Cryptic Tolerant Species and Their Potential Effect on Index of Biotic Integrity (IBI) Scores, with the Presentation of an Alternative Metric of Stream Health

by

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Abstract

Metrics such as the Index of Biotic Integrity (IBI) are often used by management agencies to estimate the abstract property of stream health. These metrics are usually predicated on the belief that certain fish species are tolerant to environmental perturbation while others are sensitive. Species are usually designated as either tolerant or sensitive in these analyses based on inherent ecological or taxonomic characteristics. However, previous literature has shown that certain species from ecological or taxonomic "sensitive" groups experience increased abundance in degraded streams. We term such species "cryptic tolerants". Using a stream fish assemblage dataset of 433 unique sample locations across the state of Alabama and the National Landcover Dataset (NLCD) (Dewitz 2021), our objectives were to 1) identify the most common cryptic tolerant species, 2) investigate how cryptic tolerant species might inflate metrics of stream health, and 3) compare an alternative metric of stream health in which species are statistically defined rather than defined using the traditional trait-based approach. We identified cryptic tolerants using Nonmetric Multidimensional Scaling in each ecoregion. A series of regressions revealed that the proportion of cryptic tolerant species decreased in response to the proportion of forested land in catchments while the proportion of true sensitives increased in all ecoregions except for the Cumberland Plateau. A metric that simply used the percentage of statistically defined, non-tolerant species generally had lower p-values and higher r^2 values than IBI scores when both were regressed against percentage of forest in catchment. However, both metrics had low degrees of correlation with expected disturbance, indicating a univariate metric may be inadequate to characterize stream health. Our results highlight a potential issue with applying the

IBI to diverse southeastern systems, which may be alleviated by designating species sensitivity based on empirical response to disturbance rather than taxonomic or ecological characteristics.

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List of Abbreviations

ADWFF   Alabama Division of Wildlife and Freshwater Fisheries

DEM   Digital Elevation Model

ESRI   Environmental Systems and Research Institute, Inc.

IBI   Index of Biotic Integrity

NAD   North American Datum

NED   National Elevation Dataset

NLCD   National Land Cover Database

NMDS   Nonmetric Multi-Dimensional Scaling

USGS   United States Geological Survey

Introduction

Biodiversity sustains resilient ecosystems (Oliver et al. 2015). The loss of ecological functional groups within an ecosystem may compromise ecosystem functions (Cardinale et al. 2012, Leduc et al. 2015). Within fish assemblages, certain ecological functional groups are more vulnerable to localized extirpation than others. Typically, resource specialists are the most vulnerable to extirpation and are often replaced by generalist species (i.e., species that have large geographic ranges, occur in both highland and lowland habitats, and are typically more tolerant of temporal and spatial variation in habitat or food type) (Karr et al. 1986, Weaver and Garman 1994, Scott and Helfman 2001, Scott 2006).

Within the southeastern United States, the center of aquatic biodiversity in the temperate world (Warren et al. 2000), declines of fish species in sensitive functional groups have been well documented (Onorato et al. 2000, Phillips and Johnston 2004a, Johnston and Maceina 2009, Johnston et al. 2013, Lawson and Johnston 2016, Dunn and Angermeier 2019). Declines of these species result in lasting assemblage changes via faunal homogenization (Johnston and Maceina 2009, Lawson and Johnston 2016). Habitat alteration is usually implicated as the cause of such fish assemblage homogenizations (Freeman and Marcinek 2006, Freedman et al. 2014, Perkin and Bonner 2016, Montag et al. 2018). Specifically, previous studies have linked shifts in stream fish assemblages to change in hydrologic regime (Freeman and Marcinek 2006, Lawson and Johnston 2016, Stiles 2016), presence of in-stream impoundments and loss of aquatic habitat connectivity (Phillips and Johnston 2004b, Catalano et al. 2007, Perkin et al. 2017, Reuter et al.

2019), and land use change (Weaver and Garman 1994, Lobón-Cerviá et al. 2016, Paller et al. 2016, Montag et al. 2018).

Land use has been shown to be a reliable predictor of stream fish assemblage structure (Weaver and Garman 1994, Wang et al. 2000, Wang et al. 2001, Allan 2004, Sawyer et al. 2004, Helms et al. 2005, Paller et al. 2016, Morrill et al. in press). Stream catchments with high percentages of forested landcover typically foster intact fish assemblages, while catchments dominated by urbanization, agriculture, or deforested landscapes tend to have assemblages dominated by tolerant habitat generalists (Weaver and Garman 1994, Scott and Helfman 2006, Wang et al. 2001, Scott 2006). The latter landscapes tend to produce erosion and sedimentation which reduce suitable habitat for benthic lithophilic species as the interstitial spaces between sediments are filled with fine sediment (Berkman and Rabeni 1987, Scott and Helfman 2001, Walters et al. 2003, Dunn and Angermeier 2019). Additionally, catchments dominated by high percentages of impervious surfaces associated with urban landcover experience flashier hydrology and lower baseflows which create conditions unfavorable for many lotic species (McMahon et al. 2003, Roy et al. 2005, Johnston and Maceina 2009). The extent of faunal homogenization in response to land use may vary with respect to physiographic region and within-species responses can even differ based on physiographic context (Utz et al. 2010).

Reliable assessment of assemblage structure is crucial for monitoring trends in fish communities. A range of metrics have been proposed by ecologists to attempt to accurately assess structure of fish assemblages (Fausch et al. 1990). Often, assessment of fish assemblages has the implied goal of quantifying the abstract property of "ecosystem health"—a concept that places high value on natural systems and low value on altered ones (Karr 1999). Diversity indices were historically used for measuring stream health but are now rarely used by fish

ecologists because they weight all species equally and fail to consider species identity and nativity (Karr 1981, Karr et al. 1986, Fausch et al. 1990). Moreover, species diversity may actually increase as a result of environmental degradation as native cosmopolitan species invade new habitats (Scott and Helfman 2001, Paller et al. 2016). Similarity indices are often employed where temporal data exist (Ross et al. 1985, Phillips and Johnston 2004a). These metrics are useful when investigators are interested in assessing the degree of faunal change in a particular system over time. Likewise, these indices do not consider species identity but are often paired with ordination or rank-abundance analyses to further identify taxon-specific patterns in assemblage shifts (Johnston and Maceina 2009, Lawson and Johnston 2016).

The Index of Biotic Integrity (IBI) attempts to unify population, diversity, and individual considerations and presents a score reflecting the abstract property of assemblage "health" (Karr 1981, Karr et al. 1986). Unlike diversity or similarity indices, the IBI attempts to account for both diversity and species identity. A suite of properties (usually 12) is measured from the fish assemblage and assigned a 1, 3, or 5 based on its degree of similarity to an expected reference assemblage for a particular ecological context. A single number is obtained by summing each of these constituent metrics. A qualitative rating (i.e., very poor, poor, fair, good, or excellent) is subsequently derived from the numeric score. The IBI has many localized adaptations that attempt to account for expected assemblage differences based on physiography and natural faunal diversity (e.g., Schleiger 2000, Compton et al. 2003, Morris et al. 2007, Whittier et al. 2007, Paller et al. 2017).

The IBI is used extensively by state and federal management agencies in the eastern United States to monitor stream health. This method allows for the distillation of a complex, abstract ecological property ("stream health") into a simple score that can be understood by

policy makers and the public. However, the IBI initially faced criticism primarily on philosophical and statistical grounds (Suter 1993, Norris 1995, Norris and Hawkins 2000). The points of criticism are numerous, but the primary categories of critique are: 1) methodological and predictive inferiority to multivariate methods, 2) problems with data distribution and variance of IBI scores, 3) problems intrinsic to the multi-metric approach (i.e., meaning of constituent metrics is lost when all are summed together; the potential of low-scoring metrics to be compensated for by high-scoring metrics in the overall score), 4) concerns over the selection of candidate constituent metrics (e.g., potential redundancy of metrics which could lead to exaggerated estimates of effect), and 5) lack of the use of independent methods of verification to assess the IBI rendering it unfalsifiable (often expressed as tautological/circular justification, i.e., the IBI is used to assess stream health which is in turn used to assess the quality of the IBI) (criticisms outlined in Suter 1993, Norris 1995, Norris and Hawkins 2000; rebutted in Karr 1999, Simon 1999a).

In addition to the philosophical and statistical concerns raised by these authors, other authors have mentioned practical concerns regarding the contribution of various species to IBI scores (e.g., Scott and Helfman 2001, Johnston and Maceina 2009). Certain native species increase in abundance when subjected to land use change or other environmental perturbations (Scott and Helfman 2001). In fact, some species that experience such increases in response to disturbance are members of taxonomic or ecological groups considered "sensitive" in IBI calculations (Johnston and Maceina 2009, Lawson and Johnston 2016). These taxa typically have large geographic ranges (Scott and Helfman 2001), but nevertheless meet the ecological or taxonomic criteria of "sensitive" species and therefore contribute to higher IBI scores. We term such species "cryptic tolerant species" because their expected response to disturbance is

negative, but in actuality they thrive in degraded streams. This phenomenon has been documented in *Percina nigrofasciata* (Johnston and Maceina 2009, Lawson and Johnston 2016). In theory, this species represents a sensitive species in multiple respects: it is a darter, an insectivore, benthic, requires a fluvial habitat, and shows preference for erosional substrata where available (Mathur 1973, Boschung and Mayden 2004, Henry and Grossman 2008). Nevertheless, this species tends to replace more sensitive forms in degraded streams and can even be the most numerically abundant fish in these systems (Johnston and Maceina 2009, Lawson and Johnston 2016). A similar phenomenon has been documented with *Cyprinella venusta* (Johnston and Maceina 2009, Lawson and Johnston 2016), an invertivorous leusiscid and crevice spawner that by most documented traits should be a sensitive species (Hambrick and Hibbs 1977, Heins 1990). These species — though tolerant — may masquerade as sensitive species in approaches that categorize taxa based on ecological or taxonomic traits. Clearly, our understanding of the traits that drive the sensitivity-tolerance gradient among stream fishes needs to be revised. Furthermore, this phenomenon implies that cryptic tolerant species could compensate for or even replace truly sensitive species. This replacement would be masked in IBI scores because cryptic tolerant species are categorized by broad ecological and taxonomic traits rather than actual response to disturbance.

Using a statewide dataset of sample sites from Alabama, we had the following objectives: 1) identify cryptic tolerant species, 2) investigate how the proportion of cryptic tolerant species and truly sensitive species change in relation to disturbance and how combining these groups in metrics like the IBI might hide ecologically important information, and 3) compare the IBI to a metric that relies on statistically defined (rather than ecologically or taxonomically defined) sensitive and tolerant taxa.

Methods

Data Collection and Calculation of IBI Scores

Four hundred and thirty-three stream sites were sampled state-wide by the Alabama

Department of Conservation of Natural Resources from 2008–2020 (Figure 1a). All fish were

collected using the 30+2 method (described in detail in ADWFF 2010). In this method,

personnel use backpack electrofishing units to shock in an upstream direction along the

shoreline. Other personnel follow closely behind with dip nets and net stunned fish. Riffles, runs,

and pools are frequently sampled by shocking into a kick seine. "Thirty" refers to 30 seine

samples collected from the riffle, run, and pool habitats (10 each) and "+2" is the number of

shoreline samples collected using backpack shockers with dipnet personnel following shortly

behind. Collected fishes are identified to species, counted, and released. Specimens that are

difficult to identify in the field are preserved in 10% formalin and identified in a laboratory.

IBI scores were calculated for all sites according to the state-standard methods (examples

for two regions in Alabama include O'Neil and Shepard 2009; 2011a,b). As in most IBI versions,

each constituent metric is mathematically assigned a score of 1, 3, or 5 based on similarity to the

expected ideal condition for that stream (see Karr 1981 for a detailed explanation). Formulas are

altered based on watershed area and zones of ichthyofaunal similarity that approximate

physiographic region to account for expected natural differences in fish assemblages (e.g.,

O'Neil and Shepard 2011a,b). For sites with multiple collections, we averaged IBI score and

species abundances to obtain single values for the site to avoid duplicate observations for our

statistical analyses.

Calculation of Land Cover

We conducted spatial analyses of land use in ArcMap version 10.3.1 (ESRI 2015). We obtained raster data detailing land use from the National Land Cover Database (NLCD) (Dewitz 2021). We used land cover data associated with the year a collection was conducted. When land cover data was not available for a particular year, we used the nearest available year. The following land cover categories are included in the NLCD layer and were used in analysis (variable abbreviations in parentheses): 1) Open water ("OpenWater"), 2) Developed—open space ("DevOpen"), 3) Developed low intensity ("DevLow"), 4) Developed medium intensity ("DevMed"), 5) Developed high intensity ("DevHigh") , 6) Barren land ("Barren"), 7) Deciduous forest ("Decid"), 8) Evergreen forest ("Everg"), 9) Mixed forest ("MixFor"), 10) Shrub/scrub ("Shrub"), 11) Herbaceous ("Herb"), 12) Hay/pasture ("Hay"), 13) Cultivated crops ("Crops"), 14) Woody wetlands ("Wwetl"), and 15) Emergent herbaceous wetlands ("Ewetl"). Open water land cover is usually indicative of artificial farm ponds and reservoirs in the southeastern United States.

We calculated percentage of each land cover type for each upstream catchment that has an associated fish collection. We used the Digital Elevation Model (DEM) methodology available in the hydrology toolbox extension in ArcMap to delineate catchments upstream of sample locations. We used the National Elevation Dataset (NED) (USGS 1999) 30-meter raster as the elevation input for this analysis because of its accuracy and accessibility (Tighe and Chamberlain 2009). After raster catchment delineations were returned from the DEM, we used the tabulate area tool in the spatial analyst toolbox to calculate areas (m^2) of each land use category that fell within the catchment. We subsequently converted these areas to percentages of

catchment area. All calculations were done using the NAD 1983 UTM zone 16 coordinate
system.

Statistical Analysis

To reduce subjectivity in determining cryptic tolerant species and to visualize species
associations with environmental variables, we ran separate Non-Metric Multidimensional
Scaling (NMDS) ordinations of species-specific counts in samples in each of 6 zoogeographic
regions we determined based on patterns of ichthyofaunal diversity (Figure 1b). Because the
Coastal Plain makes up >60% of the land area in the state of Alabama (Boschung and Mayden
2004), we partitioned the Coastal Plain into two separate sub-regions (Eastern and Western) for
the purposes of analysis. The final ecoregions we used for analysis were 1) the Piedmont, 2) The
Ridge and Valley, 3) Tennessee Valley, 4) The Mobile Portion of the Cumberland Plateau, 5)
The Eastern Coastal Plain, and 6) The Western Coastal plain (Figure 1b). For each region's
NMDS, we used the Bray measure of pairwise distances between samples and retained two
dimensions for each ordination (stress seldom exceeding 0.20 for the set of ordinations, range
0.144–0.231). We then visualized association of the 15 landcover variables (arcsin-square root
transformed) as well as catchment area (log transformed, abbreviated "Area" on biplots) as
environmental vectors in ordination space. We displayed species scores in ordination space for
the top 20 most abundant species on separate accompanying biplots. A list of species included in
analyses and their abbreviations are provided in Table 1. We considered cryptic tolerant species
to represent those species that are traditionally considered "sensitive" by multiple metrics in the
IBI but that either load highly on well-accepted environmental predictors of poor stream health
(e.g., developed land, barren land) and cluster around well-established tolerant species (e.g.,

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Lepomis spp. [sans *L. megalotis*], *Gambusia* spp.). We considered truly sensitive species to represent those that loaded high on well-accepted environmental predictors of healthy streams (e.g., deciduous forest for regions above the fall line, total forest cover for regions below the fall line) or that displayed repulsion in multivariate space from well-accepted tolerant species.

Once we identified the cryptic tolerant and true sensitive species based on their locations in multivariate space, we regressed each group (proportion of total catch) against percentage of forested landcover (deciduous forest in the case of upland regions and total forest in the case of coastal plain regions) to visualize changes in the abundance of the two groups. Because the IBI does not explicitly discriminate between cryptic tolerants and true sensitives, we also investigated changes in "total fluvials" by summing the percentage of cryptic tolerants and percentage of true sensitives to obtain a single value. We regressed proportion of total fluvials against percentage of forested landcover.

We used the species we identified from our NMDS analyses to test a new metric of stream health that uses percentage of non-tolerant species (all species not delimited as tolerant in ordinations) as opposed to the multi-metric approach of the IBI (see discussion for justification). As pointed out by Suter (1993), there is a danger that one falls into the trap of post-hoc justification when assessing the effectiveness of metrics of stream health. To reduce this risk, we regressed both IBI and the new percent non-tolerant species metric against forested landcover for each region. We used forested landcover because this represents the primary undisturbed state for the southeastern United States, whereas using a single disturbed landcover type (e.g., total developed land) would be excluding alternative modes of impairment. We assumed percentage of forested landcover serves as a proxy for the inverse of total disturbance, meaning that the

better metric of stream health should have a stronger positive correlation with forested landcover.

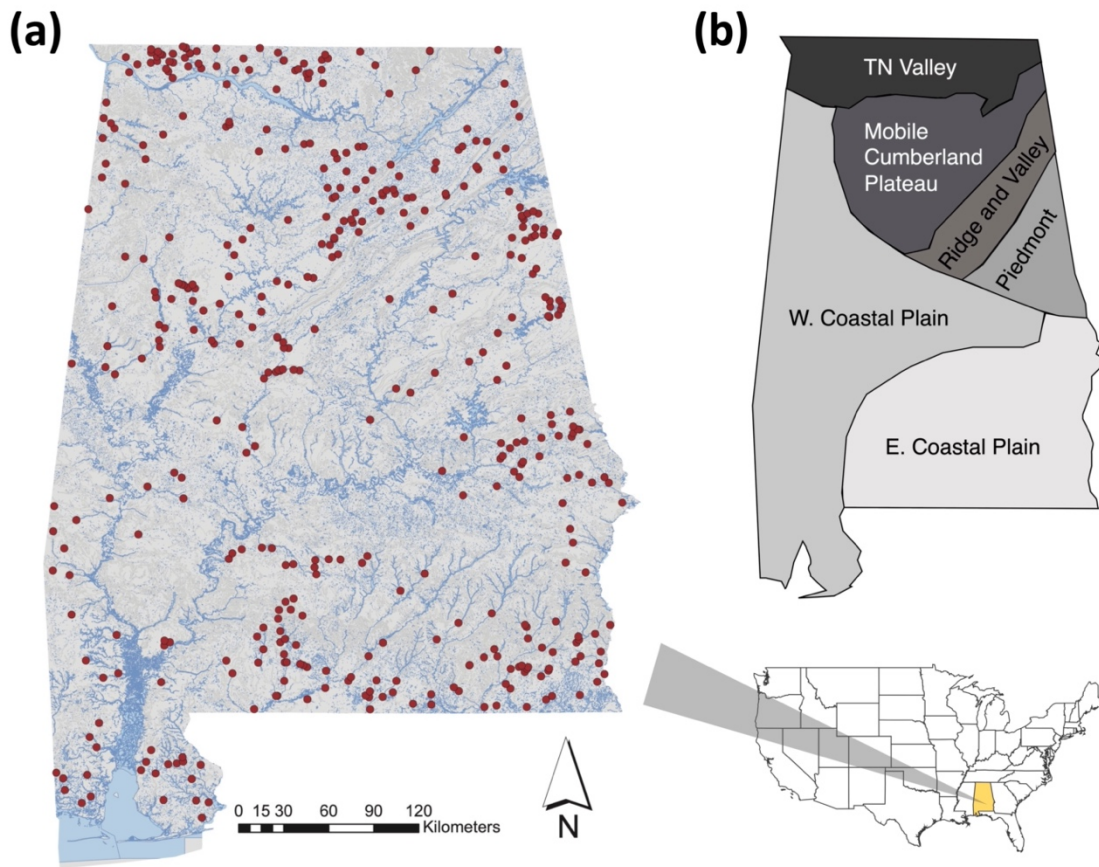All statistical analyses were conducted in RStudio 2022.02.0.

Figure 1. (a) Stream sample sites ($n = 433$) used to identify cryptic tolerant species and assess their potential effect on the Index of Biotic Integrity (IBI). These sites were also used to compare the IBI to a %non-tolerant native-species-in-catch metric. Sites are located in Alabama, USA (inset). (b) Regions used for partition of NMDS analyses and subsequent regressions for a statewide stream fish assemblage dataset in Alabama, USA. Regions were delineated according to broad patterns of ichthyofaunal diversity and to account for manageable sample sizes for analysis.

Table 1. List of species and abbreviations used in NMDS analyses.

Species	Abbrev.	Species	Abbrev.
Leuciscidae		Catostomidae	
Alburnops asperifrons	A.asperi	*Hypentelium etowanum*	H.etowa
Alburnops baileyi	A.baile	Ictaluridae	
Alburnops texanus	A.texan	*Noturus leptacanthus*	N.lepta
Alburnops xaenocephalus	A.xaeno	Aphredoderidae	
Campostoma oligolepis	C.oligo	*Aphredoderus sayanus*	A.sayan
Clinostomus funduloides	C.fundu	Fundulidae	
Coccotis zonistius	C.zonis	*Fundulus olivaceus*	F.oliva
Cyprinella callistia	C.callis	Poeciliidae	
Cyprinella galactura	C.galac	*Gambusia affinis*	G.affin
Cyprinella gibbsi	C.gibbs	Cottidae	
Cyprinella trichroistia	C.trich	*Cottus carolinae*	C.carol
Cyprinella venusta	C.venus	*Cottus tallapoosae*	C.talla
Ericymba amplamala	E.ampla	Centrarchidae	
Hybopsis amblops	H.amblo	*Lepomis auritus*	L.aurit
Hybopsis cf. *winchelli*	H.cf.winch	*Lepomis cyanellus*	L.cyane
Hybopsis lineapunctata	H.linea	*Lepomis macrochirus*	L.macro
Hydrophlox chrosomus	H.chros	*Lepomis megalotis*	L.megal
Luxilus chrysocephalus	L.chryso	*Lepomis miniatus x punctatus*	L.mi.x.pu.
Lythrurus alegnotus	L.alegn	*Micropterus henshalli*	M.hensh
Lythrurus atrapiculus	L.atrap	Percidae	
Lythrurus bellus	L.bellu	*Etheostoma artesiae*	E.artes
Lythrurus fasciolaris	L.fasci	*Etheostoma caeruleum*	E.caerul
Lythrurus roseipinnis	L.rosei	*Etheostoma colorosum*	E.color
Miniellus ammophilus	M.ammo	*Etheostoma coosae*	E.coosa
Miniellus longirostris	M.longi	*Etheostoma duryi*	E.duryi
Notropis stilbius	N.stilb	*Etheostoma flabellare*	E.flabe
Paranotropis cahabae	P.cahab	*Etheostoma simoterum*	E.simot
Paranotropis volucellus	P.voluc	*Etheostoma stigmaeum*	E.stigm
Pimephales notatus	P.notat	*Etheostoma swaini*	E.swain
Pimephales vigilax	P.vigila	*Etheostoma tallapoosae*	E.talla
Pteronotropis hypselopterus	P.hypse	*Nothonotus douglasi*	N.dougl
Pteronotropis merlini	P.merlin	*Nothonotus jordani*	N.jorda
Pteronotropis signipinnis	P.signi	*Nothonotus rufilineatus*	N.rufi
Rhinichthys obtusus	R.obtus	*Percina kathae*	P.katha
Semotilus atromaculatus	S.atrom	*Percina nigrofasciata*	P.nigro
Semotilus thoreauianus	S.thore	*Percina palmaris*	P.palma
		Percina smithvanizi	P.smith

Results

NMDS Ordinations

The NMDS analyses revealed cryptic tolerant and true sensitive species in each region. In the Piedmont ordination (stress = 0.144, 2 dimensions), *Alburnops baileyi*, *Ericymba amplamala*, *Percina nigrofasciata*, *Cyprinella venusta* and *Hypentelium etowanum* were associated with developed land use vectors and were located near the well-established tolerant species *Lepomis auritus* and *Lepomis macrochirus* in multivariate space (Figure 2a). *Cottus tallapoosae*, *Cyprinella gibbsi*, *Hybopsis lineapunctata*, *Percina smithvanizi*, and *Lythrurus bellus* were negatively correlated with developed land use vectors and instead were associated primarily with deciduous forest landcover (Figure 2a). In the Ridge and Valley ordination (stress = 0.159, 2 dimensions), *P. nigrofasciata*, *Etheostoma stigmaeum*, *Cyprinella venusta*, and *Campostoma oligolepis* were associated with developed land use vectors, correlated negatively with deciduous forest landcover, and were located near *L. macrochirus*, *L. auritus*, and *L. cyanellus* in multivariate space (Figure 2b). Conversely, *Alburnops xaenocephalus*, *Cottus carolinae*, *Etheostoma coosae, Luxilus chrysocephalus*, and *Hydrophlox chrosomus* were associated with deciduous landcover (Figure 2b). In the Tennessee Valley ordination (stress = 0.185, 2 dimensions), *C. oligolepis* and *Cyprinella galactura* were associated with high and medium intensities of developed land and open water and were located near the well-accepted tolerant species *Gambusia affinis* in multivariate space (Figure 3a). Additionally, *Lepomis auritus* (nonnative in the Tennessee River system) loaded highly on these land use vectors, even more so than other *Lepomis* spp. *Etheostoma duryi* was centered in the middle of the plot indicating a

lack of bias towards any landcover type. Therefore, we considered this species to represent a cryptic tolerant species in this context as well. *Clinostomus funduloides*, *Etheostoma flabellare*, *Etheostoma caeruleum*, *Etheostoma simoterum*, *Lythrurus fasciolaris*, *C. carolinae*, *L. chrysocephalus*, and *Hybopsis amblops* were negatively correlated with developed landcover vectors and instead associated with "undisturbed" land cover vectors — deciduous, evergreen, herbaceous and shrub (Figure 3a). In the Cumberland Plateau ordination (stress = 0.206, 2 dimensions), *C. oligolepis, C. venusta*, *H. etowanum*, and *P. nigrofasciata* were associated with cropland, hay pasture, and all intensities of developed land. These species also fell adjacent to *Lepomis* spp. (except for *Lepomis megalotis*) in multivariate space (Figure 3b). *Etheostoma artesiae*, *Alburnops asperifrons*, *L. megalotis*, *Percina kathae*, *Etheostoma stigmaeum*, and *L. bellus* were considered to represent true sensitives as they were associated primarily with mixed forest, herbaceous, woody wetland, evergreen, and Shrub land use vectors. In the eastern Coastal Plain ordination (stress = 0.231, 2 dimensions), *P. nigrofasciata*, *Miniellus longirostris*, *Ericymba amplamala*, *and Semotilus thoreauianus* were associated with "disturbed" land use types (all intensities of developed land, open water [ponds and reservoirs], and cropland) and were located near *L. auritus* in multivariate space (Figure 4a). *Cyprinella venusta* and *Alburnops texanus* were associated with catchments dominated by high percentages of barren land and hay pasture. These two leuciscids were located near *L. macrochirus* in multivariate space. Additionally, these species were associated with high catchment area (large watersheds). *Pteronotropis hypselopterus*, *Pteronotropis merlini*, *Fundulus olivaceus*, *Etheostoma colorosum*, *Lythrurus atracpiculus*, and *Lepomis megalotis* were associated with "undisturbed" lands: evergreen-forested land cover, shrubs, and herbaceous plants. In the western Coastal Plain ordination (stress = 0.179, 2 dimensions), *P. nigrofasciata* and *A. texanus* were associated

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primarily with disturbed land types (all intensities of developed land, barren, and crops), as well as with woody wetlands (Figure 4b). *Lythrurus roseipinnis* and *Noturus leptacanthus* fell somewhat near these species in multivariate space but were associated with those land use variables to a lesser degree. Therefore, we considered these species to represent "borderline" taxa that did not fall well into either cryptic tolerants or true sensitives. Unexpectedly, *Pteronotropis signipinnis* and *Pteronotropis hyspelopterus* showed association with developed land, barren land, and crops. More intuitively, these two species were associated with high amounts of woody wetlands in the catchment. We did not consider these *Pteronotropis* spp. to represent cryptic tolerants in future analysis as both species are often patchily distributed, locally abundant, and tend to be habitat specialists on small wetland creeks (Boschung and Mayden 2004). *Pimephales notatus* and *Semotilus atromaculatus* were associated with high amounts of open water in the catchment and were located near *Lepomis macrochirus* and *Gambusia affinis* in multivariate space. *Aburnops baileyi*, *L. chrysocephalus*, *Miniellus ammophilus*, *Lythrurus bellus*, and *Etheostoma stigmaeum* were associated with deciduous forest and mixed forest, and to lesser extent herbaceous plants and large watershed area. Interpretation of the western Coastal Plain biplot was more complicated than previous biplots with regards to the tolerance-sensitivity spectrum. Nevertheless, we considered *P. nigrofasciata*, *P. notatus*, *A. texanus*, and *S. atromaculatus* to represent cryptic tolerant species while *A. baileyi, L. chrysocephalus, M. ammophilus, L. bellus,* and *E. stigmaeum* were considered to represent true sensitive species for this region in subsequent analyses.

Change in Proportion of Cryptic Tolerant, True Specialist, and Total Fluvial Species

As expected, the proportion of cryptic tolerants decreased in all regions with respect to forested landcover whereas the proportion of true sensitives increased, and this relationship was statistically significant in all regions except for the Cumberland Plateau (Figure 5, Table 2). The change in proportion of total fluvials (Σ cryptic tolerants + true specialists, all considered sensitive in some aspect of the IBI) varied considerably among regions but only had a statistically significant change in the Western coastal plain (Figure 5, Table 2).

Comparison of Metrics of Stream Health

Both the IBI and percentage of non-tolerants in the collection generally correlated positively with percentage of forest in the catchment (Figure 6, Figure 7). However, the IBI failed to correlate positively with percent forest in the Eastern Coastal Plain (Figure 7). All regressions using percent non-tolerants had significant relationships at the $\alpha = 0.05$ level, but the IBI lacked a significant positive relationship in two regions (Figure 6, Figure 7). Percentage of non-tolerants in the catchment generally correlated more strongly (lower p-values and higher r^2) with percentage of deciduous forest in catchment than did the IBI. However, the reverse was true in the western Coastal Plain (figure 10).
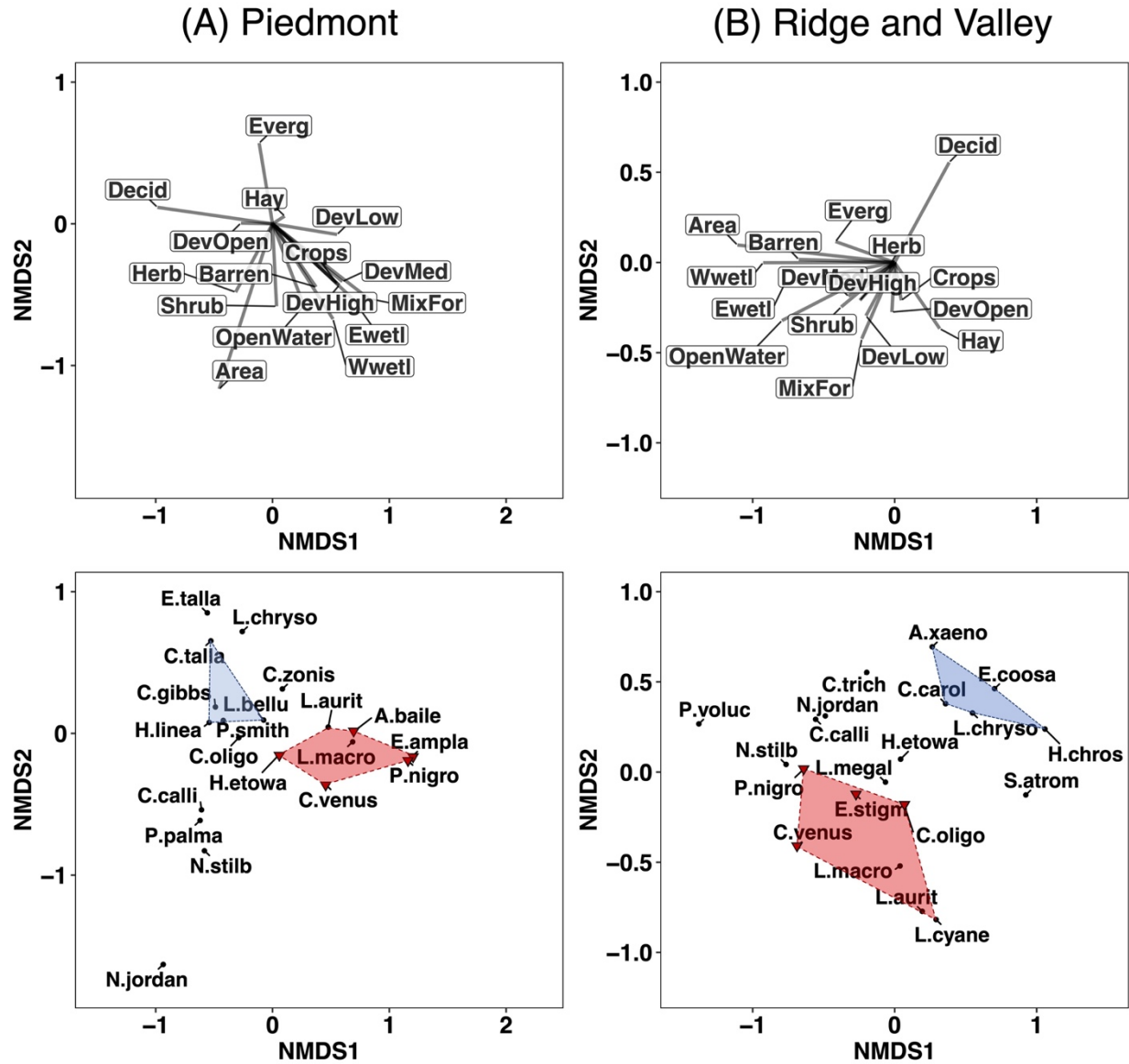
Figure 2. NMDS ordination of top 20 most abundant species in the Piedmont (stress = 0.144) and

Ridge and Valley (stress = 0.159) regions of Alabama, USA. The upper biplots display the

loadings of 15 landcover variables from the National Land Cover Database (NLCD) and

watershed area upstream of each collection site. The lower biplots displays species scores for

each respective region. The blue polygons delineate species we interpreted as sensitive and the

red polygons delineate species we interpreted as tolerant. Cryptic tolerant species are indicated

by downward-facing triangles. Species abbreviations are summarized in Table 1.
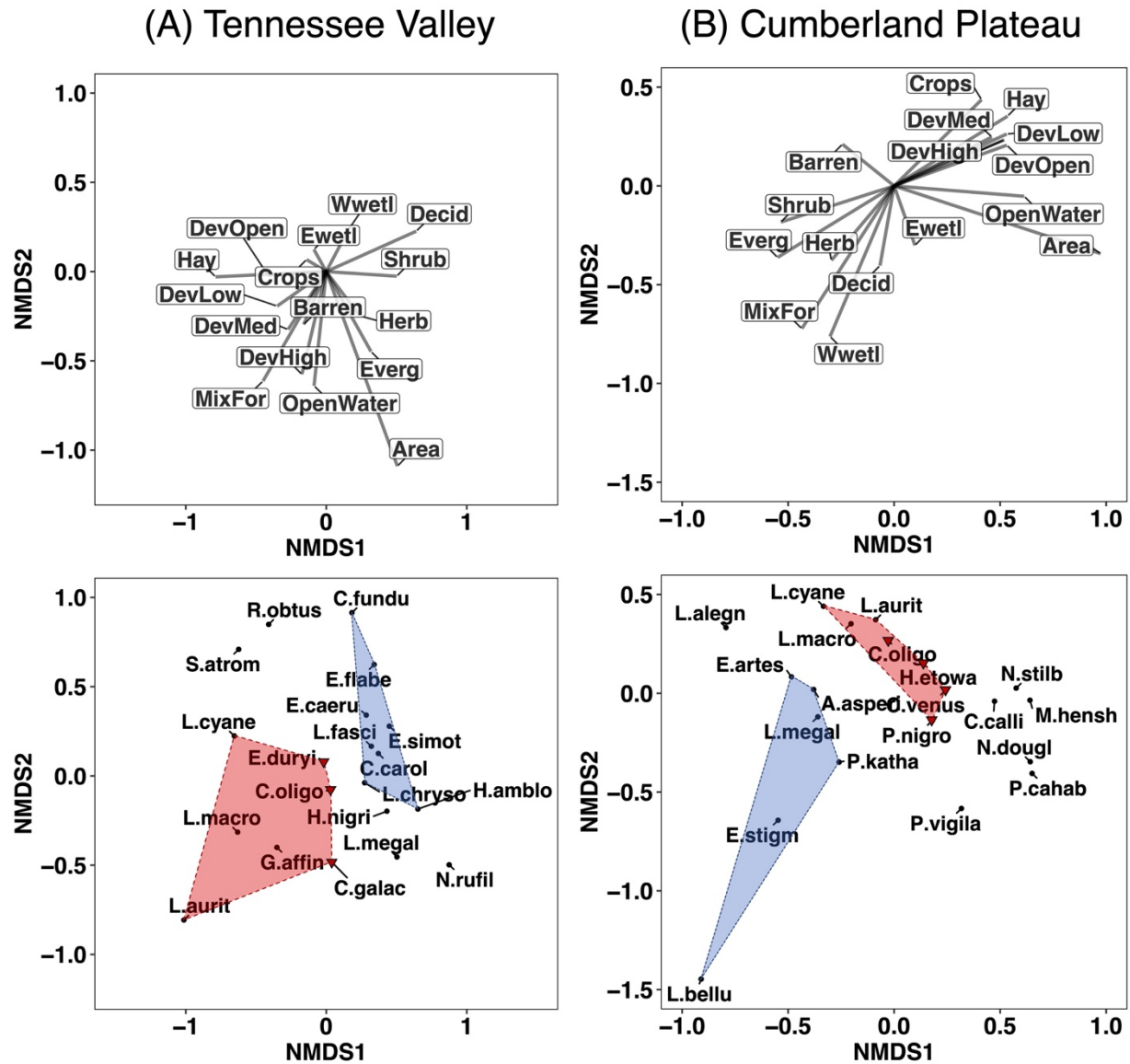
Figure 3. NMDS ordination of top 20 most abundant species in the (A) Tennessee Valley (stress = 0.185) and (B) portion of the Cumberland Plateau drained by the Mobile Basin (stress = 0.206) in Alabama, USA. The upper biplots display the loadings of 15 landcover variables from the National Land Cover Database (NLCD) and watershed area upstream of each collection site. The lower biplots displays species scores for each respective region. The blue polygons delineate species we interpreted as sensitive and the red polygons delineate species we interpreted as tolerant. Cryptic tolerant species are indicated by downward-facing triangles. Species abbreviations are summarized in Table 1.
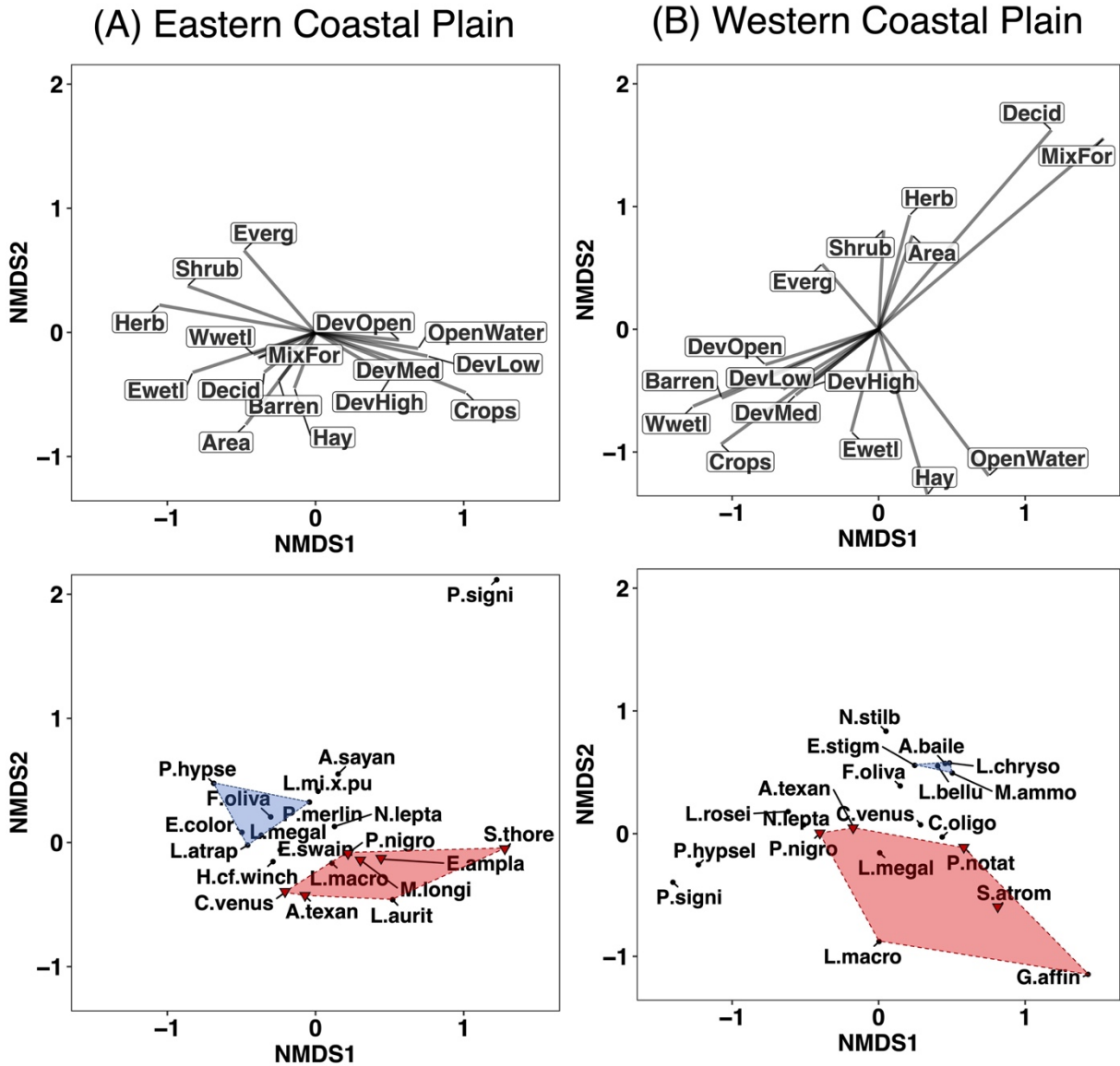
Figure 4. NMDS ordinations of top 20 most abundant species in the (A) Eastern (stress = 0.231) and (B) Western portions (stress = 0.179) of the Coastal Plain region in Alabama, USA. The upper biplot displays the loadings of 15 landcover variables from the National Land Cover Database (NLCD) and watershed area upstream of each collection site. The lower biplots display species scores for each respective region. The blue polygons delineate species we interpreted as sensitive and the red polygons delineate species we interpreted as tolerant. Cryptic tolerant species are indicated by downward-facing triangles. Species abbreviations are summarized in Table 1.
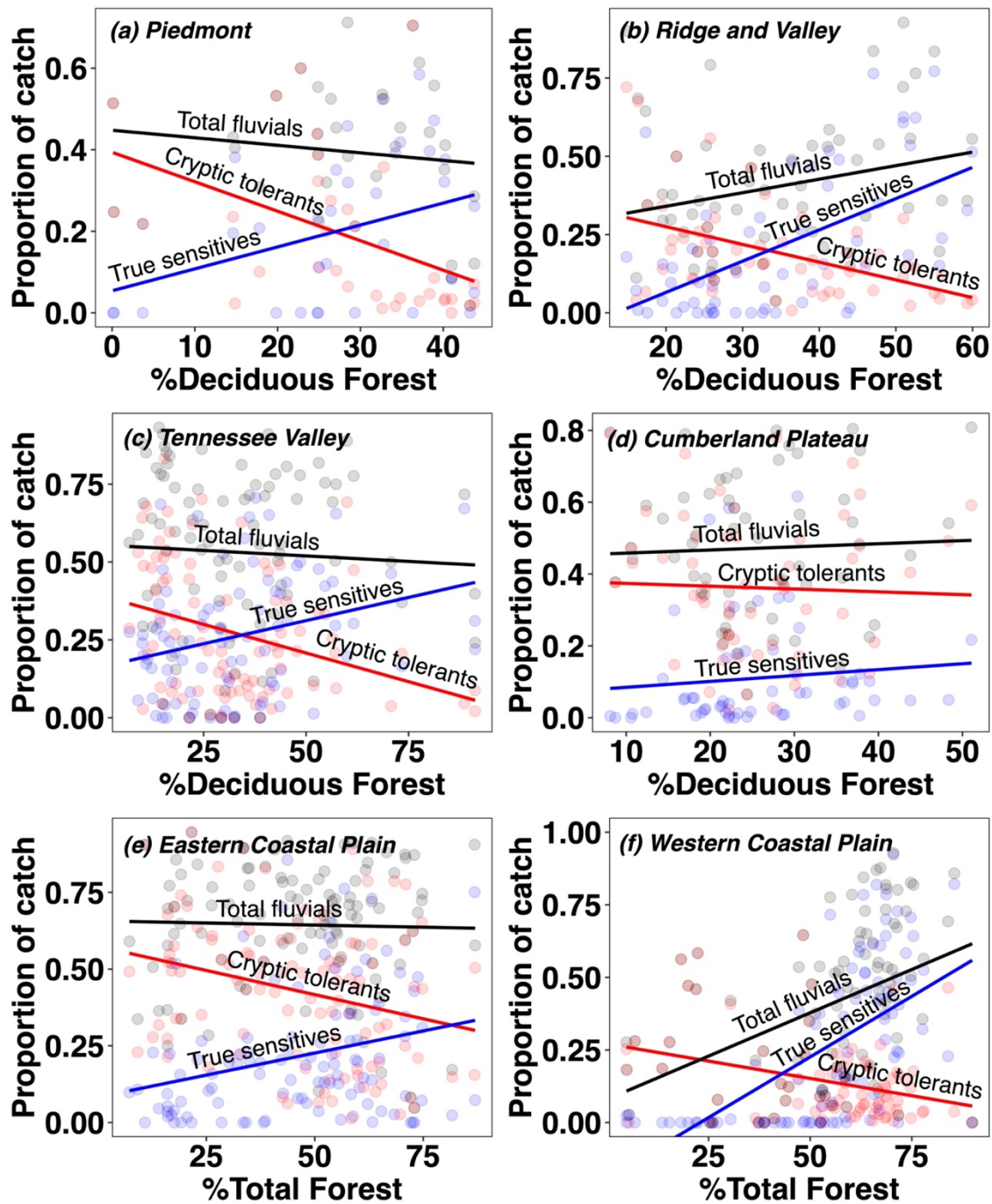
Figure 5. Regressions showing the change in the proportion of cryptic tolerants, true sensitives, and total fluvials identified in 6 NMDS analyses in Alabama stream fish assemblages. P-values and r^2 values are reported in table 2.
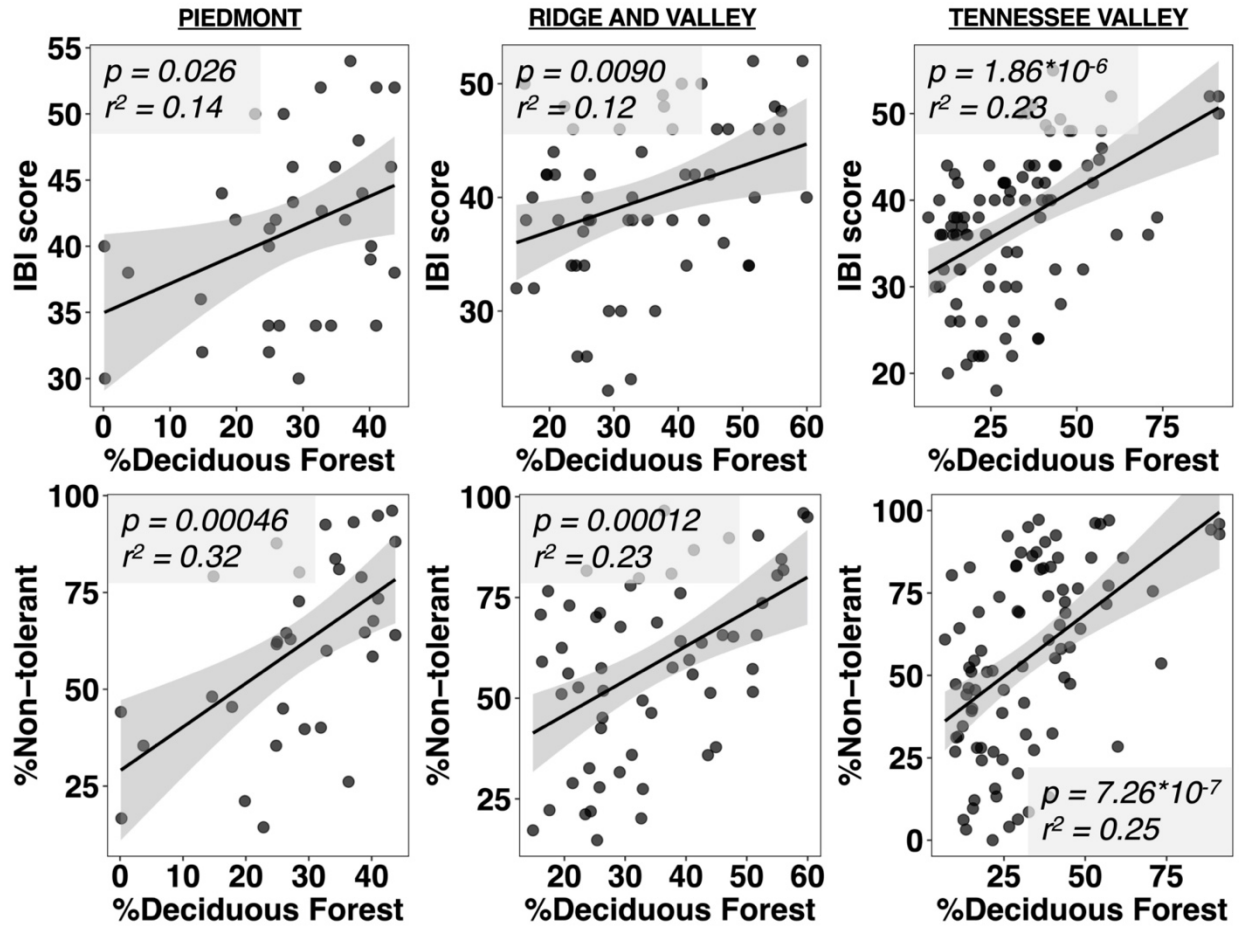
Figure 6. Correlation of Index of Biotic Integrity (IBI) scores versus statistically identified %Non-tolerant species with percentage of deciduous forest in watershed in the Piedmont, Ridge and Valley, and Tennessee Valley Regions of Alabama, USA. Shaded gray regions around the represent 95% confidence intervals. Clusters of points darker in shade represent overlap among points.

Figure 7. Correlation of Index of Biotic Integrity (IBI) scores versus statistically-identified %Non-tolerant species with percentage forested landcover in the watershed in the Mobile portion of the Cumberland Plateau as well as the Eastern and Western portions of the Coastal Plain in Alabama, USA. Percent deciduous forest was used for regions above the fall line whereas percent total forest was used for Coastal Plain regions. Shaded gray regions represent 95% confidence intervals. Points darker in shade represent overlap among points.

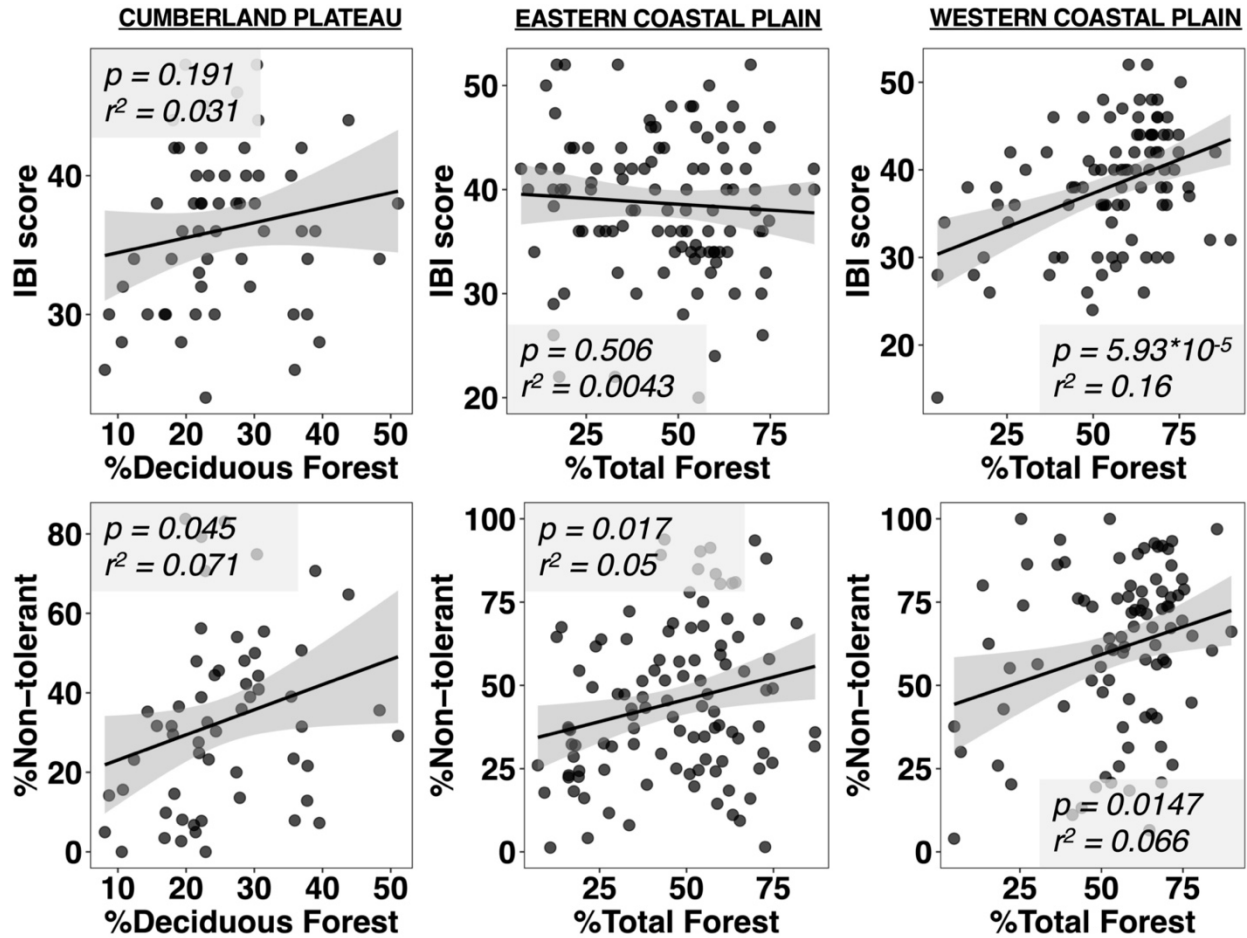Table 2. P-values, r^2, and slopes for each regression in figure 5 assessing the change in proportion of cryptic tolerants, true sensitives, and total fluvials (both groups combined) in response to percentage of forested landcover in the catchment upstream of each collection (*p<0.05, **p<0.01, ***p< 0.001). Percentage deciduous forest was used for regions above the fall line whereas percentage of total forest was used for regions below the fall line.

		Cryptic Tolerants	True Sensitives	Total Fluvials
Piedmont	*p-value*	0.0088 **	0.045 *	0.47
	r2	0.20	0.12	0.016
	slope ± 95% CI	-0.0072 ± 0.0053	0.0054 ± 0.0052	-0.0019 ± 0.0052
Ridge and Valley	*p-value*	0.00023 ***	3.53*10^-6 ***	0.059
	r2	0.22	0.32	0.062
	slope ± 95% CI	-0.0057 ± 0.0029	0.010 ± 0.0039	0.0043 ± 0.0045
Tennessee Valley	*p-value*	0.0022 **	0.0069 **	0.64
	r2	0.10	0.082	0.0025
	slope ± 95% CI	-0.0037 ± 0.0023	0.0030 ± 0.0021	-0.00070 ± 0.0030
Cumberland Plateau	*p-value*	0.78	0.44	0.76
	r2	0.0014	0.011	0.0018
	slope ± 95% CI	-0.00078 ± 0.0056	0.0016 ± 0.0042	0.00086 ± 0.0056
Eastern Coastal Plain	*p-value*	0.0046 **	0.0041 **	0.079
	r2	0.075	0.077	0.00071
	slope ± 95% CI	-0.0032 ± 0.0022	0.0029 ± 0.0019	-0.00027 ± 0.0020
Western Coastal Plain	*p-value*	0.0024 **	5.11*10^-10 ***	2.19*10^-5 ***
	r2	0.099	0.35	0.18
	slope + 95% CI	-0.0024 ± 0.0015	0.0084 ± 0.0024	0.0060 ± 0.0026

Discussion

Our results highlight a potential issue with applying the standard trait- and taxonomy-based IBI to diverse southeastern fish assemblages. We have shown that many species that appear to share taxonomic affinities or ecological similarities can respond differently to disturbance, yet these forms are often treated as interchangeable components under metrics of stream health that use a trait-based classification system. In fact, we identified a suite of species that are associated with degraded streams yet contribute to higher IBI scores in several constituent metrics (Table 3). The southeastern United States has both extensive aquatic diversity and geographic heterogeneity, which makes the application of a simple metric of stream health difficult. Additionally, many southeastern native fishes lack basic biological and natural history studies which also may create difficulties in appropriately assigning species to ecological guilds under this method (Goldstein and Simon 1999, Simon 1999b).

Using percent non-tolerant species to estimate stream health may provide a more accurate picture of stream health because it relies on statistical definition of tolerant and sensitive taxa (empirical response) rather than trait-based definition of taxa (theoretical response). This method also removes several of the methodological concerns raised by previous authors (Suter 1993, Norris 1995, Norris and Hawkins 2000). Specifically, this approach eliminates many concerns surrounding multi-metric indices (eclipsing, redundancy of constituent metrics, etc.) and represents a real property of fish assemblages with continuous units (percent composition) as opposed to the abstract and unitless nature of the IBI (Suter 1993). Therefore, the better performance of the %Non-tolerant metric may be due in part to the elimination of issues with

multi-metric indices and not due solely to the reduction of cryptic tolerant species in the stream health metric.

The IBI and % non-tolerant metrics are both vulnerable to circular justification (a philosophical bane that plagues most metrics of stream health) outside of costly and time-intensive lab-based species tolerance studies (Suter 1993). The %Non-tolerant metric also devalues all native tolerant species which should be present in low abundances even in pristine systems. Additionally, this method requires statistical definition of sensitive and tolerant species prior to analyses, which may be more time intensive than the trait-based approach the IBI uses. Any metric of stream health will require initial development and calibration, and the multivariate methods required for visualizing species associations in our method are more easily implemented today than they were at the debut of the IBI in the early 1980s. Even if the two methods yield comparable results, one could choose to use the uni-metric as opposed to multi-metric approach as this is the more parsimonious and arguably more direct method (Behnke 1987).

Nevertheless, the IBI does have merits over a uni-metric approach. It measures a suite of assemblage properties which may better reflect the complexity of ecological systems (Karr et al. 1986). The IBI also has extensive testing and use in the literature and often shows correlation with disturbance variables (Schleiger 2000, Wang et al. 2000, McCormick et al. 2001, Morris et al. 2007, Paller et al. 2017, this study). It is unclear whether the comparatively poor performance of the IBI is due to idiosyncrasies of the Alabama version of the IBI or due to fundamental issues with the IBI as a whole. Many of Karr's (1981, 1999) original cautions have been disregarded in current applications of the IBI which may represent a problem in execution rather than a methodological shortcoming of the IBI itself (Seegert 2000). Additional testing with other datasets in other geopolitical and physiographic regions could help ascertain whether statistically

identified percent non-tolerants is a consistently better predictor of disturbance than the IBI. An IBI could be developed that accounts for the presence of cryptic tolerants, but the trait-based framework would have to be adjusted to incorporate statistical definitions of species tolerance in addition to (or instead of) the current trait-based approach that bases species classifications on ecological guilds and taxonomic groups. If the multi-metric approach is desired, constituent metrics could consist of species "response groups" to different categories of disturbance (urban landcover tolerance, degree of chemical tolerance, etc.) rather that taxonomic or ecological groups.

Though percent non-tolerant species generally correlated more strongly with land-cover disturbance in our analyses, both metrics had low degrees of correlation with expected disturbance. While forms of disturbance other than land use may be present, this may also indicate that univariate approaches are limited in their ability to accurately characterize stream health. Multivariate metrics were proposed early in the history of stream health indices to account for the n characteristics (dimensions) of assemblages measured by environmental monitoring programs (Suter 1993, Norris 1995). These methods were initially impractical to use but streamlined computer programs now make the widespread use of multivariate methods relatively straightforward. However, an ideal metric of stream health should be easily interpreted by non-experts (O'Connor and Dewling 1986). Policymakers may struggle to understand the esoteric nature of raw multivariate outputs and may prefer a single number or a qualitative rating. The metric we present represents a compromise because it is a univariate metric but nevertheless informed by empirical multivariate species associations.

In addition to calculating IBI scores, management agencies could also use the same fish assemblage data to calculate similarity indices which would be a more robust measure of

assemblage change through time than the IBI (Johnston and Maceina 2009). Both numbers could be considered together to evaluate stream health and stability. This would require repeated sampling of streams as opposed to the one-time-sample approach that is used by many management agencies. Using the IBI as a surrogate to measure temporal assemblage change may fail to register the replacement of true sensitives by cryptic tolerants because these species are considered interchangeable components by the IBI during calculation, and both contribute to higher IBI scores. Therefore, the decline of endemic, truly sensitive fishes could be masked by the associated increase in cryptic tolerants when trait-based multi-metric indices are used, a phenomenon which could be termed "cryptic assemblage change" (but see Karr et al. 1987 for an IBI that appears sensitive to temporal assemblage change in two northern US states).

Scott and Helfman (2001) recognized that increased abundances of specific native fishes (even minnows and darters) have the potential to indicate assemblage homogenization and stressed that highland endemic species are the best indicators of assemblage health in southern Appalachian streams. Many of the true sensitives we identified are fishes restricted to the upland region of one or two river systems (e.g., *H. lineapunctata, P. smithvanizi, E. coosae*). Conversely, many of the cryptic tolerant species we identified are taxa that occur both above and below the fall line and have large geographic ranges (e.g., *P. nigrofasicata, A. texanus, C. venusta, E. amplamala*). Interestingly, some species that were identified as cryptic tolerant species in one region were identified as true sensitives in another (e.g, *E. stigmaeum*, *A. baileyi*). These results suggest that a given species' response to disturbance may vary based on physiographic context—an idea that is supported by previous research (Utz et al. 2010).

In addition to fishes, the IBI has been adapted for use with other taxa including aquatic macroinvertebrates (Klemm et al. 2003, Weigel and Dimick 2011), birds (Bryce et al. 2002) and

even plants (Mack 2007). Cryptic tolerants may be present in these versions as well because taxa are defined based on ecological or taxonomic characteristics rather than measured response.

Reliably assessing ecosystem health is crucial to monitoring localized faunal homogenizations and in turn implementing actionable management initiatives. Here we have identified some of the potential pitfalls of trait-based metrics of stream health and provided a method for mitigating these pitfalls. In summary, if agencies are interested in developing metrics of stream health that more accurately measure similarity to natural assemblage structure, defining sensitive and tolerant taxa based on quantified response to disturbance rather than based on ecological or taxonomic traits of organisms may provide a more accurate picture of ecosystem health.

Table 3. Cryptic tolerant species identified from NMDS analyses and their contributions to "sensitive" metrics (higher scores) in the IBI.

Species	TAXONOMIC				ECOLOGICAL					COMBINATION
	Shiner	Sucker	Darter	leuciscid	Native	Intolerant	Invertivore	Lithophil	Simple misc.	Insectivorous leuciscid
A. baileyi	x			x	x			x		x
A. texanus	x			x	x				x	x
C. oligolepis				x	x			x		
C. galactura	x			x	x				x	x
C. venusta	x			x	x	x			x	x
E. amplamala	x			x	x			x		x
M. longirostris	x			x	x			x		x
P. notatus				x	x					
S. atromaculatus				x	x			x		
S. thoreauianus				x	x			x		
H. etowanum		x			x			x		
E. duryi			x		x				x	
E. stigmaeum			x		x			x		
P. nigrofasciata			x		x			x		

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