Ichthyology & Herpetology 111, No. 4, 2023, 584–596

Distinguishing and Employing Two Species of Fish in Assessment of Stream Quality

Fred Van Dyke[1,2], Benjamin W. van Ee[3], Seth Harju[4], Joshua W. Budi[5,6], Thomas B. Sokolowski[7], and Brian Keas[1,8]

Biotic indices (bioindicators) can be individual species, species groups, or communities of species used to assess habitat quality. But, to be used effectively, managers require basic information on species used as indicators, including species distribution, differentiation between similar species, and environmental conditions associated with species presence. We addressed these problems concurrently in two related species, the Mottled Sculpin (*Cottus bairdii*) and the Slimy Sculpin (*Cottus cognatus*), as habitat quality indicators in the Manistee River in Michigan, USA. We determined the abundance and distribution of these species and related their presence to concurrent in-stream measurements of temperature, dissolved oxygen, pH, conductivity, turbidity, and stream quality score based on macroinvertebrate diversity. Cladistic analyses of CO1 supported recognition of Mottled Sculpin and Slimy Sculpin as distinct species and confirmed initial field identification to species using morphological characteristics. Both species were most abundant in headwater regions, decreased downstream, and were sympatric at 5 of 12 (42%) locations. Mottled Sculpin were associated with lower conductivity, pH, and stream quality scores. Slimy Sculpin were associated with higher levels of DO and lower levels of turbidity. As a management indicator species of the US Forest Service, Mottled Sculpin alone may be ineffective as a habitat quality indicator, but concurrent use of Mottled Sculpin and Slimy Sculpin as a related-species complex might allow sufficient coverage to permit assessment of stream quality if species-specific differences in environmental tolerances are precisely determined.

BIOTIC indices are widely used for monitoring health of ecosystems and quality of habitat (Dos Santos et al., 2011; Pander and Geist, 2013). Multi-metric and multi-species indices offer comprehensive assessments of environmental conditions and have been used with success in aquatic environments (Lydy et al., 2000; Moya et al., 2007). Fishes are commonly used in aquatic environmental evaluations (Hossein et al., 2015; Caetano et al., 2016). One of the oldest and most widely employed aquatic assessment tools, the index of biological integrity (IBI), is entirely fish-based, with metrics including species composition, richness, tolerance, hybridization, trophic measures, health condition, age structure, growth, and recruitment (Karr, 1981; Pander and Geist, 2013).

A single species whose function, population trends, or status can be used to determine ecosystem performance or environmental change can also act as a biotic indicator (bioindicator), or "indicator species" (Dziock et al., 2006; Pander and Geist, 2013). Such indicators can provide cost-effective tools for short- and long-term monitoring of environmental and ecosystem integrity (Neumann et al., 2003)—a significant consideration when funding is limited and scientists are asked to give rapid and reliable judgments about the quality of habitat at the request of government agencies or private institutions (Dos Santos et al., 2011). Indicator species should exhibit a well-defined ecological range, rapid response to environmental change, well-defined taxonomy for reliable identification, wide area or regional distribution, and low-cost sampling (Bellinger and Sigee, 2010). Species that meet these criteria can act as surrogates for delimiting the presence or abundance of other species or particular environmental states and offer simple techniques for habitat and species inventory in complex landscapes (Smale et al., 2011).

The use and interpretation of the term "indicator species" requires careful definition. There are at least seven kinds of indicator species described in scientific literature, each "indicating" something different (Lindenmayer et al., 2000; Van Dyke, 2008). Such species may be: (1) indicators of the presence or absence of other species; (2) indicators of ecosystem conditions to which they contribute or which they help to maintain ("keystone" or "function-based" species); (3) indicators of human activity altering environmental conditions (pollution indicators); (4) a dominant species that contributes disproportionate biomass or numbers of individuals to a system; (5) a species indicative of particular environmental conditions in a relatively stable ecosystem; (6) a species particularly sensitive to changes in ecosystem conditions that provides advance indication of environmental changes (a "sentinel" species providing "warning"

---

[1] Au Sable Institute, Mancelona, Michigan 49659; ORCID: (FV) 0000-0001-7903-8180; Email: (FV) vandykefred400@gmail.com. Send correspondence to FV.

[2] Present address: 6981 Rainbow Lake Road NE, Mancelona, Michigan 49659.

[3] Departamento de Biología, Universidad de Puerto Rico, Call Box 9000, Mayagüez, Puerto Rico 00681-9000; Email: bvanee@uwalumni.com.

[4] Heron Ecological LLC, P.O. Box 235, Kingston, Idaho 83839; Email: seth@heronecological.com.

[5] Department of Biology, Calvin University, 3201 Burton St. SE, Grand Rapids, Michigan 49546; Email: joshuabudi16@gmail.com.

[6] Present address: Puspita Loka B2 no. 31, BSD, Tangerang Selatan, Indonesia 15310.

[7] Department of Biology, Wheaton College, 501 College Avenue, Wheaton, Illinois 60187-5501; Email: tommy.sokolowski@my.wheaton.edu.

[8] Present address: Department of Entomology, Michigan State University, Natural Science Building, 288 Farm Lane Room 243, East Lansing, Michigan 48824; Email: keasbrian@gmail.com.

Submitted: 22 November 2021. Accepted: 6 September 2023. Associate Editor: M. P. Davis.

DOI: 10.1643/i2021132

of environmental change); or (7) a "management indicator species" that reflects and can be used to assess the effectiveness of management actions intended to preserve particular conditions (Lindenmayer et al., 2000; Zacharias and Roff, 2001; Van Dyke, 2008).

The last category, the management indicator species (MIS), is an organism whose characteristics, such as presence or absence, population density, dispersion, or reproductive success can be used as an index of environmental or biological attributes too difficult, inconvenient, or expensive to measure (Landres et al., 1988). Thorough knowledge of environmental tolerances and preferences of these species is essential if MIS are to be used in habitat assessment and facilitate fast and judicious decisions at minimal cost (Lindenmayer et al., 2000). If qualitative environmental preferences of the species can be reliably associated with specific sites or environmental conditions, its designation as an indicator species is both useful and warranted (De Cáceres et al., 2010). The management indicator species may also merit additional designation as a "sentinel species" (Gray et al., 2018) if changes in its abundance provide advance warning of environmental danger or hazard associated with changing environmental conditions.

An alternative to individual species is the use of closely related species. Species relatedness can be an advantage in environmental assessment because both inter-specific competition and differences in habitat quality could affect presence and abundance (Weinstein et al., 2019). Sculpin (*Cottus* spp.) populations have been observed to be more strongly affected than salmonids or other larger fishes by a variety of conditions (Besser et al., 2009). Two such species are the Mottled Sculpin (*Cottus bairdii*) and the Slimy Sculpin (*Cottus cognatus*). Both are used as MIS and sentinel species because of their geographically widespread but demographically stable populations (NatureServe Explorer, 2021; US Geological Survey, 2021) and their occurrence in cold-water streams (Besser et al., 2007; Gray et al., 2018). Such cold-water streams may be more limited in occurrence in many areas and more sensitive to changes in habitat (such as logging or other types of vegetation disturbances which reduce shade) or climate (such as global warming).

Like other sculpin species, Mottled Sculpin and Slimy Sculpin lack a swim bladder and inhabit benthic habitats, reducing mobility and increasing site fidelity (Petty and Grossman, 2004; Breen et al., 2009). This coupling of strong site fidelity with widespread occurrence makes Mottled Sculpin and Slimy Sculpin potentially site-specific MIS and sentinel species. In terms of environmental conditions, sculpin species have an estimated upper lethal water limit between 23 and 25°C (Symons et al., 1976; Otto and Rice, 1977), but Slimy Sculpin are rarely found in waters with sustained temperatures greater than 19°C. In some North American streams, Mottled Sculpin have shown even colder upper thermal limits (Quist et al., 2004), and the distribution of different sculpin species in the same stream is strongly influenced by temperature differences (Adams et al., 2015), with increasing downstream temperatures associated with lower sculpin densities (Lessard and Hayes, 2003). Because sculpin are also associated with higher levels of dissolved oxygen (DO), higher (more alkaline) pH, and lower levels of turbidity, these variables also have been suggested to influence sculpin presence, abundance, and ecology (Waite and Carpenter, 2000; Lessard and Hayes, 2003; Adams and Schmetterling, 2007). These associations make Mottled Sculpin and Slimy Sculpin indicative of conditions which management agencies, such as the US Forest Service (USFS), associate with high quality stream habitats. Both species have shown sensitivity to toxic metals (Dubé et al., 2005; Besser et al., 2007; Allert et al., 2009), and Slimy Sculpin have demonstrated declines in populations when exposed to contaminants associated with agriculture (Gray et al., 2002; Brasfield et al., 2015), coal mining (Miller et al., 2015), pulp and paper operations (Galloway et al., 2003), and sewage (Arciszewski et al., 2011), and are more affected by heavy metals than salmonid species (Maret and MacCoy, 2002).

The Slimy Sculpin has been chosen as a sentinel species in Canada because of its sensitivity to environmental pollutants (Gray et al., 2018), and in the United States, the Mottled Sculpin was declared an MIS of stream quality by the USFS for the Huron-Manistee National Forests (HMNF) in Michigan, USA in 2013 (USFS, 2013). Both species occur in the Manistee River in Michigan, where the Manistee Unit of the HMNF is the principal land use manager in the approximately 4,660 km^2 watershed, with state forest and private land comprising the remainder of land ownership. Some sections of the Manistee River have been designated as a Blue-Ribbon Trout Stream, a National Recreational River, and a National Wild and Scenic River. Portions of the river also flow through lands of a federally recognized Indigenous nation, the Little River Band of Ottawa Indians (LRBOI), and the river is of cultural significance to this Tribe. Given the importance of this river, with the designation of the Mottled Sculpin as an MIS and the presence of the environmentally sensitive Slimy Sculpin, we sought to determine whether these species could be reliable indicators of environmental conditions in the HMNF and non-HMNF lands in the upper Manistee River watershed.

Criteria established by the USFS for the selection of the Mottled Sculpin as one of six MIS for the HMNF include known distribution, well-documented response to stream alteration, and an important ecological role in the habitat (USFS, 2013). These characteristics can also apply to the Slimy Sculpin (Gray et al., 2018). The similarity of environmental criteria for these two species is particularly relevant, as other species of sculpin are suggested to have different water quality tolerances (Waite and Carpenter, 2000). Mottled Sculpin and Slimy Sculpin also exhibit morphological similarity, such that ambiguity in field identification potentially limits the usefulness of using either species alone as a biological indicator. The two species can be difficult to distinguish because they are comparable in size, have similarly large heads, stout bodies, wide gapes, and are dorsoventrally compressed (Resetarits, 1995). Various morphological traits have been proposed to separate them (McAllister, 1964; Bailey et al., 2004), but these traits can still lead to uncertain discrimination in the field. Genetic analysis may be needed to resolve species identification (Baker et al., 2001; Gray et al., 2018) and to determine the relationships between morphological and molecular differences, which have not been thoroughly investigated.

To aid managers in assessment of stream quality and evaluate the reliability of MIS and sentinel species at a local scale, with implications for applicability to larger regional and national assessments, we addressed these fundamental questions: (1) can Mottled Sculpin be reliably distinguished from Slimy Sculpin?; (2) are distributions of the Mottled Sculpin and Slimy Sculpin in the HMNF sufficiently ubiquitous and sufficiently distinct for habitat assessment?; and (3) is the presence and abundance of Mottled Sculpin and Slimy Sculpin associated with stable abiotic stream conditions or more complex metrics of stream quality scores (SQS)? Specifically, we investigated the distribution of the Mottled Sculpin and the Slimy Sculpin in the upper Manistee River and its tributaries in the Manistee National Forest portion of the HMNF, distinguished the species using genetic data, and identified environmental features associated with the abundance of each species.

MATERIALS AND METHODS

Study sites and sampling procedures.—We selected 12 sites in the Manistee River watershed (hereinafter, primary sampling sites) for electrofishing surveys, beginning near the headwaters of the Manistee River in Otsego and Kalkaska Counties, and progressing downstream through Crawford, Grand Traverse, and Wexford Counties (Fig. 1, approximately 44°29′ to 44°54′N, 84°50′ to 85°37′W). Six sites were located on the Main Branch of the Manistee River (sites MB1–6) beginning near the headwaters of the Main Branch (MB1) and moving progressively downstream, but all upstream of the conjunction of the Main Branch of the Manistee River with its North Branch. On the North Branch of the Manistee, three sites were selected (NB7–9), with site identification numbers again increasing with downstream distance. Downstream from the conjunction of the Main Branch and North Branch, three sites were selected in tributary streams (Little Cannon Creek, TS10; Manton Creek, TS11; and Anderson Creek, TS12) near their entrance into the Manistee River. Sampled sites were 5–20 km apart in lateral distance within each branch. Each sampling reach was 50 m of main channel, a length we found sufficient to contain ≥ 2 geomorphic channel units (Lyons, 1992; Meador et al., 1993) or ≥ 2 different channel habitat units (Bisson et al., 2017). We also obtained sculpin samples for additional molecular analyses from six additional sites (not shown in Fig. 1) in the nearby Au Sable (AS1), Boardman (AS2), Jordan (AS3), and Rapid Rivers (AS4), as well as from the upper Manistee River at its intersection with the Cameron Bridge Road (AS5) and near its mouth in the lower Manistee River (AS6).

At the 12 sites on the MB, NB, and TS of the Manistee River, we collected sculpin with single-pass electrofishing during June and July 2015 using a Smith Root LR-24 Backpack Electrofisher with a voltage setting ranging between 200 to 280 V and a duty cycle set at a constant of 17 percent. Although more intensive sampling methods, such as three-pass removal sampling, are perceived as more accurate, statistical comparisons of single-pass and three-pass electrofishing have shown that capture levels associated with the two methods are highly correlated, and the power to detect temporal trends in abundance are similar when ≥ 5 sites were considered (Hanks et al., 2018), as was the case in our study where we examined 12 sites. We sampled in June and July as these months provide the most stable summer conditions for Mottled Sculpin and Slimy Sculpin and are less susceptible to temperature and discharge fluctuations common in earlier and later months. Collection time was recorded in seconds by the electrofisher during operation and then converted to minutes to compute sculpin abundance (sculpin per unit effort [min] = Catch Per Unit Effort [CPUE]) as an index of relative abundance. We collected and identified sculpin at each site with visual examination using a Vantage 10X double hand lens. We preserved sculpin on site in plastic jars containing 95% ethanol for further assessment in the laboratory. We selected ten sculpin individuals from each site, the minimum number we considered necessary for more detailed morphological and DNA analysis and identification. Using net sampling, we also collected sculpin from the six AS sites, along with Central Mudminnow (*Umbra limi*), Brook Trout (*Salvelinus fontinalis*), Brook Stickleback (*Culaea inconstans*), and Round Goby (*Neogobius melanostomus*) in similar minimum numbers from these and the other three AS sites, with these non-sculpin species serving as controls for DNA identification. We preserved specimens in the field at each site, following methods described in guidelines of the American Fisheries Society (Jenkins et al., 2014) and approved by the LRBOI Inland Fisheries Program, Manistee, Michigan, USA. We did not estimate CPUE at AS sites.

Morphological and molecular procedures.—We used Fisher Scientific Stereomaster 7–40X zoom magnification binocular dissecting microscopes to re-identify collected sculpin specimens in the laboratory that were previously visually examined and identified in the field. For sculpin identification, we used Bailey et al. (2004), which relies on the number of pelvic-fin rays as the primary means to distinguish Mottled Sculpin from Slimy Sculpin. We then vouchered each sculpin into individual sample jars filled with 95% ethanol for preservation. We used a DNeasy Blood and Tissue Kit (Qiagen) for DNA extraction from caudal fins.

We used the polymerase chain reaction (PCR) to amplify the cytochrome oxidase subunit 1 (CO1) portion of the mitochondrial genome. We used the primers VF2_1, FishF2_t1, FishR2_t1, and FR1d_t1 for amplification (Ivanova et al., 2007), with expected bands for this primer combination being approximately 780 base pairs (bp). We conducted PCR in 50 μl reactions of 5 μl of 10X supplied buffer, 4 μl dNTP mixture (2.5 mM each), 1 μl Titanium *Taq* DNA Polymerase, 1 μl unquantified template DNA, 1 μl of each of the four primers (10 mM each), 35 μl of sterilized, distilled water, and a drop of mineral oil in a Perkin Elmer Cetus thermal cycler with 35 cycles of 94°C for 30 seconds, 52°C for 40 seconds, and 68°C for 60 seconds. To determine if amplification was successful, we loaded 5 μl of PCR product onto a precast FlashGel and electrophoresed it according to the manufacturer's recommendations. We used ExoSAP-IT to enzymatically clean the amplified DNA. Functional Biosciences (Madison, WI) performed the capillary sequencing using primers M13F and M13R, which are embedded within the amplification primers (Ivanova et al., 2007).

We edited chromatograms of the sequenced DNA using Sequencher v. 5.2.4 (GeneCodes, Ann Arbor, MI) and conducted a BLAST search of the GenBank database with each obtained sequence and used max score to identify species matches. We then aligned the sequences into a matrix using Mesquite v. 2.75 (Maddison and Maddison, 2011). We used the alignment visualized in Mesquite and results of the preliminary phylogenetic analyses to identify samples with identical sequences. We verified differences between haplotypes by consulting the chromatograms in Sequencher. Once chromatograms had been reexamined to confirm each of the identified haplotypes, we

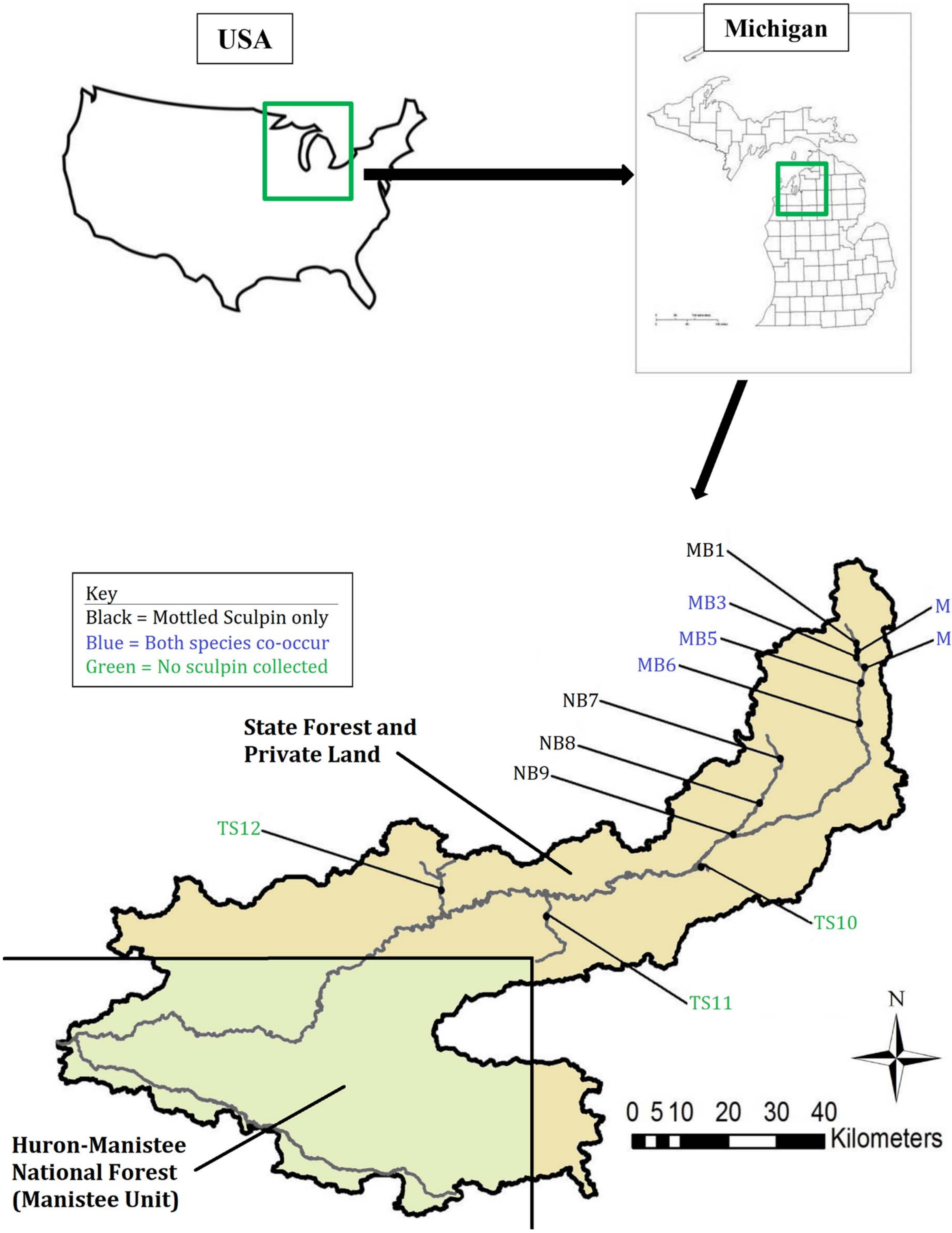

Fig. 1. Locations of 12 sampling sites on the Main Branch (MB), North Branch (NB), and tributary streams (TS) of the Manistee River examined for presence and absence of Mottled Sculpin (*Cottus bairdii*) and Slimy Sculpin (*C. cognatus*) associated with local environmental stream conditions in the upper Manistee River and its tributaries in Michigan (USA), June and July, 2015, approximately 44°29″ to 44°54″N, 84°50″ to 85°37″W. Bold black lines in the southwest portion of the figure denote the northern (horizontal) and eastern (vertical) boundaries of the Manistee Unit of the Huron-Manistee National Forests in this study area. The numerical sequence of sites is ordered to reflect descending elevation. Six associated sites (AS) on other nearby rivers (four sites) and the upper Manistee (one site) and lower Manistee River (one site) where sculpin were also collected are not shown in this figure. See Table 1.

excluded all but one sample of each haplotype to generate a matrix of the haplotypes and non-sculpin outgroups. We removed outgroups and constructed a minimum spanning network (Bandelt et al., 1999) in PopART (population analysis with reticulate trees; https://popart.maths.otago.ac.nz). We analyzed the dataset in both a distance and maximum parsimony framework in PAUP* v.4.047 (Swofford, 2002) and conducted a heuristic search with distance as the criterion, as well as a full heuristic

Table 1. Mottled Sculpin (*Cottus bairdii*) and Slimy Sculpin (*C. cognatus*) species and haplotypes represented at the collection sites on the Manistee River, Michigan (USA) and associated sites (AS) on other nearby rivers, June and July 2015. The numbers in square brackets in the Haplotypes present column are the GenBank accession numbers for the CO1 sequences of each of the 12 *Cottus* haplotypes. The accession number is given only the first time the haplotype is listed. Sites MB1 through MB6 are on the Main Branch of the Manistee River and are arranged from upstream to downstream. Sites NB7 through NB9 are on the North Branch of the Manistee River, and are also arranged from upstream to downstream. Additional sites (AS) are from neighboring rivers (AS1–4) or from the upper (AS5) or lower portion of the Manistee River (AS6). AS5 is placed to indicate its position on the Main Branch of the Manistee River between MB5 and MB6. No sculpin of either species were found in the three lowest elevation sites, Tributary Streams TS1–3.

Site	Species present	Haplotypes present
MB1	*Cottus bairdii*	1 (H2 [MW280588])
MB2	*Cottus bairdii, C. cognatus*	3 (H2, H9 [MW280595], H10 [MW280599])
MB3	*Cottus bairdii, C. cognatus*	5 (H2, H6 [MW280597], H7 [MW280598], H9, H11)
MB4	*Cottus bairdii, C. cognatus*	3 (H2, H9, H11 [MW280594])
MB5	*Cottus bairdii, C. cognatus*	4 (H2, H8 [MW280593], H9, H11)
AS5—Upper Manistee River	*Cottus cognatus*	1 (H9)
MB6	*Cottus bairdii, C. cognatus*	3 (H2, H3 [MW280590], H11)
NB7	*Cottus bairdii*	2 (H1 [MW280589], H2)
NB8	*Cottus bairdii*	1 (H2)
NB9	*Cottus bairdii*	1 (H2)
AS1—Au Sable River	*Cottus bairdii*	1 (H5 [MW280591])
AS2—Boardman River	*Cottus bairdii, C. cognatus*	2 (H2, H11)
AS3—Rapid River	*Cottus cognatus*	1 (H11)
AS4—Jordan River	*Cottus cognatus*	1 (H9)
AS6—Lower Manistee River	*Cottus bairdii, C. cognatus*	3 (H4 [MW280592], H5, H12 [MW280596])

bootstrap search of 10,000 replicates, including groups compatible with the 50% majority-rule consensus, starting with stepwise addition, simple taxon addition, and tree bisection and re-connection (TBR) branchswapping.

Sculpin and stream quality analyses.—We recorded temperature (°C), DO (mg/L), pH, conductivity (ms/cm), and turbidity (nephelometric turbidity units, NTU) with a Hydrolab HL4 (Hydrolab) at all primary sampling sites (Supplemental Table 1; see Data Accessibility). We did not control for water temperature variation relative to time of day or distance from headwaters, as the Manistee River is a groundwater-fed stream in which groundwater seepage influences temperature by providing a relatively cool input in summer and warm input in winter. In such streams, groundwater seepage affects provision of suitable water temperatures for aquatic biota and moderates other effects that could cause more extreme temperature changes (Kaandorp et al., 2019), such that air temperature–stream temperature relationships are strongly related to and largely controlled by groundwater input (Driscoll and Dewalle, 2006). We generated an estimate of elevation of each site by inserting GPS coordinates into Google Earth and used elevation as an index of the site's downstream distance in the watershed.

We collected macroinvertebrates at all primary sampling sites excluding MB2–4. The sampling protocol followed procedures specified by Michigan Clean Water Corps (MiCorps; MCWC, 2006) in which a stream is sampled by slowly walking a prescribed 100 m section of its length. We collected samples from all available habitats within the stream reach using a dip net with a 1-millimeter (mm) mesh working upstream while sampling. We sorted macroinvertebrates to order and used their presence, abundance, and diversity to compute a stream quality score (SQS; MCWC, 2006; Supplemental Table 1; see Data Accessibility), a metric that uses presence and abundance of different orders of stream macroinvertebrates, based on their association with water quality and tolerance to pollution, to generate a numerical index that can be associated with various categories of stream conditions.

Statistical analyses.—Given that elevation is a variable that aligns strongly with stream hydrogeography to the point that it has become foundational to the acquisition of hydrogeography data (Olusina and Ikwuni, 2013; Terziotti and Archuleta, 2020), we used Poisson regression to examine the relationship between elevation and CPUE for each sculpin species. We included log (seconds) as an offset to account for unequal sampling windows. For data modeling, values for site elevation were correspondingly shifted so that, to emphasize relative changes over the study area, designated elevation for the first site upstream was 0 m; in this regard, elevation difference between sites did not change in the analysis. For variables defining in-stream conditions, we compared distributions of raw data relating Mottled Sculpin and Slimy Sculpin presence to temperature, DO, conductivity, pH, NTU, and SQS using violin plots and then performed a series of logistic regressions. Each logistic regression contained fish species, a single water quality parameter, and interaction between the two as predictors in the regression. Water quality parameters were modeled separately to deal with maximum likelihood convergence issues due to quasi-complete separation of predictors and responses in the input dataset. We also compared equality of observed distributions for each water quality parameter between presence and absence locations, separately for each species, using Kolmogorov-Smirnov tests. We conducted all analyses in R v. 4.0.5 (R Core Team, 2021).

RESULTS

Field differentiation, genetic analysis, DNA sequencing, and haplotypes.—We found that Mottled Sculpin could be distinguished from Slimy Sculpin using both morphological and molecular evidence. We achieved a high degree of accuracy in the field in correctly identifying adult sculpin through visual identification with hand lens inspection, with subsequent reconfirmation of field identifications with low-power microscopic examination in the laboratory. BLAST searches differentiated Mottled Sculpin and Slimy Sculpin, supporting morphological distinction based on numbers of pelvic-fin rays. Although we found differentiation between these species to be relatively simple and reliable, it was best to observe individuals under a dissecting microscope, which we accomplished by preserving them in alcohol.

We DNA barcoded 121 sculpin. All fish samples for which we attempted DNA sequencing were successful. We edited the CO1 sequences to an aligned length of 671 bp, including two single base pair insertions in the sequence of one outgroup (*Umbra limi*). The alignment was otherwise completely unambiguous. Excluding outgroups, of the 669 bp obtained from the sculpin samples, 22 (3.3%) were variable and 18 (2.7%) were parsimony informative.

All sculpin samples from which we extracted DNA yielded CO1 sequences adequate for assigning the sample to one or the other species. Of 121 DNA-barcoded sculpin samples, we determined 60 as Slimy Sculpin, and 61 as Mottled Sculpin. We recovered 12 distinct CO1 haplotypes, five of Mottled Sculpin (H1–H5) and seven of Slimy Sculpin (H6–H12; Table 1). We deposited the CO1 sequence of each sculpin haplotype in GenBank (Table 1), as well as the newly generated outgroup CO1 sequences, which are: *Culaea inconstans* (MW280587); *Neogobius melanostomus* (MW280584); *Salvelinus fontinalis* (MW280586); and *Umbra limi* (MW280585). Of the 15 sites from which sculpins were sampled, five had only Mottled Sculpin, three had only Slimy Sculpin, and seven had both species (Table 1). Seven sites contained a single CO1 haplotype. Eight sites had between two and five haplotypes (Table 1).

The minimum spanning network we obtained from PopART (Fig. 2) gave visual depiction of 11 base pairs that consistently differentiated Mottled and Slimy Sculpin. Each of the seven Slimy Sculpin haplotypes differed from their most similar haplotype by a single base pair, and the maximum any haplotype differed from another was 4 bp (Fig. 2). We found a similar pattern for Mottled Sculpin, except that two haplotypes, H2 and H5, differed by 4 bp (Fig. 2), suggesting that, with further sampling, more Mottled Sculpin haplotypes might be recovered in the Manistee River. Both the distance-based phylogeny and maximum parsimony-based bootstrap consensus tree (Fig. 3) recovered haplotypes of both species as reciprocally monophyletic groups. Slimy Sculpins were recovered with 99% bootstrap support, but Mottled Sculpins were recovered with only 45% support (Fig. 3).

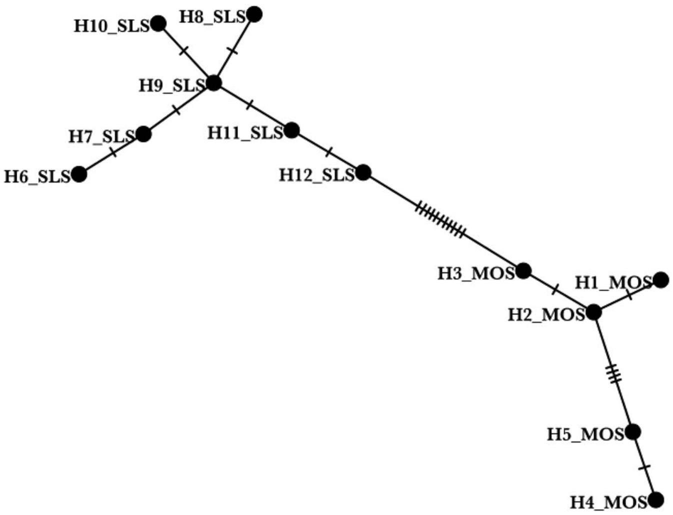

Fig. 2. Minimum spanning network of the 12 *Cottus* haplotypes from the Manistee River, Michigan (USA), June and July 2015. Each dash on the branches represents a base pair difference. Circles represent haplotypes and are labeled with the haplotype number and "MOS" for Mottled Sculpin and "SLS" for Slimy Sculpin.

Factors affecting sculpin presence.—Abundance of both Mottled and Slimy Sculpin declined with elevation (i.e., increasing downstream distance), with a decrease of 8.5% (95% CI 5.5–11.3%) Mottled Sculpin with each 1 m decline in elevation ($P < 0.001$) and a slower decline of 4.6% (3.4–5.7%; $P < 0.001$) Slimy Sculpin with each 1 m decline in elevation (Fig. 4). Over approximately 10 km of stream length in the Manistee River, species-specific relative abundance shifted from 100% Mottled Sculpin at MB1, the most upstream site, to a predominance of Slimy Sculpin at the medium elevation sites, and back to 100% Mottled Sculpin at the lowest elevation occupied sites in the North Branch (NB7, NB8, and NB9; Figs. 1, 4). We captured no sculpin in any of the three Tributary Streams (TS10, TS11, and TS12) at the three lowest elevation sites (Figs. 1, 4; Supplemental Table 1; see Data Accessibility).

Visual inspection of violin plots showed that sites with Mottled Sculpin had lower conductivity, pH, stream quality, and a narrower range of colder temperatures than sites without Mottled Sculpin, but these distributional differences were only statistically significant (at $\alpha = 0.05$) for pH ($D = 01.0$, $P = 0.009$; Fig. 5). In contrast, sites with Slimy Sculpin had higher levels of DO and narrower ranges of conductivity, pH, temperature, and turbidity than sites without Slimy Sculpin, but these distributional differences were only statistically significant (at $\alpha = 0.10$) for DO ($D = 0.75$, $P = 0.085$) and turbidity ($D = 0.75$, $P = 0.073$; Fig. 5). Due to limited sample sizes, all but one of the water quality parameters mean values were not statistically different in presence versus absence sites for either species (all $P > 0.115$). The exception was pH for Mottled Sculpin, where complete separation (i.e., all present sites had lower pH than all absent sites) prevented model convergence.

DISCUSSION

Field differentiation, genetic analyses, DNA sequencing, and haplotypes.—Species identifications are difficult within the genus *Cottus*, which has long been recognized as taxonomically challenging (Rowsey and Egge, 2017). There have been many proposals for new species classifications (Neely et al., 2007; Rudolfsen et al., 2019), as well as ongoing and unsettled debates as to phylogenetic relationships (Yokoyama and Goto, 2005). The tendency of different species to hybridize with one another (Strauss, 1986; Rudolfsen et al., 2019) and the proclivity to form new species in individual river systems (Lemoine et al., 2014) adds to challenges in identification of different sculpin species. Slimy Sculpin have also shown ability to overcome both upstream and downstream barriers to movement (Weinstein et al., 2019), which could increase their overlap with Mottled Sculpin. At sites where sculpin were present, overlapping distribution of sculpin species

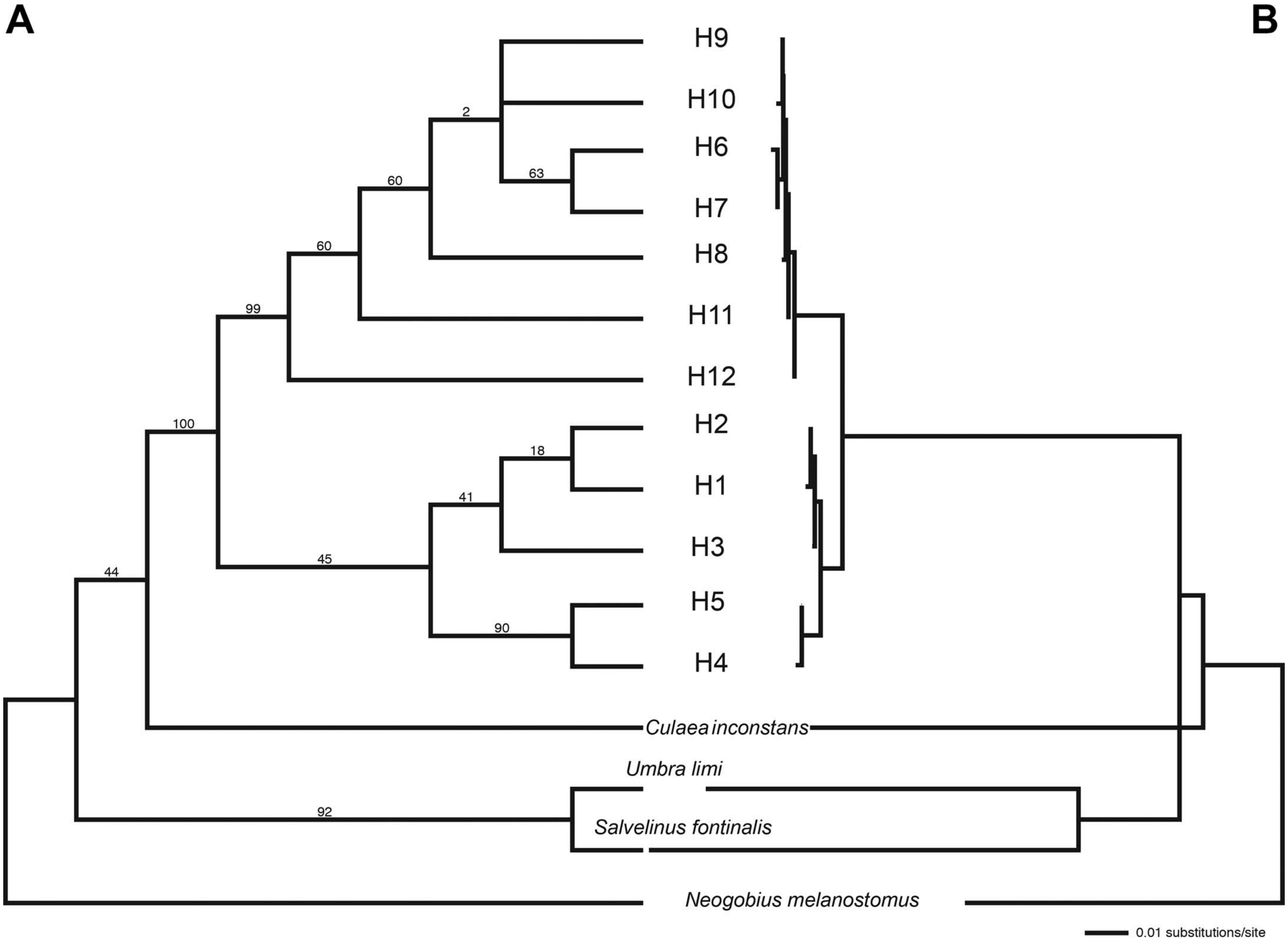

Fig. 3. (A) Maximum parsimony bootstrap consensus tree of 10,000 bootstrap replicates of the same 12 *Cottus* CO1 haplotypes and 4 outgroups of other fish species (*Culaea inconstans*, *Neogobius melanostomus*, *Salvelinus fontinalis*, and *Umbra limi*) computed in PAUP*. Numbers above branches represent the percentage of 10,000 replicates in which that clade was recovered. Haplotypes are labeled with the haplotype number; haplotypes 1–5 are Mottled Sculpin, and 6–12 are Slimy Sculpin. (B) Phylogram of 12 *Cottus* CO1 haplotypes and 4 outgroups of other fish species (*Culaea inconstans*, *Neogobius melanostomus*, *Salvelinus fontinalis*, and *Umbra limi*) computed in PAUP* with distance as the criterion from the upper Manistee River, Michigan (USA), June and July 2015. Branch lengths are proportional to the genetic distance along them. Given the number of base pairs included in the analysis, a single base pair difference is equivalent to 0.001 substitutions per site on this phylogenetic tree.

identified a region of sympatry in the upper Manistee River at 5 of the 12 electrofished sites, but our accuracy in differentiating Mottled Sculpin and Slimy Sculpin using either morphological or genetic characteristics was high.

Although we demonstrated accuracy in species differentiation, the broader problems that can occur where morphologically similar species are sympatric should not be minimized. In such cases, risk of error in identification increases risk of error in management decisions (Hammond et al., 2001; Minhós et al., 2013; Vantieghem et al., 2016). Although we found this risk to be low in the upper Manistee River and its tributaries, the sympatry of Mottled and Slimy Sculpin could still be problematic to the value of using either species alone as an MIS or sentinel species.

The observed sympatry between Mottled Sculpin and Slimy Sculpin in a portion of the Main Branch of the Manistee River's total stream length offered opportunity for hybridization. Such hybridization could lead to ambiguity in identification. In our study, we did not find any individuals of obvious intermediate morphology, which would be expected if hybridization were occurring. This absence of such hybridization, as well as the failure to find individuals of intermediate form, supports the use of both species as MIS, but remains a relevant concern in the HMNF because Slimy Sculpin have been shown to hybridize with Mottled Sculpin (Strauss, 1986) and Rocky Mountain Sculpin, a species once classified in the *Cottus bairdii* complex (Rudolfsen et al., 2019). Phylogenetic studies that can add to the CO1 sequences generated here might be needed to determine conclusively if hybrid sculpin are present in areas of sympatry (Kinziger and Raesly, 2001).

Our phylogenetic results obtained from the minimum spanning network distance and maximum parsimony suggested a down-to-up river phylogeographic pattern for the Slimy Sculpin, with haplotypes from further upstream being successively derivative of more downstream haplotypes. The phylogeographic pattern for Mottled Sculpin was less obvious but suggested that some Mottled Sculpin haplotypes were missing. We predict that these could be detected with greater sampling.

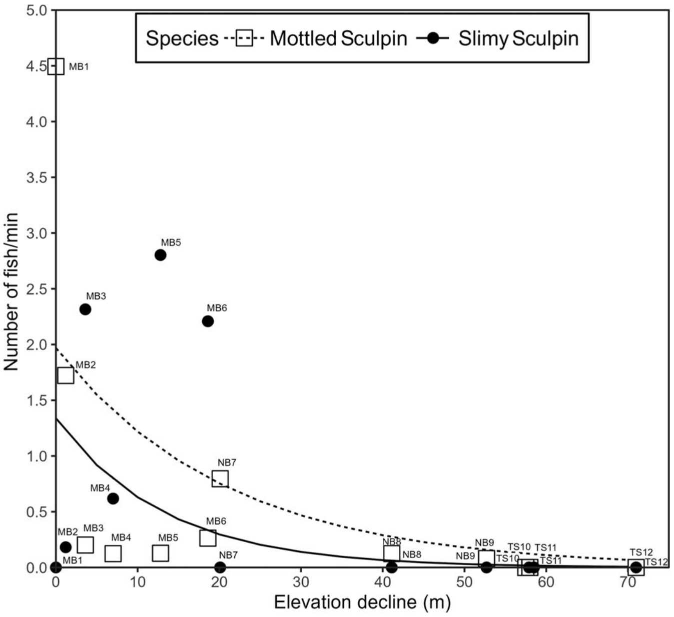

Fig. 4. Relationship (Poisson regression) between catch per unit effort (CPUE) and elevation decline (m) from highest elevation for sites with Mottled Sculpin (*Cottus bairdii*) or Slimy Sculpin (*C. cognatus*) present in the upper Manistee River and its tributaries, Michigan (USA), June and July 2015. Dashed line indicates decline in abundance with elevation for Mottled Sculpin. Solid line indicates decline in abundance with elevation for Slimy Sculpin.

Distribution of Mottled Sculpin and Slimy Sculpin.—Various studies have identified multiple factors influencing sculpin presence and abundance, and many such factors indicate covariance and integration. Population stability, for example, has been positively associated with habitat stability and, correspondingly, with fine-scale (30–50 m) spatial processes rather than broad-scale spatial processes (Grossman et al., 2006). This relationship is predictable given the relatively low movement and dispersal patterns of sculpins, particularly as species of benthic habitats with home areas ranging from only 1–50 m of stream length (McCleave, 1964; Hill and Grossman, 1987; Petty and Grossman, 2007). In more specific analyses of habitat influence, sculpin abundance has been variously related to changes in channel width, overall stream size, and hydrological variability (Rudolfsen et al., 2019). In a comprehensive study of multiple datasets comprising all sculpins species native to Idaho (USA), Higens and Scarnecchia (2021) found that sculpins were more likely to be present and in higher densities in streams with abundant riffle microhabitats that were mostly free of sediment (Higgins and Scarnecchia, 2021). Such patterns of habitat-related abundance support an underlying hypothesis of this study, that sculpin species may indeed be reliable environmental indicators given their sedentary nature and extreme site faithfulness providing indices of highly site-specific conditions.

In our study, abundance of Mottled Sculpin and Slimy Sculpin declined with downstream distance, supporting and consistent with the view of sculpin as a headwater species more abundant in the less modified and less variable stream conditions associated with headwaters in the Manistee River compared to its downstream environments. Stream elevation also appeared to affect the sympatric and allopatric characteristics of Mottled and Slimy Sculpin in this study. In the Manistee River, the distribution of Mottled Sculpin and Slimy Sculpin was sometimes sympatric, but Mottled Sculpin exhibited allopatry at sites with the highest and lowest elevations, suggesting that fine-scale, site-specific variations in habitat may be contributing determinants of species presence. The observed allopatry of Mottled Sculpin in the North Branch of the Manistee, where we did not find Slimy Sculpin, also suggested that geomorphic factors other than elevation might influence distribution, even in streams in close spatial proximity to one another. Our data cannot address hypotheses regarding specific physical stream characteristics directly, but geomorphic factors like stability in channel width, stream size, and flow levels may all be influenced by stream elevation (Rudolfsen et al., 2019).

Regions of elevational sympatric distribution of Mottled Sculpin and Slimy Sculpin also suggested that some instrument of niche separation might exist between these species. One mechanism could be the territorial behavior of Mottled Sculpin, which are known to compete against and exclude other species for feeding sites (Petty and Grossman, 2007). Such territorial behavior would have increased effect on species such as the Mottled and Slimy Sculpin, in which reduced mobility and high levels of site fidelity (Petty and Grossman, 2004; Breen et al., 2009) could increase the frequency of interactions with other species using or attempting to use the same habitat.

Physical characteristics of streams are not the only influence on sculpin distribution. Multiple studies have shown that temperature may be an overarching determinant of both presence and distribution (Adams et al., 2015) that may in turn influence effects of other factors. For example, in a fully factorial laboratory experiment on the Slimy Sculpin, Pennock et al. (2021) determined that, although growth rates were higher in high food environments, the magnitude of difference to contrasting low food environments was dependent on temperature (Pennock et al., 2021). Temperature, with other factors, also influenced the response of Mottled Sculpin to re-inhabiting restored streams from which they had been previously exterminated (Shirey et al., 2016). Additionally, presence of and negative interactions with other fish species, especially non-native species, might also influence distribution, even resulting in site-specific extinctions (Janssen and Jude, 2001).

Should the Mottled Sculpin be used as an MIS for the Huron-Manistee National Forests?—Our analysis found few environmental variables that differed between stream sites where these species were present or absent. Mottled Sculpin were absent from more alkaline streams (higher pH values) and Slimy Sculpin from streams with DO levels less than 9.0 ppm and greater turbidity. However, given the small number of variables unequivocally defining presence or absence in either species, our results do not fully support the USFS's designated use of the Mottled Sculpin alone as an MIS for stream habitat quality. The SQS measurements of all 12 of our primary sampling sites were high and may not have encompassed the range of SQS variation needed to produce more significant results. However, sites at which one or both Mottled Sculpin and Slimy Sculpin were present tended to have a narrower range of values for most variables associated with water quality, suggesting that the Mottled Sculpin and Slimy Sculpin may be more sensitive

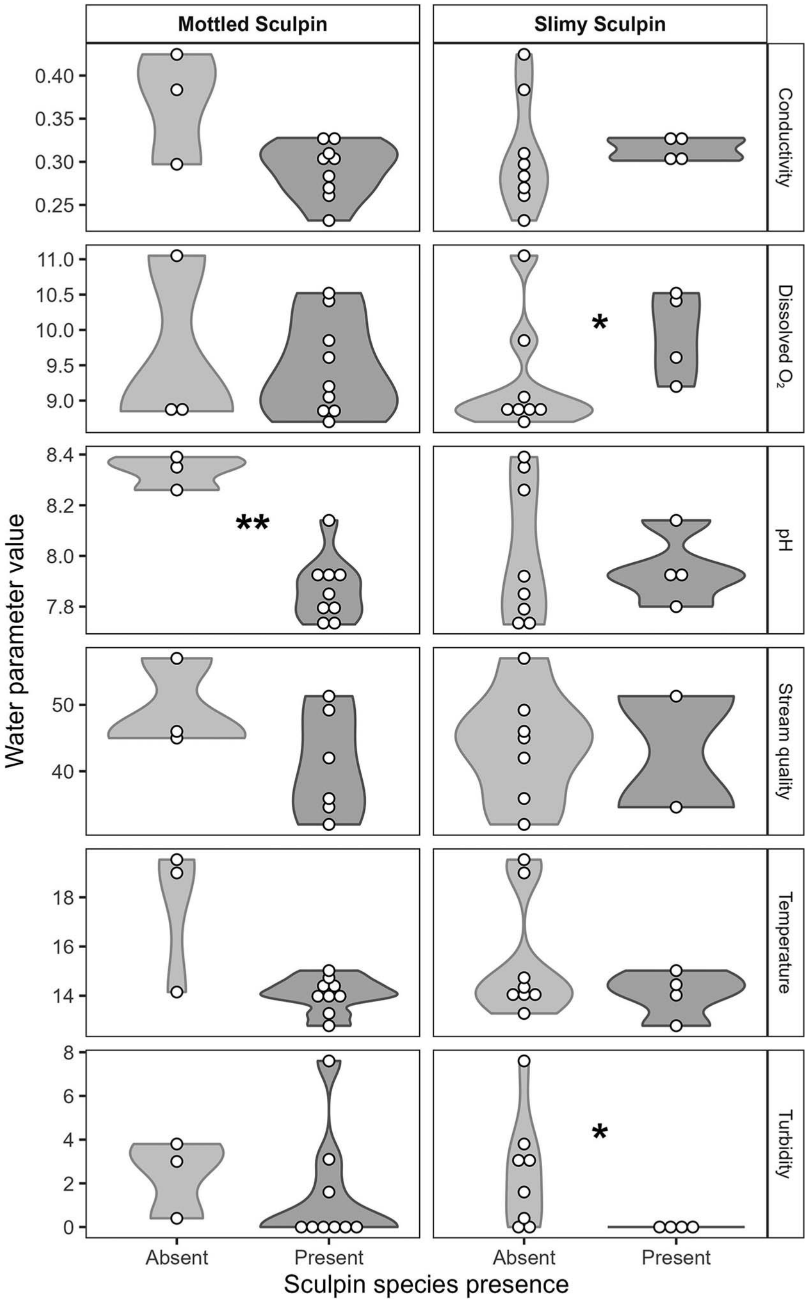

Fig. 5. Violin plots of relationships between water quality parameters and absence or presence of Mottled Sculpin (*Cottus bairdii*) and Slimy Sculpin (*C. cognatus*) in the upper Manistee River, Michigan (USA), June and July 2015. Measurements of conductivity in ms/cm, dissolved oxygen (DO) in mg/L, pH, temperature in °C, and turbidity in nephelometric turbidity units (NTU). Stream quality scores were based on orders of macroinvertebrates discovered, with the presence of less tolerant macroinvertebrates elevating the score. >48 = Excellent, 34–48 = Good, 19–33 = Fair, <19 = Poor (Michigan Clean Water Corps, 2006). Comparisons with asterisks had significantly different distributions at $P < 0.10$ (*) and $P < 0.05$ (**).

to such variables in ways that SQS values alone might obscure. The traditional conception of high-quality freshwater streams as having clear, cold, highly oxygenated water with expectedly diverse stream macroinvertebrate communities (and correspondingly high SQS; Environmental Protection Agency, 2019) matched some, but not all, conditions associated with the presence of both species in this study. Water temperature at sites with Mottled Sculpin, Slimy Sculpin, or both was always cold, but sites where either or both species were absent included both colder and warmer waters. Selection of cold-water temperatures is conducive to high growth rates in sculpin, although the selected mean temperature of 14°C in this study was two degrees warmer than that selected by Slimy Sculpin in laboratory experiments in which fish could select from a range of temperatures (Pennock et al., 2021).

Previous studies have suggested, when multiple studies are evaluated, that these two species of sculpin could provide sensitive indicators of multiple water parameters and thus function in concert as an MIS. In particular, Mottled Sculpin have shown increases in population size over time in restored stream sites compared to unrestored sites in the same stream (Shirey et al., 2016), making this species potentially and especially valuable as an indicator of the effectiveness of restorations designed to improve stream habitat. However, imperfect associations between individual indicator species and the habitats or conditions they have been presumed to indicate can be routinely expected (Barry et al., 2006). In this regard, previous investigators have shown that sculpin are sensitive to many variables indicative of water conditions to a varying, and not always predictable, extent (Quist et al., 2004), or that some effects of stream quality, such as sediment levels, affect sculpin through sex-specific, physiologic responses, such as gonad development in males, rather than in abundance (Erdozain et al., 2021).

We found Mottled Sculpin mainly at sites with water parameter variables and SQS values characteristic of high-quality streams, but we did not find Mottled Sculpin in every high-quality stream we sampled (Supplemental Table 1; see Data Accessibility). However, such a deficiency might reflect our small sample size and limited analysis of environmental variables. Slimy Sculpin were also found in cold water streams with similar stream quality scores, but otherwise displayed differences in abundance relative to different variables such as DO and turbidity. This distribution, coupled with declining abundance of both species in the lower reaches of the Manistee River (M. Holtgren, pers. comm., LRBOI, 2014), suggests that these species might be indicators of higher quality stream conditions. However, if headwater habitats are periodically colonized by Mottled Sculpin and Slimy Sculpin, then patch dynamics might produce variability in abundance without meaningful differences in habitat quality.

As previously noted, sculpins are known for their restricted movements and high levels of site fidelity as adults, and seasonal change does not influence movement (Breen et al., 2009). In Michigan streams, however, Mottled Sculpin have been shown to move up to 511 m/yr, with up to 16% of adults moving > 100 m/yr. Past studies in northwest Michigan streams have documented that 86–100% of both Mottled and Slimy Sculpin adults did not remain within 30 m stretches of stream over a single season (Shetter and Hazzard, 1939), and studies from other areas have demonstrated that individual sculpin may move relatively large distances (Schmetterling and Adams, 2004; Clarke et al., 2015; DeBoer et al., 2015). Such findings suggest that sculpin may have the capacity to be more selective of stream habitat, even once established as adults, than previously suspected (Breen et al., 2009).

However, species associations with particular sites or habitats might not necessarily be indicators that the site or habitat reflects the niche of the species, and therefore the species may be an imperfect indicator of stream habitat quality. As De Cáceres et al. (2010) point out, such associations may be (1) a random event, (2) reflections of historical events (environmental perturbations in the system) or non-niche-related species characteristics (population fluctuations or dispersal patterns), or (3) genuine associations reflecting the species preferences, but not ones the manager is assuming or even aware of. The third problem may be reflected in factors we did not examine, including and especially habitat structure. Bond and Jones (2015) noted the importance of longitudinal gradients in rivers with respect to fish abundance and distribution, with longitude serving as an integrator of changes in velocity, temperature, food resources (including and especially macroinvertebrates), and coarse particulate matter. Quist et al. (2004) determined that Mottled Sculpin were absent from reaches in mountain streams with high velocity and large rocky substrates, suggesting that they required slower-water habitats for a portion of their development. Similarly, some streams in the Manistee River watershed that did not contain both species might be healthy streams that did not provide sufficient habitat juxtaposition for adequate niche separation. Such findings might explain why no sculpin were found at our three most downstream sites, despite those sites possessing similar water quality in most variables and higher stream quality scores compared to other sites.

We cannot offer an inference as to why Mottled Sculpin were present at sites with lower SQS scores, which are primarily reflections of composition and diversity of the stream macroinvertebrate community, a metric that many view as the single best biotic indicator of stream condition (Lydy et al., 2000). Other studies have noted that the relationship between sculpin and macroinvertebrates is not a correlation between independent assessments of water quality, but as an expression of trophic linkage (McGinley et al., 2013). Petty and Grossman (1996) observed that macroinvertebrate prey abundance influenced patch selection by sculpin more than physical microhabitat characteristics. Dynamics of macroinvertebrate populations and habitat structure influencing sculpin distribution are not well understood, nor were they examined in our study. It is possible that sculpin abundance in the Manistee River and similar streams does not correlate with macroinvertebrates in ways related to SQS, but to specific distributions and abundances of individual macroinvertebrate species.

Conclusions.—Our data are limited to one river and watershed, should not be assumed to apply to other watersheds without further study, and are inconclusive with regard to whether the Mottled Sculpin and Slimy Sculpin, together or separately, may be fully discriminating indicators of stream habitat quality. Nevertheless, we offer some general insights from this investigation that might have wider applicability. There is warrant for using both the Mottled Sculpin and Slimy Sculpin together as indicators, and thus we recommend using an MIS complex of these two species in the Manistee watershed, rather than using only Mottled Sculpin as an MIS, as it has been previously designated. Using Mottled Sculpin alone could lead to a disregard of high-quality waters where this species was not present, and Mottled Sculpin lack a sufficiently ubiquitous distribution to ignore this risk. Inclusion of the Slimy Sculpin would permit coverage of a greater proportion of the watershed and reduce concerns regarding incorrect identification at the species level given that such distinction would not need to be made. Alternatively, there is warrant to consider using SQSs, such as those generated by estimation of stream macroinvertebrate diversity, and to incorporate consideration of variables affecting stream habitat structure, as well as water conditions. At a more systemic scale, managers should continue to refine and use quantitative models of species–site associations to, whenever possible, develop more precise understanding of underlying causative factors of such associations and what such associations indicate.

DATA ACCESSIBILITY

The data supporting the results of this study have not been published on any website, but all data generated or analyzed during this study are included in this manuscript and its supplementary information file (https://www.ichthyologyandherpetology.org/i2021132). Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

ACKNOWLEDGMENTS

Funding for this study was provided by the Little River Band of Ottawa Indians, a Native American tribal nation indigenous to Michigan (USA), as part of their more comprehensive efforts in research and rehabilitation of the Lake Sturgeon (*Acipenser fulvescens*), a species of high cultural significance to the Tribe. The findings, opinions, and recommendations expressed within this publication are those of the authors and not necessarily those of the Bureau of Indian Affairs (BIA) or of the Tribe. C. Riley provided guidance in the development of the original research proposal. M. Holtgren provided assistance and encouragement throughout the study. Au Sable Institute (Michigan, USA) provided room, board, and support for the research team. H. W. Garris, M. Holtgren, and R. E. Westerhof reviewed preliminary drafts of the manuscript.

LITERATURE CITED

Adams, S. B., and D. A. Schmetterling. 2007. Freshwater sculpins: phylogenetics to ecology. Transactions of the American Fisheries Society 136:1736–1741.

Adams, S. B., D. A. Schmetterling, and D. A. Neely. 2015. Summer stream temperatures influence sculpin distribution and spatial partioning in the upper Clark Fork River basin, Montana. Copeia 103:416–428.

Allert, A. L., J. F. Fairchild, C. J. Schmitt, J. M. Besser, W. G. Brumbaugh, and S. J. Olson. 2009. Effects of mining-derived metals on riffle-dwelling benthic fishes in southeast Missouri, USA. Ecotoxicology and Environmental Safety 72:1642–1651.

Arciszewski, T. J., K. A. Kidd, and K. R. Munkittrick. 2011. Comparing responses in the performance of sentinel populations of stoneflies (Plecoptera) and slimy sculpin (*Cottus cognatus*) exposed to enriching effluents. Ecotoxicology and Environmental Safety 74:1844–1854.

Bailey, R. M., W. C. Latta, and G. R. Smith. 2004. An atlas of Michigan fishes with keys and illustrations for their identification. Miscellaneous Publications of the Museum of Zoology. No. 192. University of Michigan, Ann Arbor.

Baker, R. L., L. M. Chandler, and T. T. Eckdhal. 2001. Identification of *Cottus* species in Montana using mitochondrial RFLP analysis. Bios 72:87–91.

Bandelt, H., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Barry, D., R. A. Fischer, K. W. Hoffman, T. Barry, E. G. Zimmerman, and K. L. Dickson. 2006. Assessment of habitat values for indicator species and avian communities in a riparian forest. Southeastern Naturalist 5:295–310.

Bellinger, E. G., and D. C. Sigee. 2010. Freshwater Algae: Identification and Use as Bioindicators. Wiley, West Sussex, UK.

Besser, J. M., W. G. Brumbaugh, A. L. Allert, B. C. Poulton, C. J. Schmitt, and C. G. Ingersoll. 2009. Ecological impacts of lead mining on Ozark streams: toxicity of sediment and pore water. Ecotoxicology and Environmental Safety 72:516–526.

Besser, J. M., C. A. Mebane, D. R. Mount, C. D. Ivey, J. L. Kunz, I. E. Greer, T. W. May, and C. L. Ingersoll. 2007. Sensitivity of Mottled Sculpins (*Cottus bairdii*) and Rainbow Trout (*Onchorhynchus mykiss*) to acute and chronic toxicity of cadmium, copper, and zinc. Environmental Toxicology and Chemistry 26:1657–1665.

Bisson, P. A., D. R. Montgomery, and J. M. Buffington. 2017. Valley segments, stream reaches, and channel units, p. 21–47. *In*: Methods in Stream Ecology. Third edition. F. R. Hauer and G. Lambert (eds.). Elsevier, San Diego, California.

Bond, M. J., and N. E. Jones. 2015. Spatial distribution of fishes in hydropeaking tributaries of Lake Superior. River Research and Applications 31:120–133.

Brasfield, S. M., L. M. Hewitt, L. Chow, S. Batchelor, H. Rees, Z. Z. Xing, and K. R. Munkittrick. 2015. Assessing the contribution of multiple stressors affecting small-bodied fish populations through a gradient of agricultural inputs in northwestern New Brunswick, Canada. Water Quality Research Journal of Canada 50:182–197.

Breen, M. J., C. R. Ruetz III, K. J. Thompson, and S. L. Kohler. 2009. Movements of Mottled Sculpins (*Cottus bairdii*) in a Michigan stream: how restricted are they? Canadian Journal of Fisheries and Aquatic Sciences 66:31–41.

Caetano, D., E. F. de Oliveira, and C. H. Zawadzki. 2016. Fish species indicators of environmental quality of neotropical streams in southern Brazil, upper Paraná River Basin. Acta Ichthyologial et Piscatoria 46:87–96.

Clarke, A. D., K. H. Telmer, and J. M. Shrimpton. 2015. Movement patterns of fish revealed by otolith microchemistry: a comparison of putative migratory and resident species. Environmental Biology of Fishes 98:1583–1597.

De Cáceres, M., R. Legendre, and M. Moretti. 2010. Improving indicator species analysis by combining groups of sites. Oikos 119:1674–1684.

DeBoer, J. A., J. M. Holtgren, S. A. Ogren, and E. B. Snyder. 2015. Movement and habitat use by mottled sculpin after restoration of a sand-dominated 1st-order stream. The American Midland Naturalist 173:335–345.

Dos Santos, D. A., C. Molineri, M. C. Reynaga, and C. Basualdo. 2011. Which index is best to assess stream health? Ecological Indicators 11:582–589.

Driscoll, M. O., and D. R. Dewalle. 2006. Stream-air temperature relations to classify stream-groundwater interactions in a karst setting, central Pennsylvania, USA. Journal of Hydrology 329:140–153.

Dubé, M. G., D. L. MacLatchy, J. D. Kieffer, N. E. Glozier, J. M. Culp, and J. J. Cash. 2005. Effects of metal mining effluent on Atlantic salmon (*Salmo salar*) and slimy sculpin (*Cottus cognatus*): using artificial streams to assess existing effects and predict future consequences. Science of the Total Environment 343:135–154.

Dziock, F., K. Henle, F. Foeckler, K. Follner, and M. Scholz. 2006. Biological indicator systems in floodplains—a review. International Review of Hydrobiology 4:271–291.

Environmental Protection Agency. 2019. Examples of watershed assessments for watershed health. https://www.epa.gov/hwp/examples-water-quality-assessments-watershed-health (accessed 15 July 2021).

Erdozain, M., K. A. Kidd, E. J. S. Emilson, S. S. Capell, T. Luu, D. P. Kreutzweiser, and M. A. Gray. 2021. Forest management impacts on stream integrity at varying intensities and spatial scales: do biological effects accumulate spatially? Science of the Total Environment 753:141968.

Galloway, B. J., K. R. Munkittrick, S. Currie, M. Gray, R. A. Curry, and C. S. Wood. 2003. Examination of the responses of slimy sculpin (*Cottus cognatus*) and white sucker (*Catostomus commersoni*) collected on the Saint

John River (Canada) downstream of pulp mill, paper mill, and sewage discharges. Environmental Toxicology and Chemistry 22:2898–2907.

Gray, M. A., R. A. Curry, T. J. Arciszewski, K. R. Munkittrick, and S. M. Brasfield. 2018. The biology and ecology of the slimy sculpin: a recipe for effective environmental monitoring. FACETS 3:103–127.

Gray, M. A., R. A. Curry, and K. R. Munkittrick. 2002. Nonlethal sampling techniques for assessing environmental impacts using a small-bodied sentinel fish species. Water Quality Research Journal of Canada 37:195–211.

Grossman, G. D., R. E. Ratajczak Jr., J. T. Petty, M. D. Hunter, J. T. Peterson, and G. Grenouillet. 2006. Population dynamics of mottled sculpin (Pisces) in a variable environment: information theoretic approaches. Ecological Monographs 76:217–234.

Hammond, R. L., W. Macasero, B. Flores, O. B. Mohanned, T. Wacher, and M. W. Bruford. 2001. Phylogenetic reanalysis of the Saudi gazelle and its implications for conservation. Conservation Biology 15:1123–1133.

Hanks, R. D., Y. Kanno, and J. M. Rash. 2018. Can single-pass electrofishing replace three-pass detection for population trend detection? Transactions of the American Fisheries Society 147:729–739.

Higens, T. A., and D. A. Scarnecchia. 2021. Abiotic and biotic factors associated with sculpin presence and density in northern Idaho streams. Northwest Science 95:173–187.

Hill, J., and G. D. Grossman. 1987. Home range estimates for three North American stream fishes. Copeia 1987:376–380.

Hossein, M., R. Schinegger, A. Melcher, K. Moder, C. Mielach, and S. Schmutz. 2015. A new fish-based multimetric assessment index for cyprinid streams in the Iranian Caspian Sea Basin. Limnologica 51:37–52.

Ivanova, N. V., T. S. Zemlak, R. H. Hanner, and P. D. N. Hebert. 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes 7:544–548.

Janssen, J., and D. J. Jude. 2001. Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbor, southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. Journal of Great Lakes Research 27:319–328.

Jenkins, J. A., H. L. Bart, Jr., J. D. Bowker, P. R. Bowser, J. R. MacMillan, J. G. Nickum, J. W. Rachlin, J. D. Rose, P. W. Sorensen, B. E. Warkentine, and G. W. Whitledge. 2014. Guidelines for Use of Fishes in Research—Revised and Expanded, 2014. Fisheries 39:415–416.

Kaandorp, V. P., P. J. Doornenbal, K. Kooi, H. P. Broers, and G. B. de Louw. 2019. Temperature buffering by groundwater in ecologically valuable lowland streams under current and future climate conditions. Journal of Hydrology 3:100031.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21–27.

Kinziger, A., and R. Raesly. 2001. A narrow hybrid zone between two *Cottus* species in Wills Creek, Potomac Drainage. Journal of Heredity 92:309–314.

Landres, P. B., J. Verner, and J. W. Thomas. 1988. Ecological uses of vertebrate indicator species: a critique. Conservation Biology 2:316–328.

Lemoine, M. L., M. K. Young, K. S. McKelvey, L. Eby, K. L. Pilgrim, and M. K. Schwartz. 2014. *Cottus schitsuumsh*, a new species of sculpin (Scorpaeniformes: Cottidae) in the Columbia River basin, Idaho-Montana, USA. Zootaxa 3755:241–258.

Lessard, J. L., and D. B. Hayes. 2003. Effects of elevated water temperature on fish and macroinvertebrate communities below small dams. River Research and Applications 19:721–732.

Lindenmayer, D. B., C. R. Margules, and D. B. Dotkin. 2000. Indicators of biodiversity for ecologically sustainable forest management. Conservation Biology 14:941–950.

Lydy, M. J., C. G. Crawford, and J. W. Frey. 2000. A comparison of selected diversity, similarity, and biotic indices for detecting changes in benthic-invertebrate community structure and stream quality. Archives of Environmental Contamination and Toxicology 39:469–479.

Lyons, J. 1992. The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. North American Journal of Fisheries Management 12:198–203.

Maddison, D., and W. Maddison. 2011. Mesquite: a modular system for evolutionary analysis. https://mesquiteproject.org (accessed 15 July 2015).

Maret, T. R., and D. E. MacCoy. 2002. Fish assemblages and environmental variables associated with hard-rock mining in the Coeur d'Alene River basin, Idaho. Transactions of the American Fisheries Society 131:865–884.

McAllister, D. E. 1964. Distinguishing characteristics for the sculpins *Cottus bairdi* and *Cottus cognatus* in eastern Canada. Journal of the Fisheries Research Board of Canada 21:1339–1342.

McCleave, J. D. 1964. Movement and population of the Mottled Sculpin (*Cottus bairdi* Girard) in a small Montana stream. Copeia 1964:506–513.

McGinley, E., R. Raesley, and W. Seddon. 2013. The effects of embeddedness on the seasonal feeding of Mottled Sculpin. The American Midland Naturalist 170:213–228.

Meador, M. R., T. F. Cuffney, and M. E. Gurtz. 1993. Methods for sampling fish communities as part of the national water-quality assessment program. U.S. Geological Survey. U.S. Department of the Interior Washington, D.C. https://water.usgs.gov/nawqa/protocols/OFR-93-104/fish5.html (accessed 21 March 2021).

Michigan Clean Water Corps (MCWC). 2006. MiCorps volunteer stream monitoring procedures. Michigan Clean Water Corps. https://micorps.net/wp-content/uploads/2021/01/VSMP-MonitoringProcedures.pdf (accessed 3 June 2020).

Miller, L. L., M. A. Isaacs, C. J. Martyniuk, and K. R. Munkittrick. 2015. Using molecular biomarkers and traditional morphometric measurements to assess the health of slimy sculpin (*Cottus cognatus*) from streams with elevated selenium in North-Eastern British Columbia. Environmental Toxicology and Chemistry 34:2335–2346.

Minhós, T., E. Wallace, M. J. Ferreira da Silva, R. M. Sá, M. Carmo, A. Barata, and M. W. Bruford. 2013. DNA identification of primate bushmeat from urban markets in Guinea-Bissau and its implications for conservation. Biological Conservation 167:43–49.

Moya, N., S. Tomanova, and T. Oberdorff. 2007. Initial development of a multi-metric index bases on aquatic macroinvertebrates to assess streams condition in the Upper Isiboro-Sécure Basin, Bolivian Amazon. Hydrobiologia 589:107–116.

NatureServe Explorer 2.0. 2021. *Cottus bairdii* Mottled Sculpin. https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.819868/Cottus_bairdii (accessed 19 May 2021).

Neely, D. A., J. D. Williams, and R. L. Mayden. 2007. Two new sculpins of the genus *Cottus* (Teleostei: Cottidae) from rivers of North America. Copeia 2007:641–656.

Neumann, M., M. Liess, and R. Schulz. 2003. An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators, Part 2: The knowledge base of LIMPACT. Ecological Indicators 2:391–401.

Olusina, J. O., and M. N. Ikwuni. 2013. Modeling hydrological functions using Digital Elevation Model (DEM) and Soil Conservation Service (SCS) Model. International Journal of Science and Emerging Technologies 6:284–293.

Otto, R. G., and J. O. Rice. 1977. Responses of a freshwater sculpin (*Cottus cognatus gracilis*) to temperature. Transactions of the American Fisheries Society 106:89–94.

Pander, J., and J. Geist. 2013. Ecological indicators for stream restoration success. Ecological Indicators 30:106–118.

Pennock, C. A., P. Budy, C. L. Atkinson, and N. Barrett. 2021. Effects of increased temperature on arctic slimy sculpin *Cottus cognatus* is mediated by food availability: implications for climate change. Freshwater Biology 66:549–561.

Petty, J. T., and G. D. Grossman. 1996. Patch selection by Mottled Sculpin (Pisces: Cottidae) in a southern Appalachian stream. Freshwater Biology 35:261–276.

Petty, J. T., and G. D. Grossman. 2004. Restricted movement by Mottled Sculpin (Pisces: Cottidae) in a southern Appalachian stream. Freshwater Biology 49:631–645.

Petty, J. T., and G. D. Grossman. 2007. Size-dependent territoriality of Mottled Sculpin in a southern Appalachian stream. Transactions of the American Fisheries Society 136:1750–1761.

Quist, M. C., W. A. Hubert, and D. J. Isaak. 2004. Factors affecting allopatric and sympatric occurrence of two sculpin species across a Rocky Mountain watershed. Copeia 2004:617–623.

R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Resetarits, W. J., Jr. 1995. Limiting similarity and the intensity of competitive effects on the Mottled Sculpin, *Cottus bairdii*, in experimental stream communities. Oecologia 104:31–38.

Rowsey, D. M., and J. J. D. Egge. 2017. Morphometric analysis of two enigmatic sculpin species, *Cottus gulosus* and *Cottus perplexus* (Scorpaeniformes: Cottidae). Northwestern Naturalist 98:190–202.

Rudolfsen, T., J. W. L. Ruppert, E. B. Taylor, C. S. Davis, D. A. Watkinson, and M. S. Poesch. 2019. Habitat use and hybridisation between Rocky Mountain Sculpin (*Cottus* sp.) and Slimy Sculpin (*Cottus cognatus*). Freshwater Biology 64:391–404.

Schmetterling, D. A., and S. B. Adams. 2004. Summer movements within the fish community of a small montane stream. North American Journal of Fisheries Management 24:1163–1172.

Shetter, D. S., and A. S. Hazzard. 1939. Species composition by age groups and stability of fish populations in sections of three Michigan trout streams. Transactions of the American Fisheries Society 68:281–302.

Shirey, P. D., M. A. Brueseke, J. B. Kenny, and G. A. Lamberti. 2016. Long-term fish community response to a reach-scale stream restoration. Ecology and Society 21:11.

Smale, D. A., T. J. Langlois, G. A. Kendrick, J. J. Meeuwig, and E. S. Harvey. 2011. From fronds to fish: the use of indicators for ecological monitoring in marine benthic ecosystems, with case studies from temperate Western Australia. Reviews in Fish Biology and Fisheries 21:311–337.

Strauss, R. E. 1986. Natural hybrids of the freshwater sculpins *Cottus bairdi* and *Cottus cognatus* (Pisces: Cottidae): electrophoretic and morphometric evidence. American Midland Naturalist 115:87–105.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Sinauer, Sunderland, Massachusetts.

Symons, P. E. K., J. L. Metcalfe, and G. D. Harding. 1976. Upper lethal and preferred temperatures of the slimy sculpin, *Cottus cognatus*. Journal of the Fisheries Research Board of Canada 33:180–183.

Terziotti, S., and C. M. Archuleta. 2020. Elevation-derived hydrography acquisition specifications: U.S. Geological Survey Techniques and Methods, book 11, chap. B11. https://doi.org/10.3133/tm11B11

US Forest Service (USFS). 2013. Huron-Manistee Forests Plan and Environmental Impact Statement. Appendix G. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fsm8_046666.pdf (accessed 14 January 2015).

US Geological Survey (USGS). 2021. *Cottus bairdii* Mottled Sculpin. https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.819868/Cottus_bairdii (accessed 2 February 2021).

Van Dyke, F. 2008. Conservation Biology: Foundations, Concepts, Applications. Second edition. Springer, Dordrecht, The Netherlands.

Vantieghem, P., D. Maes, A. Kaiser, and T. Merckx. 2016. Quality of citizen science data and its consequences for the conservation of skipper butterflies (Hesperiidae) in Flanders (northern Belgium). Journal of Insect Conservation 21:451–463.

Waite, I. R., and K. D. Carpenter. 2000. Associations among fish assemblage structure and environmental variables in Willamette Basin streams, Oregon. Transactions of the American Fisheries Society 129:754–770.

Weinstein, S. Y., J. A. Coombs, K. H. Nislow, C. Riley, A. H. Roy, and A. R. Whiteley. 2019. Evaluating the effects of barriers on Slimy Sculpin (*Cottus cognatus*) movement and population connectivity using novel sibship-based and traditional genetic metrics. Transactions of the American Fisheries Society 148:1117–1131.

Yokoyama, R., and A. Goto. 2005. Evolutionary history of freshwater sculpins, genus *Cottus* (Teleostei: Cottidae) and related taxa, as inferred from mitochondrial DNA phylogeny. Molecular Phylogenetics and Evolution 36:654–668.

Zacharias, M. A., and X. C. Roff. 2001. Use of focal species in marine conservation and management: a review and critique. Aquatic Conservation Marine Freshwater Ecosystems 11:59–76.