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Environmental impact assessment and conservation planning of a Middle-Eastern River basin using a fish-based tolerance index

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Abstract

The tolerance of aquatic organisms to stressors is widely used to monitor and evaluate the condition of freshwater ecosystems. Tolerance values (TV) derived from analyses of the relationship between species and their environment are considered to be more objective than those that rely on expert opinion. We used principal component analysis (PCA) to derive a generalized stressor gradient based on physicochemical characteristics and physical habitat quality and structure. Scores of the first principal component axis (PC1) were used to estimate TV for 37 fish species collected from 54 sites in the Karun River basin, Iran. PCA of 17 variables identified stressors that were influential such as total phosphate, total nitrogen, total coliform, and habitat and morphological score. The species were separated into three categories on the stressor gradient: sensitive (18.9%), semi-tolerant (48.6%), and tolerant species (32.4%). Based on these results we developed the Karun Fish Tolerance Index (KFTI) and demonstrated that it performed well in separating the least, moderate, and most disturbed sites in the study area. The discrimination efficiency of the KFTI was 82.5%, which makes it a robust management tool for the protection and conservation of streams and rivers in the Karun River watershed. TV developed here reflect objective characteristics of the sensitivity of fish species to the predominant stressors in the Karun and similar systems.

KEYWORDS

biodiversity, ecosystem management, stressor gradient, stressor sensitivity

1 | INTRODUCTION

Humans receive essential goods and services from river ecosystems which include drinking water, fisheries, irrigation, and wastewater

processing (Postel & Richter, 2003; Vörösmarty et al., 2005). Many of these benefits depend directly on the existence of healthy, well-performing ecosystems with a variety of species and functioning food webs (Postel & Richter, 2003). However, the unsustainable use of these goods and services impairs the integrity of these ecosystems and exerts pressures on their biodiversity (Collares Pereira &

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Cowx, 2004; Dudgeon et al., 2006; Vörösmarty et al., 2010). Globally, one-third of freshwater fish species are at risk of extinction (Abell et al., 2000; Freyhof & Brooks, 2011; IUCN, 2013). Therefore, there is an urgent need to evaluate the ecological status of freshwater ecosystems and quantify the impact of human activities.

Aquatic organisms are widely used as bioindicators of ecosystem conditions (O'Brien et al., 2016) because they provide robust and meaningful information about site conditions that is not included in the monitoring of physicochemical characteristics only (Ogren, 2014). Organisms most commonly used as indicators include algae/diatoms, macroinvertebrates, and fish. The sampling and analysis of these organism groups and comparison with established reference conditions allows objective assessments of ecosystem conditions (Flotemersch et al., 2006; Stoddard et al., 2006). Fish communities provide useful bioindicators because of their diversity and range of habitats used in different life stages. They are relatively long-lived and include many species that are highly mobile, thus integrating effects over larger spatial and temporal scales compared to other organism groups. Additionally, life histories and ecological requirements are well understood for many species, thus enhancing the interpretation of their presence or absence. Many fish species are also pursued for recreational or human consumption and therefore provide an assessment metric directly related to ecosystem services. However, fish stocking may alter fish communities in many regions, and complicate interpretations of fish-based bioindicators. From a field logistics standpoint, fish are generally considered easier to identify at the species level than other indicator organisms, and most can be identified in the field and released unharmed (Flotemersch et al., 2006).

The degree to which organisms can be exposed to pollution or other disturbances (stressors) and maintain their capacity for survival and reproduction is known as their stressor tolerance. More sensitive (i.e., less tolerant) species are less likely to be well-represented in habitats with strong impacts of human activities such as physicochemical degradation, including low oxygen, high turbidity, high concentrations of contaminants, and/or degraded physical habitat (Bryce et al., 2002; Stolte & Mangis, 1992). Therefore, their tolerance characteristics can serve as a useful indicator of habitat and ecosystem quality (Segurado et al., 2011). Differences among fish species in tolerance to anthropogenic stressors (Bressler et al., 2006; Chevin et al., 2010; Yuan, 2004) can be used to inform on stressor intensity. The concept of using species tolerances in aquatic ecosystem monitoring was introduced in the early 20th century, with notable advances made in associating them with organic contamination and/or low dissolved oxygen (Chutter, 1972; Hilsenhoff, 1977). Information on tolerance characteristics is usually available only for a subset of species occurring in a river system (Oberdorff et al., 2002). However, the improvement of regional and national datasets collected using standard methods has greatly expanded the understanding of how aquatic species react to a variety of stressor gradients (Segurado et al., 2011). Methods for the development and formulation of biotic indices are well-developed and readily available (Borja et al., 2000, 2007; Devlin et al., 2007; Karr et al., 1986). The first steps in optimizing the performance of biotic indices include selecting and defining relevant stressor gradients and objective

development of tolerance values (TV) based on observed fish community composition (Whittier et al., 2007a; Whittier & Van Sickle, 2010).

Quantifying the tolerance of species in fish communities increases the understanding of their autecology and allows for the interpretation of community composition on stressor gradients. Such knowledge is particularly important in arid and semi-arid regions of the globe where the scarcity of freshwater resources results in severe competition among the various uses, often reducing the quantity of water allocated to ecosystems and increasing the inputs and concentration of pollutants, which results in stress on species, biodiversity, and ecosystem functions (Fathi, et al., 2022b; Vörösmarty et al., 2010). Efforts to document biological conditions are still hampered by a lack of comparative and quantitative assessment of fish tolerance to multiple stressors in less-studied areas such as Iran (Alonso & Castro-Díez, 2008; Scott et al., 2007; Vila Gispert et al., 2005).

This study aimed at quantifying the sensitivity and tolerance of fish species to stressor gradients in the Karun River basin (Western Iran) and to use this information to develop a fish-based tolerance index, the Karun Fish Tolerance Index (KFTI), that can serve as a practical management tool. We also tested if native, endemic, and non-native fish species differ in tolerance to the range of stressors that occur in the Karun River system. The KFTI complements a multi-metric index (KFMMI) based on the same data developed in a companion study (Zare Shahraki, et al., 2022b). The KFTI has the potential to provide a less complex yet robust, fish-based rapid assessment tool that can be used in studies where resources may be limited. Overall, while both the KFTI and the KFMMI provide important information about freshwater ecosystem health, they differ in their approach, scope, and level of detail, and are best used in combination to gain a more comprehensive understanding of ecosystem health and identify potential threats and stressors.

2 | METHODS AND MATERIALS

2.1 | Study area

The Karun River (Figure 1) is the largest and longest (950 km) freshwater resource in southwestern Iran. It covers seven provinces and has a total drainage area of about 67,000 km². The sources of the Karun are in the Zagros Mountain range at an elevation of >4000 m. As it flows toward the Persian Gulf, it drains mountainous, foothills, and desert areas fluctuating from hot and dry conditions in summer with temperatures >50°C to cold winters with temperatures <0°C. The total annual rainfall in this basin ranges from 150 mm in the plains to 1200 mm in the mountains (Fathi et al., 2022a). These factors collectively result in flows that exhibit high intra- and inter-annual discharge variability; with final discharge volumes to the Persian Gulf of about 21×10^9 m³/year (Afkhami et al., 2007). The variable nature of the flows is modified by water withdrawal and dam operations (Khosravi et al., 2017). This study was limited to wadeable, upper portions of the watershed where backpack electrofishing equipment

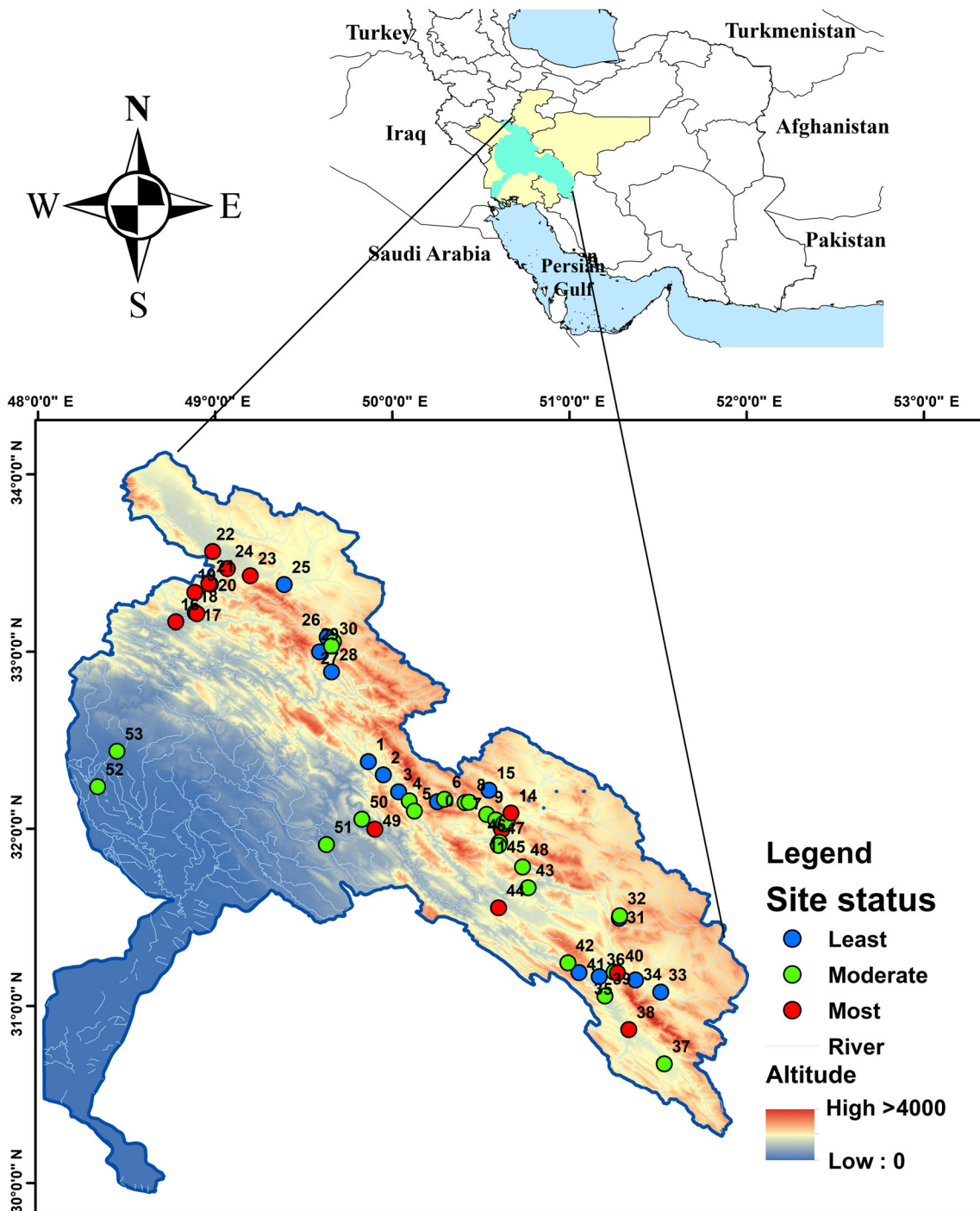


FIGURE 1 Sampling location throughout the Karun River basin. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/tra.4233)]

could be used (Figure 1) (Zare Shahraki et al., 2022b; Zare Shahraki et al., 2022a).

2.2 | Sampling

From July to August 2019, 54 sites were sampled with a Samus backpack electrofishing unit (Model: 1000) through multiple habitats

(Barbour et al., 1999; Flotemersch et al., 2006; Zare Shahraki et al., 2022b; Zare Shahraki et al., 2022a). Sampling reaches were approximately 200 m long and included different mesohabitats (e.g., riffles, runs, and pools) (Barbour et al., 1999). We standardized catch per unit effort on distance rather than time because the time required to safely navigate the stream habitats varied greatly among sites. Species detection curves were used to affirm the adequacy of sampling (Fisher et al., 1943) (Figure A1). Fish were identified at the

species level using regionally appropriate identification keys (Coad, 2020; Froese & Pauly, 2019; Jouladeh Roudbar et al., 2020; Keivany et al., 2016) prior to being released at the location of capture.

2.3 | Environmental variables measurement

We measured 22 physicochemical parameters (Zare Shahraki et al., 2021; Zare Shahraki et al., 2022b) (Table A1), using standard methods (Rice et al., 2017) simultaneously with fish sampling. At each sampling site, the physical structure was determined as river order, a summary of riparian vegetation characteristics, measurements of instream parameters such as width, channel slope, reach length, depth, flow velocity, high water mark, surface substrate, and the presence of dams (Barbour et al., 1999; Zare Shahraki et al., 2021; Zare Shahraki et al., 2022b; Zare Shahraki et al., 2022a).

3 | DATA ANALYSIS

3.1 | TV calculation

The development of TV required the identification of a stressor gradient for the basin. We used principal component analysis (PCA) to reduce the number of variables and to develop a representative stressor gradient (Bressler et al., 2006; Fathi et al., 2022b). Because

single, isolated stressors are rare, the resulting stressor gradient represents several physicochemical and habitat features (Bressler et al., 2006; Fathi et al., 2022b). Species-specific TV were calculated based on their relative abundance along the stressor gradient (Bressler et al., 2006). We also specifically compared TV among the categories of endemic, native, and non-native species.

3.2 | Stressor gradient

We based the stressor gradient on the concept of least disturbed conditions (LDC) (Stoddard et al., 2006), which was developed in companion papers using the same dataset (Zare Shahraki et al., 2021; Zare Shahraki et al., 2022b). Environmental variables including physicochemical and habitat variables were transformed to approximate normal distributions, and of highly correlated variables one was discarded. The variable to discard was chosen based on redundancy and efficiency of measurement (Fathi et al., 2022b). The PC1 values as the primary stressor gradient (Blocksom & Johnson, 2009) used in this study were developed with standardized and centered variables (17 variables, Table A1) by Zare Shahraki et al. (2021) (Table 1). Based on the direction of the stressor along PC1, sites falling within the less-impacted quartile of the axis were classified as least disturbed, those within the most-impacted quartile as most disturbed, and sites between the two as moderately disturbed (Blocksom & Johnson, 2009).

| Indicators | Eigenvalue | Proportion | PC1 eigenvector |
|--------------------------------|------------|------------|-----------------|
| PC1 | 4.60 | 0.27 | |
| PC2 | 2.82 | 0.16 | |
| Biological oxygen demand | | | 0.3991 |
| Chemical oxygen demand | | | -0.0508 |
| Total phosphate | | | 0.6555 |
| Dissolved oxygen | | | -0.3119 |
| Electrical conductivity | | | 0.8652 |
| Hardness | | | 0.6683 |
| Alkalinity | | | 0.7295 |
| Total nitrogen | | | 0.7196 |
| Total coliform | | | 0.6312 |
| Total solid | | | 0.9555 |
| Turbidity | | | 0.9369 |
| Habitat score | | | -0.9036 |
| Instream score | | | -0.4911 |
| Morphological score | | | -0.9865 |
| Riparian score | | | 0.4650 |
| Clay | | | 0.6483 |
| Sand | | | 0.7196 |

TABLE 1 Environmental variables used in principal component analysis (PCA), in the Karun River Basin, Iran.

Note: Parameters highlighted in bold (eigenvector >0.5 or <-0.5) are considered influential for ecosystem quality.

3.3 | Calculation of TV using relative abundance-weighted averaging

Preliminary TV (TV_p) were calculated using weighted abundance (WA) following Equation (1).

$$TV_p = \frac{\sum_{i=1}^n RA_i * PCA_i}{\sum_{i=1}^n RA_i} \quad (1)$$

Relative abundance (RA_i) is the proportion of species p within the sample from the i th, and PCA_i is the scaled PCA score for the i th site (Zare Shahraki et al., 2022b). This procedure weights TV_p -values on the basis of the position along the stressor gradient where each species shows the highest RA values (Bressler et al., 2006). TV can range from 0 to 10, with 0 representing the greatest stressor sensitivity (intolerance) and 10 representing the greatest tolerance. Final TV (TV_i) were evaluated by rescaling TV_i between the 2nd and 98th percentiles of the stressor gradient total range; this resulted in a distribution of final values across the 10-point scale. After scaling, all values below zero or above 10 were set to 0 and 10, respectively (Fathi et al., 2022b).

3.4 | Biotic index formulation

We developed the KFTI based on (Hilsenhoff, 1987) with

$$KFTI = \sum \frac{TV_i \times n_i}{N} \quad (2)$$

where TV_i is the tolerance value of each fish species, n is the number of species per site, n_i is the abundance of each species per site, and N is the total abundance of all species per site.

3.5 | Quality classification of the KFTI

The KFTI-based quality classification thresholds were determined using the 25th percentile of the stressor gradient (PC1), with four equal subdivisions (Barbour et al., 1996; Reynoldson et al., 1997). This approach is consistent with the one used in the European Water Framework Directive with some modifications in scores, categories, and interpretation (Fathi et al., 2022b).

Then we evaluated the ability of the KFTI to detect impairment based on its discrimination efficiency (DE) (Equation 3; Bressler et al., 2006):

$$DE = \frac{a}{b} \times 100, \quad (3)$$

where a is the number of common sites characterized as stressed sites based both on the KFTI and PCA results and b is the number of

stressed sites, based on the PCA results (Zare Shahraki et al., 2022b). A higher DE indicates a stronger performance of the biotic index and the capacity to distinguish between disturbed and reference sites (Bressler et al., 2006). In water quality assessment, an index with $DE > 80\%$ is broadly considered robust and efficient for the detection of biological degradation (Bressler et al., 2006). Validation of the KFTI was performed using (i) boxplots of index values stratified by stressor gradient categories (least, moderate, and most disturbed sites) to verify index sensitivity (Fathi et al., 2022b) and (ii) correlation analysis of the KFTI with 22 environmental parameters, PC1, and the Revised Iranian Water Quality Index (RIWQI) (Fathi et al., 2022c), to determine how well the KFTI distinguished among individual stressors.

3.6 | Statistical analysis

We used Spearman correlation to examine the relationship among different variables (KFTI, RIWQI, PC1, and environmental parameters). One-way analysis of variance and Tukey HSD tests were used to analyze the differences in fish tolerance classifications. Box plots were used to indicate differences in impacted and non-impacted sites and to compare TV of various fish categories. The significance level was set at $\alpha < 0.05$. Statistical analyses and graphics were done using the R software (v. 4.0.4, R Core Team, 2020), the R packages *vegan* (v. 2.5-6) and *ggplot2* (v. 2.2.0), and Excel 2020.

4 | RESULTS

4.1 | Stressor gradient

The PC1 was compared to variables related to stream size and position within the watershed (e.g., wetted width, stream order, and elevation). Correlation analysis indicated that the stressor gradient was independent of the longitudinal position of the site in the stream network (Zare Shahraki et al., 2021). We specified LDC reference sites as those falling below the 25th percentile along this gradient and stressed sites as those above the 75th percentile of PC1.

4.2 | Fish TV

A total of 37 fish species representing 12 families and 27 genera were collected from the 54 sites (5241 individuals) (Zare Shahraki et al., 2022a). TV were calculated for all species (Table 2). Three general categories of species were evident in the $RA \times PC1$ plots: sensitive (18.9%), semi-tolerant (48.6%), and tolerant (32.4%) (Figure 2, Table 2). Differences in TV between endemic, native, and non-native fish species were significant with TV of non-native fish species being significantly higher than those of the other two categories (i.e., native and endemic species; Figure 4; Tukey HSD).

TABLE 2 Tolerance values of fish species in the Karun River basin, Iran.

| Family | Subfamily | Genus-species | Presence status in the Karun Basin | TV | TVC | |
|-----------------|--------------------------|------------------------------------|------------------------------------|---------------|---------------|---------------|
| Cyprinidae | Cyprininae | <i>Capoeta coadi</i> | Endemic | 4.4 | Sensitive | |
| | | <i>Capoeta pyragyi</i> | Endemic | 5.8 | Semi-tolerant | |
| | | <i>Capoeta aculeata</i> | Endemic | 4.2 | Sensitive | |
| | | <i>Capoeta trutta</i> | Native | 5.8 | Semi-tolerant | |
| | | <i>Carassius gibelio</i> | Alien | 6.6 | Tolerant | |
| | | <i>Arabibarbus grypus</i> | Native | 8 | Tolerant | |
| | | <i>Cyprinus carpio</i> | Alien | 5.1 | Semi-tolerant | |
| | Barbinae | <i>Barbus lacerta</i> | Native | 5.9 | Semi-tolerant | |
| | | <i>Barbus karunensis</i> | Endemic | 6.1 | Semi-tolerant | |
| | | <i>Luciobarbus barbulus</i> | Native | 5.1 | Semi-tolerant | |
| | | <i>Carasobarbus luteus</i> | Native | 6.4 | Tolerant | |
| | | <i>Carasobarbus kosswigi</i> | Native | 3.5 | Sensitive | |
| | | - | <i>Cyprinion macrostomus</i> | Native | 7.2 | Tolerant |
| | | Labeoninae | <i>Garra rufa</i> | Native | 5.5 | Semi-tolerant |
| | <i>Garra gymnothorax</i> | Endemic | 6.1 | Semi-tolerant | | |
| Xenocyprididae | Squaliobarbinae | <i>Ctenopharyngodon idella</i> | Alien | 10 | Tolerant | |
| | Cultrinae | <i>Hemiculter leucisculus</i> | Alien | 6.1 | Semi-tolerant | |
| Leuciscidae | Leuciscinae | <i>Chondrostoma regium</i> | Native | 3.9 | Sensitive | |
| | | <i>Squalius berak</i> | Native | 4.7 | Semi-tolerant | |
| | | <i>Squalius lepidus</i> | Native | 4.5 | Semi-tolerant | |
| | | <i>Acanthobrama marmid</i> | Native | 4.4 | Sensitive | |
| | Alburninae | <i>Alburnus sellal</i> | Native | 4.9 | Semi-tolerant | |
| | | <i>Alburnus caeruleus</i> | Native | 5.3 | Semi-tolerant | |
| | | <i>Alburnoides idignensis</i> | Endemic | 5.5 | Semi-tolerant | |
| | | <i>Alburnus doriae</i> | Endemic | 3.6 | Sensitive | |
| Nemacheilidae | | <i>Sasanidus kermanshahensis</i> | Endemic | 8.6 | Tolerant | |
| | | <i>Turcinoemacheilus saadii</i> | Endemic | 6.6 | Tolerant | |
| | | <i>Turcinoemacheilus hafezi</i> | Endemic | 3.5 | Sensitive | |
| | | <i>Oxyoemacheilus freyhofi</i> | Endemic | 4.6 | Semi-tolerant | |
| Sisoridae | Glyptosterninae | <i>Glyptothorax silviae</i> | Endemic | 4.6 | Semi-tolerant | |
| Mugilidae | | <i>Planiiza abu</i> | Native | 8.3 | Tolerant | |
| Aphanidae | Cyprinodontina | <i>Aphanius vladkovi</i> | Endemic | 4.8 | Semi-tolerant | |
| Mastacembelidae | | <i>Mastacembelus mastacembelus</i> | Native | 7.2 | Tolerant | |
| Salmonidae | | <i>Oncorhynchus mykiss</i> | Alien | 5.2 | Semi-tolerant | |
| Oxudercidae | Gobionellinae | <i>Rhinogobius lindbergi</i> | Alien | 8.7 | Tolerant | |
| Gobionidae | Gobioninae | <i>Pseudorasbora parva</i> | Alien | 8.7 | Tolerant | |
| Poeciliidae | | <i>Gambusia holbrooki</i> | Alien | 8.7 | Tolerant | |

Abbreviations: TV, tolerance value; TVC, tolerance value category.

4.3 | Formulating and validating the KFTI

KFTI achieved an impressive 82.5% discrimination efficiency, as shown in Table 3 for the four quality classes.

Box plots of KFTI values for least, moderate, and most disturbed sites indicated differences among all stress categories (Figure 3). In the least and moderately disturbed sites, the KFTI was significantly lower than for the most disturbed sites. The KFTI was also strongly

correlated ($p < 0.05$ and $p < 0.01$) with several abiotic parameters, PC1, and RIWQI (Table 4).

5 | DISCUSSION

Bioassessment of human impacts on stream ecosystems has often been based on the sensitivity of benthic macroinvertebrate

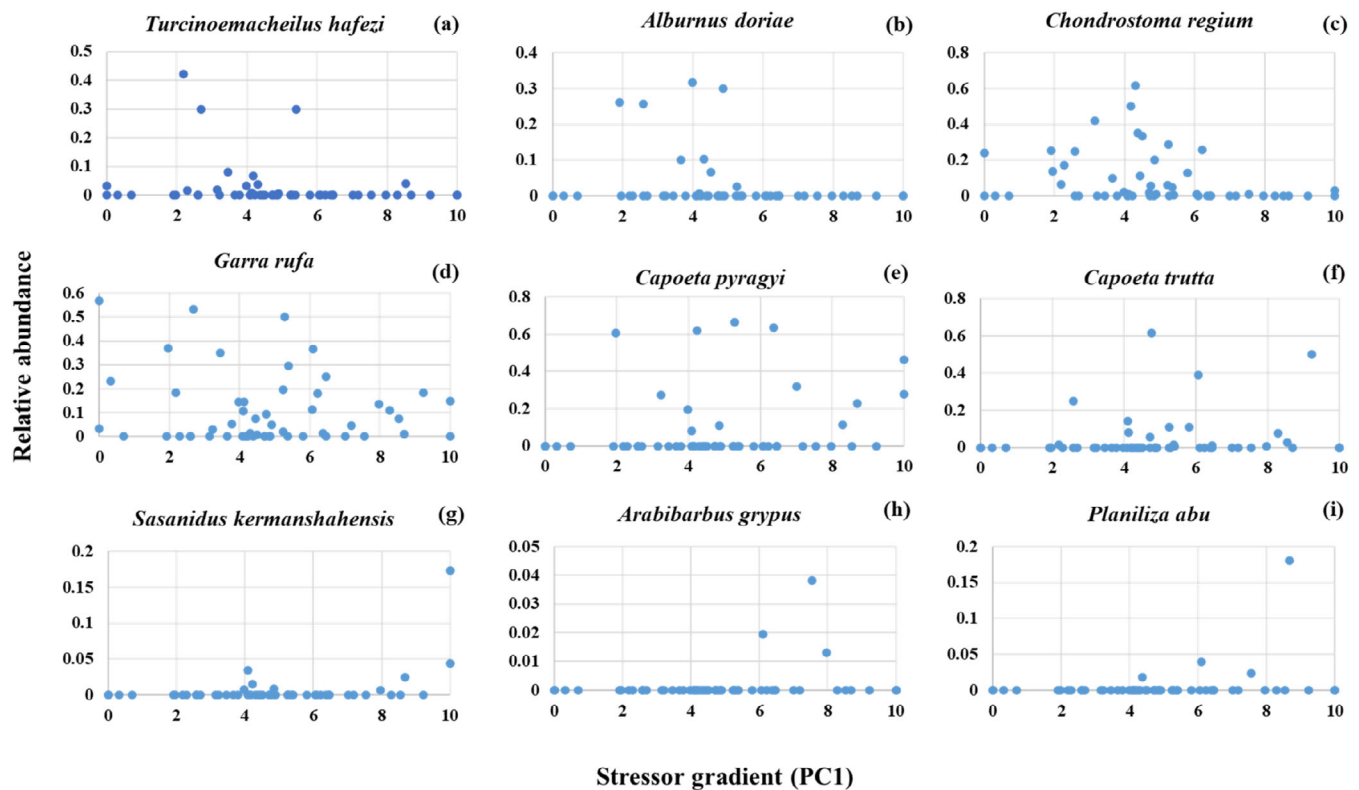


FIGURE 2 Examples of sensitive (a–c), semi-tolerant (d–f), and tolerant (g–i) fish species, illustrated by their relative abundance versus increasing the stressor gradient intensity (first principal component [PC1]). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/tra.4233)]

TABLE 3 Numerical values of Karun Fish Tolerance Index (KFTI) into quality classes adapted for the rivers in Karun Basin, Iran.

| Quality class | KFTI score |
|---------------|------------|
| Good | 0–4.50 |
| Fair | 4.51–5.50 |
| Poor | 5.51–6.7 |
| Very poor | 6.71–10 |

communities (Armitage et al., 1987; Bressler et al., 2006; Carlisle et al., 2007; Yuan, 2006). A companion study has applied this approach to the Karun River (Fathi et al., 2022b). However, there have been fewer efforts to similarly analyze freshwater fish communities and no such efforts have been conducted in Iran. This study represents the first empirically derived TV for riverine fishes of the Karun River basin in Iran and allows quantitative assessments of their sensitivity to environmental stressors. Such objective knowledge of fish species' sensitivity to stressors is important for detecting and interpreting the effects of human stressors on fish communities and developing ecological indicators, such as community tolerances indices, needed for evaluating ecosystem quality (e.g., community tolerance indices) (Whittier et al., 2007a; Whittier & Van Sickle, 2010). Our findings support the use of fish communities as bioindicators of the ecological conditions of the Karun and similar rivers.

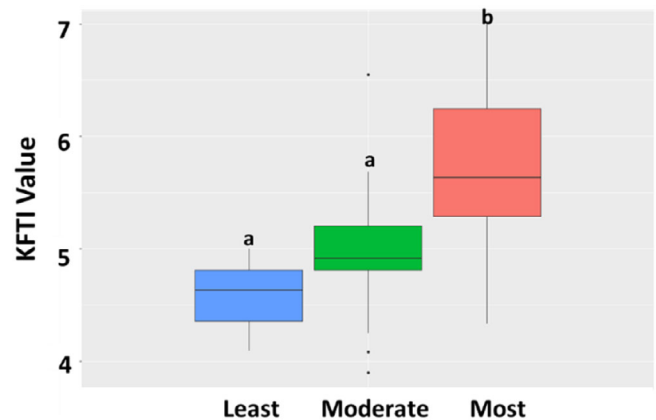


FIGURE 3 Karun Fish Tolerance Index index within least, moderate, and most disturbed site categories. Different lower-case letters (“a” and “b”) represent statistically significant differences ($p < 0.05$). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/tra.4233)]

5.1 | Stressor gradients

Quantifying a general stressor gradient is the basis for developing species TV (Whittier et al., 2007b). We identified the most important stressor gradients based on PCA analysis of environmental and physico-chemical parameters measured at river sites (Zare Shahraki et al., 2021). It is worth mentioning that most of the less-impacted sites in our study are located in upstream portions of the Karun River basin (Zare Shahraki

et al., 2021). Generally, defining human stressor gradients and assessing how humans affect ecosystems is also complicated by the covariance between human activities, natural gradients (e.g., elevation, slope, and precipitation), and biological assemblages (Whittier & Stoddard, 2006). Whittier et al. (2007c) pointed out that as elevation and slope increase, human presence and activities decrease as does the productivity of the natural ecosystem. Similarly, the river continuum concept (Vannote et al., 1980) illustrates that larger rivers at lower elevations and slopes would naturally be warmer, more turbid, and more productive and, often be subjected to increased human stressors. This is not different in the Karun River system. As a consequence, the problem of adequately characterizing reference conditions for large rivers remains largely unresolved (Whittier et al., 2007a), including for our study.

5.2 | TV of fish species

We included physical habitat parameters in our analyses to investigate changes in fish species abundance relative to physical habitat index values, similar to responses observed by Ferreira and Casati (2006) where distributions of some species were optimal relative to habitat conservation efforts. A subsequent study by Casatti et al. (2012) specifically showed that sensitive fish species are gradually replaced by tolerant species with increasing degradation of riparian vegetation, leading other researchers (e.g., Fedorenkova et al., 2013) to endorse considering physical parameters in developing fish TV. Results of the current study corroborate these findings in that the PCA demonstrates the importance of physical habitat condition as part of the stressor gradient. Indeed, the KFTI had a significant negative correlation with habitat scores (Table 3).

In our study, almost all of the most sensitive species occurred in mountainous ecoregions, and nearly all of the most tolerant species were characteristic of the plains and it was consistent with other studies (Hermoso et al., 2009; Whittier et al., 2007a). Species that were recorded less frequently in our study, such as *Pseudorasbora parva* and *Gambusia holbrooki* (each present at only one site) were considered tolerant species, consistent with the results of Mostafavi et al. (2015). Other results were less consistent, for example, Mostafavi et al. (2015) classified *Hemiculter leucisculus* and *Barbus lacerta* as tolerant and intolerant species, respectively, but according to our method, we classified them as semi-tolerant species. *Carasobarbus kosswigi* was the most sensitive species to the stressor gradient in our study corroborating findings from Hermoso et al. (2009), who classified another species of the same genus in the Mediterranean basin as a sensitive species.

5.3 | Tolerance of endemic, native, and non-native species

We acknowledge that tolerance classifications based on expert opinion are ambiguous and may result in over- and underestimation of TV for some species (Wang et al., 2018; Whittier et al., 2007a). This

TABLE 4 Significant correlations coefficients (r) between the Karun Fish-based Tolerance Index (KFTI), abiotic data, and Revised Iranian Water Quality Index (RIWQI) in the Karun River basin (asterisks [** and *] indicate significant correlations at $p < 0.01$ and 0.05 level, respectively).

| Variables | KFTI (r) |
|-------------------------|--------------|
| Electrical conductivity | 0.370** |
| Hardness | 0.308* |
| Nitrate | 0.305* |
| Total nitrogen | 0.308* |
| Alkalinity | 0.286* |
| Total dissolved solids | 0.349** |
| Total phosphate | 0.332* |
| Total solid | 0.491** |
| Total suspended solid | 0.484** |
| Turbidity | 0.484** |
| Dissolved oxygen | -0.351** |
| Water temperature | 0.710** |
| Width | 0.319* |
| Depth | 0.402** |
| Elevation | -0.466** |
| Slope | -0.532** |
| Velocity | -0.327* |
| Habitat score | -0.500** |
| Morphological score | -0.560** |
| RIWQI | -0.470** |
| Riparian score | 0.274* |
| Clay | 0.290* |
| Sand | 0.308* |
| PC1 | -0.27* |

apparent ambiguity may be related to other factors that must be considered for more precise interpretations. These include evidence that the tolerance range of individual species varies regionally (Whittier & Hughes, 2011) in response to different environmental conditions and types, and extent of environmental stressors (Kennard et al., 2005; Maceda Veiga & De Sostoa, 2011; Whittier & Hughes, 2011). Also, we have shown different tolerance ranges among the life-history strategies of fish, in particular, relative to whether they are endemic, native, or non-native.

Our analysis revealed a significantly higher tolerance to stressors of non-native species in comparison to native (Figure 4). In contrast, Fedorenkova et al. (2013) found no significant difference in tolerance to multiple abiotic stressors between native and non-native fish species in the Rhine delta (northwestern Europe), which they interpreted as a consequence of introduction pathways since many of the fish species in the Rhine River were intentionally introduced in the catchment (Leuven et al., 2009). Most of the non-native fish species in the Karun were also introduced and have then dispersed in the basin through interconnected waterways. The competitive advantage of non-native species in the Karun River watershed probably reflects

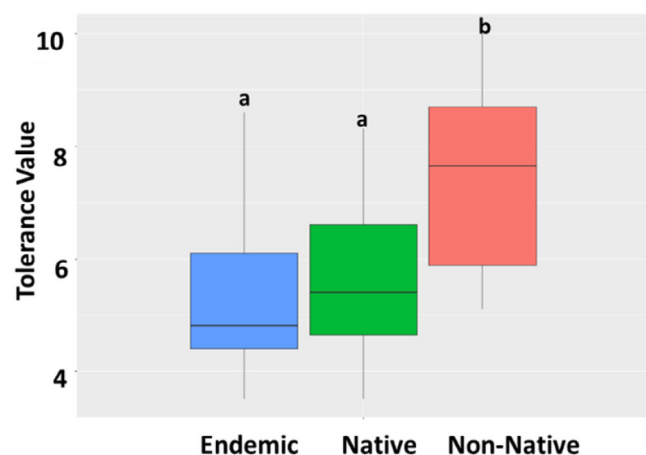


FIGURE 4 Fish tolerance values for endemic, native, and non-native species. Different lower-case letters (“a” and “b”) represent statistically significant differences ($p < 0.05$). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/trn.4233)]

their wider environmental and physiological tolerance as suggested by our data and that of others (Piscart et al., 2011; Whittier et al., 2007a).

5.4 | The KFTI

In general, species are considered sensitive to human disturbance if their abundance or range decreases in response to human disturbances, and tolerant if decreases are not observed. We quantified these responses with TV and the KFTI. The KFTI had $DE > 80\%$, which makes it suitable for use in the Karun River and similar systems. The formula has an identical structure to that of the HBI (Barbour et al., 1999; Hilsenhoff, 1987) developed for macroinvertebrate communities, which has been efficient for a macroinvertebrate tolerance index developed in a companion study (Fathi et al., 2022b). PC1 was positively correlated ($p < 0.05$) with KFTI values, representing biological responses to the disturbance gradient (Table 4). A strong negative correlation ($p < 0.01$) was also detected between KFTI and RIWQI developed in a companion study, thus manifesting a biological response to water quality (Table 4). In addition, the KFTI was significantly correlated ($p < 0.05$ and $p < 0.01$) with multiple abiotic parameters (Table 4). These results help validate the usefulness of the KFTI due to observed responses to gradients in water quality and disturbance. Further, the KFTI was able to separate different sites according to human disturbance as well as to water and habitat quality, and showed high compatibility between various stress categories.

6 | CONCLUSIONS

The large geographical area and heterogeneity of rivers in the Karun River basin covered in this study allowed us to assess the tolerance of fish species to different human disturbances. In general, TV were

significantly related to major stressors occurring in the region. The KFTI was developed using these TV, performing well in differentiating among sites as least, moderate, and most disturbed sites. It also showed a significant negative response to increased anthropogenic impacts. Our results highlight the value of developing region-specific TV for fish-based bioassessment in supporting the assessment of ecological condition, and subsequent management, of streams and rivers in Iran. Of course, the TV accuracy would further improve with an increased number of sites sampled (including more sites with references and very poor conditions). These TV effectively and efficiently reflect the relationships between fish species and environmental gradients and consequently represent a powerful tool supporting effective management of Iranian freshwater resources. The two complementary fish-based indices developed based on this dataset, that is, KFTI (this study) and KFMMI (Zare Shahraki et al., 2022b) are now available for use by stakeholders, managers, and scientists to evaluate the quality of freshwater ecosystems in Iran and elsewhere.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A

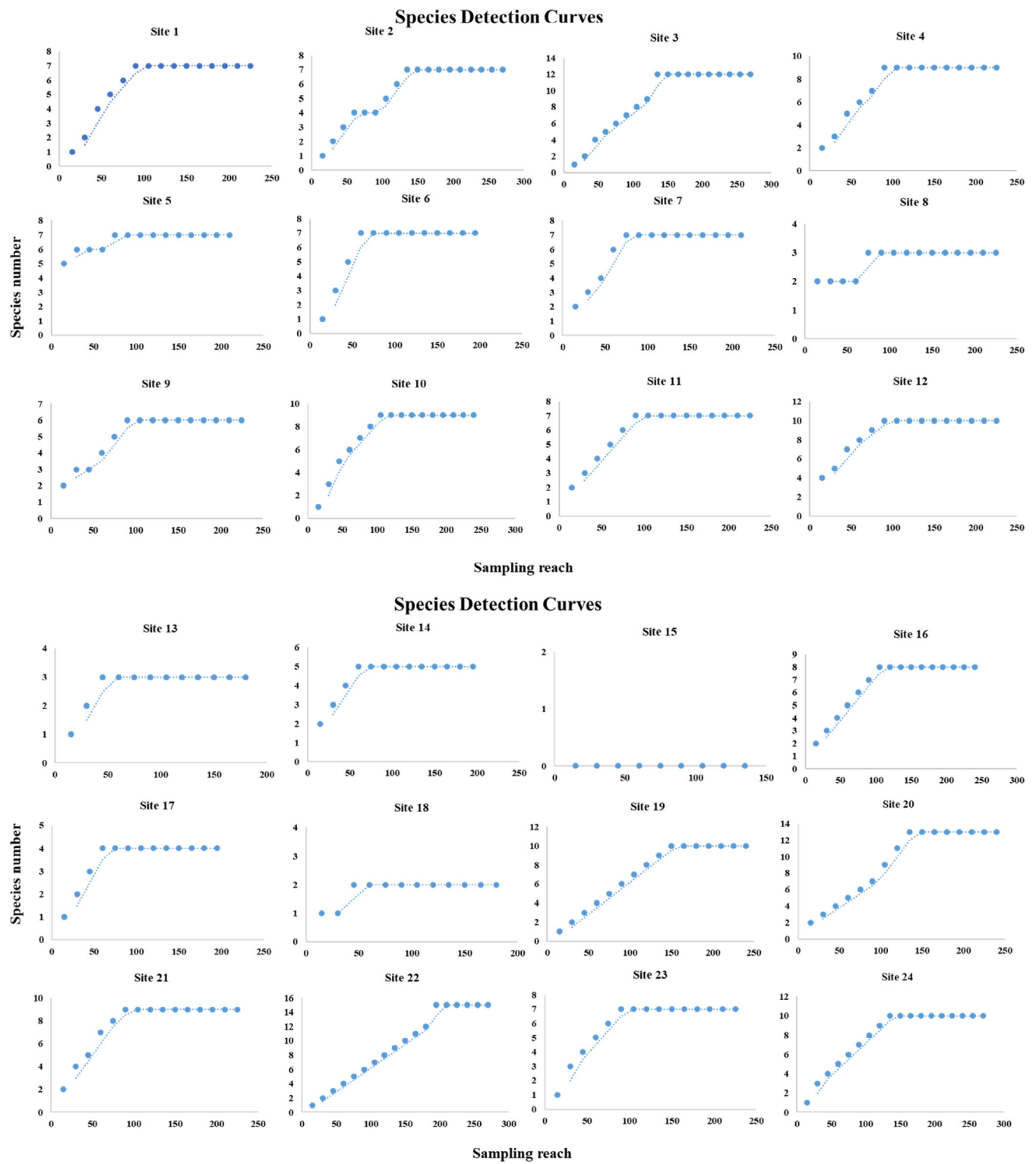


FIGURE A1 Species detection curves for sampling sites in the Karun River basin, Iran. [Color figure can be viewed at wileyonlinelibrary.com]

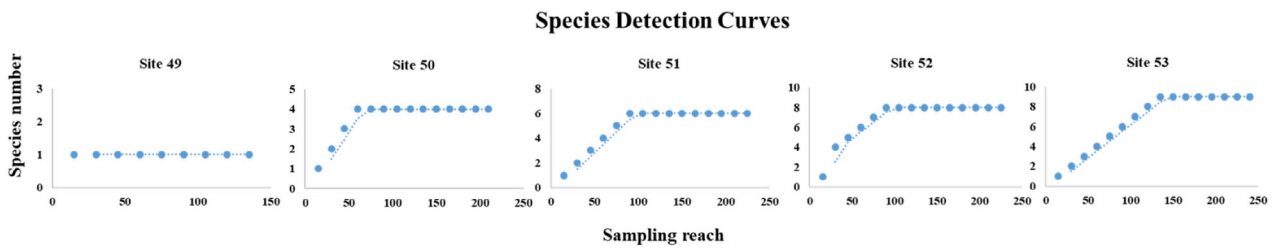
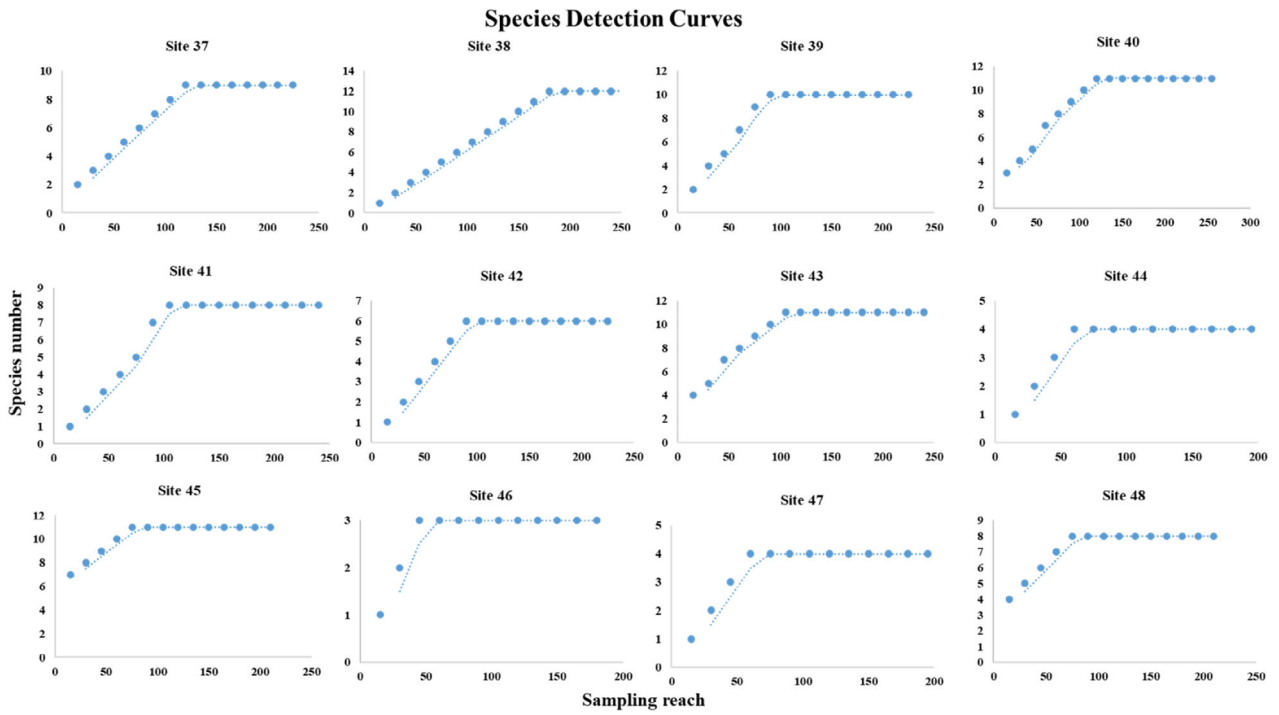
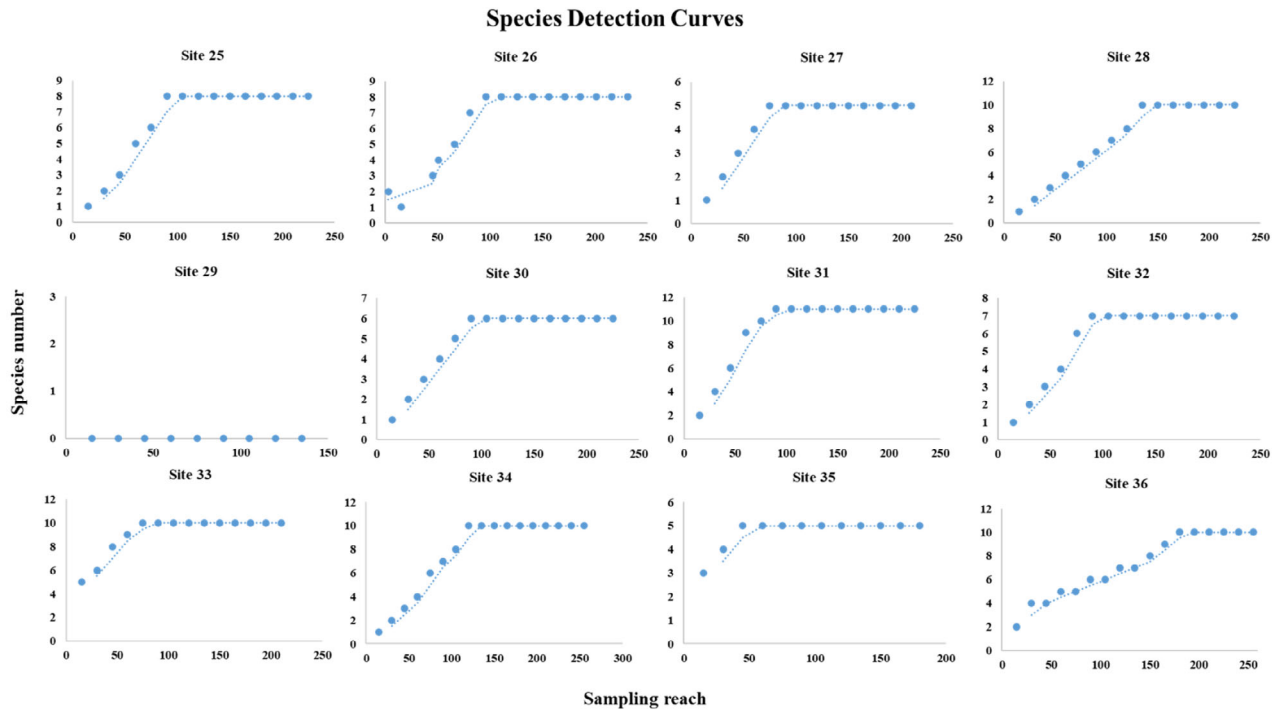


FIGURE A1 (Continued)

TABLE A1 Variables related to environmental features applied for the selection of reference sites in the Karun River Basin, Iran (Zare Shahraiki et al., 2021).

| Assessment category | Assessed features | Unit | Scale of measurement |
|----------------------------|--|------------------------|--------------------------|
| Physical habitat | *River bank alteration | - | Reach |
| | *River channel alteration | - | |
| | *Vegetative protection | - | |
| | Riparian vegetative zone width | - | |
| | Channel slope | Degree and percentage | |
| | Elevation | Meter above sea level | |
| | Width | m | |
| | High water mark | m | |
| | Depth | cm | |
| | Reach length | m | |
| | Flow velocity | cm/s | |
| | Meso habitat types (% run, %riffle, and %pool) | - | |
| | % Gravel | % | |
| | % Cobble | - | |
| | *% Clay and silt | | |
| | *% Sand | | |
| | Sediment deposition | | |
| | *Total habitat score | | |
| | *Instream score | | |
| | *Morphological score | | |
| *Riparian score | | | |
| Physico-chemical variables | Biological oxygen demand (BOD) | mg/L | Site (spot measurements) |
| | Chemical oxygen demand (COD) | mg/L | |
| | <i>Escherichia coli</i> (<i>E. coli</i>) | n/100 mL | |
| | Fecal coliform (FC) | n/100 mL | |
| | Electrical conductivity (EC) | µmho/cm | |
| | Total hardness (TH) | mg/L CaCO ₃ | |
| | pH | - | |
| | Total alkalinity (TA) | mg/L CaCO ₃ | |
| | Phosphate (PO ₄) | mg/L | |
| | Total phosphorus (TP) | mg/L | |
| | Nitrate (NO ₃) | mg/L | |
| | Nitrite (NO ₂) | mg/L | |
| | Total ammonia nitrogen (TAN) | mg/L | |
| | Total Kjeldahl nitrogen (TKN) | mg/L | |
| | Total nitrogen (TN) | mg/L | |
| | Total dissolved solids (TDS) | mg/L | |
| | Total suspended solid (TSS) | mg/L | |
| | Total solid (TS) | mg/L | |
| | Turbidity (NTU) | mg/L | |
| | Temperature (T) | °C | |
| | Oxygen saturation (DO %) | % | |
| | Dissolved oxygen (DO) | mg/L | |
| | Land use | % Agriculture | |
| % Forest | | | |
| % Range | | | |
| % Developed | | | |
| % Dry river bed | | | |
| % Water | | | |
| % Wetland | | | |

Note: Parameters marked with an asterisk were calculated based on the rapid bioassessment protocol (Barbour et al., 1999); parameters in bold were used in the PCA.