

Quantifying tolerance indicator values for common stream fish species of the United States

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Abstract

The classification of fish species tolerance to environmental disturbance is often used as a means to assess ecosystem conditions. Its use, however, may be problematic because the approach to tolerance classification is based on subjective judgment. We analyzed fish and physicochemical data from 773 stream sites collected as part of the U.S. Geological Survey's National Water-Quality Assessment Program to calculate tolerance indicator values for 10 physicochemical variables using weighted averaging. Tolerance indicator values (TIVs) for ammonia, chloride, dissolved oxygen, nitrite plus nitrate, pH, phosphorus, specific conductance, sulfate, suspended sediment, and water temperature were calculated for 105 common fish species of the United States. Tolerance indicator values for specific conductance and sulfate were correlated ($\rho = 0.87$), and thus, fish species may be co-tolerant to these water-quality variables. We integrated TIVs for each species into an overall tolerance classification for comparisons with judgment-based tolerance classifications. Principal components analysis indicated that the distinction between tolerant and intolerant classifications was determined largely by tolerance to suspended sediment, specific conductance, chloride, and total phosphorus. Factors such as water temperature, dissolved oxygen, and pH may not be as important in distinguishing between tolerant and intolerant classifications, but may help to segregate species classified as moderate. Empirically derived tolerance classifications were 58.8% in agreement with judgment-derived tolerance classifications. Canonical discriminant analysis revealed that few TIVs, primarily chloride, could discriminate among judgment-derived tolerance classifications of tolerant, moderate, and intolerant. To our knowledge, this is the first empirically based understanding of fish species tolerance for stream fishes in the United States.

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Keywords: Fish; Tolerance values; Biological assessment; Streams

1. Introduction

Fish assemblage characteristics have been used for over 100 years to assess ecosystem conditions

(Simon, 1999). The use of fish assemblage characteristics has accelerated greatly over the past 30 years with implementation of the Clean Water Act of 1972, requiring protection and restoration of biological integrity as part of water-quality standards. A focus on biological integrity led to the development and use of biological criteria, and a variety of quantitative

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indices such as the widely used Index of Biological Integrity (IBI; Karr, 1981). The perception of fish species tolerance to environmental disturbance was integral to the development of the IBI (Fausch et al., 1990) and three general classes of fish tolerance to environmental disturbance – tolerant, moderate, and intolerant – are widely recognized (Halliwell et al., 1999).

The classification of fish species tolerance to environmental disturbance, and thus, its use as a component of the IBI may be problematic because the approach to tolerance classification is based on subjective judgment of overall tolerance to disturbance — including both physicochemical water quality and physical habitat. Assignment of fish species into tolerance classes has generally been based on professional judgment in terms of a general application of Shelford's law of tolerance (Shelford, 1912), which states that the growth and distribution of organisms can be limited both by too little or too much of an essential factor. When occasional differences of opinion have occurred among ecologists, the problem generally has been solved by expanding the three classes into four, for example — tolerant, moderately tolerant, moderately intolerant, and intolerant. Whittier and Hughes (1998), in developing a quantitatively derived tolerance classification for lake-dwelling fishes, noted that they could find no evidence that quantitative methods had been applied in assigning fish species to overall tolerance classes.

A related problematic issue may be the assignment to a tolerance classification based on fish species relations to general environmental disturbance. For example, tolerance classifications are often based on an understanding of the cumulative tolerance to “water-quality changes” and “habitat alteration” (Jester et al., 1992). Whittier and Hughes (1998) suggested that tolerance classifications to such general environmental disturbance may have been relatively successful in the Midwestern United States, where the IBI was originally developed, because of a perceived relative homogeneity of environmental conditions in this region. Thus, tolerance classifications based on cumulative understanding to general environmental disturbance may have limited utility across a broad geographic area beyond detecting significant levels of disturbance. The U.S. Environmental Protection Agency has produced a dataset of commonly used

tolerance classifications for selected fish species in the United States (Barbour et al., 1999), based on a classification for each species from selected literature sources. However, such tolerance classifications seem to beg the question, “tolerant to what”? The application of tolerance classifications across multiple geographic scales would require a greater understanding of tolerance to specific environmental stressors.

Fish assemblage structure is characterized as part of an integrated physical, chemical, and biological assessment of the Nation's water quality in the U.S. Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) Program. Data collected as part of the NAWQA Program provided the opportunity to quantify fish species tolerances to selected physicochemical variables. Specifically, our objectives were to (1) calculate fish species tolerance indicator values (TIVs) to selected physicochemical variables from a national-scale dataset, (2) examine relations among TIVs, (3) assess variation in TIVs among fish species, and (4) assess the ability of TIVs to discriminate among judgment-based tolerance classes.

2. Methods

The NAWQA Program's design focuses on major river basins across the United States (Gilliom et al., 1995). Together, these areas account for 60–70% of the Nation's population and cover about one-half of the Nation's land area. Major river basins were selected based on several factors including population and water use, importance of water-quality issues, and geographic distribution. River basin selection focused on agricultural and urbanized basins and used forested basins as a type of control to reflect undisturbed land use. Whereas the river basins are geographically distributed across the United States, their locations are biased towards areas where population, water use, and agricultural land uses are greater than average. The sites were not selected to be a statistically representative sample of the Nation's streams. Data for this analysis were collected from 773 stream sites sampled from 1993 to 2004. Combined, these sites are located downstream of 43% of the total kilometers of streams and rivers in the Nation and represent a wide range of environmental settings (Table 1).

Table 1
Statistical summary of environmental and physicochemical variables sampled by the NAWQA Program (1993–2004) and used in this study

Variables	Mean	Range	N
Environmental variables			
Drainage area (km ²)	4680	3–220,908	773
Elevation (masl)	420	0–2673	773
Agricultural land use (%)	31	0–98	773
Forested land use (%)	40	0–100	773
Urban land use (%)	12	0–98	773
Physicochemical variables			
Ammonia (mg/L)	0.08	0.01–17.0	1432
Chloride (mg/L)	27.9	0.1–315.5	1368
Dissolved oxygen (DO; mg/L)	8.2	0.3–15.1	1581
Nitrite/nitrate (NITRATE; mg/L)	1.22	0.01–13.95	1432
pH	7.7	3.4–9.6	1603
Specific conductance (COND; μ S/cm at 25 °C)	427	13–3,915	1667
Sulfate (mg/L)	55.4	0.1–2300	1411
Suspended sediment (SUSPSED; mg/L)	66	0–2645	1336
Total phosphorus (PHOS; mg/L)	0.17	0.01–4.78	1426
Water temperature (TEMP; °C)	20.4	0–34.8	1723

Abbreviations for variables are in capital letters.

2.1. Fish sampling

Fishes were collected during summer low-flow periods, using a standard sampling protocol based on a combination of two-pass electrofishing and seining (Meador et al., 1993). Operators of electrofishing gear received training in the sampling protocol and in electrofishing principles, such as power transfer theory in order to help standardize electrofishing efforts and increase the efficiency of electrofishing operations (Reynolds, 1996). Fish were identified to species and counted. Fish that could not be identified in the field were retained for identification and processing in the laboratory (Walsh and Meador, 1998). Taxonomic identifications in the field and laboratory were made or supervised by trained ichthyologists (Walsh and Meador, 1998).

Sampling sites were typically located near USGS gaging stations, and a sampling reach was identified at

each site. Reach lengths were determined based on the presence of a variety of habitat types (pools, riffles, and runs) and meander wavelength. Attempts were made to include at least two examples each of two different habitat types. When this was not possible, reach length was determined based on a distance of 20 times the mean channel width, roughly equivalent to one meander wavelength (Fitzpatrick et al., 1998). A minimum reach length of 150 m and a maximum reach length of 300 m were established prior to sampling for wadeable sites. Minimum and maximum reach lengths for non-wadeable sites were 300 and 1000 m, respectively.

2.2. Chemical sampling

Physicochemical water-quality variables were sampled during summer low-flow periods, using standardized methods (Shelton, 1994). Physicochemical data collection at each site included suspended sediment (mg/L), nitrite plus nitrate (nitrite/nitrate; mg/L), total phosphorus (mg/L), sulfate (mg/L), chloride (mg/L), ammonia (mg/L), dissolved oxygen (mg/L), pH, water temperature (°C), and specific conductance (μ S/cm at 25 °C). Several liters of water were composited from depth or width integrated samples. From this single composited sample, 250–1000 mL splits were extracted and preserved in the field for laboratory analyses of unfiltered nutrients, ions, and suspended sediment concentrations. Data quality was maintained through adherence to quality assurance plans and quality control samples (e.g., replicates, field blanks, spikes) (Shelton, 1994). Dissolved oxygen and water temperature were measured directly from the stream using hand-held probes. Attempts were made to sample physicochemical water-quality variables concurrent with fish sampling. When this did not occur, only physicochemical data collected within 14 days of fish sampling were included in this analysis.

2.3. Data analysis

We calculated tolerance indicator values (related to species optima) as predictors of physicochemical water-quality (WQ) variables, using weighted averaging (WA) inference models similar to those used by Potapova et al. (2004) and Carlisle et al. (2007).

Only those species collected from greater than 60 samples and represented by more than 100 individuals total (all sites combined) were included in the analysis. Species TIVs for each WQ variable were calculated using the formula:

$$u_j = (Y_1X_1 + Y_2X_2 + \dots + Y_nX_n)/(Y_1 + Y_2 + \dots + Y_n)$$

where u_j is the weighted average of fish species j , Y the species abundance in samples 1, ..., n , and X is the value of the WQ variable in samples 1, ..., n .

Preliminary analyses were used to determine the effect of censoring on WA estimates because data for ammonia, nitrite/nitrate, and total phosphorus were censored by values below reporting limits. We substituted censored values with half the reporting limit (Patton and Truitt, 2000; Fishman, 1993) and recalculated WA estimates. Although this procedure biases estimates of central tendency, the effect diminishes as the number of observations increases (Newman, 1995). Estimates of WA from censored and uncensored data were highly correlated for ammonia ($N = 111$, $R^2 = 0.99$), nitrite/nitrate ($N = 111$, $R^2 = 1.00$), and total phosphorus ($N = 108$, $R^2 = 0.99$). Therefore, we concluded that WA estimates were unaffected by censoring and we used censored data for all further analyses.

In order to facilitate comparisons between empirically derived TIVs and judgment-based tolerance classifications, we constructed a tolerance classification based on the TIVs. First, we transformed WA estimates to ordinal ranks. The ordinal rank (1–10) of each species was assigned based on the percentiles of TIVs across all species for each WQ variable. Ranks for each WQ variable were ordered such that a rank of 1 reflected the lowest 10% of TIVs whereas a rank of 10 reflected the highest 10% of TIVs. The ordinal ranks for all 10 WQ variables were then averaged. An overall tolerance classification was assigned based on the average scores as follows: 1–4 = intolerant; >4 to <7 = moderate; 7–10 = tolerant.

We used cluster analysis to examine relations among TIVs. A correlation matrix of the TIVs for all 10 WQ variables for all fish species was constructed using Pearson correlation coefficients. The Pearson coefficients were used as measures of similarity, and clusters were determined by using unweighted pair-group averaging. The results of the cluster analysis

were used to produce a dendrogram from which clusters of similar WQ TIVs could be identified. Clusters of two or more WQ variables were considered similar when Pearson coefficients were greater than 0.75.

Principal components analysis (PCA) of the correlation matrix was conducted to assess variation in TIVs among species. Tolerance indicator values were standardized to mean = 0 and standard deviation = 1 before analysis. The number of PCA axes examined was determined by Kaiser's rule, which states that the minimum eigenvalue should be 1 when correlation matrices are used (Legendre and Legendre, 1983).

To assess the ability of TIVs to discriminate among judgment-based tolerance classes, we used multivariate analysis of variance (MANOVA), analysis of variance (ANOVA), and canonical discriminant function analysis (CDA). The judgment-based tolerance classifications (hereafter referred to as EPA-RBP) were obtained from Barbour et al. (1999) and are available at http://www.epa.gov/owow/monitoring/rbp/app_c-1.html. For MANOVA, statistical differences were determined based on Wilk's lambda and Mahalanobis distance. For ANOVA, Tukey's Studentized range test was used to compare mean values. All data were tested for normality using the Kolmogorov–Smirnov test. When non-normality was indicated, data were transformed to improve normality by using $\log_{10}(x + 1)$ and the transformed data were retested. Tests indicated that transformations improved normality.

The CDA was used to derive linear combinations of TIVs that have the highest possible multiple correlations with the EPA-RBP tolerance classifications to maximally separate tolerant, moderate, and intolerant classes. We initially included all 10 WQ variables in the CDA. However, because our intent was to derive canonical variates that most clearly separate the defined tolerance classes, and to keep our ratio of class sample sizes to WQ variables to the recommended 3–1 (Williams and Titus, 1988), we excluded variables that were not significant and those with low explanatory power (those with univariate F statistics <4). Pearson correlation analysis was used to examine relations between canonical variates and WQ variables. All differences were declared to be statistically significant when P was less than 0.05.

3. Results

A total of 1734 fish assemblage samples was collected from the 773 sites (Fig. 1) from 1993 to 2004. The number of water-quality samples for each of the 10 WQ variables ranged from 1336 for suspended sediment to 1723 for water temperature (Table 1). Data were examined for 583,666 individuals representing 485 fish species. Tolerance indicator values were calculated for 105 fish species representing a total of 457,882 individuals. An electronic file containing a list of the species and their respective TIVs can be found at <http://water.usgs.gov/nawqa/ecology/>. Tolerance indicator value data for six selected fish species – mosquitofish (*Gambusia affinis*), bowfin (*Amia calva*), cutthroat trout (*Onchorhynchus clarkii*), brook trout (*Salvelinus fontinalis*), largemouth bass (*Micropterus salmoides*), and bluegill (*Lepomis macrochirus*) – are provided as examples (Table 2). Using ordinal ranks to construct an empirically derived tolerance classification, 25 species

were classified as tolerant (23.8%), 63 as moderate (60%), and 17 as intolerant (16.2%).

Cluster analysis of TIVs for the 10 WQ variables indicated the presence of a cluster of two similar ($\rho = 0.87$) WQ TIVs-specific conductance and sulfate. No other clusters of two or more similar WQ TIVs were detected. Tolerance indicator values for water temperature were least similar to TIVs for all other WQ variables ($\rho = 0.01$).

The PCA was conducted using nine WQ variables, based on the results of the cluster analysis. Because of the high correlation between specific conductance and sulfate, sulfate was excluded from the PCA. The PCA ($N = 105$) produced three significant axes (eigenvalues > 1) that collectively represented 79.0% of the variation in TIVs among species. Factors loading $\geq |0.40|$ on axis 1 (41.4% of the variation) included specific conductance, suspended sediment, total phosphorus, and chloride; on axis 2 (25.5% of the variation) dissolved oxygen, pH, and water temperature; on axis 3 (12.1% of the variation), ammonia,

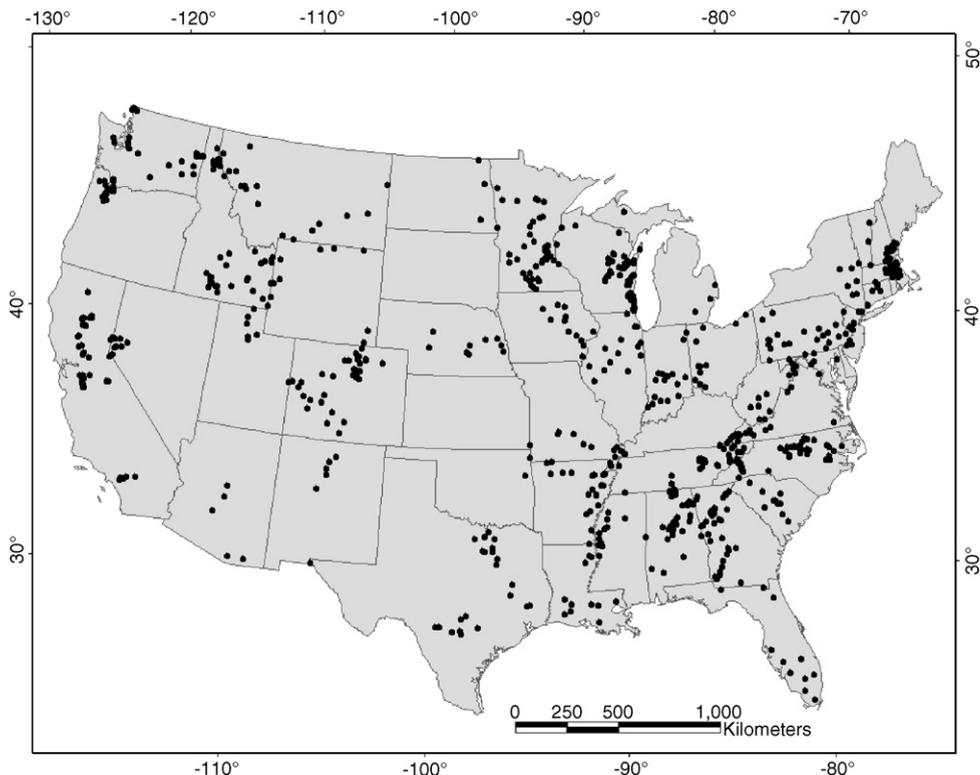


Fig. 1. Map of 773 sites sampled as part of the NAWQA Program (1993–2004) and included in this study.

Table 2

Tolerance indicator values for selected physicochemical water-quality variables and six selected fish species

	<i>Gambusia affinis</i>	<i>Micropterus salmoides</i>	<i>Lepomis macrochirus</i>	<i>Amia calva</i>	<i>Salvelinus fontinalis</i>	<i>Onchorhynchus clarkii</i>
EPA-RBP tolerance classification	Moderate	Moderate	Moderate	Moderate	Moderate	Intolerant
Ammonia (mg/L)	0.13	0.14	0.07	0.06	0.03	0.02
Chloride (mg/L)	32.2	38.7	30.4	23.4	3.1	4.4
Dissolved oxygen (mg/L)	6.8	7.4	7.0	4.9	8.9	9.6
Nitrite/nitrate (mg/L)	1.06	1.15	0.80	0.30	0.67	0.49
pH	7.4	7.5	7.3	7.2	7.5	7.7
Specific conductance ($\mu\text{S}/\text{cm}$ at 25 °C)	440	441	325	328	198	141
Sulfate (mg/L)	54.5	46.4	25.6	12.3	13.2	10.5
Suspended sediment (mg/L)	135	31	36	24	11	6
Total phosphorus (mg/L)	0.35	0.23	0.15	0.12	0.03	0.03
Water temperature (°C)	26.3	22.9	23.0	25.7	15.5	12.8

The judgment-based tolerance classification (EPA-RBP; tolerant, moderate, or intolerant) from Barbour et al. (1999) is provided for reference.

Table 3

Principal components analysis physicochemical variable loadings for axes 1, 2, and 3 ($N = 105$). Bold values are considered high $\geq |0.40|$

Physicochemical variable	Axis 1	Axis 2	Axis 3
Ammonia	-0.311	0.097	0.569
Chloride	-0.415	0.131	-0.159
Dissolved oxygen	0.007	-0.614	0.053
Nitrite/nitrate	-0.370	-0.230	0.279
pH	-0.190	-0.507	-0.413
Specific conductance	-0.430	-0.163	-0.360
Total phosphorus	-0.417	0.084	0.245
Suspended sediment	-0.428	0.160	-0.059
Water temperature	-0.125	0.477	-0.454

water temperature, and pH (Table 3). Using the empirically derived tolerance classification to illustrate variation in TIVs among species, the PCA revealed that most of the variation between tolerant and intolerant species occurred along axis 1, whereas variation in species classified as moderate was represented along axis 2 (Fig. 2).

Comparisons of empirically derived tolerance classifications with EPA-RBP classifications indicated 97 species in common with this study. Empirically derived tolerance classifications for eight species in this study – telescope shiner (*Notropis telescopus*), reidside shiner (*Richardsonius balteatus*), whitetail shiner (*Cyprinella galactura*), blacktail shiner (*Cyprinella venusta*), warpaint shiner (*Luxilus coccogenis*), Alabama hog sucker (*Hypentelium etowanum*), red-line darter (*Etheostoma rufilineatum*), and black-banded darter (*Percina nigrofasciata*) – were not EPA-

RBP classified. Overall, 57 of the 97 species (58.8%) classified through WQ TIVs had a matching classification with the classification provided by the EPA-RBP. The percent matches between our empirically derived classifications and EPA-RBP classifications were 24.0% for tolerant, 76.7% for moderate, and 33.3% for intolerant. Of the 97 fish species, EPA-RBP tolerance classifications revealed that 11 were classified as tolerant (11.3%), 72 as moderate (74.2%), and 14 as intolerant (14.4%).

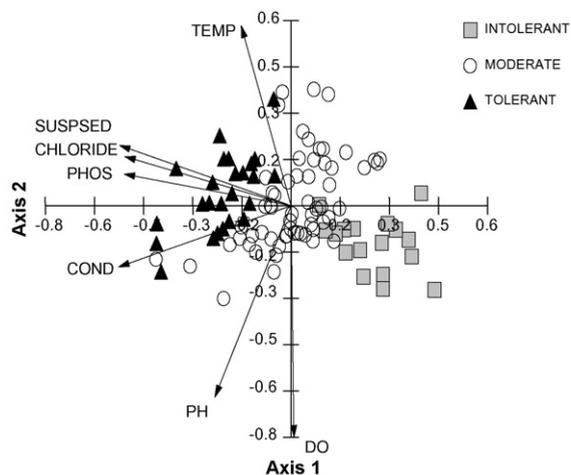


Fig. 2. Principal components analysis (PCA) plot of axes 1 and 2 based on a PCA of tolerance indicator values for 105 fish species classified as tolerant, moderate, and intolerant. Classifications were empirically derived from the physicochemical tolerance indicator values. Arrows indicate loadings $\geq |0.40|$. See Table 1 for variable abbreviations.

Table 4

Analysis of variance among judgment-derived tolerant classifications of tolerant, moderate, and intolerant (Barbour et al., 1999) for 10 physicochemical water-quality variables for 97 fish species

	Tolerant	Moderate	Intolerant	F-value	P-value
Ammonia	0.09 A (0.05–0.14)	0.07 AB (0.06–0.08)	0.04 B (0.03–0.05)	3.83	0.025
Chloride	38.5 A (35.4–41.6)	27.0 B (23.2–30.8)	17.3 C (10.5–24.1)	9.42	0.001
Dissolved oxygen	8.3 AB (7.8–8.8)	7.8 B (7.5–8.0)	8.6 A (8.2–9.0)	4.12	0.019
Nitrite/nitrate	1.74 A (1.25–2.23)	1.12 B (0.97–1.27)	0.97 B (0.71–1.23)	5.20	0.001
pH	7.8 (7.6–8.0)	7.7 (7.6–7.8)	7.8 (7.7–8.0)	1.68	0.193
Specific conductance	547 (434–660)	422 (382–461)	404 (319–490)	2.67	0.075
Sulfate	76.8 (41.9–111.7)	47.8 (38.7–56.8)	51.5 (32.0–70.9)	2.38	0.098
Suspended sediment	62 A (43–81)	55 AB (46–64)	32 B (21–43)	3.24	0.043
Total phosphorus	0.21 A (0.14–0.29)	0.18 A (0.15–0.20)	0.09 B (0.07–0.13)	4.89	0.009
Water temperature	21.0 (20.0–22.0)	21.9 (21.3–22.7)	20.9 (19.3–22.6)	1.02	0.366

Means with the different letters are not significantly different (Tukey's test $P < 0.05$). Numbers are given in parenthesis means represent 95% confidence limits.

Physicochemical TIVs varied significantly among EPA-RBP tolerance classifications (MANOVA: $N = 97$, $F = 2.02$, $P = 0.009$). Mahalanobis distances indicated that WQ TIVs varied significantly between intolerant and moderate classifications ($P = 0.020$) and between tolerant and intolerant classifications ($P = 0.003$), but did not vary significantly between moderate and tolerant classifications ($P = 0.124$). Analysis of variance indicated that mean TIVs for pH, water temperature, sulfate, and specific conductance did not vary significantly among the three EPA-RBP tolerance classes (Table 4). Mean TIVs for all other WQ variables did vary significantly among the three classes. Only TIVs for chloride significantly distinguished among all three individual tolerance classes (Table 4).

Based on the univariate F statistics in Table 4, the CDA was conducted with four WQ variables — chloride, total phosphorus, dissolved oxygen, and nitrite/nitrate. The first canonical axis (CDA1) significantly discriminated among the three classes (squared multiple correlation = 0.21, $P = 0.001$). Additional canonical variate axes were not significant. Chloride was positively correlated with CDA1 ($\rho = 0.90$, $P = 0.001$), followed by total phosphorus ($\rho = 0.65$, $P = 0.001$), and nitrite/nitrate ($\rho = 0.60$, $P = 0.001$). Dissolved oxygen was not positively correlated with CDA1 ($\rho = -0.20$, $P = 0.049$).

4. Discussion

To our knowledge, this study provides the first empirically derived set of fish species TIVs for stream

fishes in the United States. The approach provides quantitative support to the process of evaluating tolerance of fish species to specific physicochemical stressors. This approach may aid biologists in identifying suites of species sensitive or tolerant to specific stressors, and thus, aid in diagnosing potential causes of biological impairment (e.g., Norton et al., 2000).

Though the dataset used in this study may not include the full range of physicochemical conditions and fish species distributions, the TIVs appear to agree with data from other studies. For example, in the present study the TIVs for brook trout for pH and water temperature were 7.5 and 15.5 °C, respectively. These values are comparable to those reported by Raleigh (1982) who indicated that the optimal pH range for brook trout is 6.5–8.0, with a tolerance range of 4.0–9.5. Raleigh (1982) reported that whereas brook trout can tolerate an overall range in water temperature of 0–24 °C, the optimal range is 11–16 °C. Similarly, the TIVs for largemouth bass for pH and water temperature were 7.5 and 22.9 °C, respectively. These values also are comparable to those reported by Stuber et al. (1982) who indicated that though largemouth bass can survive limited exposures to pH levels of 3.9–10.5, the optimal pH range is 6.5–8.5. Stuber et al. (1982) also reported that largemouth bass can tolerate a wide range of water temperatures that vary with consideration of survival, growth, and spawning, however, the optimal range for growth is 24–30 °C. Thus, we believe that the dataset of TIVs for 105 stream-dwelling fish species to 10 WQ variables provides valuable information related to the

biology of these species and a realistic foundation from which to assess tolerance.

Although the approach used in this study provides substantial quantitative support to the understanding of tolerance classifications, judgment is still a component of the process of tolerance classification. As noted by Whittier and Hughes (1998), there is considerable variability among the published literature in the number of tolerance classes, ranging in most cases from one class (for example, intolerant) to five overall classes. We chose to use three classes for comparability to three-class EPA-RBP tolerance designations, which similarly attempt to describe fish species tolerances at a large geographic scale. There are no standardized criteria for selecting which species fall into a specific tolerance class, and thus considerable variation in approaches exists (Whittier and Hughes, 1998). Karr et al. (1986) suggested that the most sensitive 5–10% of species should be classified as intolerant. In the present study, we designated 17 species as intolerant (16.2%). This is comparable to intolerant designations of 16.6% reported by Whittier and Hughes (1998).

Empirically derived tolerance classifications provide greater understanding of species tolerances and help to address questions such as, “tolerant to what”? Results of the PCA indicate that the distinction between empirically derived designations of tolerant and intolerant was determined largely by tolerance to suspended sediment, specific conductance, chloride, and total phosphorus. Tolerance indicator values for specific conductance and sulfate were strongly correlated, and thus, fish species may be co-tolerant to these WQ variables.

Factors such as water temperature, dissolved oxygen, and pH may not be as important in distinguishing between tolerant and intolerant classifications, but may segregate species classified as moderate. For example, results of the PCA revealed four species classified as moderate – bowfin, pirate perch (*Aphredoderus sayanus*), threadfin shad (*Dorosoma petenense*), and spotted gar (*Lepisosteus oculatus*) – that were associated with increased water temperature, and decreased dissolved oxygen and pH. These species may be tolerant to these WQ variables and less tolerant to increased nutrients. For example, the water temperature TIV for bowfin was 25.7 °C, within the 90th percentile of all water temperature

TIVs. Conversely the TIVs for bowfin for sulfate and nitrite/nitrate were 12.3 and 0.30 mg/L, respectively, less than the 10th percentile for the respective TIVs. Thus, consideration of water temperature, dissolved oxygen, and pH specifically may give quantitative support for refinement of classifications of moderate such as “moderately tolerant” and “moderately intolerant”.

Examination of WQ TIVs relative to judgment-based tolerance classifications indicated that there may be relatively little quantitative evidence for the distinction among the three judgment-based tolerance classes based on physicochemical data alone. The 58.8% match between the empirically derived classification and the EPA-RBP classification indicates that, based on consideration of physicochemical tolerances alone, using expert judgment to classify species into tolerance categories is slightly better than 50%. Examination of the data from Table 2 indicates that TIVs were relatively similar between brook trout (EPA-RBP classified as moderate) and cutthroat trout (EPA-RBP classified as intolerant). However, there appeared to be considerable variation in TIVs among mosquitofish, largemouth bass, bowfin, bluegill, and brook trout, all EPA-RBP classified as moderate.

Results of this study indicate that whereas suspended sediment, chloride, ammonia, total phosphorus, dissolved oxygen, and nitrite/nitrate were significantly distinct among the three EPA-RBP tolerance classes, the significance was primarily based on the ability of these variables to distinguish between tolerant and intolerant species. Of these variables, only chloride varied significantly between all possible pairwise comparisons of classes. Tolerance indicator values did not appear to be useful in distinguishing between tolerant and moderate classes. Given that judgment-based tolerance classifications are derived from a general understanding of tolerance to a combination of influences including water chemistry and habitat, it may not be surprising that physicochemical TIVs provide limited ability to discriminate among tolerance classes. These results, however, may indicate that factors other than physicochemical influences, such as streamflow and physical habitat, may be driving distinctions among judgment-based tolerance classifications.

There are some limitations to widespread use and interpretation of the underlying TIVs and comparisons

with judgment-based tolerance classifications. Interpretations of TIVs for individual WQ variables should be made with caution because chemical and physical stressors can co-vary. Also, the TIVs may be biased by the non-random sampling design. We suggest that the large-scale geographic scope, the wide range of environmental conditions sampled, and the ordinal ranking approach employed are fairly robust to geographic variation. Further, the empirically derived tolerance classification used for comparison with a judgment-based tolerance classification was derived from a limited suite of physical and chemical components of water quality. Judgment-derived tolerance classifications are based on a cumulative understanding of water chemistry and habitat alterations. Discrimination among judgment-based tolerance designations may be enhanced by a better, more quantitative understanding of fish species tolerances to stream physical habitat conditions. Regardless, we believe that the results of this study provide a greater understanding of fish species tolerances to specific physicochemical stressors across a wide range of environmental conditions.

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