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Evaluating functional diversity conservation for freshwater fishes resulting from terrestrial protected areas

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Abstract

1. Protected areas are one of the hammers in conservation toolkits, yet few protected areas exist that were designed to protect freshwater ecosystems. This is problematic as freshwater ecosystems are among the most threatened systems on earth. Nonetheless, terrestrial protected areas (TPAs) may afford spillover benefits to freshwater ecosystems

included within their boundaries, but evaluations of these potential benefits for the protection of freshwater fish diversity are lacking.

2. Using fish community data from 175 lakes inside, outside, or bordering TPAs in Ontario, Canada, we sought to determine if TPAs preserve fish functional diversity. We focused on functional diversity because previous work indicated no taxonomic differences between these lakes, but a difference in normalized-length size-spectra slopes inside versus outside TPAs (indicator of unique predator-prey ratios and trophic energy transfer). We expected that communities inside TPAs would show greater functional diversity (i.e., functional dispersion and functional richness) and have more extreme trait combinations (i.e., functional divergence) than communities outside or bordering TPAs. As well, we tested for differences in the rarity of species-specific functional traits between fish communities inside, outside, or bordering TPAs, between thermal guilds, and across average body size and overall prevalence of the species.
3. Our results indicated no significant differences in functional diversity among lake fish communities inside, outside, or bordering TPAs. However, fish communities inside TPAs had more extreme trait combinations than outside TPAs because abundant species in lake communities outside TPAs had more ubiquitous trait combinations than abundant fishes inside TPAs.
4. Small-bodied species showed greater functional rarity than large-bodied species indicating that small-bodied fishes fill functionally unique roles while the most prevalent, large-bodied species possess a more generalist set of traits.
5. Overall, the similarity of functional diversity metrics for lake fish communities inside, outside, or bordering TPAs in Ontario suggests that TPAs capture the functional diversity of Ontario's lake fish communities. However, we encourage similar evaluations in regions where environmental conditions and stressors are more distinct across TPA boundaries than they are in Ontario, as these types of evaluations will inform guidelines for the design of freshwater protected areas and monitoring of their effectiveness in the future.

1. Introduction

Freshwater ecosystems provide society with many ecosystem services such as safe drinking water, food, and places for recreation (Aylward et al., 2005); however, they are also among the most threatened on our planet (Dudgeon et al., 2006; Carpenter et al., 2011; Dudgeon, 2014; Reid et al., 2018), risking the delivery of these services to society. Examples of the disturbances impacting freshwater ecosystems include physical and chemical habitat degradation (Smith et al., 1999; Arthington et al., 2016; Dugan et al., 2017), invasive species (Dextrase & Mandrak, 2006), microplastics (Eerkes-Medrano et al., 2015), and global climate change (Poesch et al., 2016; Myers et al., 2017). These disturbances can interact and their effects can accumulate in downstream systems (Schindler, 2001; Jackson et al., 2016; Nõges et al., 2016). As a result, extinction rates of freshwater biota are estimated to be 112 to 855 times higher than rates for terrestrial species (Collen et al., 2014; Tedesco et al., 2017). For example, between 1896 and 2006, 57 North American freshwater fish species went extinct (Burkhead, 2012) and in Canada, 71 of the 207 native fish species are considered at risk by the Committee on the Status of Endangered Wildlife in Canada (Dextrase & Mandrak, 2006; Lamothe et al., 2019). As such, improvements to the protection of freshwater ecosystems and continued restoration is needed to sustain future freshwater ecosystem services provisioning.

The need to protect and conserve freshwater ecosystems is particularly relevant in Canada, which contains approximately seven percent of all the renewable liquid freshwater (i.e., lakes and streams) on Earth (Environment and Climate Change Canada, 2017a). Protection of this resource is primarily achieved through federal legislation related to environmental protection (e.g., *Canada Water Act*; Government of Canada, 1985a), fisheries (e.g., *Fisheries Act*; Government of Canada, 1985b), and protection of species at risk of extinction (*Species at Risk Act*; Government of Canada, 2002). Additionally, terrestrial protected areas (TPAs), or areas where the land and water are protected for the purpose of conserving nature (Environment and Climate Change Canada, 2017b), can provide protection for freshwater ecosystems (Dudley, 2008). For example, areas within TPAs have been shown to have more species than areas outside of TPAs (Gray et al., 2016) and their designation can lead to fewer anthropogenic disturbances in an area (Bruner et al., 2001). However, due to the connectivity of freshwater ecosystems, occurrence of freshwater species within TPAs does not necessarily imply protection (Pimm et al., 2014; Harrison et al., 2016).

Compared to terrestrial organisms, there have been relatively few studies investigating the degree of protection TPAs may provide freshwater fishes. Chu et al. (2018) recently demonstrated no statistically significant differences in species-level diversity (i.e., Shannon's index) or catch-per-unit effort (CPUE) between fish communities inside, bordering, or outside TPAs in Ontario, Canada. However, CPUE and species diversity was generally higher inside TPAs than outside. In addition, the normalized-length size-spectra slopes, an indicator of predator-prey ratios and trophic-energy transfer of fish communities, were significantly steeper in fish communities outside TPAs compared to communities situated inside TPAs. This difference in size spectra slope was attributed to a greater abundance (albeit statistically insignificant) of small-bodied, pollution and turbidity tolerant species in lakes outside TPAs and differences in turbidity and angling pressure in lakes outside versus inside TPAs. Chu et al. (2018) concluded that taxonomic indicators may not be the most useful indicators for evaluating the effectiveness of TPAs for protecting freshwater ecosystems.

In some cases, traditional taxonomic-based diversity metrics fail to respond to changes in community structure resulting from environmental disturbances, for example, in the case of species invasions (e.g., Sagouis et al., 2016). As such, incorporating multiple diversity metrics into conservation strategies can provide a more robust and systematic framework for conservation resource allocation (Strecker et al., 2011). Functional diversity metrics, for example, relate the characteristics of individuals or species to the structures and functions of ecosystems and have been used to identify mechanisms of biodiversity loss (Cardinale et al., 2012) and vulnerability of biotic communities to future disturbance (Mouillot et al., 2014; Lamothe et al., 2018a). Recent research suggests that although extinction rates for riverine fishes are on the rise, functional diversity at the river-basin scale has increased by approximately 150% because of the introduction of non-native species and their unique functional characteristics that were historically absent, particularly in low-diversity systems (Toussaint et al., 2018).

Few studies have explored the effectiveness of conservation programs for protecting functional diversity and the relative degree of protection that TPAs provide to freshwater fish species. Britton et al. (2017) found that cichlid communities in water adjacent to TPAs had more herbivorous and specialist species, and more similar fish assemblages than areas farther from the TPAs. Similarly, Wilkinson et al. (2018) found greater local fish functional richness in headwater streams surrounded by protected areas compared to streams coursing through logged forests or oil-palm plantations. As such, our objectives were to determine if TPAs preserve functional diversity of lake fish communities by comparing community-level and species-specific functional diversity metrics between fish communities in lakes inside, outside, and bordering TPAs. We expected that functional diversity (i.e., functional dispersion and functional richness) may be more sensitive than taxonomic diversity (examined by Chu et al., 2018). Given sufficient time since protection and stress outside of protected areas, we expected fish communities inside TPAs to show greater functional diversity than communities outside or bordering TPAs. Further, given the recent findings of Toussaint et al. (2018), we expected higher functional divergence (i.e., more extreme trait combinations) outside TPAs compared to lake communities inside or bordering TPAs.

2. Methods

2.1 Study lakes

Ontario, Canada spans a large geographic area, covering more than 100 million ha with approximately 250,000 freshwater lakes (Lester et al., 2003). Four freshwater ecoregions are represented in Ontario (Figure 1; St. Lawrence, Laurentian Great Lakes, Southern Hudson Bay, and English-Winnipeg Lakes; Abell et al., 2008) with the Laurentian Great Lakes and St. Lawrence ecoregions having the highest freshwater fish species richness in Canada (Chu et al., 2015). Fish species richness in Ontario lakes ranges from 113 species in southern lakes to fewer than 10 species in northern lakes (OMNRF, 2015). As with other north temperate regions of the world, species richness and productivity are influenced by postglacial recolonization, climate, lake morphometry, and water chemistry (Mandrak, 1995). Anthropogenic disturbances are greatest along the shorelines of the Great Lakes and decrease at higher latitudes with relatively pristine conditions in the central and northern regions of the province (Chu et al., 2015). There are currently 661 federal or provincial TPAs in Ontario that cover approximately 10% of the province (IUCN, UNEP-WCMC, 2016).

To control for the impacts of lake and regional environmental factors, which were not the focus of this study but are known to impact fish diversity and abundance (e.g., Chu et al., 2016), we paired lakes inside, bordering, and outside TPAs with similar abiotic characteristics. We used the database of paired lakes and their respective fish communities from Chu et al. (2018) to investigate differences in functional diversity characteristics inside, bordering, or outside TPAs (Figure 1). 'Inside' lakes are completely contained inside a TPA, 'outside' lakes are completely outside TPAs, and 'border' lakes are those with part, but not all, of their shoreline inside a TPA. Lakes were paired using single-linkage nearest-neighbour cluster analysis based on total precipitation, growing-degree days above 5°C, lake surface area, and mean depth (Table 1), followed by three tests of robustness to confirm pairing decisions (Chu et al., 2018). The selection process generated 40 pairs of lakes for inside-outside comparisons, 15 pairs of lakes for the inside-border comparisons, and 42 pairs of lakes for border-outside comparisons for a total of 194 lakes. However, 16 lakes were paired twice in either inside-outside, inside-border, or border-outside group, two were paired twice within the border-outside group, and one was paired twice in the border-inside group. Therefore, the number of unique lakes was 175. Lake characteristics varied widely across the 175 study lakes, with total annual precipitation ranging from 679 – 1135 mm, growing degree days above 5°C from 1234 – 2218, lake area from 33 to 17,402 ha, and mean depths from 1.1 – 40.1 m.

2.2 Fish sampling

Fishes were sampled using the Ontario Ministry of Natural Resources and Forestry's Broad-scale Monitoring for Inland Lakes protocol, which outlines standardized methods for sampling fishes, invertebrates, water quality, and angler activities across lakes (see Sandstrom et al., 2011). North American standard large-mesh multi-panel gill nets and Ontario standard small-mesh multi-panel gill nets were used to catch large-bodied (e.g., Lake Trout *Salvelinus namaycush* Salmonidae and Walleye *Sander vitreus* Percidae) and small-bodied fishes (e.g., Fathead Minnow *Pimephales promelas* Leuciscidae or juveniles of large-bodied species), respectively. Gill nets were set following a randomized, depth-stratified design for approximately 18-hour time intervals. Each sampled fish was identified to species, counted, and total lengths were measured.

2.3 Functional trait space

Fish trait variables (*n* = 17) were gathered from various sources (Coker et al., 2001; Frimpong & Angermeier, 2009; Holm et al., 2009; Eakins, 2017) to characterize the ecological niche of each species (Givan et al., 2017; Lamothe et al., 2018a) including components of the species' diet, modes of reproduction, substrate associations, and habitat-depth relationships (Table 1). Diet traits were sourced from the literature and included binary variables representing a preference for algae, phytoplankton, or filamentous algae, macrophytes and vascular plants, detritus or unidentifiable vegetative matter, fish, crayfish, crabs, or frogs, and eggs of fish or other organisms (Frimpong & Angermeier, 2009; Table 1). Mode of reproduction for each species was characterized by two binary variables based on whether fishes guard their brood and spawn on open substrate or hide or create nests for their brood (Table 1). Substrate associations included seven binary variables indicative of preferences for muck substrate, clay or silt substrate, sand substrate, gravel substrate, cobble substrate, boulder substrate, and bedrock (Table 1). Habitat-depth relationships were measured in the field and represent the depth at which species were caught including median depth, maximum depth, and minimum depth (Table 1). Finally, we calculated the average maximum total lengths (mm) of the top 5% of individual fish captured per species after removing the top 2% (to remove any true or false giants that may skew the distribution), and we retrieved data on the thermal preference of species (cold-water, cold/cool-water, cool-water, cool/warm-water, and warm-water; Coker et al., 2001) for post-hoc comparisons.

To build the functional trait space, we first performed four PCAs to combine traits from within each of the four trait type categories (diet, substrate, habitat, and reproduction – Table 1) into respective trait dimensions (Lamothe et al., 2018a). By combining the traits into their respective trait dimensions, we place similar weights on the various trait types when building the functional trait space. Hellinger transformations were performed on the trait data (Ochiai, 1957; Hubálek, 1982; Legendre & Gallagher, 2001) and nontrivial axes were determined through permutation analysis (Peres-Neto et al., 2003, 2005) where we permuted each column of the Hellinger-transformed species composition matrix and performed subsequent PCAs 9,999 times (Lamothe et al., 2018b). Axes were retained if the proportion of variance explained in the empirical data exceeded 95% of the permuted PCAs for that component. In total, one axis was extracted from each of the reproduction, diet, substrate, and habitat analyses, totaling four dimensions for each species. We then performed a principal coordinates analysis (PCoA) on the Euclidean distances of the four trait dimensions to define the functional trait space (Laliberté et al., 2010; Winemiller et al., 2015).

2.4 Functional diversity metrics

We calculated three functional diversity metrics for each fish community: functional dispersion (Anderson, 2006; Laliberté & Legendre, 2010), functional richness (Mason et al., 2005; Villéger et al., 2008), and functional divergence (Mason et al., 2005; Villéger et al., 2008).

Functional dispersion is the average distance of each species in functional trait space to the centroid of all species in a community (Laliberté & Legendre, 2010); greater functional dispersion indicates a more functionally diverse community. Functional dispersion was calculated using all available PCoA axes and were weighted by the relative CPUE of each species. Functional richness was calculated as the convex hull area of each community (Villéger et al., 2008), where greater functional richness indicates greater functional diversity. We calculated a convex hull area using the first two axes of the PCoA because one of the lakes in the analysis contained only three species, and therefore two axes is the maximum number of axes that allows for the number of species to be greater than the number of traits (Villéger et al., 2008). Finally, functional divergence describes the position of species relative to the border of the functional trait space (Villéger et al., 2008) and provides a measure of how the abundance of a community is distributed toward the extremities of occupied trait space (Mouchet et al., 2010; Mason et al., 2012). Functional divergence approaches zero when abundant species are close to the centre of functional trait space and it approaches one when abundant species are distant from the centre of functional trait space (Mouillot et al., 2011). Functional divergence should increase when niche complementarity enhances species' relative abundances (Mason et al., 2012).

We also calculated two species-specific measures of functional rarity from the Euclidean distance trait matrix: functional distinctiveness and functional uniqueness (Violle et al., 2017). Functional rarity describes the degree to which particular species possess traits that are rare or unique to the assemblage. Functional distinctiveness (*D*) is calculated as the average distance of each species to all other species within a local community, whereas functional uniqueness (*U*) describes the distance of each species to the nearest neighbour within the regional species pool (Buisson et al., 2013; Mouillot et al., 2013; Violle et al., 2017).

2.5 Statistical analyses

We used paired *t*-tests to test for differences in functional dispersion, functional richness, and functional divergence between lakes inside versus outside TPAs, lakes inside versus bordering TPAs, and lakes bordering versus outside TPAs. Significance was assessed at $\alpha = 0.01$ to account for multiple comparisons. Functional diversity can increase with species richness and saturation in this relationship has been used as an indicator of functional redundancy (Micheli & Halpern, 2005; Lamothe et al., 2018a). Therefore, we built generalized additive models (GAMs) to investigate the relationship between functional dispersion, functional richness, and functional divergence with species richness for lake communities inside, bordering, and outside TPAs following the procedures of Rose et al. (2012). We fit interaction models of functional diversity as:

$$y_{ij} = \alpha_0 + \alpha_{1j} Category_{ij} + f_j(R_i) + \varepsilon_i, \varepsilon_i \sim N(0, \sigma^2)$$

where α_0 is the model intercept, α_1 is the difference between the mean response for the *j*th lake category (communities inside, bordering, or outside TPAs) and α_0, $f_j()$ are centered, smooth functions of species richness (R) for the *j*th lake category, and ε are the Gaussian distributed residuals with mean 0 and variance σ^2. We compared the fitted smooth functions between lake categories by first building a prediction matrix $\mathbf{X_p}$ related to the fitted values of functional diversity ($\hat{y}_p$) for a set of new data points **p**. The rows of $\mathbf{X_p}$ were then subtracted from one another for pairwise comparisons between lakes inside, outside, or bordering TPAs. Approximate 95% confidence intervals for the differences between pairs of smooth functions were then generated and plotted (Rose et al., 2012); areas where the confidence interval overlaps zero indicates no pairwise difference in smooth functions between lake categories.

We used the 'FD' (Laliberté & Legendre, 2010; Laliberté et al., 2014), 'funrar' (Grenié et al., 2016; Grenié et al., 2017), 'ggplot2' (Wickham, 2009), mgcv (Wood, 2004, 2006), and 'vegan' (Oksanen et al., 2018) packages in the R Statistical Software (R Core Team, 2018) for graphing and analyses.

3. Results

3.1 Taxonomic diversity

A total of 71 species were sampled from the study lakes (Table 2). Fishes of the Leuciscidae family (formerly Cyprinidae; Tan & Armbruster, 2018) were the most prevalent family among the studied lakes with 19 species represented. On average, each lake contained 11.88 ± 3.98 SD species, with a minimum of three species per lake and a maximum of 24 species (median: 12 species). Total CPUE per lake ranged from 11.72 to 1,478.52.

3.2 Functional diversity

Single axes were extracted from each of the reproduction, diet, substrate, and habitat PCAs, explaining 80.0%, 40.9%, 32.4%, and 91.2% of the variation, respectively. To build our multidimensional functional trait space, two axes were extracted from a PCoA of the four PCA trait axes providing a moderate reduced-space quality (59.0% of the total variation; Figure 2). Removing the single lake with three species (and its paired lake) from the analysis and using three PCoA axes to build trait space did not change our results but produced a higher quality functional trait space. We chose to include the three species lake community in the analysis because three and four species lakes are common in the region. Fitting the PCA vectors to the PCoA biplot indicated that the first component was primarily related to habitat depth and reproduction traits while the second component was influenced by substrate and diet preferences (Figure 2).

There were no significant differences in functional dispersion or functional richness across pairwise comparisons of fish communities inside versus outside TPAs, inside versus bordering TPAs, or bordering versus outside TPAs (Table 3). However, fish communities inside TPAs had substantially higher functional divergence than fish communities outside TPAs, indicating that fish communities inside TPAs may show greater niche complementarity than communities outside TPAs.

No significant differences were found in the relationship between functional richness and species richness or functional divergence and species richness across pairwise comparisons of lakes that border TPAs, are inside TPAs, or are outside TPAs (Figures S1, S2). In contrast, significant differences were observed in the relationships between functional dispersion and species richness (Figure 3A); lake communities outside TPAs showed significantly greater functional dispersion than lake communities bordering TPAs at species richness levels less than 12, and significantly lower functional dispersion at species richness greater than 12 (Figure 3C). There were no differences in the functional dispersion and species richness relationship between lake communities inside versus outside TPAs (Figure 3B) or bordering versus inside TPAs (Figure 3D).

Species that were captured in the greatest number of lakes had the least distinct (β = -0.55 ± 0.10 SE; t = -5.51, p < 0.001; Figure 4A) and least unique (β = -0.14 ± 0.05 SE; t = -2.65, p = 0.01; Figure 4D) ecological niches. Similarly, large-bodied species were less distinct (β = -1.13 ± 0.30 SE; t = -3.74, p < 0.001; Figure 4B) than small-bodied species, but there was no significant pattern observed between uniqueness and body size (β = -0.09 ± 0.16 SE; t = -0.59, p = 0.56; Figure 4E). Bridle Shiner *Notropis bifrenatus* (Leuciscidae), Threespine Stickleback *Gasterosteus aculeatus* (Gasterosteidae), and Green Sunfish *Lepomis cyanellus* (Centrarchidae) showed the highest average functional rarity across rarity measures (i.e., uniqueness and distinctiveness; Figure 5) and were clustered on the negative end of the first functional trait space axis (Figure 2). Bridle Shiner and Green Sunfish were only sampled inside TPAs and were rare (average CPUE: 0.003 and 0.076, respectively), whereas Threespine Stickleback was only sampled in a single lake bordering TPAs (CPUE: 0.250). In contrast, Trout-Perch *Percopsis omiscomaycus* (Percopsidae), Cisco *Coregonus artedi* (Salmonidae), and Lake Whitefish *Coregonus clupeaformis* (Salmonidae) showed the lowest functional rarity (Figure 5) and were clustered on the positive side of the first component, near the origin (Figure 2). Trout-perch, Cisco, and Lake Whitefish were among the most abundant species and occurred across all lake types. There were no significant differences in uniqueness or distinctiveness across thermal preference groups (distinctiveness: $F_{4,66}$ = 0.90, p = 0.47; uniqueness: $F_{4,66}$ = 0.20, p = 0.94; Figure 4C, F).

4. Discussion

Similar to results based on taxonomic indices (Chu et al., 2018), we found few differences in functional diversity of lake fish communities inside, bordering, or outside TPAs. Of the differences we did find, our results indicated that lake fish communities inside TPAs had greater functional divergence than communities outside TPAs, regardless of species richness. This result indicates that, on average, abundant species in fish communities inside TPAs had more extreme (i.e., divergent) trait values than abundant species outside TPAs. According to the principle of limiting similarity (MacArthur & Levins, 1967), species with limited functional overlap with the rest of an assemblage should increase in abundance within TPAs because they are less limited by competition than species with common functions. For example, Mouillot et al. (2008) observed increases in the abundance of functionally original fishes after protection of a reserve in the Mediterranean Sea. In our study, the abundance distribution of species was more heavily skewed in lakes outside TPAs compared to lakes inside TPAs and fish communities inside TPAs also had greater Bray-Curtis dissimilarity than communities outside TPAs (by pairwise permutational analysis of variance, not shown), suggesting greater differences in community composition among lakes inside TPAs than outside. The greater abundances of generalist species in lakes outside TPAs is similar to Britton et al. (2017) who found more generalist species in areas of Lake Tanganyika that were farther from protected areas.

We also found differences in functional dispersion between lake communities bordering versus outside TPAs, where in the most speciose lakes (>12 species), functional dispersion was higher among fish communities in lakes bordering TPAs compared to those outside TPAs. Lakes bordering TPAs had a similar taxonomic composition to lakes outside TPAs but had a higher CPUE (Chu et al., 2018). As well, although not statistically significant, fish communities in lakes bordering TPAs that contained 12 or more species had a greater mean convex hull volume (8.23 ± 0.08 SE) than fish communities in lakes outside of TPAs (7.79 ± 0.07 SE), indicating greater spread across functional trait space in lakes bordering TPAs. It seems possible that species with divergent trait values, which are abundant inside TPAs are spilling over (e.g., from marine reserves; Roberts et al., 2001) in to hydrologically-connected lakes bordering TPAs leading to these patterns, but this hypothesis has yet to be tested directly.

From a species-specific perspective, our results indicate that smaller-bodied species are characterized by the greatest functional distinctiveness, that is, they fill functionally unique roles in our study lakes. This makes intuitive sense given that smaller-bodied species are inherently restricted by gape limitations and swimming capacity due to size alone. This result is in contrast with Lamothe et al. (2018a), who found no differences in distinctiveness with average total body length across fishes in Ontario lakes; however, the habitat traits used in our study, namely depth and body size, are based on empirical measures from the field whereas Lamothe et al. (2018a) used data based on literature sources, making comparisons difficult. Finally, our results confirmed expectations that the most prevalent species across the studied lakes had the lowest average functional distinctiveness and uniqueness, suggesting that common species possess a more generalist set of traits, forming an average ecological niche.

There are several potential reasons why we found few differences in functional diversity of lake fish communities inside, bordering, and outside TPAs. First, many of the lakes paired for the inside and outside TPA comparisons are in areas of low disturbance and where environmental conditions and anthropogenic stresses are similar across TPA boundaries (Chu et al., 2015, 2018). This homogeneity makes the benefits of protections implemented in most TPAs (see OMNR, 2011) less noticeable or only obvious if and when stresses (e.g., forestry or industrial development) outside TPAs increase. Second, differences in functional diversity may not be detectable as a result of our study design. Pairing lakes based on ecologically relevant abiotic characteristics allowed us to examine the potential impact of TPAs while controlling for other factors that are known to impact functional diversity, but also limited our sample size. Third, functional diversity measures are influenced by which traits are included in their calculation (Cadotte et al., 2011). Here, we chose to include traits related to diet, substrate, depth, and reproduction to broadly encompass the ecological niche of fishes. Significant differences may have been observed had we defined trait space differently. Finally, sampling bias may have played a role in our inability to detect differences between lake communities. We found that the smallest species were the most functionally distinct, but these species are also least likely to be captured in the gill nets. Given their body sizes, they are not as likely as large-bodied species to encounter the nets (Minns, 1995) and they are susceptible to only the smallest mesh sizes (13 and 19 mm stretch mesh) used in the BsM protocol. The effects of size-based sampling could be magnified in abundance-weighted diversity metrics, but running our analysis with presence-absence data did not change our results.

Functional similarity of fish communities inside and outside TPAs suggests that lakes within designated TPAs capture representative samples of lake fish diversity and the services these fishes provide. We suggest that functional diversity should be considered in future monitoring of these lake communities as a means to assess the effectiveness of protection for fish diversity. As fish species distributions and changes in relative abundance have already been observed with climate change, it will be particularly interesting to document how increasing lake temperatures impact functional diversity (Chu et al., 2005; Alofs et al., 2014; Hansen et al., 2017). Based on our results, and those of Lamothe et al. (2018a), there seems to be little relationship between functional rarity and thermal preference in Ontario lake fishes. However, we found small-bodied species showed the greatest functional rarity and large, common species showed the most average functional niches. Of the 21 species in Ontario listed for protection under the federal Canadian *Species at Risk Act* (Government of Canada 2002) as *Special Concern*, *Threatened*, *Endangered*, or *Extirpated*, nine (~43%) have an average total length of less than 10 cm (Holm et al., 2009); this includes Bridle Shiner and Deepwater Sculpin *Myoxocephalus thompsonii* (Cottidae) which were sampled in the studied lakes. Taken together, conservation of small-bodied species should be prioritized to maintain the functional diversity of Ontario lakes.

Almost 15% of the terrestrial landscape is protected (World Bank Group, 2019), yet few explicitly freshwater protected areas have been established worldwide (Saunders et al., 2002). Although we detected few differences in functional diversity, we encourage similar evaluations in high contrast areas where environmental conditions and stressors are distinct across TPA boundaries and in different types of freshwater ecosystems. For example, Wilkinson et al. (2018) found greater local species richness, greater functional richness, and no difference in functional divergence in protected streams in Borneo. This was driven by the presence of several endemic, specialist species in streams within protected areas, which is not consistent with our findings for lakes. More evaluations of the effectiveness of existing protected areas for freshwater ecosystems will improve our understanding of the benefits and shortcomings of them and inform the development of guidelines for freshwater protected area designs and for monitoring their ecosystem services and success in the future.

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Figure Captions

Figure 1. Location of the 175 study lakes sampled to compare the functional diversity of fish assemblages in Ontario, Canada. Dark borders within Ontario demarcate the four freshwater ecoregions represented in the province.

Figure 2. Functional trait space generated from a principal coordinates analysis of species traits. Species codes are presented in Table 2.

Figure 3. A) Fitted generalized additive model for functional dispersion and species richness for lakes bordering, outside, and inside of terrestrial protected areas (TPAs). B) Difference between fitted smooth functions (difference in trends; solid line) and approximate 95% pointwise confidence intervals (shaded region) on this difference for functional dispersion and species richness between lake communities inside and outside of TPAs, C) bordering and outside of TPAs, and D) bordering and inside of TPAs.

Figure 4. Functional distinctiveness (A-C) and uniqueness (D-F) across log-transformed species prevalence, log-transformed average maximum total length (cm), and thermal preference groups.

Figure 5. Functional rarity measures for 71 observed species. Species are ordered based on the average of the scaled and centered functional rarity measures (i.e., uniqueness, distinctiveness) across lake communities.

Table 1. Lake characteristics and fish traits descriptions.

Variable	*Units/Variable type*	*Description*
Lake characteristics		
Surface area	ha	Surface area of lake
Mean depth	m	Mean depth of lake
Total precipitation	mm	Total precipitation based on 1981-2010 climate normals
Growing degree days	Continuous	Growing degree days above 5°C based on 1981-2010 climate normals
TDS	mg/L	Concentration of total dissolved solids
Elevation	m	Mean elevation of lake
Depth		
Mean depth	m	Mean depth of species in water column
Maximum depth	m	Maximum depth of species in water column
Minimum depth	m	Minimum depth of species in water column
Diet		
Algae	Binary	Feeds on algae, phytoplankton, or filamentous algae
Macrophyte	Binary	Feeds on macrophytes and vascular plants
Detritus	Binary	Feeds on detritus or unidentifiable vegetation
Fish	Binary	Feeds on larger fish, crayfish, frogs

Eggs	Binary	Feeds on eggs of fish, frogs, etc.
Substrate		
Muck	Binary	Associates with muck substrate
Clay/silt	Binary	Associates with clay or silt substrates
Sand	Binary	Associates with sand substrate
Gravel	Binary	Associates with gravel substrate
Cobble	Binary	Associates with cobble substrate
Boulder	Binary	Associates with boulder substrate
Bedrock	Binary	Associates with bedrock substrate
Reproduction		
Guarder	Binary	Guards or does not guard brood
Spawning substrate	Binary	Open substrate spawners vs hiding or nesting

Table 2. Species captured across study lakes and their thermal preference group (Coker et al., 2001) and average maximum total lengths (TL; cm) of the top 5% of individual fish captured per species after removing the top 2%. Temperatures defining the thermal groups are; cold (<19 ºC), cool (19-25 ºC), and warm (>25 ºC) with cold/cool and cool/warm species having thermal preferences that straddle the boundaries.

Genus	**Species**	**Common name**	**Spp. code**	**Thermal group**	**TL**
Acipenser	*fulvescens*	Lake Sturgeon	LaStu	cold/cool	90.8
Alosa	*pseudoharengus*	Alewife	Ale	cold	19.7
Ambloplites	*rupestris*	Rock Bass	RoBa	cool	21.4
Ameiurus	*natalis*	Yellow Bullhead	YeBul	warm	32.9
Ameiurus	*nebulosus*	Brown Bullhead	BrBul	warm	31.2
Amia	*calva*	Bowfin	Bow	warm	63.4
Catostomus	*catostomus*	Longnose Sucker	LnSuc	cold	46.5
Catostomus	*commersonii*	White Sucker	WhSuc	cool	53.5
Chrosomus	*eos*	Northern Redbelly Dace	NoRD	cool/warm	7.9
Coregonus	*artedi*	Cisco	Cisco	cold	34.1
Coregonus	*clupeaformis*	Lake Whitefish	LaWhi	cold	54.5
Cottus	*bairdii*	Mottled Sculpin	MoScu	cold	7.5

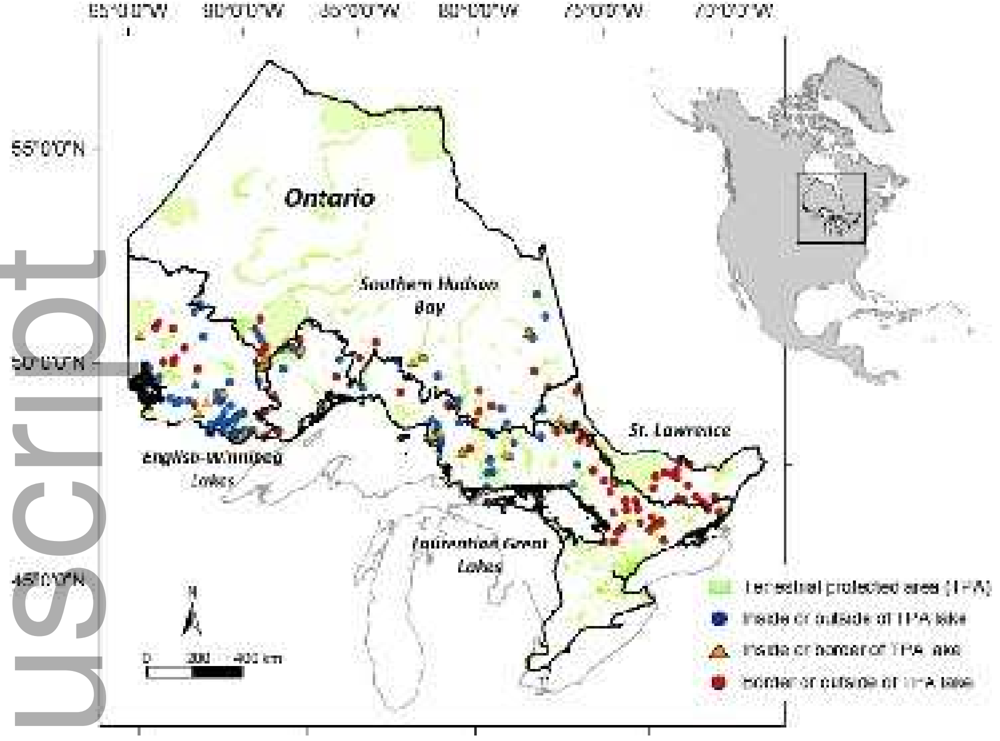
Cottus	*cognatus*	Slimy Sculpin	SlScu	cold	7.4
Cottus	*ricei*	Spoonhead Sculpin	SpScu	cold	8.1
Couesius	*plumbeus*	Lake Chub	LaCh	cold	12.0
Culaea	*inconstans*	Brook Stickleback	BrSt	cool	4.7
Cyprinus	*carpio*	Common Carp	CoCar	warm	72.3
Dorosoma	*cepedianum*	Gizzard Shad	GiSh	cool	16.5
Esox	*lucius*	Northern Pike	NoPik	cool	82.8
Esox	*masquinongy*	Muskellunge	Musk	warm	86.3
Etheostoma	*exile*	Iowa Darter	IoDar	cool	6.0
Etheostoma	*nigrum*	Johnny Darter	JoDar	cool	6.2
Fundulus	*diaphanus*	Banded Killifish	BaKil	cool	7.2
Gasterosteus	*aculeatus*	Threespine Stickleback	ThrSti	cold	5.5
Hiodon	*alosoides*	Goldeye	Gold	warm	44.6
Hiodon	*tergisus*	Mooneye	Moon	cool/warm	28.6
Ictalurus	*punctatus*	Channel Catfish	ChCat	warm	72.8
Labidesthes	*sicculus*	Brook Silverside	BrSil	cool/warm	7.3
Lepisosteus	*osseus*	Longnose Gar	LnGar	warm	109.5
Lepomis	*cyanellus*	Green Sunfish	GrSun	warm	13.6
Lepomis	*gibbosus*	Pumpkinseed	Pump	warm	17.6
Lepomis	*macrochirus*	Bluegill	Blue	warm	18.1
Lepomis	*peltastes*	Northern Sunfish	NoSun	warm	13.6
Lota	*lota*	Burbot	Burb	cold/cool	55.4
Luxilus	*cornutus*	Common Shiner	CoShi	cool	13.1
Margariscus	*nachtriebi*	Northern Pearl Dace	NoPD	cold/cool	8.5
Micropterus	*dolomieu*	Smallmouth Bass	SMB	warm	44.0
Micropterus	*salmoides*	Largemouth Bass	LMB	warm	33.6
Moxostoma	*anisurum*	Silver Redhorse	SiRed	cool	57.2
Moxostoma	*macrolepidotum*	Shorthead Redhorse	ShRed	warm	50.2
Moxostoma	*valenciennesi*	Greater Redhorse	GrRed	cool/warm	51.0
Myoxocephalus	*thompsonii*	Deepwater Sculpin	DeScu	cold	9.5
Notemigonus	*crysoleucas*	Golden Shiner	GoShi	cool	14.5
Notropis	*atherinoides*	Emerald Shiner	EmShi	cool	8.9
Notropis	*bifrenatus*	Bridle Shiner	BrShi	cool	5.8
Notropis	*heterodon*	Blackchin Shiner	BcShi	cool/warm	6.5

Notropis	*heterolepis*	Blacknose Shiner	BnShi	cool/warm	6.7
Notropis	*hudsonius*	Spottail Shiner	SpShi	cold/cool	9.4
Notropis	*rubellus*	Rosyface Shiner	RoShi	warm	6.6
Notropis	*stramineus*	Sand Shiner	SaShi	warm	8.8
Notropis	*volucellus*	Mimic Shiner	MiShi	warm	6.4
Oncorhynchus	*mykiss*	Rainbow Trout	RaTro	cold	38.5
Oncorhynchus	*tshawytscha*	Chinook Salmon	ChSal	cold	82.7
Osmerus	*mordax*	Rainbow Smelt	RaSm	cold	14.9
Perca	*flavescens*	Yellow Perch	YePer	cool	22.9
Percina	*caprodes*	Logperch	Log	cool/warm	8.1
Percopsis	*omiscomaycus*	Trout-Perch	TrPer	cold	9.2
Pimephales	*notatus*	Bluntnose Minnow	BnMin	warm	7.3
Pimephales	*promelas*	Fathead Minnow	FaMin	warm	7.7
Pomoxis	*nigromaculatus*	Black Crappie	BlCra	cool	24.7
Prosopium	*cylindraceum*	Round Whitefish	RoWh	cold	36.7
Pungitius	*pungitius*	Ninespine Stickleback	NiSti	cold	6.0
Rhinichthys	*atratulus*	Blacknose Dace	BnDac	cool	4.7
Rhinichthys	*cataractae*	Longnose Dace	LnDac	cool	7.3
Salvelinus	*fontinalis*	Brook Trout	BrTro	cold	43.3
Salvelinus	*namaycush*	Lake Trout	LaTro	cold	72.5
Sander	*canadensis*	Sauger	Saug	cool	37.9
Sander	*vitreus*	Walleye	Wall	cool	64.2
Semotilus	*atromaculatus*	Creek Chub	CrCh	cool	17.7
Semotilus	*corporalis*	Fallfish	Fall	cool	42.4
Umbra	*limi*	Central Mudminnow	CeMud	cool/warm	9.1

Table 3. Summary statistics and paired *t*-test results for functional dispersion, functional evenness, and functional richness for lakes inside versus outside of TPAs, inside versus bordering TPAs, and bordering versus outside of TPAs.

	Functional dispersion	*Functional divergence*	*Functional richness*
Inside versus outside (n = 40)			
Mean inside (SD)	1.07 (0.27)	0.66 (0.17)	5.13 (2.62)

Mean outside (SD)	0.96 (0.26)	0.60 (0.15)	5.59 (2.97)
|Mean difference|	0.10	0.06	0.46
|*t*|	1.68	2.03	0.86
p-value	0.10	0.05	0.40
Inside versus bordering (*n* = 15)			
Mean inside (SD)	1.02 (0.27)	0.66 (0.16)	4.82 (1.97)
Mean bordering (SD)	1.06 (0.27)	0.67 (0.10)	5.43 (2.41)
|Mean difference|	0.04	0.01	0.62
|*t*|	0.43	0.21	1.41
p-value	0.67	0.84	0.18
Bordering versus outside (*n* = 42)			
Mean bordering (SD)	1.07 (0.34)	0.60 (0.13)	6.59 (2.68)
Mean outside (SD)	1.06 (0.28)	0.63 (0.14)	6.72 (2.73)
|Mean difference|	0.01	0.03	0.13
|*t*|	0.23	1.02	0.30
p-value	0.82	0.31	0.77



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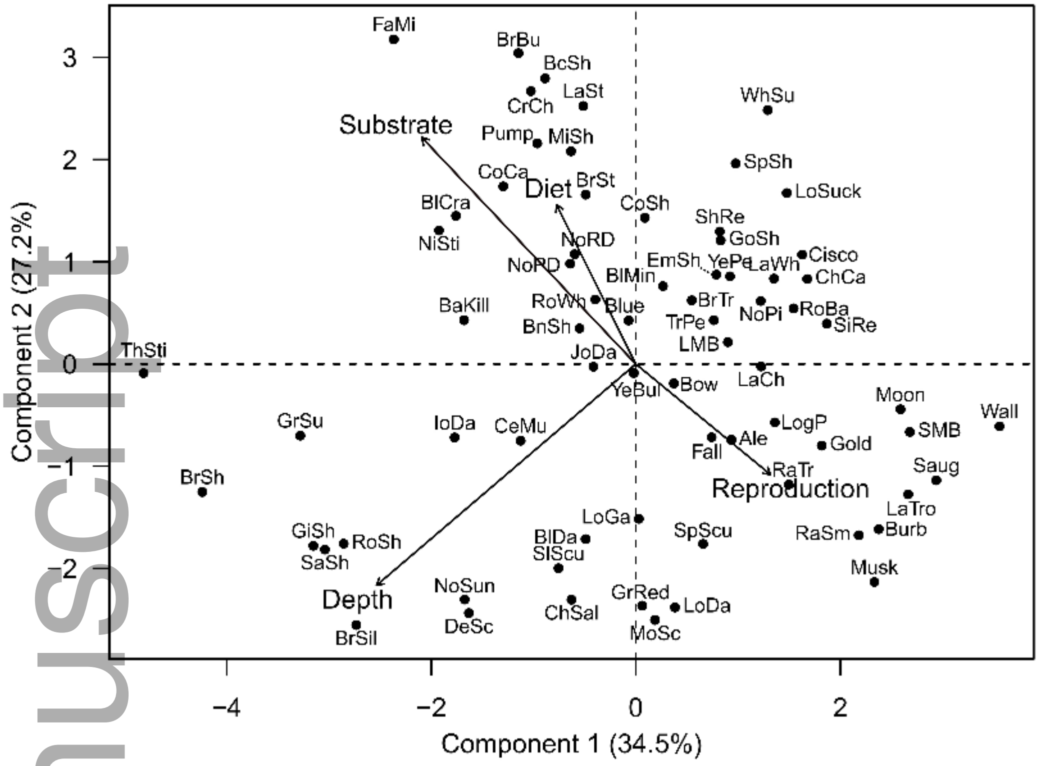

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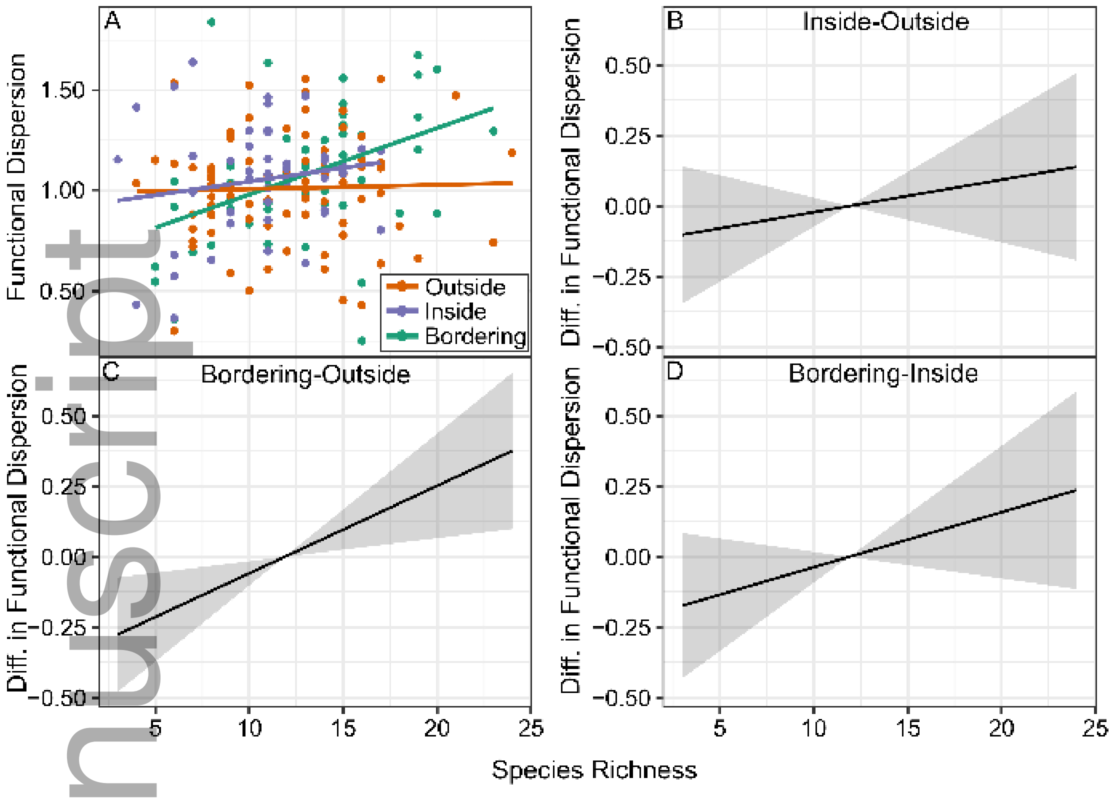

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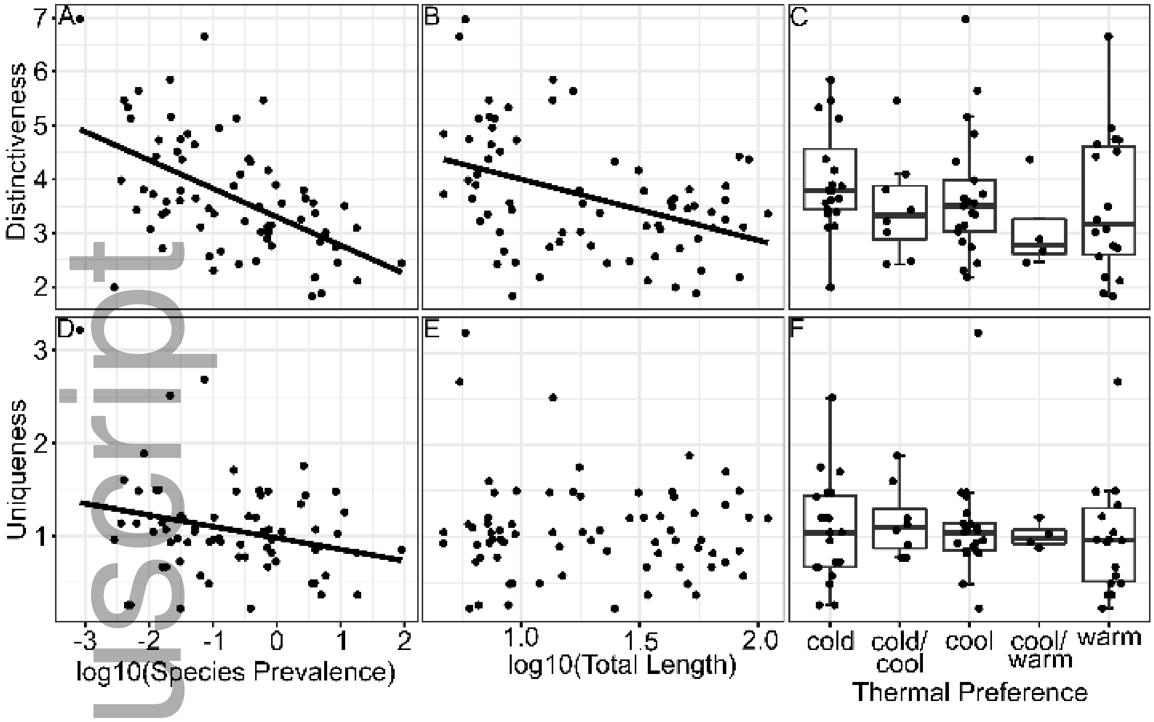

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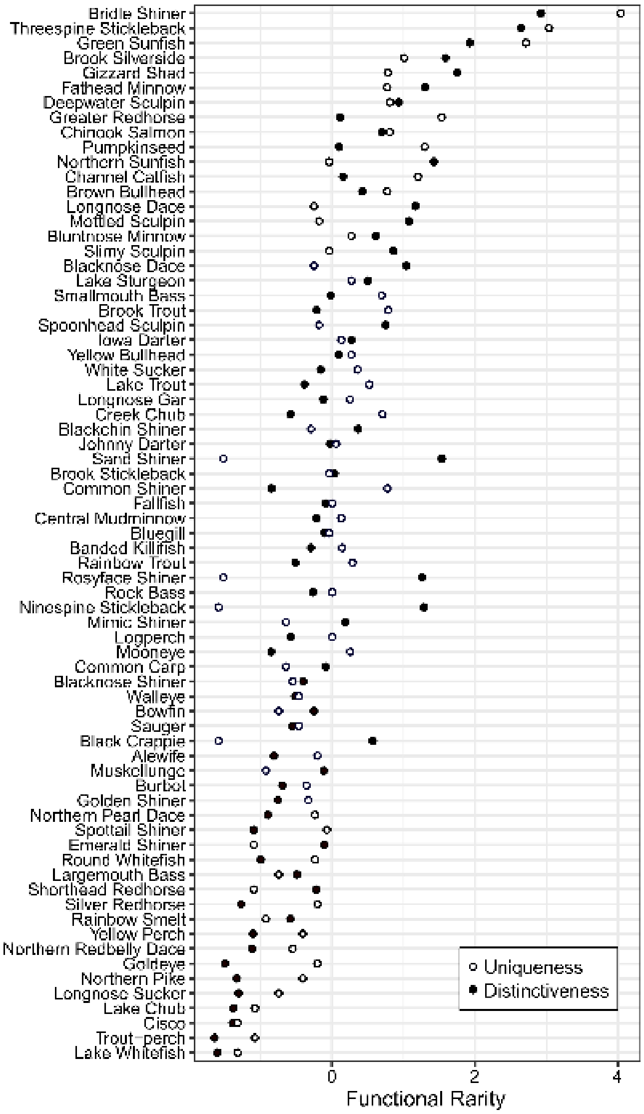

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