). Species richness of Great Lakes are from Roth et al. (2012). Watershed boundaries (grey lines) are based on third level of the Water Survey of Canada (1977) hierarchical watershed classification. Maps are in Canada Lambert Conformal Conic projection. [Colour online.]

181x181mm (300 x 300 DPI)

Fig. 3. Results of variance partitioning, showing the contributions environmental, historical, anthropogenic, and spatial explanatory variable groups for explaining richness variation of native, non-native, and at-risk freshwater fish species richness in 985 watersheds of Canada. Each ellipse represents the total variation in richness explained by each variable group. Overlapping sections represent shared variations attributable to two or more variable groups, while non-overlapping sections represent variations uniquely attributable to each variable group. Fractions are expressed as percentages of total variation (based on adj. R2); *p < 0.05. [Colour online.]

Fig. 4. Relative independent importance of environmental, historical, anthropogenic and spatial predictor variable groups in explaining native, non-native, and at-risk freshwater fish species richness in 985 watersheds of Canada based on LMG metric. The relative independent importance of each predictor variable group is expressed as the percentage of total variation (based on R^2) in each case; Vertical bars represents 95% bootstrap confidence intervals; Letters indicate the ranks covered by bootstrap confidence intervals for each species category.

179x109mm (600 x 600 DPI)

Fig. 5. Relative independent importance of environmental, historical, anthropogenic and spatial predictor variable groups in explaining foreign and translocated non-native freshwater fish species richness in 985 watersheds of Canada based on LMG metric. The relative independent importance of each predictor variable group is expressed as the percentage of total variation (based on R2) in each case; Vertical bars represents 95% bootstrap confidence intervals; Letters indicate the ranks covered by bootstrap confidence intervals for each species category.

80x104mm (600 x 600 DPI)

Fig. 6. Relative independent importance of individual environmental, historical and anthropogenic factors in explaining native, non-native, and at-risk freshwater fish species richness in 985 watersheds of Canada based on LMG metric. The relative independent importance of each predictor is expressed as the percentage of total variation (based on R^2) in each case; Horizontal bars represents 95% bootstrap confidence intervals; * variables with statistically significant ($p < 0.05$) regression coefficient; [+] / [-] represents direction of the relationship (based on sign of the regression coefficient); For variable codes, see Table 1.

179x189mm (600 x 600 DPI)

Fig. 7. Relative independent importance of individual environmental, historical and anthropogenic factors in explaining foreign and translocated non-native freshwater fish species richness in 985 watersheds of Canada based on LMG metric. The relative independent importance of each predictor is expressed as the percentage of total variation (based on R2) in each case; Horizontal bars represents 95% bootstrap confidence intervals; * variables with statistically significant ($p < 0.05$) regression coefficient; [+] / [-] represents direction of the relationship (based on sign of the regression coefficient); For variable codes, see Table 1.

129x189mm (600 x 600 DPI)