




RESEARCH ARTICLE

A fish-based multi-metric assessment index in the Karun River basin, Iran

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Abstract

Large river systems are one of the most important water resources for human societies. However, the ecological integrity of large rivers has been altered greatly by human activities. To monitor and manage these ecosystems, multimetric indices (MMI) are considered as efficient tools. This study aimed to develop and validate a fish-based multimetric index for the Karun River basin, Iran (Karun fish-based multimetric index [KFMMI]). Eighteen rivers and 54 sites in the basin were sampled in July–August 2019, and physico-chemical and habitat characteristics were used to identify reference sites based on principal components analysis (PCA). Of the 54 sites, 14, 26, and 14 sites were classified as least, moderate, and most disturbed sites, respectively. Fifty-four candidate metrics were evaluated for range, responsiveness, and redundancy with other metrics. This resulted in the identification of eight metrics (relative abundance of native and endemic taxa, relative richness of migratory taxa, relative richness of Leuciscidae taxa, relative richness of herbivorous taxa, relative abundance of cyprinid taxa, relative richness of vegetative inhabitant taxa, relative abundance of slow water flow, and relative richness of edge inhabitant taxa) that informed on species richness and composition, migratory status, functional feeding groups, and habitat preferences. The KFMMI showed excellent performance in separating least, moderate, and most disturbed sites in our study area. Regarding water quality, the KFMMI was classified 16, 5, and 29 sites as good, moderate, and bad, respectively. The discrimination efficiency of KFMMI was 81.6%, which makes it an effective management tool for directing restoration actions at most disturbed sites and intensifying protection of least disturbed sites.

KEYWORDS

biological indicator, fish metrics, multimetric index, reference site, water quality

1 | INTRODUCTION

Freshwaters are among the most disturbed ecosystems on the Earth (Carpenter et al., 2011; Carpenter, Stanley, & Zanden, 2011; Gonino, Benedito, Cionek, Ferreira, & Oliveira, 2020; Grimm, Pickett, & Redman, 2000; Reid et al., 2019). In recent decades, the rate of biodiversity loss in freshwater ecosystems has been far greater than of

terrestrial (Carpenter, Cole, et al., 2011; Carpenter, Stanley, & Zanden, 2011; Curtean-Bănăduc, Bănăduc, & Blaga, 2007; Saunders, Meeuwig, & Vincent, 2002), and human impacts on streams and rivers are further increasing worldwide (Lima, Wrona, & Soares, 2017; Nel et al., 2009; Strayer & Dudgeon, 2010). Factors such as overexploitation, pollution, flow modification, degradation of habitat and invasion by exotic species are leading causes of population declines of

freshwater organisms (Dudgeon et al., 2006; Reid et al., 2019). To support rational policy and regulatory decisions, managers require information on the ecological conditions of ecosystems as well as the pressures and stressors driving changes (Barbour et al., 2000; Yoder & Barbour, 2009). This necessitates the use of efficient and effective tools for the assessment of biotic communities and ecosystem condition (Ruaro, 2019).

Multimetric indices (MMIs) are common tools for assessing ecosystem condition (Ruaro, Gubiani, Hughes, & Mormul, 2020; Schoolmaster, Grace, & William Schweiger, 2012). MMIs are tools for assessing environmental quality and include metrics that individually provide limited information but, when integrated, serve as a broad indicator of the biological status of a specific environment (Harrison & Whitfield, 2004). MMI metrics typically characterize a wide range of biological features (e.g., species composition and richness of focal communities and environmental factors) and responses to natural gradients and human disturbances (Ruaro, 2019). The first MMI for the assessment of freshwater resources was published by Karr (1981) and used fish population characteristics to assess a US river. Since then, the use of MMI has become commonplace in rivers and other ecosystems (Linke, Bailey, & Schwindt, 1999; Miller, Bradford, & Peters, 1986; Stoddard et al., 2008) and has expanded to include use of other taxonomic groups such as macroinvertebrates (e.g., Kerans & Karr, 1994), macrophytes (e.g., Beck, Hatch, Vondracek, & Valley, 2010), plankton (e.g., Abbasi & Chari, 2008), and periphyton (e.g., Fore & Grafe, 2002).

Ruaro (2019) states that evaluation based on bioassessment in Asian regions (with an area of 4.43 million square kilometers) has only recently begun, and cites work in India (Abhijna & Kumar, 2017), Pakistan (Qadir & Malik, 2009), China (Gu et al., 2015; Jia, Sui, & Chen, 2013; Li, Li, Xu, & Li, 2016; Miao, Ni, Borthwick, & Yang, 2011; Wan, Bu, Zhang, & Meng, 2013; Wu, Xu, Yin, & Zuo, 2014), and Iran (Aazami, Esmaili Sari, Abdoli, Sohrabi, & Brink Van Den, 2015; Mostafavi et al., 2015; Mostafavi, Teimori, Schinegger, & Schmutz, 2019). While recognizing this progress, there is still an urgent need to provide more tools based on the biotic components of Iranian freshwater ecosystems because Iran, with an area of 1,629,807 km², has complex and diverse climates, topography, and geology. These features have resulted in aquatic ecosystems with varied and unique biodiversity that resource managers are working to maintain (Jouladeh-Roudbar, Ghanavi, & Doadrio, 2020; Mostafavi et al., 2015).

Many water quality monitoring programs in Iran largely rely on physico-chemical data for the assessment of conditions (Fadaei & Sadeghi, 2014; Fathi, Dorcheh, Mirghaffari, & Esmaeili, 2015; Nikaeen et al., 2016; Sharifinia, Ramezanzpour, Imanpour, Mahmoudifard, & Rahmani, 2013). Two exceptions are fish-based multimetric assessment indices recently developed by Mostafavi et al. (2015, 2019) in the Iranian Caspian Sea Basin, which is designed for cold-water and cyprinid streams of this basin. These indices have empowered resource managers with useful information. Regardless, adequate biotic indices are still lacking for many other Iranian water basins and ecosystem types. The long-term protection of Iranian rivers would be

aided by the development of MMI indices tailored to their specific biological and environmental conditions.

The objective of this research was to develop a fish community-based MMI specifically adapted to the climatic conditions, topography, geology, and land-use patterns of the Karun River basin in Iran. The Karun River has great cultural (Jafarzadeh, Rostami, Sepehrfar, & Lahijanzadeh, 2004) and economic importance as Iran's largest navigable river (Ghadiri & Afkhami, 2005). It is also environmentally significant as it contains many endemic and native fish species and communities that differ from those observed in comparable systems in Europe and Asia (Abdoli & Naderi Jolodar, 2009; Jouladeh-Roudbar et al., 2020; Mostafavi et al., 2015, 2019). We chose to focus our efforts on development of a fish-based MMI as fish are excellent bio-indicators, both at the local and basin scale, that respond to different disturbances (e.g., organic and nutrient pollution, hydro-morphological alterations, land use patterns) and thereby provide complete information on ecological status of any freshwater system (Birk et al., 2012; Hering et al., 2006; Hering, Borja, Carvalho, & Feld, 2013). Compared to other organism groups, fish are long-lived and mobile and can therefore reflect effects within the whole ecosystem (Karr et al., 1986; Oosterhout & Velde, 2015). Different impacts, such as acute toxicity (loss of species) and effects of stress (depressed growth and reproductive success), can be assessed as they impact fish populations (Abbasi, 2012; Karr et al., 1986).

A precise evaluation of recruitment and growth dynamics of different groups of fishes through time (i.e., years) can help identify periods of stress (Karr, 1981). For example, decline in insectivorous fish communities usually indicates the degradation of the macroinvertebrate food base of rivers and is therefore a good predictor of river integrity (Gonino et al., 2020; Karr et al., 1986). The presence of alien species in degraded conditions and consequently their impacts on native fish assemblages have also been shown to yield useful information (Brown, 2000; Ferreira et al., 2012). Thus, the use of indices based on fish assemblages may provide a more complete and integrated view of ecosystem condition than assemblages of more local distribution and shorter life spans, such as biofilm and macroinvertebrates (De Bikuna et al., 2017). Fish are also of interest to the public because of cultural, recreational, and economic interests (Barbour, Gerritsen, Snyder, & Stribling, 1999; Flotemersch, Stribling, & Paul, 2006; Linke et al., 1999; McCormick et al., 2001). In our efforts to develop the KFMMI, we followed the steps of: (1) measuring human disturbances at different spatial scales; (2) identifying the least disturbed condition (LDC) based on water quality and habitat parameters; (3) identifying responsive fish metrics to human disturbances; and (4) integrating these metrics into an MMI.

2 | METHODS

2.1 | Study area and site selection

The Karun River basin (Figure 1) is the largest freshwater resource in Iran with a drainage area of approximately 67,000 km² covering seven

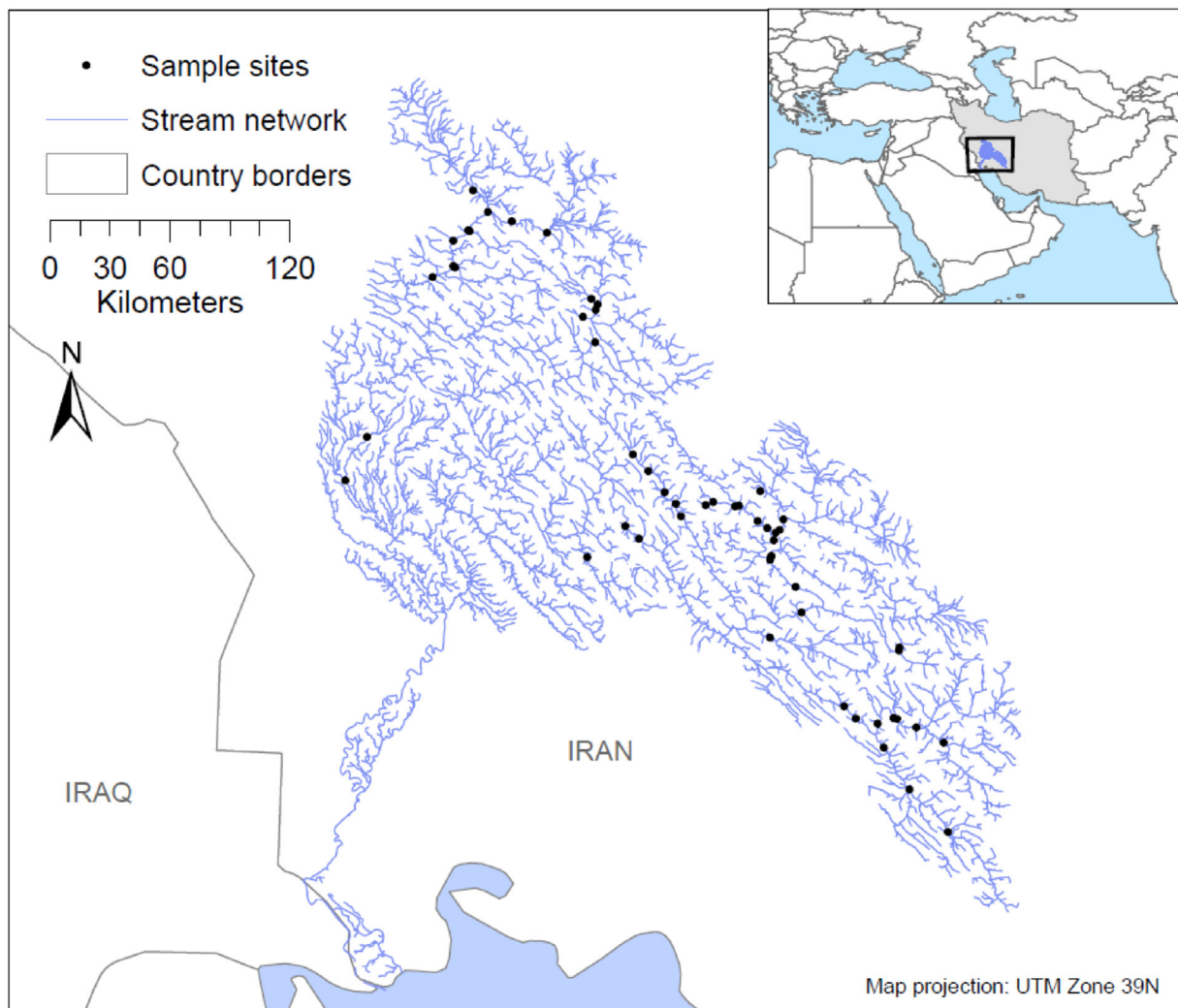


FIGURE 1 Map of the Karun River basin and location of the sampling sites (black dots). *Source:* Topographic map (scale: 1/25000) prepared from the Iranian forests, rangelands, and watershed management organization [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

provinces (Chaharmahal-and-Bakhtiari, Fars, Isfahan, Khuzestan, Kohgiluyeh-and-Boyer-Ahmad, Lorestan, and Markazi). As the longest river in Iran (950 km), the Karun originates at an elevation of 4,221 m in the Zagros Mountains and flows through a variety of landscapes and mesohabitats, eventually discharging into the Persian Gulf ($21 \times 10^9 \text{ m}^3/\text{year}$) (Afkhami, Shariat, Jaafarzadeh, Ghadiri, & Nabizadeh, 2007). The Karun has an average slope of 3% and is also the only navigable waterway in Iran (Pirasteh, Woodbridge, & Rizvi, 2009). About 75% of the basin consists of mountains and highlands, while plains and low elevation areas cover about 25% of the basin (Bakhsipoor, Ashrafi, & Adib, 2019). Lowland areas of the Karun basin are mainly composed of recent alluvial sediments (Yousefi, Pourghasemi, Hooke, Navartil, & Kidova, 2016). Temperatures in the basin range from hot and dry conditions in summer (i.e., $>50^\circ\text{C}$) to cold winter temperatures of less than 0°C . (Khosravi, Siadatmousavi, Yari, & Azizpour, 2017). Mean annual precipitation ranges from 400 to 1,200 mm in the central Zagros range to less than 200 mm in the arid zone of the lower Khuzestan plains (Woodbridge, Parsons, Heyvaert, Walstra, & Frostick, 2016). With about 50 small and large

ivers, the Karun River system is a significant resource for various uses including agriculture, industry, drinking water, and recreation (Afkhami et al., 2007; Bagherian Marzouni, Mohammad, & Moazed, 2014; Naddafi, Honari, & Ahmadi, 2007). In recent decades, the river system has become increasingly modified with the construction of many large dams (for water storage, energy production, and irrigation) (Salarjazi, 2012), agriculture, industry and urban development (Zare Shahraki et al., 2021).

Sampling in the rivers and streams was limited to wadable portions of the Karun River basin where wadable electrofishing gear could be employed. This concentrated the sampling sites in the upper part of the basin (Figure 1). Criteria considered for site selection included topography (e.g., high and low altitude), channel morphology (e.g., width, depth, slope), climate (e.g., cold, mild and hot), basin size, accessibility, and safety. These factors resulted in the identification of 54 sites distributed across 18 sub-basins (Bazoft, Kouhrang, Behesht Abad, Sabz Kouh, Armand, Abvanak, Komeh, Marber, Boshar, Khersan, Tireh, Marbare, Dare takht, Sezar, Gholyan, Bakhtiari, Dez, and Karun) (Figure 1).

2.2 | Sampling methods

2.2.1 | Fish sampling data

Fish sampling was performed in low flow conditions in summer (July–August 2019). This season coincides with the presence of different size classes of fish, a less stressful period for fish, the longest sampling season under which most sites can be sampled, and is generally considered safer (Barbour et al., 1999). The sampled reach at each site was 200 m long and included sampling of all available mesohabitats (e.g., riffles, runs, and pools). Fish samples were collected using a Samus (Model: 1000) backpack electrofishing instrument. The species detection curve was used to ensure sampling adequacy (Fisher, Corbet, & Williams, 1943). The sampling process was equivalent to approximately 90 min of fishing effort at each sampling site. All individuals were identified to species using regionally relevant identification keys (Abdoli & Naderi Jolodar, 2009; Coad, 2020; Esmaeili, Coad, Gholamifard, Nazari, & Teimori, 2010; Froese & Pauly, 2019; Jouladeh-Roudbar et al., 2020; Keivany, Nasri, Abbasi, & Abdoli, 2016), inspected for disease, parasites, and deformities and then released back into the stream (Kamdem Toham & Teugels, 1999).

2.2.2 | Environmental data sampling

In situ measures of dissolved oxygen, oxygen saturation, and temperature were performed using a portable multimeter (Model: oxi, 3,205. WTW). Water samples for laboratory analysis were collected in triplicate at a depth of 10–15 cm using 1-L plastic containers. After being sealed and labeled, samples were stored in cooled containers and transported to the Laboratory at Isfahan University of Technology for analysis. In the laboratory, samples were stored at 4°C until analysis of 22 variables (Table A1) according to standard methods (Baird & Bridgewater, 2017). At each sampling location, physical structure was characterized as river order, a summary of riparian vegetation features, measurements of instream parameters such as width, channel slope, reach length, depth, flow velocity, high water mark, surface substrate, presence, and size of barriers and dams (Table A1). Physical structure data were used to calculate habitat score, instream, morphological, and riparian/bank condition based on the Rapid Bioassessment Protocols (Barbour et al., 1999).

2.3 | Data analysis

2.3.1 | Identifying the disturbance gradient

The first step for developing the Karun Fish MMI (KFMMI) was to characterize the disturbance gradient. An LDC approach was used to establish the upper end of the disturbance gradient (Stoddard, Larsen, Hawkins, Johnson, & Norris, 2006). In brief, the LDC approach establishes reference based on best observed conditions in the basin. Variables with nonnormal distributions, as identified by Kolmogorov–Smirnov ($p > .05$) and Levene tests, were transformed using natural

logarithm and Box-Cox transformations. Prior to principal components analysis (PCA), linear regressions were used to identify strongly correlated variables ($R^2 > .7$) of which one was removed. Then, PCA was run with standardized and centered data of 17 parameters (Table 1). The first principal component (PC1) was used as the stressor gradient to establish least, moderate, and most disturbed conditions. The 25th and 75th percentiles of PC1 were used to classify stress categories (Zare Shahraki et al., 2021). Sites falling below the 25th percentile were defined as most disturbed, those above the 75th percentile as least disturbed, and those in between as moderately disturbed (Blocksom & Johnson, 2009).

2.3.2 | Calculation and evaluation of metrics and index development

Sampling sites where only one species was collected were excluded to avoid outliers. We then calculated a total of 54 metrics based on species richness and composition, migratory status, functional feeding groups, habitat preferences, and reproduction status (Tables A2 and A3). Proposed metrics were calculated using the following equations:

$$\text{“Relative abundance metrics (PIND)”}: \text{PIND} = \sum \frac{i}{n} \times 100.$$

$$\text{“Richness metrics (NTAX)”}: \text{NTAX} = \sum j$$

$$\text{“Relative richness metrics (PTAX)”}: \text{PTAX} = \sum \frac{j}{m} \times 100$$

where (i) the total species abundance with a specific feature per site, (n) the total fish abundance per site, (j) the number of species with a specific feature (Table A3) per site, and (m) the total number of species per site.

Metrics were evaluated for range, responsiveness, screening, and redundancy with other metrics to select the most responsive metrics to the disturbance gradient and to reduce the number of redundant metrics. A stepwise process was used to reduce the number of candidate metrics after each stage of evaluation. For range tests, we eliminated relative richness and abundance metrics with a range of 15% or less, richness metrics with a range of 4 or less, and metrics with 50% or more of the same value (Blocksom & Johnson, 2009). For responsiveness, Spearman correlation between candidate metrics and stressors (all physico-chemical, habitat variables, and PC1) was used to test their relationships. Then, those correlations with p value $\leq .01$ were maintained for further analysis, and other metrics were removed. For screening, retained metrics were examined through box-plots of various stress categories and only those that showed moderate to strong separation were selected (i.e., neither median overlapping with the interquartile range [IQR]; i.e., 25th to 75th percentile range, of the other group: box-plot score of 2; or no overlap of IQRs at all: score of 3) (Blocksom & Johnson, 2009). Kruskal–Wallis test was also used to compare metrics at least, moderate, and most disturbed sites. At this step, metrics have remained with p value $\leq .01$ and those with box plot scores of 2 or 3. Furthermore, Spearman

TABLE 1 Abiotic variables used in principal components analysis (PCA), along with transformations to reduce skewness, distributional characteristics (minimum, maximum, median)

Parameter	Transform	Min, max	Median	PC1 (27.08%)	PC2 (16.62%)
Biological oxygen demand		2.16, 5.25	3.51	0.39	-0.55
Total phosphate		0.23, 4.49	1.74	0.65	0.05
Dissolved oxygen		6.05, 10.5	8.52	-0.31	-0.72
Chemical oxygen demand	Natural log(X)	2.67, 65.09	20.86	-0.05	0.23
Electrical conductivity	Natural log(X)	194, 2,250	419	0.86	-0.27
Total nitrogen		2.74,12.02	6.71	0.71	-0.19
Hardness	Natural log(X)	106.33, 466.66	186.66	0.66	-0.6
Alkalinity	Natural log(X)	84.66, 253	146	0.72	-0.33
Total coliform	Natural log(X + 6)	0, 1,500	63	0.63	-0.64
Total solids	Natural log(X)	176.66, 1980	460	0.95	-0.6
Turbidity	Natural log(X)	15.34, 148.84	39.96	0.93	-0.53
Total habitat score		92, 177	125	-0.90	-0.87
Instream score		31, 56	47	-0.49	-0.26
Morphological score		37, 78	54	-0.98	-0.62
Riparian score		18, 54	25.5	0.46	0.92
% Clay and silt	Box cox	0, 14	4	0.64	0.55
% Sand	Natural log(X + 2)	0, 20	4	0.58	0.24

Note: The eigenvalue of each axis is represented.

correlation was performed on the remaining metrics from previous steps and PC1, and then metrics were chosen with p value $\leq .01$.

A first step in crafting the KFMMI was to standardize, or score, all evaluated metrics to the 0–10 value range (Blocksom & Johnson, 2009; Hughes et al., 1998; Minns, Cairns, Randall, & Moore, 1994). This method reduces variances when metric values differing by a value ≤ 1 are scored as different categories. These changes, by offering more precise depiction of the data, make MMIs more reliable and more easily understood (Blocksom, 2003; Hughes & Oberdorff, 1998; Minns et al., 1994). Metrics were encountered that related to disturbances in both positive and negative directions. We calculated continuous scores as $(\text{value} - \text{floor}) / (\text{ceiling} - \text{floor}) \times 100$ for positive metrics and as $(\text{ceiling} - \text{value}) / (\text{ceiling} - \text{floor}) \times 100$ for negative metrics. The ceiling (maximum) and floor (minimum) were calculated as the ninth and fifth percentiles, respectively, across the entire distribution of sites (Blocksom & Johnson, 2009). This continuous approach is advocated for by Blocksom (2003), Hughes and Oberdorff (1998), and Minns et al. (1994), and has been shown to have better performance characteristics (particularly variability) compared to discrete scoring or scoring using thresholds only based on least disturbed sites (Blocksom, 2003).

Finally, we combined metrics into subsets and calculated a series of multimeric indices using various sets of 4, 6, and 8 metrics, following the “best subsets” approach (Magee, Blocksom, & Fennessy, 2019). To calculate the index for each combination of metrics, we averaged the metric scores and multiplied by 10 to rescale the index to a 100-point scale for consistency and ease of interpretation. This approach randomly selects a given number of metrics from those that passed screening, creates an index, and calculates statistics for that index. The

process was iterated with different numbers of metrics (10'000 repetitions for each number of metrics) to include a large number of possible combinations of metrics and also examine indices based on larger and smaller numbers of metrics. For each combination of metrics, the KFMMI values were calculated on a 100-point scale, and then a series of statistical tests were performed. This included a sensitivity analysis, defined in Van Sickle (2010) as the percent of most disturbed sites with index scores statistically below the fifth percentile of least disturbed sites (an approach first described in Kilgour, Somers, and Matthews (1998)). We also calculated the mean and maximum correlations among metrics in a given index and mean and standard deviation of index scores among least disturbed sites. We filtered the 30,000 results by first ordering them based on the sensitivity and then keeping only results with maximum correlation among metrics (max.corr) of $<.75$ and mean correlation (mean.corr) of $<.50$ (Van Sickle, 2010). We then selected the final set of metrics for the KFMMI from among the top performing results, based on ease of calculation and ecological interpretation.

2.3.3 | Validation

In response to the limited number of sampling sites in the current study, data from all sites were used in the creation process of the KFMMI, and we did not have a separate set of data for validation. We created box plots of KFMMI values at least, moderate, and most disturbed sites and used an ANOVA (Duncan method) to test significant differences. Spearman correlation was used to examine the relationship between the KFMMI and physico-chemical and habitat

parameters, National Sanitation Foundation-Water Quality Index (NSFWQI), and PC1 to determine how well the KFMMI distinguished individual stressors.

2.3.4 | Discrimination efficiency

An index is considered discriminating if it can detect impairment, which can be quantitatively assessed using discrimination efficiency (DE). DE is a numerical description of the degree of separation between index value distributions of reference and impacted sites, defined using abiotic criteria and was calculated according to the equation (Bressler, Stribling, Paul, & Hicks, 2006):

$$DE = 100 \times \frac{a}{b}$$

where a is the number of common's moderate and most disturbed sites based on the KFMMI and PCA results and b is the number of moderate and most disturbed sites, based on the PCA results. A higher DE indicates a stronger performance of an index and the capacity to distinguish between impacted and nonimpacted sites (Bressler et al., 2006).

All statistical analyses were set at a level of significance lower than .01, to detect strong patterns (Ramos-Merchante & Prenda, 2018). They were performed using the R software (v. 4.0.4, R Core Team, 2020) and vegan (v. 2.5-6) and ggplot2 (v. 2.2.0) for analyses and graphics. A flow chart describing the procedure of KFMMI development is shown in Figure 2.

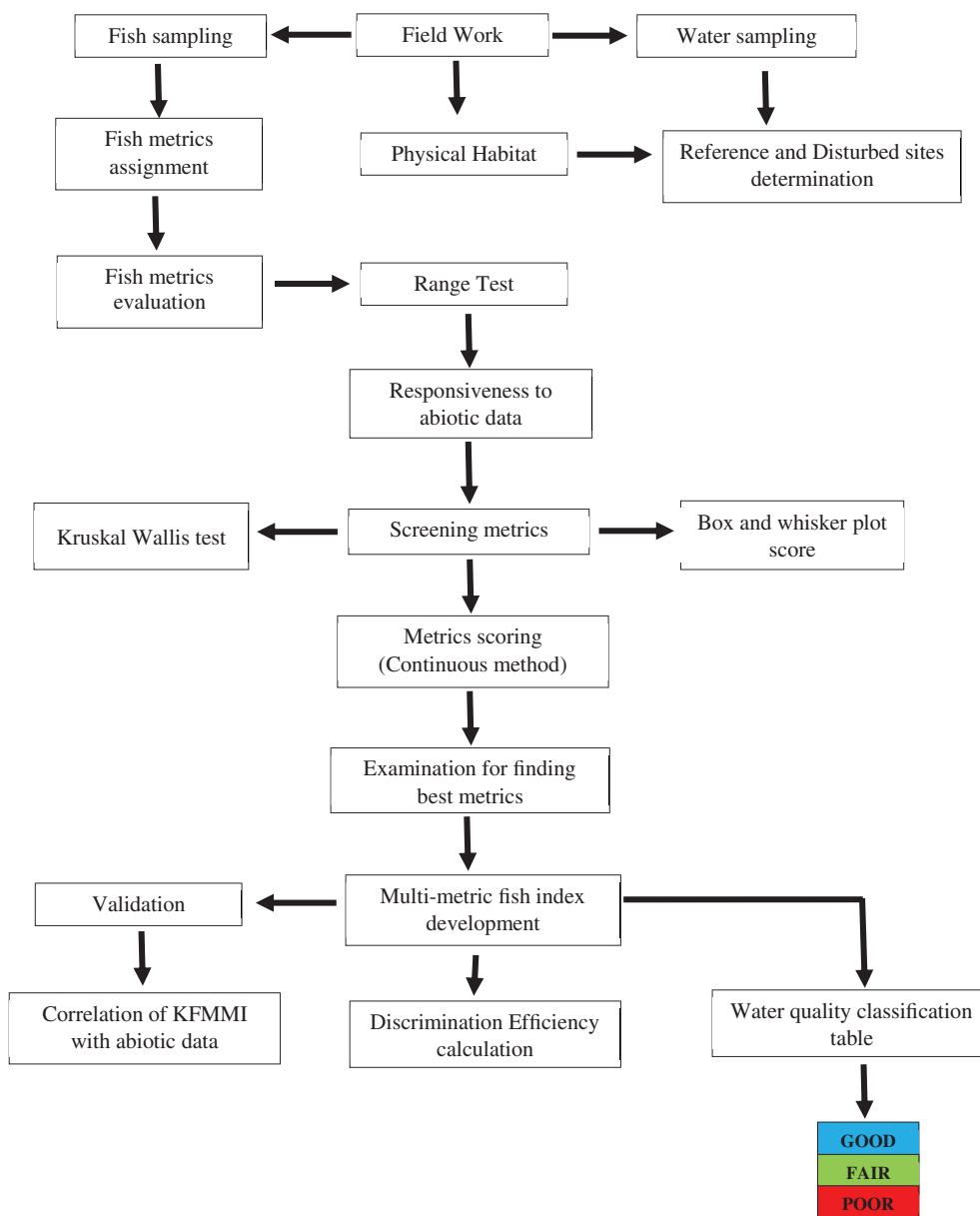


FIGURE 2 Flow chart describing the procedure of KFMMI development [Color figure can be viewed at wileyonlinelibrary.com]

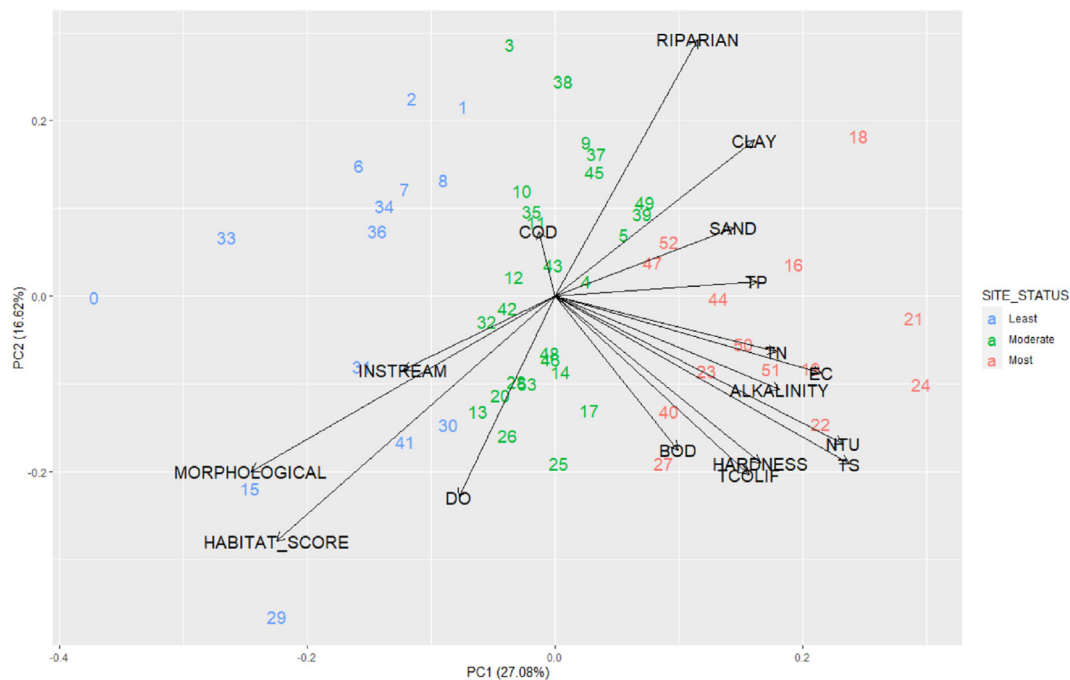


FIGURE 3 Principal component analysis plot in the Karun River basin, Iran [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

3 | RESULTS

3.1 | Identifying the least disturbed condition

A human-influenced disturbance gradient was evident in PC1. The first and second axis accounted for 27% and 16% of the variance in the data set, respectively. PC1 reflected a gradient from sites with higher total habitat scores, morphological score at the negative end, and less nutrients, clay, and sand at the positive end (Figure 3 and Table 3). PC2 did not indicate a clear disturbance gradient, thus, only PC1 was used as a disturbance gradient to identify least, moderate, and most disturbed sites. We identified LDC reference sites as those falling below the 25th percentile along these gradient and stressed sites as those above the 75th percentile of PC1. Out of 54 sites, 14, 26, and 14 sites were identified as least, moderate, and most disturbed sites, respectively. Most of least disturbed sites are located in upstream sections of the basin (Figure 4). These least-impacted upstream sites may not be appropriate for defining reference conditions at larger, higher order (>7) sites further downstream in the catchment, which were not considered for the current study.

3.2 | Metric evaluation and KFMMI development

Before metric calculation, sites 0, 15, and 29 were removed because no fish were detected at these sites. Also, site 49 was removed because it had only one fish species (*Hemiculter leucisculus*). Of the 54 proposed metrics, nine metrics had limited

ranges of values and were removed in the range test. Of those remaining, 28 metrics had significant Spearman correlation with abiotic variables (Table A4), 17 metrics had a p value $\leq .01$ in the Kruskal–Wallis test or showed the responsiveness in the box plot (Data S1 and Table A5) and 6 metrics had significant Spearman correlation with PC1 (Table A4). Finally, according to all the performed analyses, 15 metrics were selected for further consideration. In proportion to the response of metrics to abiotic parameters, positive (HERBPTAX, LEUCPTAX, LITHPIND, MIGRPTAX, NATPIND, NATPTAX, and ROCKPTAX) and negative (VEGP TAX, SLOWPTAX, SLOWPIND, SLOWNTAX, EDGENTAX, EDGE PIND, EDGEPTAX, and CYPRPIND) metrics were scored by the continuous method. The final index was selected according to the best subset of retained metrics. We looked at the various statistics in the best performing KFMMIs and selected the one that performed best. The best KFMMI includes 8 metrics (NATPIND, CYPRPIND, MIGRPTAX, VEGPTAX, LEUCPTAX, HERBPTAX, SLOWPIND, and EDGENTAX). The thresholds (ceiling and floor) and formula for scoring metrics included in the KFMMI are presented in Table 2.

3.3 | Validation

Box plots of KFMMI values at least, moderate, and most disturbed sites (Figure 5) revealed significant difference between the stress categories. KFMMI values and abiotic parameters revealed significant correlations (Table 3).

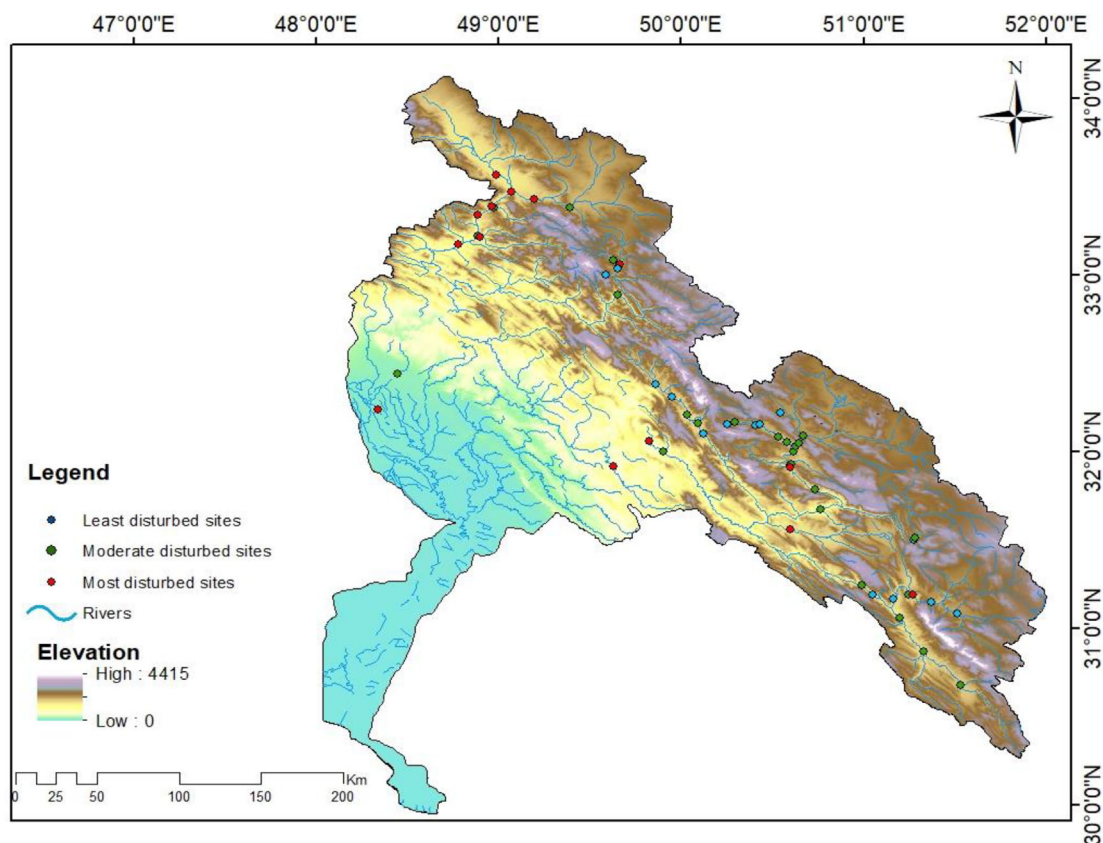


FIGURE 4 Location of least, moderate, and most disturbed sites on the Karun River basin, Iran [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Thresholds (ceiling and floor) and formula for scoring metrics included in the Karun fish-based multi-metric index (KFMMI)

Metric	Direction	Ceiling (95th percentile)	Floor (5th percentile)	Scoring formula
NATPIND	Positive	100	79.72	$(X - 79.72)/(100 - 79.72) \times 10$
MIGRPTAX	Positive	14.29	0	$(X - 14.29)/(14.29 - 0) \times 10$
LEUCPTAX	Positive	50	0	$(X - 50)/(50 - 0) \times 10$
HERBPTAX	Positive	38.87	9.22	$(X - 38.87)/(38.87 - 9.22) \times 10$
CYRPIND	Negative	94.72	19.28	$(94.72 - X)/(94.72 - 19.28) \times 10$
VEGPTAX	Negative	33.33	0	$(33.33 - X)/(33.33 - 0) \times 10$
SLOWPIND	Negative	49.26	0	$(49.26 - X)/(49.26 - 0) \times 10$
EDGENTAX	Negative	4.1	0	$(4.1 - X)/(4.1 - 0) \times 10$

$$\text{KFMMI} = \sum \text{metric score} \times 1.25$$

3.4 | Discrimination efficiency

This coefficient is calculated according to the performance of KFMMI in identifying the least and most disturbed sites. In this study, the number of moderate and most disturbed sites according to the PCA analysis was 38 sites, whereas the KFMMI identified 34 sites, and 31 sites were common to both methods. Therefore, the DE of the KFMMI was estimated as 81.6%.

3.5 | Bioassessment of study sites using the KFMMI

After calculating the KFMMI for all sites, the 5th and 25th percentiles of the least disturbed sites were used to separate the most disturbed from the moderate disturbed sites and the moderate disturbed from the least disturbed sites, respectively. Due to the limited number of sites, creating more than three quality

FIGURE 5 KFMMI values within various stress categories (least, moderate, and most disturbed sites). The letters above boxes indicate statistically different groups

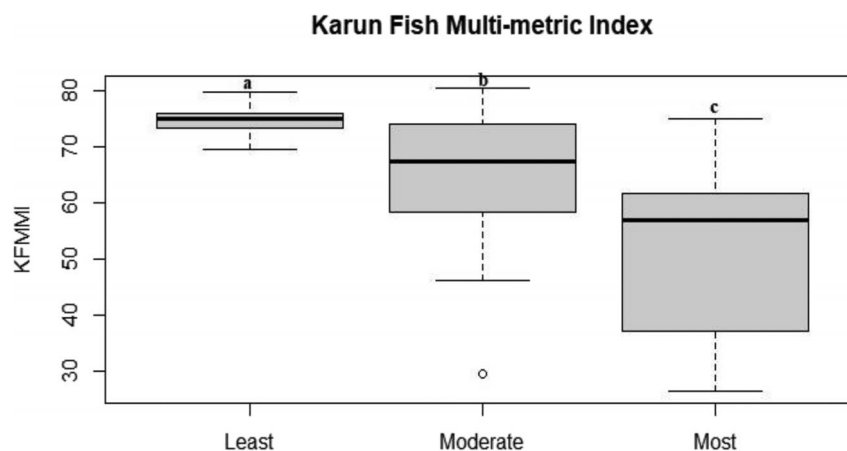


TABLE 3 The results of significant correlations between Karun fish-based multi-metric index (KFMMI) and abiotic data in the Karun River basin

Row	Column	Correlation value
KFMMI	EC	-.4
KFMMI	Hardness	-.45
KFMMI	Alkalinity	-.33
KFMMI	TS	-.52
KFMMI	NTU	-.44
KFMMI	HS	.35
KFMMI	Flow velocity	.29
KFMMI	PC axis 1	.52
KFMMI	NSFWQI	.3

Abbreviations: NSFWQI, National Sanitation Foundation-Water Quality Index; NTU, nephelometric turbidity unit; TS, total solid.

TABLE 4 Water quality classification table using KFMMI

Water quality class	KFMMI score
Good	73–100
Moderate	69–72
Poor	0–68

Abbreviation: KFMMI, Karun fish-based multi-metric index.

classes (good, moderate, and poor) was not considered useful (Table 4).

4 | DISCUSSION

Freshwater ecosystems provide a wide range of services such as water supply, fish production, biogeochemical cycles, energy, and recreation to humans (Ruaro, 2019). As a consequence of these uses, the ecological integrity of freshwater ecosystems is often compromised (Dudgeon, 2006; Grizzetti, Lanzanova, Lique, Reynaud, & Cardoso, 2016). Numerous additional anthropogenic alterations

directly affect the physico-chemical conditions and habitat structure of freshwater ecosystems and can strongly impact the organisms living therein (Ebrahimi Dorche, Shahraiki, Farhadian, & Keivany, 2018; Fathi, Ebrahimi, Mirghafary, & Esmaeili Ofoogh, 2016; Keivany et al., 2016; Mostafavi et al., 2015). Together, these impacts cause the current decline in freshwater biodiversity and in population sizes of many freshwater fish species (Reid et al., 2019). Biodiversity is critical to ecosystem health, and species extinction threatens important freshwater ecosystem functions such as productivity, material cycles, and sustainability (Jouladeh-Roudbar et al., 2020; Reid et al., 2019). MMIs, like the KFMMI, can serve as powerful tools for quantifying and characterizing the impact of these and other activities on aquatic ecosystems (Machado, Venticinqu, & Penha, 2011; Meador, Whittier, Goldstein, Hughes, & Peck, 2008; Mostafavi et al., 2015; Pont et al., 2006; Ruaro et al., 2020; Schmutz, Cowx, Haidvogel, & Pont, 2007). Although specifically developed for the Karun River basin, the KFMMI and its underlying methodology may prove useful (possibly with some adaptation of metrics) for other rivers in Iran and its biome where local indices are lacking.

4.1 | Description of reference site selection

The establishment of reference condition is a critically important step in index development (Stoddard et al., 2006). Given that rivers or river sections in undisturbed condition are globally rare (Bozzetti & Schulz, 2004; Wu et al., 2014), the establishment of reference condition for river index development often must rely on data from local least disturbed sites (Herlihy et al., 2008; Stoddard et al., 2006; Tejerina-Garro, Merona, Oberdorff, & Hugueny, 2006; Terra, Hughes, Francelino, & Araujo, 2013; Whittier, Stoddard, Larsen, & Herlihy, 2007; Wu et al., 2014). The Karun River basin has not been spared from the influence of human activities as shown in the rarity of reference conditions (Coad, 1996; Ghadiri & Afkhami, 2005; Khoshnood & Khoshnood, 2016; Woodbridge et al., 2016). Consequently, and similar to other research efforts (Bozzetti & Schulz, 2004; Fausch, Karr, & Yant, 1984; Gonino et al., 2020; Li, Huang, Jiang, & Wang, 2018; Liu, Zhou, Li, & Lan, 2010; Oosterhout & Velde, 2015;

Ramos-Merchante & Prenda, 2018; Stoddard et al., 2006; Wu et al., 2014), we used the concept of LDC to establish reference condition (Zare Shahraki et al., 2021). Similarly, Casatti, Ferreira, and Langeani (2009) selected reference sites based on the Physical Habitats Index, which identified good physical habitats as reference sites and Pei, Niu, and Xu (2010) selected reference sites based on water quality and habitat. The lack of information about historical conditions in Iran has also led to the acceptance of some degree of degradation, as access to the original conditions is not possible, a challenge which also speaks for the use of LDC (Soga & Gaston, 2018; Stoddard et al., 2006). Most of the LDC sites were located in the higher altitude of the Karun River basin because of less human access to these areas (Figure 4). This is a common finding of survey studies (Chen et al., 2014; Li et al., 2016; Pinto, Carvalho, & Gerson, 2007; Qadir & Malik, 2009; Wu et al., 2014) and requires some caution when interpreting the results.

4.2 | The performance of Karun fish-based multi-metric index

The suite of metrics selected supports assessment of the system from the perspectives of species richness and composition, migratory status, functional feeding groups, and habitat preferences. This combination is suitable to reflect the heterogeneity of environmental conditions, biological assemblages, and human pressures (De Bikuna et al., 2017; Hughes et al., 1998; Karr, 1981; Marzin et al., 2012; Mostafavi et al., 2015; Pont et al., 2006). Performances of individual metrics do not reliably predict the joint performance of their summed index (Blocksom, 2003; Van Sickle, 2010) but can show disturbances differently, as described below for some important metrics of the KFMMI.

The relative abundance of native and endemic species in the species pool (NATPIND) was used as a positive metric in KFMMI (higher values related to better conditions). This metric has been very informative in ours and other indices (Aparicio, Carmona-Catot, Moyle, & Garcia-Berthou, 2011; Gonino et al., 2020; Mostafavi et al., 2015; Oberdorff & Hughes, 1992; Petesse, Petrere, & Agostinho, 2014; Pinto, Araujo, & Hughes, 2006; Ramos-Merchante & Prenda, 2018). Simonson and Lyons (1995) have shown that native species decrease with increasing disturbances while invasive species increase. In our study sites, nonnative species were represented by *Carassius gibelio*, *Ctenopharyngodon idella*, *Hemiculter leucisculus*, *Gambusia holbrooki*, *Oncorhynchus mykiss*, *Pseudorasbora parva*, and *Rhinogobius lindbergi*. These species are very tolerant to environmental degradation (Jouladeh-Roudbar et al., 2020) and thus can reflect the degraded state of ecosystems. In addition to an indication of ecosystem condition, nonnative species cause important threats to the conservation of freshwater fish diversity and ecosystem health through impacts such as predation, competition, and disease transmission (Adamczyk et al., 2017; Choi, Kumar, Han, & An, 2011; Gonino et al., 2020; Ramos-Merchante & Prenda, 2018; Ticiani & Delariva, 2020; Vitule, Freire, & Simberloff, 2009). Considering nonnative species in an index for ecosystem condition in addition to suitable physico-chemical and

habitat conditions thus seems informative (Hermoso, Clavero, Blanco-Garrido, & Prenda, 2010; Hermoso, Clavero, Blanco-Garrido, & Prenda, 2011; Ramos-Merchante & Prenda, 2018).

Relative richness of Herbivorous taxa (HERBPTAX) is a positive metric, which has also been considered in other MMIs (Cetra & Ferreira, 2016). The functional composition of fish communities using trophic guilds can reflect the ecological quality of the system (Choi et al., 2011) as disturbances tend to negatively affect species with specific feeding behaviors (Scott & Hall, 1997; Vehanen, Sutela, & Korhonen, 2010) but less so generalist species (Noble, Cowx, Goffaux, & Kestemont, 2007). In our study, omnivorous species were present with high abundance in disturbed sites, which corroborate the findings of other studies (Carvalho et al., 2017; Cetra & Ferreira, 2016; Choi et al., 2011; Kamdem Toham & Teugels, 1999; Karr, 1981). This is probably because they can feed on different types of resources such as plant, animal, detritus, and organic debris when a preferred resource becomes unavailable due to human disturbances (Karr, 1981; Oosterhout & Velde, 2015; Pinto et al., 2006; Shetty, Venkateshwarlu, & Muralidharan, 2015). For example, the % carnivorous individuals' metric was used in some indices, and it indicates a good state of river health because it depends on a well-structured trophic network (Choi et al., 2011; Kamdem Toham & Teugels, 1999; Karr, 1981; Oberdorff & Hughes, 1992). Instead, in our index, the HERBPTAX metric may have been selected because it reflects habitat diversity that supports herbivorous species (Balvanera et al., 2006) that are affected by one of the dominant stressors in the catchment, that is, deposited and suspended fine sediment. In degraded conditions, fine sediment causes instability of the substrate, preventing the accumulation or attachment of periphyton and thus reducing the abundance of resources for taxa relying on this resource (Cetra & Ferreira, 2016; Ferreira, Caiola, Casals, Oliveria, & Sostoa, 2007).

The higher presence of the Leuciscidae taxa (LEUCPTAX) in the LDCs can be related to access to different habitats and food resources in the river margins because most of the species of this family prefer habitats with macrophytes and reed (Caetano, Oliveira, & Zawadzki, 2016; Terra et al., 2013). For instance, riverine vegetated habitats, directly and indirectly, play important roles in providing food sources for different species, for example, periphyton that serves as a vital food resource and shelter for many fish in their young life stages (Ferreira et al., 2012). Degraded conditions are associated with a decrease in aquatic vegetation near the river margins and more channel erosion with low potential for the presence of species depending on these habitats (Gonino et al., 2020; Terra et al., 2013).

The relative abundance of Cyprinid taxa individuals (CYPRPIND) had been considered as a negative metric and increases with impairments as in other MMIs (Li et al., 2016). Several species of the Cyprinidae family were widely present from upstream and downstream sites. The dominance of cyprinids is due to their high potential for adaptation (Johnson & Arunachalam, 2009) and flexibility in the use of heterogeneous habitats and resources (Bhat, 2003; Li et al., 2016; Shetty et al., 2015). Most cyprinid species are tolerant and have high compatibility to human impacts including loss of habitat connectivity, habitat degradation, and warming of rivers systems (Lange, Bruder, Matthaei,

Brodersen, & Paterson, 2018; Persson, Diehl, Johansson, Andersson, & Hamrin, 1991; Vehanen et al., 2010). Cyprinids usually have greater presence at disturbed sites in our and other studies (Harig & Bain, 1998; Lange et al., 2018; Li et al., 2016; Raburu & Masese, 2012).

The relative abundance of individuals preferring slow-flowing waters (SLOWPIND) was also considered as the negative metric. Shallow, slow-moving sections of streams are prone to temperature increases and decreased oxygen levels due to high decomposition and respiration rates, thereby stressing fish and favoring tolerant species (Tsfay Gebrekios, 2016). In a river assessment context, areas dominated by slow-flow species often indicate impairment to the natural flow regime by some structure (e.g., dams), which then favors species that are adapted to survive if not thrive in those conditions (Townsend, 1996).

4.3 | Validation and discrimination efficiency

In this study, the KFMMI showed an excellent ability to separate least and most disturbed sites in our study area and had significant differences in various stress categories (Figure 5). The index was validated using three types of control variables; (a) physical habitat features, (b) water chemistry and NSFQI, and (c) the main environmental gradient obtained after PC1. Results of the index represented a significant correlation with control variables (Table 3). This indicates that the KFMMI is sensitive to dominant impacts on physico-chemical and habitat quality. It is therefore suitable to detect human disturbances in river ecosystems. However, more multiple-stressor studies are needed on a wider variety of fish taxa to better understand the role of stressor co-tolerance for interaction outcomes (Segner, Schmitt-Jansen, & Sabater, 2014). Overall, its sensitivity to disturbance was similar to that of other indices (Casatti et al., 2009; dos Santos & Esteves, 2015; Hughes et al., 1998; Vehanen et al., 2010). As an example, human alterations of water flow velocity and turbidity often have negative impacts on MMIs (Alonso, Garcia De Jalon, & Marchamalo, 2011; Ramos-Merchante & Prenda, 2018). In our study, a significant negative correlation was observed between the KFMMI and turbidity and a significant positive correlation between KFMMI and flow velocity. Flow velocity changes can alter the biological function of native and endemic species in a variety of ways, for example, by altering resource availability, reproduction, recruitment, and abundance that lead to reduced population and distribution (Aparicio, Vargas, & Olmo, 2000; Bruder, Salis, Jones, & Matthaei, 2017; Clavero, Blanco-Garrido, & Prenda, 2004; Freeman, Bowen, Bovee, & Irwin, 2001; Lytle & Poff, 2004). The DE of the KFMMI was calculated to be 81.6%, which reflects a high potential for the detection of disturbance. Moderate and most disturbed sites identified by PCA based on abiotic data were also detected by the KFMMI.

5 | CONCLUSION

Karun River basin is an important water resource in Iran, and therefore its assessment should include a variety of bioindicators. In this study,

the least disturbed sites were identified by descriptors physico-chemical parameters and habitat characteristics used in statistical analysis. EC, total solids, turbidity, total habitat score, and morphological score were the most influential parameters in the study area. Based on these analyses, the KFMMI was developed using data on fish composition, functional feeding, habitat preference, and migratory status. It performed well in distinguishing between the least, moderate, and most disturbed sites and showed a significant negative response to increased human stressors. Based on its performance, the KFMMI should have utility in other basins of similar composition in the region and the biome for which indices have yet to be developed. However, indices will likely differ between regions with such diverse biomes and taxa richness as in Iran. For instance, the relative abundance of native species was the only common metric with the study of Mostafavi et al. (2015). They developed a fish-based multi-metric index of cyprinid streams (MMICS) in the Caspian Sea Basin, Iran (Mostafavi et al., 2015). The differences in metrics between our KFMMI and the MMICS are likely a function of the different study areas and their communities (including biogeography and land-use history), sample size, stream size, and metric selection process that is stated by other researchers as well (Carvalho et al., 2017). The development of KFMMI and other indices provide an opportunity to support national biomonitoring programs and inform river management in Iran.

Future research should focus on the sampling and assessment of non-wadable components of the Karun River basin. This would support a more complete understanding of the ecology of the system, incorporate more geological features, and help expand the suite of standards available for sampling wadable and nonwadable sections of Iranian rivers. Future applications of the KFMMI should consider that the index was developed for use in wadable sections of the Karun River basin. It may be applicable in other areas, but interpretations need to be conducted with caution. Similarly, due to seasonal changes in the abundance of individual species at different times (Lyons, 2006), this index is most suitable for the interpretation of data collected during summer. Last, as fish sampling methods are not currently standard in Iran, we recommend use of our KFMMI or American-European standard methods (Barbour, Diamond, & Yoder, 1996; CEN, 2003; Flotemersch et al., 2006) as the sampling methodology can impact the validity of index scores (Simon & Sanders, 1999). This biological tool could be utilized by managers to direct restoration actions for the most disturbed river ecosystems and fish communities and to strengthen the conservation of the least disturbed ones.

DATA AVAILABILITY STATEMENT

Author elects to not share data, research data are not shared.

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APPENDIX A.

TABLE A1 Variables considered for the Karun River basin, with respective scales of measurement

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	
	Altitude	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)	–	
	*Surface substrate size (gravel, cobble, clay and silt, and 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)		

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

TABLE A2 List of proposed fish metrics

Metric code	Metric code	Metric description
Richness and species composition		
1	TOTLNIND	Total number of individuals per site
2	TOTLNTAX	Total number of taxa per site
3	FAMNTAX	Total number of families per site
4	CYPRNTAX	Total number of Cyprinid taxa
5	CYPRPIND	Relative abundance of Cyprinid taxa
6	CYPRPTAX	Relative richness of Cyprinid taxa
7	INTRNTAX	Total number of introduced taxa
8	INTRPIND	Relative abundance of introduced taxa
9	INTRPTAX	Relative richness of introduced taxa
10	LEUCNTAX	Total number of Leuciscidae taxa
11	LEUCPIND	Relative abundance of Leuciscidae taxa
12	LEUCPTAX	Relative richness of Leuciscidae taxa
13	NATNTAX	Total number of native and endemic taxa
14	NATPIND	Relative abundance of native and endemic taxa
15	NATPTAX	Relative richness of native and endemic taxa
Functional feeding groups		
16	HERBNTAX	Total number of herbivorous taxa
17	HERBPIND	Relative abundance of herbivorous taxa
18	HERBPTAX	Relative richness of herbivorous taxa
19	INVENTAX	Total number of invertivorous taxa
20	INVEPIND	Relative abundance of invertivorous taxa
21	INVEPTAX	Relative richness of invertivorous taxa
22	OMNINTAX	Total number of omnivorous taxa
23	OMNIPIND	Relative abundance of omnivorous taxa
24	OMNIPTAX	Relative richness of omnivorous taxa
Reproduction status		
25	LITHNTAX	Total number of lithophilic spawner taxa
26	LITHPIND	Relative abundance of lithophilic spawner taxa
27	LITHPTAX	Relative richness of lithophilic spawner taxa
28	PHYNTAX	Total number of phytophilic spawner taxa
29	PHYTPIND	Relative abundance of phytophilic spawner taxa
30	PHYTPTAX	Relative richness of phytophilic spawner taxa
Migratory status		
31	MIGRNTAX	Total number of migratory taxa
32	MIGRPIND	Relative abundance of migratory taxa
33	MIGRPTAX	Relative richness of migratory taxa
Habitat preferences		
34	BENTNTAX	Total number of benthic taxa
35	BENTPIND	Relative abundance of benthic taxa
36	BENTPTAX	Relative richness of benthic taxa
37	COLDNTAX	Total number of cold-water taxa
38	COLDPIND	Relative abundance of cold-water taxa
39	COLDPTAX	Relative richness of cold-water taxa
40	EDGENTAX	Total number of edge inhabitant taxa
41	EDGEPIIND	Relative abundance of edge inhabitant taxa

TABLE A2 (Continued)

Metric code	Metric code	Metric description
42	EDGEPTAX	Relative richness of edge inhabitant taxa
43	FASTNTAX	Total number of fast waterflow inhabitant taxa
44	FASTPIND	Relative abundance of fast waterflow inhabitant taxa
45	FASTPTAX	Relative richness of fast waterflow inhabitant taxa
46	ROCKNTAX	Total number of rocky inhabitant taxa
47	ROCKPIND	Relative abundance of rocky inhabitant taxa
48	ROCKPTAX	Relative richness of rocky inhabitant taxa
49	SLOWNTAX	Total number of slow waterflow inhabitant taxa
50	SLOWPIND	Relative abundance of slow waterflow inhabitant taxa
51	SLOWPTAX	Relative richness of slow waterflow inhabitant taxa
52	VEGNTAX	Total number of vegetative inhabitant taxa
53	VEGPIND	Relative abundance of vegetative inhabitant taxa
54	VEGPTAX	Relative richness of vegetative inhabitant taxa

TABLE A3 Scientific names and characteristics of 37 fish species sampled in the Karun River basin, Iran

	Scientific name	Presence status in Karun Basin	Migratory status	Reproduction substrate preference	Feeding behaviour	Habitat type	Flow velocity preference	Substrate preference
1	<i>Acanthobrama marmid</i>	Native	No	Phytophile	Omnivore	Bank edge	Slow	Phytophile
2	<i>Chondrostoma regium</i>	Native	No	Phytophile	Omnivore	Water column	Moderate	Lithophile
3	<i>Alburnoides idignensis</i>	Endemic	No	Phytophile	Omnivore	Bank edge	Slow	Phytophile
4	<i>Alburnus caeruleus</i>	Native	No	Lithophile	Omnivore	Bank edge	Slow	Phytophile
5	<i>Squalius berak</i>	Native	No	Phytophile	Omnivore	Water column	Moderate	Lithophile
6	<i>Squalius lepidus</i>	Native	No	Phytophile	Omnivore	Water column	Moderate	Lithophile
7	<i>Alburnus doriae</i>	Endemic	No	Lithophile	Benthivore	Water column	Moderate	Lithophile
8	<i>Alburnus sellal</i>	Native	No	Lithophile	Omnivore	Water column	Moderate	Lithophile
9	<i>Capoeta aculeata</i>	Endemic	No	Phytophile	Herbivore	Water column	Slow	Lithophile
10	<i>Capoeta coadi</i>	Endemic	No	Other	Herbivore	Water column	Moderate	Lithophile
11	<i>Capoeta trutta</i>	Native	No	Lithophile	Herbivore	Water column	Moderate	Lithophile
12	<i>Carasobarbus kosswigi</i>	Native	No	Lithophile	Omnivore	Water column	Moderate	Lithophile
13	<i>Carasobarbus luteus</i>	Native	No	Lithophile	Herbivore	Bank edge	Slow	Lithophile
14	<i>Carassius gibelio</i>	Nonnative	No	Phytophile	Omnivore	Bank edge	Slow	Phytophile
15	<i>Cyprinion macrostomus</i>	Native	No	Lithophile	Omnivore	Bank edge	Moderate	Lithophile
16	<i>Cyprinus carpio</i>	Translocate	Yes	Phytophile	Omnivore	Bank edge	Slow	Phytophile
17	<i>Garra rufa</i>	Native	No	Other	Omnivore	Benthic	Fast	Lithophile
18	<i>Garra gymnothorax</i>	Endemic	No	Other	Omnivore	Benthic	Fast	Lithophile
19	<i>Luciobarbus barbulus</i>	Native	Yes	Lithophile	Carnivore	Water column	Fast	Lithophile
20	<i>Capoeta pyragyi</i>	Endemic	No	Other	Herbivore	Water column	Moderate	Lithophile
21	<i>Arabibarbus grypus</i>	Native	No	Lithophile	Omnivore	Bank edge	Moderate	Phytophile
22	<i>Barbus karunensis</i>	Endemic	No	Lithophile	Omnivore	Benthic	Moderate	Lithophile
23	<i>Barbus lacerta</i>	Native	No	Lithophile	Omnivore	Benthic	Moderate	Lithophile
24	<i>Ctenopharyngodon idella</i>	Nonnative	No	Phytophile	Herbivore	Bank edge	Slow	Phytophile
25	<i>Hemiculter leucisculus</i>	Nonnative	No	Other	Omnivore	Water column	Moderate	Lithophile
26	<i>Gambusia holbrooki</i>	Nonnative	No	Other	Omnivore	Bank edge	Slow	Phytophile
27	<i>Glyptothorax silviae</i>	Endemic	No	Lithophile	Benthivore	Benthic	Fast	Lithophile
28	<i>Mastacembelus</i>	Native	No	Lithophile	Carnivore	Bank edge	Slow	Lithophile

(Continues)

TABLE A3 (Continued)

	Scientific name	Presence status in Karun Basin	Migratory status	Reproduction substrate preference	Feeding behaviour	Habitat type	Flow velocity preference	Substrate preference
29	<i>Oncorhynchus mykiss</i>	Nonnative	No	Lithophile	Carnivore	Water column	Fast	Lithophile
30	<i>Oxynoemacheilus freyhofi</i>	Endemic	No	Lithophile	Benthivore	Benthic	Fast	Lithophile
31	<i>Sasanidus kermanshahensis</i>	Endemic	No	Lithophile	Benthivore	Benthic	Fast	Lithophile
32	<i>Turcinoemacheilus hafezi</i>	Endemic	No	Lithophile	Benthivore	Benthic	Fast	Lithophile
33	<i>Turcinoemacheilus saadii</i>	Endemic	No	Lithophile	Benthivore	Benthic	Fast	Lithophile
34	<i>Planiliza abu</i>	Native	No	Lithophile	Benthivore	Water column	Moderate	Lithophile
35	<i>Pseudorasbora parva</i>	Nonnative	No	Lithophile	Omnivore	Bank edge	Slow	Phytophile
36	<i>Rhinogobius lindbergi</i>	Nonnative	No	Lithophile	Benthivore	Bank edge	Slow	Lithophile
37	<i>Aphanius vladykovi</i>	Endemic	No	Phytophile	Omnivore	Bank edge	Slow	Phytophile

TABLE A4 Significant Spearman correlation between fish metrics and abiotic data on the Karun River basin, Iran

Row	Column	Correlation	p value
TOTLNTAX	COD	-.38532	.005722
FAMNTAX	COD	-.42455	.00212
INVENTAX	COD	-.45365	.000937
INVEPTAX	COD	-.37156	.007891
LITHNTAX	COD	-.42768	.001948
HERBPTAX	Ecol	-.42931	.001864
LEUCNTAX	Hardness	-.38493	.005775
MIGRPIND	Nitrate	-.36283	.009609
MIGRPTAX	Nitrate	-.42744	.001961
NATPIND	Nitrite	-.39706	.004303
NAT_PTAX	Nitrite	-.37429	.00741
MIGRPIND	TN	-.37735	.006903
MIGRPTAX	TN	-.44027	.001376
NATPTAX	TN	-.37259	.007706
CYPRNTAX	PH	-.45035	.001032
CYPRPIND	PH	-.40808	.003263
CYPRPTAX	PH	-.41813	.002514
LEUCPIND	PH	.415634	.002685
LEUCPTAX	PH	.371914	.007827
HERBPTAX	Phosphate	.439602	.001402
SLOWNTAX	Phosphate	-.38414	.005885
SLOWPIND	Phosphate	-.40703	.003351
SLOWPTAX	Phosphate	-.36525	.009103
NATPTAX	Alkalinity	-.3612	.009962
CYPRPIND	T-coliform	-.36613	.008923
CYPRPTAX	T-coliform	-.40285	.003725
HERBPTAX	T-coliform	-.40814	.003258
LEUCPIND	T-coliform	.395713	.004449
EDGENTAX	TDS	.395322	.004492
EDGEPTAX	TDS	.391769	.004899
PHYTPIND	TDS	-.36733	.008685

TABLE A4 (Continued)

Row	Column	Correlation	p value
SLOWNTAX	TDS	.423332	.00219
SLOWPIND	TDS	.363175	.009535
SLOWPTAX	TDS	.426105	.002033
EDGENTAX	TKN	-.39259	.004802
EDGEPIIND	TKN	-.44981	.001048
EDGEPTAX	TKN	-.41258	.002907
HERBPTAX	TKN	.505967	.000178
SLOWNTAX	TKN	-.48286	.000383
SLOWPIND	TKN	-.49646	.000246
SLOWPTAX	TKN	-.47447	.000499
LEUCPIIND	TP	-.41475	.002747
EDGENTAX	TS	.476484	.000469
EDGEPIIND	TS	.457951	.000826
EDGEPTAX	TS	.485204	.000355
SLOWNTAX	TS	.545361	4.21E-05
SLOWPIND	TS	.501407	.000208
SLOWPTAX	TS	.547261	3.91E-05
EDGENTAX	TSS	.469463	.000583
EDGEPIIND	TSS	.471517	.000547
EDGEPTAX	TSS	.477984	.000447
HERBPTAX	TSS	-.38357	.005965
NATPIIND	TSS	-.36659	.008833
SLOWNTAX	TSS	.54724	3.91E-05
SLOWPIND	TSS	.522973	9.76E-05
SLOWPTAX	TSS	.537709	5.65E-05
EDGENTAX	NTU	.469463	.000583
EDGEPIIND	NTU	.471517	.000547
EDGEPTAX	NTU	.477984	.000447
HERBPTAX	NTU	-.38357	.005965
NATPIIND	NTU	-.36659	.008833
SLOWNTAX	NTU	.54724	3.91E-05
SLOWPIND	NTU	.522973	9.76E-05
SLOWPTAX	NTU	.537709	5.65E-05
EDGENTAX	Temperature	.459793	.000782
EDGEPIIND	Temperature	.398459	.004157
EDGEPTAX	Temperature	.438612	.001442
LEUCPIIND	Temperature	-.42791	.001936
SLOWNTAX	Temperature	.404957	.003532
SLOWPTAX	Temperature	.425156	.002086
MIGRPIND	Depth	.396182	.004398
MIGRPPTAX	Depth	.371474	.007906
CYPRPIIND	Elevation	-.47641	.00047
CYPRPPTAX	Elevation	-.40528	.003504
LEUCPIIND	Elevation	.485939	.000347
LEUCPPTAX	Elevation	.459916	.000779
OMNINTAX	Slope_perc	-.37958	.006554

(Continues)

TABLE A4 (Continued)

Row	Column	Correlation	<i>p</i> value
CYPRPIND	Total Habitat Score	−.37465	.007349
OMNIPIND	Riparian	.429053	.001877
OMNIPTAX	Riparian	.430406	.001809
NATPIND	PC axis 1	.450302	.001033
NATPTAX	PC axis 1	.416034	.002657
VEGPTAX	PC axis 1	−.38431	.005862
EDGENTAX	PC axis 1	−.37097	.007998
VEGPTAX	PC axis 1	−.38431	.005862

Abbreviations: COD, chemical oxygen demand; NTU, nephelometric turbidity unit; TDS, total dissolved solid; TKN, total Kjeldahl nitrogen; TN, total nitrogen; TS, total solid; TSS, total suspended solid.

TABLE A5 The result of Kruskal–Wallis test and box plot scores for selected metrics on the Karun River basin, Iran

Metric	Box plot score	<i>p</i> value	Kruskal–Wallis test
EDGEPTAX	1	.01	6.17
EDGENTAX	1	.01	6.51
EDGEPIIND	1	.01	6.17
MIGRPIND	3	.05	3.7
MIGRPTAX	3	.01	5.62
NATPIND	1	.009	10.87
NATPTAX	1	.009	10.87
ROCKPIND	2	.04	3.91
ROCKPTAX	2	.02	5.37
SLOWPTAX	1	.01	6.02
SLOWNTAX	1	.01	6.24
SLOWPIND	1	.01	5.86
VEGPIND	1	.0023	9.22
VEGPTAX	1	.0023	9.23
CYPRPIND	1	.01	6.67
HERBPTAX	2	.01	6.05
LITHPIND	2	.27	1.19