

## Defining a disturbance gradient in a Middle-Eastern River Basin

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### ARTICLE INFO

#### Keywords:

Least-disturbed conditions  
Bioassessment  
Rivers  
Physico-chemical  
EPT

### ABSTRACT

A physical, chemical and biological characterization of river systems is needed to evaluate their ecological quality and support restoration programs. Herein, we describe an approach using water chemistry, physical structure and land use for identification of a disturbance gradient existing in the Karun River Basin. For this purpose, at each site, physical structure and physico-chemical data were collected once in each season for a total of 4 samples during the period (October 2018 - September 2019). Principal components analysis (PCA) of 17 variables identified five variables that were influential across all seasons: conductivity, total habitat score, stream morphology, clay & silt, and sand. Of the 54 sites, 14, 26 and 14 sites were classified as least, moderate and most disturbed sites, respectively. The metric Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa was used for validation of the classification. Results in different seasons showed that all the least disturbed sites ( $n = 14$ ) were significantly different from moderate and most disturbed sites ( $p < 0.01$ ). In this study the validation process presented good confirmation of *a priori* reference sites selection, showing that the proposed criteria could be considered as appropriate tools for characterization of the existent disturbance gradient in the Karun River Basin.

### 1. Introduction

In the last century, many Iranian rivers have experienced increasing pressures from human activities with detrimental effects on their condition (Ghadiri and Afkhami, 2005; Mostafavi et al., 2015; Sabbaghi and Masihi, 2012). Dominant consequences include eutrophication and degradation of aquatic and riparian habitat quality caused by channelization, in-channel gravel mining, and disruption of longitudinal connectivity and of the hydrological regime due to damming (Bagherian Marzouni et al., 2014; Woodbridge et al., 2016). Characterization of the direct and indirect effects of these anthropogenic activities on stream and river conditions is generally achieved through ecological assessment (Sanchez et al., 2009).

A frequently used approach for conducting such assessments is the comparison of site conditions to conditions at so-called “reference sites” (Stoddard et al., 2006). Reference sites ideally comprise comparable

environmental settings in the absence of human disturbances or stressors. However, this approach is often challenged by a lack of pristine sites in the study region (Woodbridge et al., 2016; Wyżga et al., 2012). Furthermore, as new information becomes available (e.g., expanded datasets, spatial surveys, predictive modeling, historical data, and/or expert judgment), local definitions of reference conditions might need to be reevaluated (Carstensen and Henriksen, 2009; Thorne and Williams, 1997). Regardless of these challenges, the reference condition (RC) approach continues to be an important tool to characterize the impacts of human activities on freshwater ecosystems and to plan management actions (Karr and Chu, 1999).

Stoddard et al. (2006) identified four different reference condition types: 1) Historical Condition, 2) Minimally Disturbed Condition, 3) Best-Attainable Condition, and 4) Least-Disturbed Condition. Historical Condition uses data collected from a stream or river before it became degraded (Stoddard et al., 2006). However, even if historical data are

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<https://doi.org/10.1016/j.limno.2021.125923>

Received 12 May 2021; Received in revised form 8 September 2021; Accepted 13 September 2021

Available online 30 September 2021

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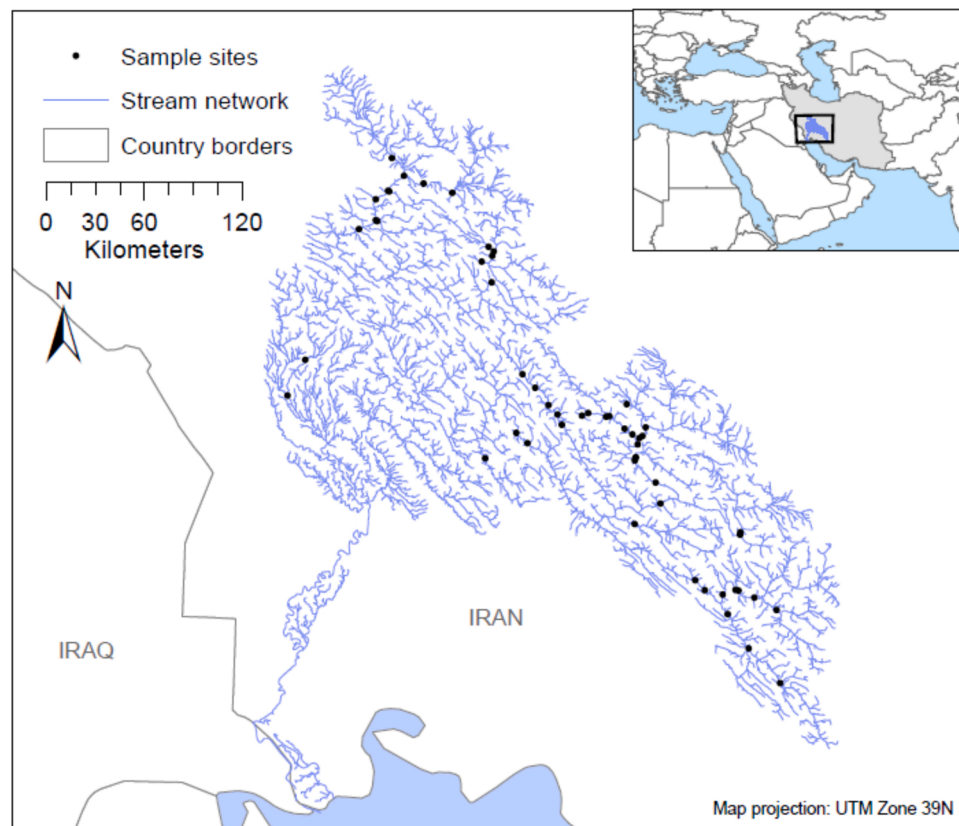


Fig. 1. Map of the Karun River Basin and location of the sampling sites (black dots).

available, these data may not represent a condition that is reasonably achievable given changes to the environment (e.g., climate change) (Cooper and Thomsen, 1988; Vant and Huser, 2000; Wyżga et al., 2012). The Minimally Disturbed Condition approach uses data from sites displaying negligible anthropogenic disturbance (Stoddard et al., 2006). However, such reference sites might be uncommon, particularly in most agriculturally productive landscapes (McDowell et al., 2013). The Best-Attainable Condition represents the ecological condition expected if best possible management practice were used (Johnson et al., 2013). Least-Disturbed Condition describes “best-available physical, chemical and biological habitat conditions given the current state of the water-body” (Hughes, 1995; Hughes et al., 1986). That is, least-disturbed sites would be where the biota is exposed to the lowest levels of the prevailing stressor gradients, thus representing an informative and realistic management goal.

We developed and analyzed a dataset of abiotic habitat conditions from seasonal sampling of 54 sites in the Karun River system, western Iran. In recent decades, the Karun has been increasingly modified with the construction of many large dams for water storage, electricity production, and irrigation (Salarijazi, 2012). These human uses have led to organic and inorganic pollution and alterations to the flow and sediment regime; however, lack of quantified characterization hinders ecosystem management and restoration. We measured physico-chemical conditions, physical habitat structure and land use and used this dataset to identify the combination of abiotic variables that best define gradients of anthropogenic stress and Least-Disturbed Condition. Specifically, our objectives were to:

- 1 Identify the most important parameters affecting water quality in the Karun River System,
- 2 Propose criteria to select least-disturbed sites, and
- 3 Identify impacted conditions in need of management or restoration.

## 2. Material and methods

### 2.1. Study area

The Karun River (Fig. 1) is the largest freshwater resource in Iran, with a drainage area of approximately 67,000 km<sup>2</sup> covering seven provinces (Chaharmahal-and-Bakhtiari, Fars, Isfahan, Khuzestan, Kohgiluyeh-and-Boyer-Ahmad, Lorestan and Markazi) of southwestern Iran. The longest river in Iran (950 km), the Karun originates at an elevation of 4,409 m in the Zagros Mountains and flows through a variety of landscapes into the Persian Gulf ( $21 \times 10^9$  m<sup>3</sup>/year) (Afkhani et al., 2007). The Karun has an average slope of 3 % and is the only navigable waterway in Iran.

The upper sections of the catchment are geologically very heterogeneous and comprised of limestone, marly-limestone, marl, shale, sandstone, and conglomerate whereas the lower parts of the catchment are dominated by recent alluvial sediments (Yousefi et al., 2016). About 74.6 % of the basin consists of mountain and highlands, while plains and low-elevation areas cover about 25.4 % (Bakhsipoor et al., 2019). The Karun Basin has three general climatic zones: the mountains, foothills, and the desert. Air temperature ranges from hot and dry conditions in summer (i.e., >50 °C) to less than 0 °C in winter (Khosravi et al., 2017). Mean annual precipitation ranges from 400–1200 mm in the Central Zagros Zone to less than 200 mm in the arid Zone of the Lower Khuzestan Plains (Woodbridge et al., 2016). With about 50 small and large tributaries, this basin is an important water source for various human uses including agriculture, industry, households and recreation (Afkhani et al., 2007; Bagherian Marzouni et al., 2014; Naddafi et al., 2007).

### 2.2. Basin assessment categories

We characterized the driving forces, pressures, and possible impacts

**Table 1**

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	
	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 & silt	–	
	*% Sand	–	
	Sediment deposition	–	
	*Total Habitat Score	–	
	*Instream Score	–	
	*Morphological Score	–	
	*Riparian Score	–	
	Biological Oxygen Demand (BOD)	(mg/L)	
	Chemical Oxygen Demand (COD)	(mg/L)	
	Escherichia coli (E. coli)	(n/100 mL)	
	Fecal Coliform (FC)	(n/100 mL)	
Physico-chemical variables	Electrical Conductivity (EC)	(µmho/cm)	Site (Spot measurements)
	Total hardness (TH)	(mg/l caco3)	
	pH	–	
	Total Alkalinity (TA)	(mg/l caco3)	
	Phosphate (PO <sub>4</sub> )	(mg/L)	
	Total Phosphorus (TP)	(mg/L)	
	Nitrate (NO <sub>3</sub> )	(mg/L)	
	Nitrite (NO <sub>2</sub> )	(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)	
Land use	Temperature (T)	°C	Catchment
	Oxygen Saturation (DO %)	%	
	Dissolved Oxygen (DO)	(mg/L)	
	% Agriculture	–	
	% Forest	–	
	% Range	–	
	% Developed	–	
	% Dry river bed	–	
	% Water	–	
	% Wetland	–	

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

that affect water quality in the Karun River Basin by compiling a list of human disturbances of rivers discussed in the literature (Appendix Table A1, Feio et al., 2014; Johnson et al., 2013; Kabore et al., 2018; Klemm et al., 2002; McDowell et al., 2013; Nijboer et al., 2004; Sanchez et al., 2009). The combination of physical structure, land use and water

chemistry provided insights into the ability of a stream to support a healthy aquatic community and to the presence of chemical and non-chemical stressors to the stream ecosystem. Also, these aspects can be easily used to check for any unexpected changes that occur in least and most disturbed conditions.

### 2.3. Sampling

Sampling in the Karun River Basin was done during four seasons and limited to wadeable sections, which resulted in a better representation of the upper part of the basin (Fig. 1) as downstream portions of the system were generally non-wadeable. The seasonal sampling was done to assure that sites selected were least impaired in all seasons. Criteria for site selection included topography, morphology, climate, and sub-catchment size, in addition to logistical constraints. Other important site selection criteria included: 1) ability to access the site, 2) security of the sampling team, 3) distance between sampling sites, 4) other studies conducted in the study area (to compare results if needed), 5) change in the type of land use within the study area and 6) the possibility of point pollutants entering the river upstream of each site. The sampling sites were located in the third to sixth order streams (order > 2) of the Karun River Basin and had variable climates and habitats. The elevation of sampling sites varied from 2087 m (site 0) to 67 m (site 52) above mean sea level with slopes ranging from 8.5 % (site 29) to 0 % (site 22).

A total of 54 sampling sites were identified across 18 rivers (Bazoft, Kouhrang, Behesht Abad, Sabz Kouh, Armand, Abvanak, Komeh, Marber, Boshar, Khersan, Tireh, Marbare, Dare takht, Sezar, Gholyan, Bakhtiari, Dez and Karun) (Fig. 1). At each site, a range of physical structure and physico-chemical data were collected once in each season (54 sites per season, a total of 216 samples in four seasons) from October 2018 to September 2019 (Table 1). Only site 0 was not sampled due to lack of access in spring and winter. Physical structure evaluation within a reach of 200–300 m included description of the river order, summary of the riparian vegetation features, and measurements of instream parameters such as width, channel slope, reach length, depth, flow velocity, high water mark, surface substrate size and elements of disturbance to river connectivity such as barriers and dams. Physical habitat quality was rated visually at each site with 10 parameters describing instream, morphological, and bank and riparian condition which were scored on a 0–20 scale (0 represented poor conditions and 20, optimal) based on the Rapid Bioassessment Protocols developed by Barbour et al. (1999). The instream score was calculated from the sum of three scores of epifaunal substrate/available cover, pool substrate characterization and pool variability. The morphological score was calculated from the sum of four scores of sediment deposition, channel flow status, channel alteration and channel sinuosity. The riparian/bank condition was calculated from the sum of three scores of bank stability, vegetative protection, and riparian vegetative zone width. A composite physical habitat score was also calculated as a sum of these three scores.

The composition of different land uses was calculated within a buffer zone upstream of each site using ArcMap 10.6. A geospatial land use/land cover layer was obtained from the Iran Ministry of Energy, which contained 108 different land use/cover categories. These categories were aggregated into 10 general categories (Table 1; Appendix Table C1). Site GPS locations were verified in Google Earth, then imported to ArcMap and snapped to the nearest streamline in the geospatial stream layer obtained from the Ministry of Energy. Snapped site locations were verified in ArcMap. The fraction of different land uses within the buffer upstream of each site was then calculated. Upstream buffer widths and distances varied by site and were based on the wetted width of the stream at each site. We used a buffer width of 10 times the wetted width of the stream, centered on the geospatial streamline, and an upstream distance of 20 times the wetted width. We also applied a minimum and maximum width and upstream distance so that buffers for all sites were at least 200 m wide and 400 m long, and no more than 1000 m wide and 2000 m long. Where two or more tributaries joined

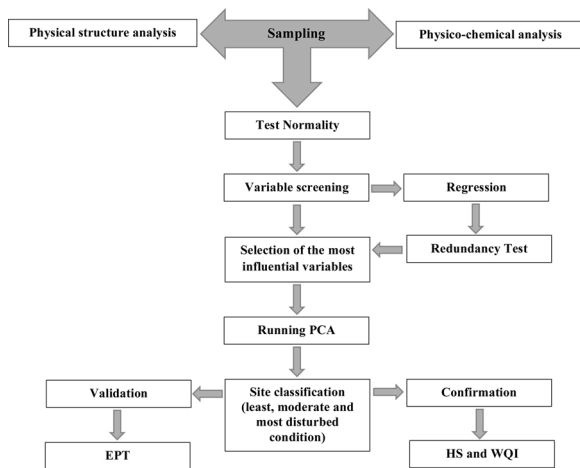


Fig. 2. Flowchart of least-disturbed sites selection in the Karun River Basin.

within the area of the upstream buffer, the buffer was extended the appropriate distance up each tributary. These dimensions were defined based on the findings of other studies (Fryirs et al., 2007; Hughes et al., 2006; Madden et al., 2007; Smith et al., 2007).

Water samples for characterizing water quality were collected in triplicate at a depth of 10–15 cm with 1-L plastic containers. The samples were then placed in a box containing ice packs and transported to the laboratory at Isfahan University of Technology and kept at a temperature of 4 °C prior to the analyses. Water samples were then analyzed for 22 variables according to Baird and Bridgewater (2017) (Table 1). Water temperature and dissolved oxygen concentration and saturation were measured in situ using a portable multiparameter probe (Model: oxi, 3205. WTW). The National Sanitation Foundation Water Quality Index (NSFWQI) was used to summarize the water quality (<https://water-research.net/index.php/water-treatment/water-monitoring/monitoring-the-quality-of-surfacewaters>). NSFWQI was obtained using 9 water quality variables, including dissolved oxygen, fecal coliform, biological oxygen demand, pH, nitrate, phosphate, temperature, turbidity and total solids.

Biological data (benthic macroinvertebrates) were collected with Surber and D-frame dip samplers (with dimensions of 30 × 30 cm and mesh size of 250 µm) on ten transects across a sampling reach (includes different mesohabitats of riffle, run and pool). The sampling effort at each site was 5 min. The collected samples were sieved using 300 and

250 µm mesh sieves and were fixed with 4 % formalin. The samples were then transported to the laboratory, washed, and then preserved in 70 % ethanol. Macroinvertebrates were sorted and identified to family level using a dissecting microscope and available identification keys (Beaty, 2016; Buck et al., 2009; Elliott et al., 1988; Epler, 2010; Hynes, 1984; Milligan, 1997; Pescador et al., 2004; Wagenhoff et al., 2017; Xliv et al., 1944).

## 2.4. Data analysis

### 2.4.1. Definition of disturbance conditions

There were no appropriate criteria available for selecting least disturbed sites in the current study area, so we followed the approach of others in defining a disturbance gradient using principal components analysis (PCA) and selecting the least disturbed sites from that gradient (Fig. 2; (Bio et al., 2011; Blocksom and Johnson, 2009; Ferreira et al., 2005; Hering et al., 2006a, 2006b; Hughes et al., 1998; Johnson et al., 2006; Klemm et al., 2002)). Multivariate statistical analysis is a valuable tool for identifying factors and sources that may affect water systems and cause changes in water quality (Chattopadhyay et al., 2012). Principal components analysis of physico-chemical and habitat data was used to reduce the number of variables by (i) calculating hypothetical main gradients of the environmental dataset and (ii) identifying redundant (co-correlating) variables. Data from all four seasons were used in separate PCAs to identify sites that were consistently among the least disturbed.

PCA is based on the assumption of multivariate normality among the distributions (Johnson and Wichern, 2008). Prior to PCA, variables with non-normal distributions, as identified by Kolmogorov–Smirnov ( $p > 0.05$ ) and Leven's tests, were transformed using standard logarithm and Box-Cox transformations. Also prior to PCA, linear regressions were used to identify strongly correlated variables ( $R^2 > 0.7$ ), of which one was removed. Then PCA was run with standardized and centered data of 17 parameters (Table 1) for the four seasons separately (autumn, winter, spring and summer).

PC axis 1 (PC1) was used to identify the primary stressor gradients in the study area (Blocksom and Johnson, 2009). The PC1 of each season was scaled 0 as the worst and 10 as the best condition according to Eqs. (1) and (2). Seasonal PC1 with negative values associated with good conditions were scaled using Eq. (1) whereas those with negative values associated with poor conditions were scaled using 2.

$$PCA_S = (PCA_{max} - PCA) / (PCA_{max} - PCA_{min}) \times 10 \quad (1)$$

$$PCA_S = (PCA - PCA_{min}) / (PCA_{max} - PCA_{min}) \times 10 \quad (2)$$

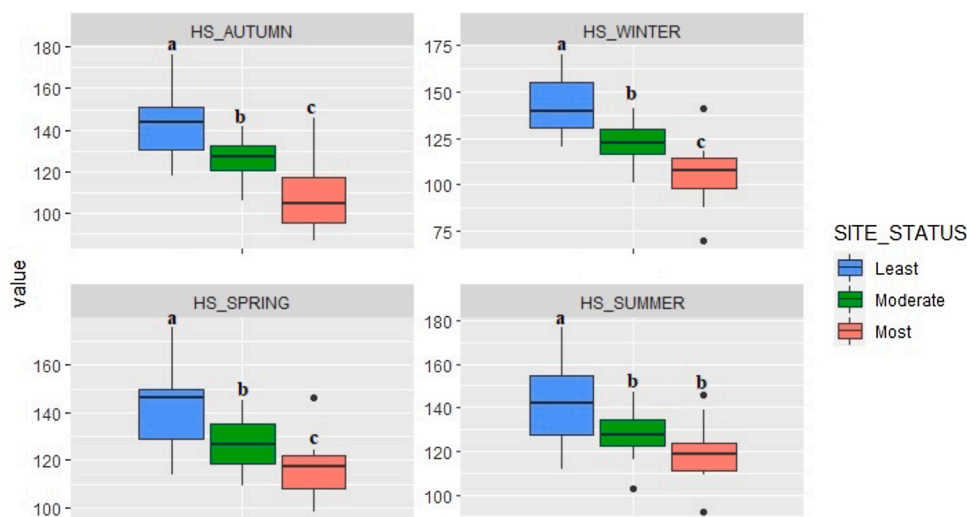


Fig. 3. Seasonal HS between Least, Moderate and Most disturbed sites. Letters above boxplots indicate statistically different groups.



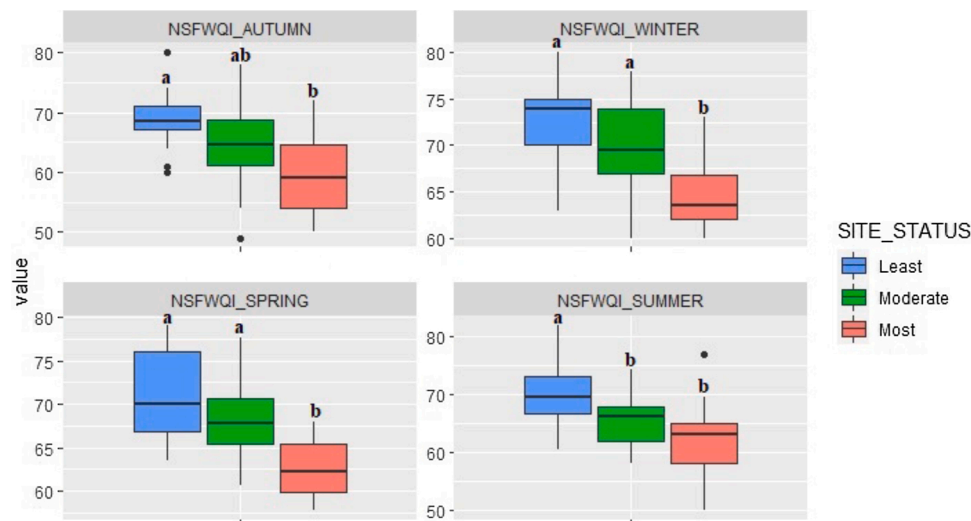


Fig. 4. Seasonal NSFQI values between Least, Moderate and Most disturbed sites. Letters above boxplots indicate statistically different groups.

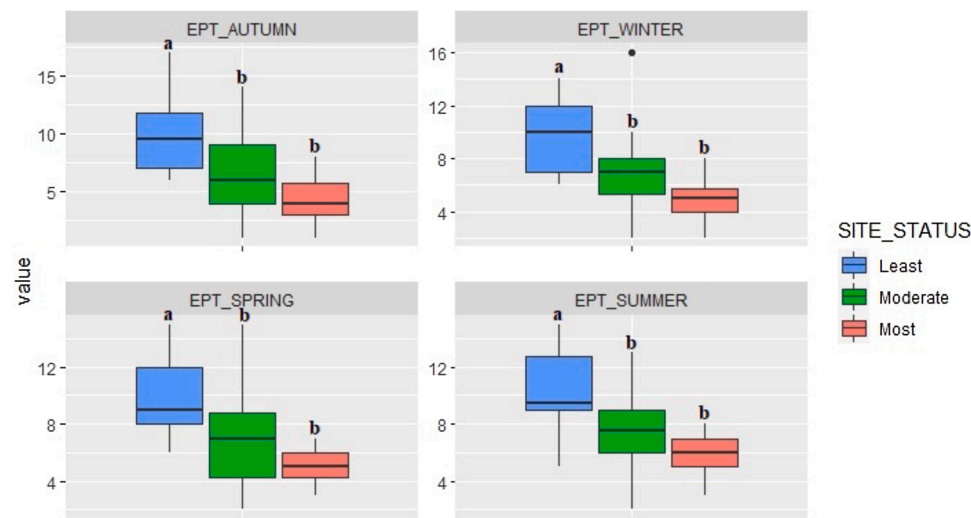


Fig. 5. Multiple box plots for EPT family richness in all seasons within various stress categories (Least, Moderate and Most disturbed sites). Letters above boxes indicate statistically different groups.

where PCA is the score being scaled and  $PCA_{min}$  and  $PCA_{max}$  are the minimum and maximum PCA axis 1 scores across all sites, respectively.  $PCA_S$  represents the scaled position of an individual site in each season. Then, after scaling individual site values we took the average of scaled PCA axis 1 ( $PCA_{AS}$ ) between all seasons (Table 3).

The 25th and 75th percentiles of the combined PC1 were used to classify sites to least, moderate and most disturbed sites: sites falling below the 25th percentile were considered most disturbed, those above the 75th percentile were considered least disturbed condition, and those in between were moderately disturbed (Blocksom and Johnson, 2009). Then, Pearson correlation between  $PCA_{AS}$  and land use data were applied to test the effects of land use on stressor gradients. All statistical analyses were performed using the Microsoft Excel 2016, SPSS v. 22 and R software (v. 4.0.4, R Core Team, (2020)) and vegan (2.5–6) and ggplot2 (v. 2.2.0) packages were used for analysis and graphics.

#### 2.4.2. Confirmation and validation of least-disturbed conditions

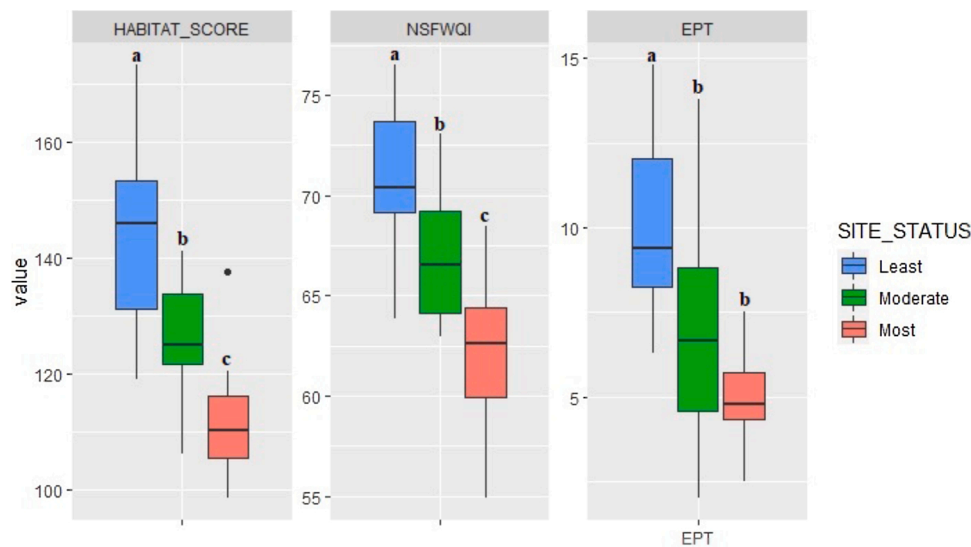
Seasonal sets of least, moderate and most disturbed sites were confirmed using the total habitat score (HS) (Barbour et al., 1999) and the National Sanitation Foundation Water Quality Index (NSFWQI) (Brown et al., 1970). The definition was then validated with a

macroinvertebrate community metric commonly used in biomonitoring, i.e., the family richness of the Ephemeroptera, Plecoptera and Trichoptera (EPT) orders sampled from each site (Sanchez et al., 2009). Differences in HS, NSFQI, and the EPT index between least, moderate, and most disturbed sites were explored using box-and-whisker plots and tested using one-way ANOVAs followed by Tukey pairwise HSD tests (Figs. 3–6).

### 3. Results

To determine if physical habitat variables used in establishing a disturbance gradient were strongly associated with the longitudinal positioning of sample sites in the system, we tested for correlations between variables associated with longitudinal position (i.e., stream order, elevation, and width) and a suite of physical habitat variables often considered to be a function of increasing stream size along the longitudinal gradient (i.e., conductivity, total habitat score, morphological score, clay and silt, and sand) (Table 2). Correlation values were not of a level that would negate the value of using these data for informing on the existence of a disturbance gradient in the study area.

Principal components analysis (PCA) of 17 variables identified five



**Fig. 6.** Comparison of averaged HS, WQI and EPT family richness between Least, Moderate and Most disturbed sites. Letters above boxplots indicate statistically different groups.

**Table 2**

Correlation between indicators of longitudinal position of sampling sites and select habitat variables.

Season	Natural gradients	Conductivity	Total Habitat Score	Morphological Score	Clay & Silt	Sand
Autumn	Stream Order	0.42	−0.24	−0.3	0.3	0.38
	Elevation	−0.44	0.23	0.19	−0.13	−0.21
	Width	0.24	−0.3	−0.28	0.22	0.25
Winter	Stream Order	0.31	−0.22	−0.43	−0.05	0.44
	Elevation	−0.41	0.21	0.3	0.18	−0.27
	Width	0.26	−0.39	−0.43	0.16	0.43
Spring	Stream Order	0.32	−0.2	−0.39	0.26	0.23
	Elevation	−0.42	0.24	0.24	−0.06	−0.21
	Width	0.27	−0.34	−0.29	0.25	0.02
Summer	Stream Order	0.34	−0.12	−0.34	0.4	0.48
	Elevation	−0.46	0.33	0.35	−0.23	−0.28
	Width	0.17	−0.46	−0.3	−0.34	−0.06

**Table 3**

Eigenvectors of physico-chemical and habitat parameters of PC1 in different seasons. Bold font highlights influential parameters.

Parameters / Season	Spring	Summer	Winter	Autumn
Biological Oxygen Demand	<b>0.62</b>	0.40	0.19	<b>0.78</b>
Chemical Oxygen demand	<b>0.99</b>	−0.05	−0.59	0.07
Dissolved Oxygen	−0.04	−0.31	0.39	−0.64
Electrical Conductivity	<b>0.75</b>	<b>0.87</b>	−0.67	<b>0.61</b>
Hardness	0.33	<b>0.67</b>	0.05	<b>0.57</b>
Total Coliform	<b>0.80</b>	<b>0.63</b>	−0.79	0.49
Total Phosphate	0.08	<b>0.66</b>	0.02	−0.53
Total Solids	0.42	<b>0.96</b>	0.25	0.31
Turbidity	0.00	<b>0.94</b>	0.47	−0.63
Alkalinity	<b>0.68</b>	<b>0.73</b>	0.29	<b>0.88</b>
Total Habitat Score	−1.08	−0.90	1.17	−1.08
Total Nitrogen	<b>0.62</b>	<b>0.72</b>	−0.55	0.45
Instream score	−0.64	−0.49	<b>0.64</b>	−0.66
Morphological score	−1.13	−0.99	<b>1.18</b>	−1.03
Riparian score	<b>0.59</b>	0.47	−0.64	0.32
Clay & Silt	<b>0.86</b>	<b>0.65</b>	−0.66	<b>0.94</b>
Sand	<b>0.84</b>	<b>0.58</b>	−0.62	<b>0.84</b>

as influential across all seasons: conductivity, total habitat score, morphological score, clay and silt, and sand (Table 3). Seasonal PC1 explained between 22.5 and 28.2 % variability, whereas PC2 explained between 13.7 and 16.6 % variability (Table 4). The results of scaled PCA axis 1 are presented in Appendix Table B1.

Of the 54 sites, 14, 26 and 14 sites were classified as least, moderate

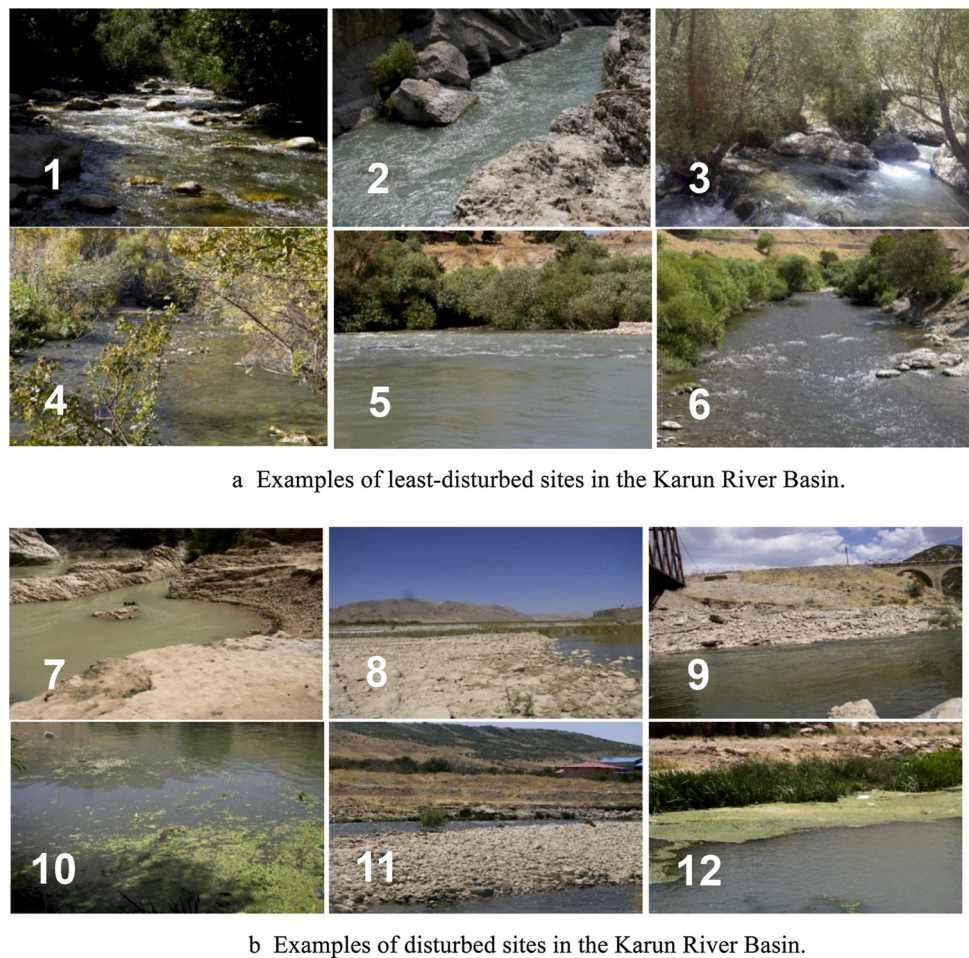
**Table 4**

Percentage of variation explained by PC1 and PC2 of seasonal PCA.

Sampling Seasons / PC	PC1 (%)	PC2 (%)
Spring	28.17	16.13
Summer	27.08	16.62
Winter	22.49	13.67
Autumn	26.77	16.47

and most disturbed sites, respectively (Appendix Fig. D1). Examples of least and most disturbed sites in this dataset are shown in Fig. 7a and b, respectively (Table 5).

Pearson correlation was used to associate different land use characteristics with PC1. No significant correlation was observed between any land-use category and PCA<sub>AS</sub>. Total habitat score (HS) and water quality (NSFWQI) indices in different seasons were higher (often significantly at  $p < 0.01$ ) in least disturbed sites compared to moderate and most disturbed sites (Figs. 3 and 4). Seasonal EPT family richness was significantly higher ( $p < 0.01$ ) in least disturbed sites compared to moderate and most disturbed sites (Fig. 5). The pattern of significantly higher HS, WQI and EPT family richness in least disturbed sites compared to moderate and most disturbed sites was also visible in the averaged dataset (Fig. 6).



**Fig. 7.** Examples of least- and most-disturbed sites in the Karun River Basin. (a) Least-disturbed sites (upper panel): 1 – chesmeh naz, 2 – gahkadeh, 3 – abshar ab-sefid, 4 – cheshmeh pire-ghar, 5 – katak, 6 – daretakht). (b) Most-disturbed sites (lower panel): 7 – entehaye kaj, 8 – tireh, 9 – cham-chit, 10 – dorud, 11 – botari, 12 – joneghan.

**Table 5**  
Site's classification according to the range of scaled PC1.

Categorization of stressors levels	Percentile Range	PCA <sub>AS</sub>	Number of sites
Least-disturbed condition	≥ 0.75	≥ 5.65	14
Moderate condition	> 0.25 and < 0.75	> 3.06 and < 5.65	26
Most disturbed condition	≤ 0.25	≤ 3.06	14

4. Discussion

4.1. Establishing least-disturbed conditions: how to choose the most suitable method

Biomonitoring projects are a relatively novel endeavor in Iran (Aazami et al., 2015a,b; Ebrahimi Dorche et al., 2019; Nemati Var-nosfaderany et al., 2010) and consequently, historical datasets are rare. Neighboring countries such as Syria, Iraq and Turkey also lack such information (Chabuk et al., 2020; Gültekin, 2019; Iraq, 2010). However, the importance of such data is increasing, in concordance to human pressures on natural resources and ecosystems, in particular in streams. In the current study, the lack of historical data precluded the application of approaches using historical, best attainable and minimally-disturbed conditions. For river types lacking historical data or where undisturbed sites cannot be located, alternative methods are required to establish reference condition (Ehlert et al., 2002; Kabore et al., 2018; Kristensen

and Hansen, 1994; Nijboer et al., 2004). The aim of this study was to define criteria (a disturbance gradient) to select least-disturbed reference sites in Middle-Eastern river basins. We applied the least-disturbed method for establishing reference conditions (Bailey et al. 2004; Ehlert et al., 2002; Jackson and Davis, 1994) for biomonitoring of the Karun River and its tributaries.

4.2. Selection of least-disturbed sites using influential criteria

Many riverine systems in Iran have been influenced by human activities with negative impacts to their water quality and quantity (Jafarzadeh et al., 2004; Sabbaghi and Masihi, 2012). For example, Mostafavi et al. (2015), in their study in the Caspian sea basin located in the north of Iran, mentioned that the intensity of pressure in their study area was higher compared to studies from Europe (Schinegger et al., 2012), with most of their sites being affected by multiple pressures (Mostafavi et al., 2015)). Different criteria have been used in a number of studies to define least disturbed conditions, including a wide range of variables related to the physical structure, water chemistry and land use characteristics (Barbour et al., 1999; Feio et al., 2014; Kabore et al., 2018; Nijboer et al., 2004; Plafkin et al., 1989; Sanchez et al., 2009) and our criteria were aligned with them.

We found evidence of anthropogenic stress to some degree in almost all study sites. The most important parameters affecting the Karun River Basin varied among seasons (Appendix Fig. D1), but in general some physico-chemical parameters of water (such as fecal coliform, total nitrogen, total phosphorus, total solids), habitat parameters and percent



clay and silt and percent sand particles were the most important and influential parameters in the study area and elsewhere (Sanchez et al., 2009). We detected increasing numbers and intensity of multiple anthropogenic pressures from the least to most disturbed condition (Figs. 3 and 4). Stoddard et al. (2006) stated that these criteria, which are developed to determine the least amount of human ambient disturbance, can vary across ecological regions in response to differing landscape features and human uses. While anthropogenic stressors can act separately, in most cases, numerous factors act jointly in affecting water and habitat quality with consequences on the biota (Aurouet et al., 2005; Munné et al., 2012). We considered key parameters such as current velocity, substrate, alkalinity, nutrients, hardness or light availability that can strongly alter communities of primary producers and invertebrates in rivers and can thus help to interpret local conditions in biomonitoring. Further quantification of the criteria could add to the standardization of reference site selection.

#### 4.3. Least-disturbed conditions in the Karun River Basin

High human population demands for drinking water, irrigation, fishing or leisure activities, along with other activities such as dam construction, have consequences in destroying the "naturalness" of the rivers in Iran (Hooke, 2006; Prat and Rieradevall, 2006). Chabuk et al. (2020) have stated that dam-building within the upper parts of the Tigris and Euphrates catchments (Turkey, Syria, and Iran) has had a significant effect on surface water further downstream in Iraq and also in Turkey (Chabuk et al., 2020; Gültekin, 2019). In addition to dam construction, Al-Ansari et al. (2018) have highlighted climate change as the most important factor affecting water quality of the Tigris and Euphrates Rivers. The dominant impacts manifested in the disturbed sites of the Karun River have been sediment deposition, channelization, bank alteration, sewage discharges, dam constructions and changes in riparian vegetation (Fig. 7b). These impacts have well-documented consequences for aquatic communities (Feio et al., 2014) as reflected in the lower EPT family richness in our study. In the study of Mostafavi et al. (2015), channelization was also one of the main morphological pressures in the Caspian Sea Basin, Iran, which was generally linked to farmland acquisition, construction of bridges or roads, and flood prevention, as well as river bed and bank erosion control (Mostafavi et al., 2015). Our results on parameters that reflect degradation of water quality are relevant to neighboring catchments (e.g., Tigris and Euphrates Basins) that have similar climatic and hydrological characteristics to our study area (Chabuk et al., 2020; Gültekin, 2019; Iraq, 2010). Zaines et al. (2004) reported that riparian vegetation loss can lead to increased bank erosion, that can contribute to more than 50 % of the sediment deposition in streams and strongly affect EPT richness. Channelization commonly causes loss of habitat for feeding, reproduction, refugia, and reduction of the retentive capacity for allochthonous inputs (Allan and Flecker, 1993; Muotka et al., 2002; Petersen and Petersen, 1991). The alteration of natural flow regimes may not be accordant with life cycles of aquatic animals and plants (Elosegi et al., 2010; Gasith and Resh, 1999). Autochthonous riparian vegetation losses may reduce litter quality and alter decomposition rates, and increased insolation and potentially result in higher primary production (Ashton et al., 2005; Gasith and Resh, 1999; Graca, 2001). We found that sites with more natural physical habitat structure (i.e., Total Habitat Score, Fig. 3) usually also have less impacted water physico-chemical quality (NSFWQI, Fig. 4), which corroborates other studies (Duran, 2006; Fierro et al., 2017; Leunda et al., 2009). In combination, these abiotic characteristics benefited EPT family richness (Fig. 6) as has been shown in other river systems (Linke et al., 1999; Wyżga et al., 2012).

It is worth noting that most of the least-disturbed sites in our study are located in upstream parts of the Karun River Basin (Appendix Fig. D1). These were clean upstream sites characterized by cobble sediment, high flow velocity, excellent habitat features and good chemical quality (Fig. 7a). These least-impacted upstream sites may not

be appropriate for defining reference conditions at larger, higher order (> 7) sites further downstream in the catchment. The higher order sites further downstream were outside of the current study area.

Our results showed no significant correlation between land use and PCA<sub>AS</sub>. This was unexpected since human land use, in particular urbanization and agriculture, have well-documented impacts on physical and chemical habitat quality in streams with consequences on freshwater biodiversity (Dudgeon, 2006; MEA, 2005; Vorosmarty et al., 2010) and overall ecosystem integrity (Allan, 2004; Feld et al., 2013, 2011; Friberg, 2014). One possible reason could be the strong relationship between land use (developed and agriculture) and geo-climatic conditions (Brucet et al., 2013; Feld et al., 2016). Natural descriptors of geographical and climatic conditions include temperature, precipitation, longitude and latitude, which greatly affect the biodiversity of freshwater. Climatic descriptors also play an important role in determining the pattern of human uses (e.g., agriculture and urbanization). This leads to a significant shared effect between natural and human impact variables which cannot be fully disentangled (Feld et al., 2016). Another possible cause might be related to forested land cover in the study area because it generally acts to retain nutrient, which suggests strong biological nutrient retention (e.g., microbial and plant assimilation and microbial denitrification) (Camara et al., 2019; Gardner and Gardner, 2009). As such, Nainar et al. (2017) in their study underlined some contributions of rainforest to the preservation of water quality and reduced erosion. Meanwhile, the forest lands in other areas of Iran are exposed to indiscriminate harvesting, with Akhani et al. (2010) noting that half of the forest in the Caspian Sea Basin was eradicated in recent decades (from 3.6 million to 1.8 million hectares). Furthermore, studied sites in the Karun Basin were not affected by intense agricultural activities with high consumption of fertilizers and pesticides and the influence of agricultural land use on water quality depends on farming management practices (Ding et al., 2015). In contrast, Mostafavi et al. (2015) have found that, in the Caspian Sea basin the extent of agriculture and urban areas have been increased in recent years and in their study, most sites were affected by land use pressure. They observed that the effluent of agriculture and some livestock, factories, slaughter houses, hospitals, restaurants, and the like is directly discharged into rivers without any treatment.

#### 4.4. Confirmation and validation of least-disturbed sites in the Karun River Basin

Site validation is important for confirming and refining selected reference sites (Barbour et al., 1999). Ideally, physical, chemical and biological attributes should all be considered in defining reference condition and establishing the class boundaries (Nijboer et al., 2004; Reynoldson et al., 1997; Sanchez et al., 2009). In our study, we used biotic data (EPT family richness) to validate our categorization of sites according to their abiotic characteristics because validation methods preferably use data other than those used to select the reference sites (Nijboer et al., 2004). Despite substantial variation of EPT families within categories, least disturbed sites for all the rivers presented significantly higher values than those of disturbed sites (Fig. 5). Like the result of our study, the EPT taxa indicated high insect diversity in the reference streams of Euphrates basin in Turkey (Gültekin, 2019). EPT taxa are sensitive to stressors produced by disturbances whose consequences might otherwise be difficult to recognize with commonly used screening methods, and have a proven capacity to reflect the influence of combined stressors on aquatic ecosystems (Karr and Chu, 1999; Piggott et al., 2015). There are many indirect stressor effects such as interactions among multiple co-occurring stressors that result in biological responses that cannot be predicted from single-stressor effects (i.e., synergisms and antagonisms). At the ecosystem level, multiple-stressor effects can be further modified by biotic interactions (Karr and Chu, 1999). Since the 14 selected least disturbed sites were confirmed in the final validation, a consistency was observed between the selection and the validation



method in determining least disturbed sites. This result implies that least disturbed sites were appropriately selected. Therefore, we conclude that our analyses of a common indicator of the macroinvertebrate community composition confirm *a priori* site selection and suggest that the criteria proposed for Karun River Basin reflect an ecologically relevant gradient of disturbance. EPT taxa, both individually and as a summary metric, may thus provide a useful basis for future studies of biodiversity and conservation in the Karun River System. Nevertheless, further work with more biotic metrics should be carried out to assess the condition of river ecosystems.

## 5. Conclusion

This comprehensive study is the first research effort to identify and characterize sites with Least-Disturbed Condition in the Karun River Basin. Such an assessment provides the foundation for the ecological status assessment of river systems in Iran and other basins in the neighboring countries of Iran such as Tigris and Euphrates. It has been widely accepted that the comparability of abiotic evaluations could be improved by the use of uniform methods and consequent joint efforts of the management and restoration of rivers with a catchment perspective. To be effective, such assessments require uniformity in the methods used to measure stressors and biological assessments. The validation process in this study was a good confirmation of the *a priori* reference sites and showed that the criteria proposed could be considered as an appropriate tool to select reference sites in the Karun River. In addition, this study might provide useful findings for river restoration and management of Karun River Basin and similar systems by (i) identifying and evaluating the major consequences of anthropogenic pressures (physico-chemical, physical structure and land use) on its biotic communities, and (ii) providing the first steps towards the selection of benchmarks in this region. Future research should be directed towards increasing the range of available data (e.g., the relationships of climatic conditions, land use and biodiversity) as well as the quality of existing data. This will provide for better understanding of the relationships between land use patterns and instream conditions, ultimately leading to improved descriptors of least-disturbed condition in the Karun River and similar stream ecosystems. However, in the absence of non-impacted sites, the base level of impact should be designated as the reference level as in our study. It is therefore important to select reference sites that are least disturbed when applying biological indicators to identify threshold values of human impacts. A second area of future research should focus on the sampling and assessment of non-wadeable river sections which would support a more complete understanding of the ecology of the Karun River Basin.

## Authors' contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by [Pejman Fathi, Mojgan Zare, Eisa Ebrahimi Dorche and Andreas Bruder]. Data analysis were performed by [Pejman Fathi, Mojgan Zare and Karen Blocksom]. The first draft of the manuscript was written by [Pejman Fathi and Mojgan Zare]. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgment

This research was financially supported by the collaborative project between Isfahan University of Technology and Swiss Leading House for South Asia and Iran (ZHAW). The authors are grateful to the Iranian Ministry of Energy and Department of Environment for their in-kind

support. We also appreciate Alireza Esmaeili for his assistance with field work. The authors would like to thank Joanna Zawiejska (Pedagogical University of Krakow) and Kate Mulvaney (United States Environmental Protection Agency) for their comments on an earlier draft that greatly contributed to the improvement of this paper. The authors also thank the anonymous reviewers for their constructive suggestions. The views expressed in this article are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.

## Appendix A

**Table A1**

List of categorical stressor variables used by different researchers worldwide.

Variables	Reference
1 Acid neutralizing capacity	Klemm et al. (2002)
2 Chloride	
3 Sulfate	Nijboer et al. (2004)
4 Total phosphorus	
5 Total nitrogen	
6 Mean RBP habitat score	
7 pH	
8 Total number of individuals counted	
1 Presence of coarse woody debris	
2 Presence of standing water bodies	
3 No bank fixation	
4 No bed fixation	
5 No migration barriers	
6 No flood protection	
7 Presence of natural floodplain vegetation	
8 Natural discharge regime	
9 No sediment retention	
10 No water diversion	Sanchez et al. (2009)
11 No point-source pollution	
12 No point-source eutrophication	
13 No diffuse impacts	
14 No acidification	
15 No liming	
16 Natural thermal conditions	
17 Natural salinity	
18 No introduced species	
1 Riparian vegetation zone	Johnson et al. (2013)
2 Introduced species	
3 Point sources of pollution	
4 Diffuse sources of pollution and land uses	
5 River morphology and habitat conditions	
6 Hydrological conditions and regulation	McDowell et al. (2013)
1 Agriculture	
2 Point sources	
3 Urbanized area	
4 Population density	
5 Other pressures	Feio et al. (2014)
1 Physical parameters	
2 Nutrients	
3 Fecal indicator bacteria count	
1 Channelization	
2 Bank alteration	Kabore et al. (2018)
3 Local habitat alteration	
4 Riparian vegetation	
5 General morphology	
6 Connectivity	
7 Stream flow	
8 Upstream dam influence	
9 Hydropeaking	
10 General hydrology	
1 Morphological pressures	
2 Habitat pressures	
3 Hydrological pressures	
4 Connectivity pressures	
5 Water quality pressures	
6 Direct pressures	
7 Riparian land use pressures	

## Appendix B

Table B1

Scaled PCA axis 1 score in all seasons and the average values.

SITE	Autumn	Winter	Spring	Summer	Average
0	10.00	–	–	10.00	<b>10.00</b>
1	8.84	6.57	7.20	5.54	<b>7.03</b>
2	8.38	5.53	5.36	6.16	<b>6.36</b>
3	8.09	5.24	4.94	4.98	<b>5.81</b>
4	4.76	4.91	3.87	4.06	4.40
5	7.21	4.24	5.06	3.59	5.02
6	6.40	4.46	4.31	6.80	5.49
7	5.26	4.54	4.92	6.24	5.24
8	6.08	3.83	5.25	5.78	5.24
9	4.88	2.59	2.90	4.05	3.60
10	3.89	1.97	2.99	4.83	3.42
11	3.43	1.47	2.44	4.66	3.00
12	5.37	2.32	3.66	4.93	4.07
13	4.40	4.81	5.98	5.37	5.14
14	1.69	0.00	1.35	4.36	1.85
15	9.12	10.00	8.89	8.12	<b>9.03</b>
16	4.71	0.61	2.06	1.54	2.23
17	5.67	5.15	3.87	4.02	4.68
18	4.07	1.72	0.98	0.76	1.88
19	0.16	3.82	2.07	1.33	1.85
20	6.11	5.79	4.39	5.10	5.35
21	4.02	2.20	2.05	0.09	2.09
22	0.00	0.75	0.00	1.21	0.49
23	2.02	3.69	2.98	2.59	2.82
24	2.75	0.31	1.30	0.00	1.09
25	6.47	7.73	6.32	4.39	<b>6.22</b>
26	7.15	6.02	6.09	5.01	<b>6.07</b>
27	7.58	4.93	4.60	3.12	5.06
28	7.85	6.04	4.40	4.89	<b>5.80</b>
29	8.51	9.61	10.01	7.80	<b>8.98</b>
30	3.64	5.44	6.23	5.72	5.26
31	6.99	8.01	5.45	6.76	<b>6.81</b>
32	5.76	4.35	3.79	5.26	4.79
33	8.60	7.39	7.18	8.40	<b>7.89</b>
34	5.78	5.83	6.16	6.50	<b>6.07</b>
35	4.97	3.94	4.44	4.71	4.51
36	5.82	5.20	5.23	6.58	<b>5.71</b>
37	3.07	2.20	3.28	3.93	3.12
38	3.41	1.81	2.01	4.34	2.89
39	3.99	2.25	3.59	3.38	3.30
40	5.66	0.91	2.57	3.05	3.05
41	7.03	5.47	7.40	6.24	<b>6.54</b>
42	5.31	4.74	4.70	5.01	4.94
43	6.09	4.14	5.61	4.46	5.07
44	2.55	3.93	1.80	2.46	2.69
45	4.48	1.53	1.64	3.95	2.90
46	5.32	5.01	4.79	4.49	4.90
47	5.09	2.75	3.75	3.24	3.71
48	6.43	4.94	3.59	4.49	4.86
49	3.35	1.98	3.17	3.35	2.96
50	3.35	3.59	4.07	2.15	3.29
51	4.99	2.90	4.69	1.81	3.60
52	2.76	3.58	4.04	3.05	3.36
53	3.40	3.69	6.22	4.77	4.52

\*Bold numbers in the average column represent least-disturbed sites.

## Appendix C

Table C1

Different land use categories in the Karun River Basin.

Land use/land cover categories used	Land use/land cover categories in original geospatial data layer
Agriculture	Agri, bagh, bagh-useless, mix(agri_bagh), mix(agri_bagh_dryfarming_follow), mix(agri_bagh_goodrange), mix(agri_bagh_lowforest), mix(agri_bagh_modrange), mix(agri_bagh_woodland1), mix(agri_dryfarming), mix(agri_dryfarming_follow), mix(agri_dryfarming_follow_goodrange), mix(agri_dryfarming), mix(agri_follow), mix(agri_goodrange), mix(agri_lowforest), mix(agri_modforest), mix(agri_modrange), mix(agri_poorange), mix(agri_wetland1), mix(agri_woodland1), mix(bagh_denseforest), mix(bagh_modrange), mix(dryfarming_follow), mix(dryfarming_follow_agri), mix(dryfarming_follow_bagh), mix(dryfarming_follow_bagh_modrange), mix(dryfarming_follow_bagh_modrange), mix(dryfarming_follow_follow), mix(dryfarming_follow_lowforest), mix(dryfarming_follow_modrange), mix(dryfarming_follow_poorange), mix(dryfarming_follow_woodland1), mix(dryfarming_followmodrange), mix(dryfarming_goodrange), mix(dryfarming_modrange), mix(dryfarming_modrange), mix(dryfarming_lowforest), Goodrange, mix(goodrange_agri), mix(goodrange_agri)), mix(goodrange_dryfarming_follow), mix(goodrange_lowforest), mix(goodrange_modforest), mix(goodrange_modrange), mix(goodrange_agri), mix(goodranange_dryfarming_follow), mix(modrange_dryfarming_follow), mix(modrange_agri), mix(modrange_bagh), mix(modrange_dryfarming), mix(modrange_dryfarming_follow), mix(modrange_lowforest), mix(modrange_modforest), mix(modrange_poorange), mix(modrange_woodland1), mix(poorange_agri), mix(poorange_bareland), mix(poorange_dryfarming_follow), mix(poorange_follow), mix(poorange_modrange), midrange, poorange,
Forest	Afforest, denseforest, lowforest, mix(lowforest_agri), mix(lowforest_bagh), mix(lowforest_goodrange), mix(lowforest_modrange), mix(modforest_agri), mix(modforest_dryfarming), mix(modforest_goodrange), mix(modforest_modrange), mix(verylowforest_modrange), mix(verylowforest_poorange), mix(woodland1_dryfarming_follow), mix(woodland1_goodrange), mix(woodland1_modrange), mix(woodland2_modrange), modforest, verylowforest, woodland1, woodland2
Aquaculture	Fisherypool
Developed	Urban
Barren	Bareland, mix(bareland_poorange), rock, saltland
Dry riverbed	abkhan
Water	Water
Wetland	wetland1, wetland2, mix(wetland1_saltland), mix(wetland2_saltland)
Aquifer	Masil

## Appendix D

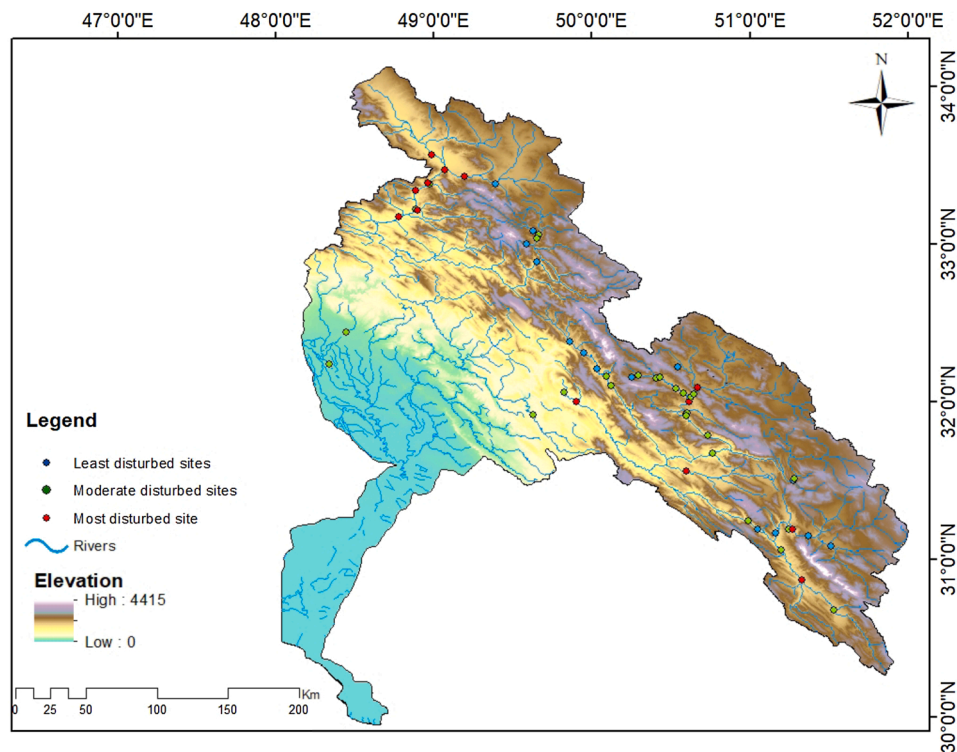


Fig. D1. The location of Least, Moderate and Most disturbed sites in the Karun River Basin.

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