ECOLOGICAL ASSESSMENTS OF IRANIAN RUNNING WATERS

Dissertation

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Vienna, November 2014

Composed in the project “Predictive Index of Biotic Integrity for running waters of Iran”, funded by the Austrian Science Fund (FWF), contract number P 23650-B17.
If the tree of human being gets the fruit of knowledge,
you can bring the whole universe down.

Wenn der Baum der Menscheit Früchte des Wissens trägt,
gehört dir das Universum.

Nasir Khusraw, the Persian poet, 11th century
Preface

This dissertation consists of three research articles and a synthesis. Moreover, five oral presentations have been extracted from this dissertation. It was written in the frame of the FWF research project “Index of Biotic Integrity for running waters of Iran”, contract number P 23650-B17, project leader Ao. Univ. Prof. DI Dr. Stefan Schmutz and was funded by the Austrian Science Fund (FWF). In addition, the Ministry of Sciences, Technology of Iran awarded a scholarship to Hossein Mostafavi as a PhD-student.
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Abstract

Freshwater ecosystems offer provisioning, regulatory, cultural and supporting services. If they are degraded, some or all of the services may be lost. Therefore it is highly necessary to protect, enhance and restore all surface waters with the aim of achieving a good ecological status, as e.g. demanded by the EU-Water Framework Directive. Biological indicators i.e. the fish fauna, benthic invertebrates, phytoplankton and macrophytes/phytobenthos are considered for the assessment of rivers in Europe, North America and in many other regions of the world, mainly as these quality components are continuous witnesses of the river's condition of health and are collectively sensitive to the whole range of potential impacts. To date, however, Iran has not yet included the fish fauna in its routine monitoring programs. Therefore, this thesis actually establishes appropriate ecological assessment methods using fish assemblages as a framework in Iran.

This dissertation focuses on (1) predicting the presence and absence of trout (Salmo trutta) for the whole Iran and (2) on developing fish-based multi-metric assessment indices for cold-water and cyprinid streams for the Caspian Sea Basin.

Overall, for predicting the presence and absence of brown trout, 1090 sites were investigated. Five different modelling techniques (Generalised Linear Models, Generalised Additive Models, Generalised Boosting Models, Classification Tree Analysis and Random Forests) which were summarised in an ensemble forecasting approach were used to explore the potential distribution of brown trout. The related results deepen the knowledge about distribution patterns of brown trout and give a basic background for the future development of assessment methods for riverine ecosystems in Iran.

For developing multi-metric fish index in cold-water streams, 88 sites (44 reference and 44 impacted), and for cyprinid streams 102 sites (50 reference and 52 impacted) were investigated. First, various human pressure types related to land use, connectivity, hydrology, morphology, water quality and biology as well as their combinations were investigated in each stream type. According to the results, most sites were affected by land use pressure in both stream types. In addition, only a few sites were affected by single pressure in both stream types while most sites in cold-water streams were affected by double and triple pressures and in cyprinid streams by multiple pressures.

Moreover, the differences of fish metrics between reference and impacted sites were quantified for each stream type according to a site-specific modelling approach. In fact, specific statistical models were used to describe metric responses to natural environmental differences in the absence of any human pressures. By including impacted sites, the residual distributions of these models described the response range of each metric to human pressures, independently from natural environmental influence.

Finally, to develop a multi-metric fish index of cold-water streams, two fish metrics were selected which showed the best ability to discriminate between impacted and reference sites. In contrast, for the development of a multi-metric fish index for cyprinid streams, seven metrics were considered.

The related results can be used for further development of a standardised monitoring tool to assess the biological condition as well as the ecological status and trends for streams of Iran and are very important for this bioregion with a complex and diverse geology and climate.
Kurzfassung


Die Dissertation fokussiert auf (1) die Vorhersage von Bachforellenvorkommen (Salmo trutta) für den ganzen Iran und (2) die Entwicklung von fischbasierten multimetrischen Indizes für Kaltwasser und Cyprinidengewässer im Einzugsgebiet des Kaspischen Meeres.

Um das Vorkommen der Bachforelle zu analysieren, wurden insgesamt 1090 Probestellen untersucht. Fünf unterschiedliche Modellierungsverfahren (Generalisierte lineare Modelle, Generalisierte Additive Modelle, Generalisierte Boostingmodelle, Entscheidungsbauanalysen und Random Forests) wurden in einem gemeinsamen Prognoseansatz kombiniert, um so das potentielle Verbreitungsgebiet dieser Fischart im Iran zu ermitteln. Die erzielten Ergebnisse vertiefen nicht nur das Wissen zum Verbreitungsmuster der Bachforelle, sondern liefern auch Grundlagen für die zukünftige Entwicklung von Bewertungsmethoden in Fließgewässern des Iran.


In weiterer Folge wurden die Unterschiede der Fischmetriks (Maßzahlen) zwischen Referenz- und beeinträchtigten Stellen je Gewässertyp entsprechend des standortspezifischen Modellierungsansatzes quantifiziert. Spezielle statistische Modelle wurden zur Beschreibung der Metrikreaktionen auf naturgegebene Umweltunterschiede ohne anthropogene Beeinträchtigungen verwendet. Unter Berücksichtigung der beeinträchtigten Stellen zeigt die Verteilung der Residuen in den Modellen den Reaktionsbereich der einzelnen Metriks auf anthropogene Beeinträchtigungen, unabhängig von veränderten Umweltgegebenheiten, auf.

Schließlich wurden zur Entwicklung eines multimetrischen Fischindex für Gewässerobläufe jene zwei Fischmetriks ausgewählt, welche am besten zwischen beeinträchtigten und
Referenzstellen unterscheiden konnten. Im Gegensatz dazu wurden bei der Entwicklung des multimetrischen Fischindex für Cyprindengewässer sieben Metriks in den Index aufgenommen.

Die erzielten Ergebnisse können für die weitere Entwicklung von standardisierten Monitoring-Instrumenten zur Ermittlung der biologischen Beschaffenheit sowie des ökologischen Zustands und des Entwicklungstrends iranischer Gewässer herangezogen werden, was für die untersuchte Bioregion (Kaspisches Meer), die sich durch eine komplexe Geologie und ein vielfältiges Klima auszeichnet, besonders wichtig ist.
1. Dissertation outline

This dissertation consists of three research articles and a summary in form of a synthesis. To date, one article has been published; another two have been submitted to scientific journals and are in review:


Moreover, five oral presentations have been achieved based on the findings of this research which were presented in different conferences and symposiums in Europe and Iran as follows:


- Mostafavi, H., Schinegger, R., Melcher, A. H., Bakhtiyari, M., Trautwein, C., Pletterbauer, F., Unfer, G., Schmutz, S (2014): Quantifying human impacts on trout streams in Southern basin of Caspian Sea at different spatial scales. [First Iranian Conference of Ichthyology, Karaj, IRAN, MAY 7-8, 2014] In: Faculty of Natural Resources, University of Tehran, مجموعة پژوهش مقالات دومین کنفرانس ماهی شناسی ایران


2. Synthesis

2.1. Introduction

Freshwater ecosystems are an integral part of human beings. As societies develop, we manipulate rivers to meet our needs. We dam and straighten them, introduce exotic species that compete with native biota, extract water to irrigate crops, and also divert them to develop urban, agricultural and recreational areas. As a result of our increasing demands, freshwater ecosystem health and ecological integrity is jeopardized in many places in the world (Bjorkland et al., 2003).

Different assessment methods have been developed for management, sustainable exploitation and conservation of aquatic ecosystems by scientists all over the world (e.g. Bailey, 1939; Karr and Chu, 1999; Jørgensen et al., 2005; Noble at al., 2007). Concern about the condition of aquatic ecosystems has expanded in the past decades with recognition that chemical standards have not protected aquatic resources sufficiently (Adler et al., 1993). As a matter of fact, chemical monitoring provides only a “snapshot” of conditions at the time of sampling and may fail to detect acute pollution events (e.g. runoff from heavy rain, spills etc.) and non-chemical degradation (e.g. habitat alteration, hydrological changes an others). In order to address the shortcomings of chemical monitoring, biological monitoring has been introduced as a supplement. It is based on the premise that biological communities are shaped by the long-term conditions of their environment and more accurately reflect the health of an ecosystem (Karr, 1981).

Ecologists have developed biological indices to monitor water quality, beginning with the pioneering efforts of Kolkwitz and Marsson (1908 and 1909). Since this early work, the concept of biological monitoring has been greatly refined with a general trend away from the indicator species concept and/or diversity indices towards an integrated, community-based approach (Fausch et al., 1990).

Many groups of organisms have been proposed as indicators of environmental quality, but no single group has emerged as the favourite (Karr, 1981). However, fishes have clear advantages as indicator organisms. According to the FAME Consortium, (2004), fishes have proved their suitability as indicators for human disturbances for many reasons:

- Fishes are present in most surface waters.
- The identification of fishes is relatively easy and their taxonomy, ecological requirements and life histories are generally better known than in other species groups.
- Fishes have evolved complex migration patterns making them sensitive to continuum interruptions.
- The longevity of many fish species enables assessments to be sensitive to disturbance over relatively long time scales.
- The natural history and sensitivity to disturbances are well documented for many species and their responses to environmental stressors are often known.
- Fishes generally occupy high trophic levels, and thus integrate conditions of lower trophic levels. In addition, different fish species represent distinct trophic levels: omnivores, herbivores, insectivores, planktivores and piscivores.
Fishes occupy a variety of habitats in rivers: benthic, pelagic, rheophilic, limnophilic, etc. Species have specific habitat requirements and thus exhibit predictable responses to human induced habitat alterations.

Depressed growth and recruitment are can be easily assessed and reflect stress.

Fishes are valuable economic resources and are of public concern. Using fishes as indicators confers an easy and intuitive understanding of cause effect relationships to stakeholders beyond the scientific community.

The principal evaluation mechanism utilizes the concept of the Index of Biotic Integrity (IBI), a fish assemblage approach developed by Karr (1981). This principle is based on the fact that fish communities respond to human alterations of aquatic ecosystems in a predictable and quantifiable manner. An IBI is a tool to quantify human pressures by analysing alterations of the structure of fish communities. The original IBI (Karr, 1981) uses several components of fish communities, e.g. taxonomic composition, trophic levels, abundance and fish health. Each component is quantified by metrics (e.g. proportion of intolerant species). A metric is a measurable variable or process that represents an aspect of the biological structure, function, or other component of the fish community and changes in value along a gradient of human influence. Depending on the underlying biological hypotheses, a metric may decrease (e.g. number of sensitive species) or increase (e.g. number of tolerant species) with the intensity of human disturbance (Karr and Chu, 1999; FAME Consortium, 2004; Noble et al., 2007).

There are numerous modifications of this first IBI, particularly in North America for the use in different regions (e.g. Fausch et al., 1984; Lyons et al., 1996; Wang et al., 2011). Due to its popularity, the application of IBIs spread to all continents with different versions for various regions and ecosystem types. In Europe, the IBI was modified and used by e.g. Oberdorff and Hughes (1992); Pont et al. (2006 and 2007); Schmutz et al. (2007 a and b); Noble et al. (2007); Hermoso et al. (2010); in Africa by Hugueny et al. (1996) in Guinea; in South America e.g. by Alexandre et al. (2010) in Brazil; in Australia e.g. by Harris (1995); in Asia e.g. by Ganasan and Hughes (1998) in India; Qadir and Malik (2009) in Pakistan; Liu et al. (2010) and Jia et al. (2013) in China. These new versions have the same multi-metric structure, but they differ from the original IBI in number, identity and scoring of metrics.

Study area and background of this study

Although biological assessment methods for aquatic ecosystems have been developed for various countries, many others as Iran have no experience in the application of these methods, in particulate fish-based indices.

Iran is the second largest country in Southwest Asia (after Saudi Arabia), has an area of 1,648,000 sq km with a population of 76,091,000 people and ranks fourteenth of the world in size (Coad, 2014) (Figure 1). This country is considered as a centre for the origin of many species. The wide ranges of geographical and geological conditions coupled with the climatologically diverse environments provide an enormous diversity. Iran lies in the Palearctic zoogeographical realm bordering the Oriental and African ones (Coad and Vilenkin, 2004) and therefore is of considerable interest. Northern and western Iran is considered as part of the Irano-Anatolian biodiversity hot spot (Majnonian et al., 2005), which contains many centres of local endemism. In total, 164 mammal species, 517 birds, 200 reptiles, 20 amphibians, 8000 plants and 203 fish species have been reported for this country representing a wide variety of ecosystem types (Jalali and Jamzad, 1999; Abdoli, 2000; Esmaeili et al., 2007; Esmaeili et al., 2010; Teimori et al., 2012; Coad, 2014).

In total, 203 fish species (180 native of which 40 are endemic and 23 exotic species) have
been found in 19 major basins in different water bodies such as lakes, streams, rivers, hot springs, caves, qanats and sacred waters. Qanats and sacred waters are unusual habitats for fishes contributing considerably to the biodiversity of Iran and are of importance for management, assessment and conversation of Iranian aquatic ecosystems (Esmaeili et al., 2007 and 2010; Coad, 2014). Qanats are essentially horizontal wells fed by groundwater and provide a continuous, low gradient flow of freshwater. Over 20% of the irrigated area of Iran is fed by qanats (Coad, 2014). Moreover, a number of springs in Iran is said to be "sacred" and the related fish population attains a degree of importance due to its inaccessibility to ichthyologists (Coad, 2014).

Man's activities have had profound, and usually negative influences on freshwater ecosystems in Iran. According to Esmaeili et al. (2007), seven fish species are categorized as endangered (EN), and another 5 as vulnerable (VU) based on the IUCN (International Union for Conservation of Nature) criteria, but it is very probable that due to insufficient data many fishes may not have been included in this list yet.

Some negative effects are due to contaminants, while others are associated with changes in hydrology, restricted water recourses, habitat modifications, hydropower plant constructions and introduced species. Current efforts to evaluate effects of man's activities on aquatic ecosystems in Iran use chemical/physical water quality parameters and macro-invertebrates (e.g. Qaneh et al., 2006; Kamali and Esmaeili, 2009). However, a more refined biotic assessment program is required for the effective protection of freshwater resources. Moreover, as fish species diversity and composition as well as population structure in Iran are different to other regions, investigating these facts for Iran is a substantial need.

Hence, this dissertation focuses on the assessment of Iranian running waters using fish in three different levels: (1) The modelling of fish species distribution for the whole Iran which brown trout (Samo trutta) was selected as a framework, and further the development of multi-metric fish indices for (2) cold-water and (3) for cyprinid streams of the Caspian Sea Basin.

At the beginning of this dissertation, it was planned to develop an IBI for the whole Iran, however, after the data collection for around 1700 sites was finished, it was observed that some main information i.e. fish abundance is missing or fish were sampled by different methods (e.g. net, electric aggregate). Moreover, the sampling method even by electric aggregates was not following standardised assessment techniques, as e.g. CEN (2003). Moreover, some existing datasets for certain rivers were sampled for different aims (i.e. to study the biology or ecology of specific species) and thus were not comparable with others. Consequently, first the dissertation focused on fish species distribution modelling, a basic assessment method that gives an overview on presence and absence of fish species. Then, in a second step, a multi-metric fish index for cold-water streams of the Caspian Sea Basin was developed, in which it was observed that fish species diversity is extremely low for reference sites (i.e. reference streams are mostly occupied by brown trout only). This low diversity resulted in only two fish metrics (related to density and population structure of brown trout) that were proposed for the fish index. However, for multi-species rivers, an IBI is understood to be a multi-metric index that integrates structure, composition, trophic ecology, and reproductive attributes of fish assemblages at multiple levels of ecological organisation (Karr and Chu, 1999). As these objectives were not principally examined in this level, the third level aimed to develop a multi-metric fish index for cyprinid streams in the Iranian Caspian Sea Basin.

Moreover, IBIs are based on the assumption that various human pressures (e.g. hydrological- and morphological alterations, connectivity disruptions, water quality problems etc.) affect riverine fish assemblages (e.g. Karr and Chu, 1999; Degerman et al., 2007; Schinegger et al., 2012). These pressures have not been quantified for Iran to date, as, all
existing studies in Iran exclusively described human pressures (except water quality), if ever, at local scale so far. However, it is of great importance to quantify different types of pressures at various spatial scales, in order to better understand the response of biota to human activities (Schinegger et al., 2012 and 2013; Trautwein et al., 2012). Therefore, not only single human pressures were analysed in this dissertation, but also additive and multiplicative consequences of these pressures were investigated.

The overall objectives of this dissertation can be summarized as follows:

1. Development of countrywide presence/absence models for brown trout.

2. Development of multi-metric fish indices for cold-water and cyprinid streams of the Caspian Sea Basin in which human pressures at different spatial scales and multiple pressure indices are quantified as well.

In total, around 150 days were spent for fish sampling and the measurement of human pressures in the Caspian Sea Basin streams on this research. Overall, 300 sites were monitored in which 190 sites were sampled. Moreover, more than six months was spent for the collecting and revision of database regarding the predicting presence/absence of brown trout in Iran.

In the following chapters, related findings from articles A 1 to A 3 are summarized and discussed.

Figure 1: Topography of Iran and its location in the world
2.2. Predicting presence and absence of trout (*Salmo trutta*) in Iran

Species distribution modelling has been a central issue in ecology in recent years (Guisan and Thuiller, 2005). An increasing number of studies in ecology, biogeography, and conservation biology have tried to build predictive models of species distribution, aiming at a better protection and management of natural resources and ecosystems (Logez et al., 2012; Filipe et al., 2013). Freshwaters of Iran are already exposed to numerous anthropogenic stressors. All pressures collectively resulted in the fact that some fish species are eliminated from many original habitats in Iran. Moreover, based on the different literature sources describing the historical zoogeography of the basins, the distribution of some species, and the phylogenetic relationships between different populations, it might be reasonable that some species also might have occurred in other Iranian basins where they are now extinct. Even if the species are absent, it is unclear whether natural physical barriers (e.g. geologic history), anthropogenic activities, or climatic changes triggered its absence in those regions (Majnonian et al., 2005).

Targeting this thematic background, A 1 represents a first country-wide presence/absence modelling for brown trout as a framework for further steps. Brown trout is a sensitive species which reacts to habitat changes induced by human pressures and its original distribution has been limited due to anthropogenic disturbance. Furthermore, modelling results were compared to the distribution described in the literature.

Overall, 1090 sites were investigated in this study. For each site seven environmental descriptors (bankfull width, wetted width, elevation, slope, mean air temperature, range of air temperature and annual precipitation (three former climatic predictors were extracted on three different spatial extents (1/5/10 km)) were estimated. For modelling five different techniques i.e. Generalised Linear Models (GLM), Generalised Additive Models (GAM), Generalised Boosting Models (GBM), Classification Tree Analysis (CTA) and Random Forests (RF) were applied and finally combined in an ensemble-forecasting framework. This study was based on a heterogeneous data set containing information from several sources, consequently, a “pseudo-absence” approach was used.

The related results show that changing the extent of climate variables had no strong influence on the model performance. The GLM had an inferior performance compared to the four other techniques, whereas RF had the highest performance values in all extents. In total, most occurrences were predicted for the Caspian, Urmia and Namak basins. The spatial pattern of predicted brown trout presences was coherent with the described distribution area (based on the literature). However, the models also identified potential sites outside the known distribution area. Those sites were found in the Tigris Basin and in the eastern part of the Caspian Basin. The variables slope, mean air temperature and range of air temperature had the highest importance values for the prediction of brown trout. According to the results of predicted occurrences it was possible to define suitable conditions for brown trout i.e. slope: 0.3‰ - 28‰, mean air temperature: 5.5 - 17 °C for and range of air temperature: 7.3 - 15.7 °C.
2.3. New fish-based multi-metric assessment indices for cold-water and cyprinid streams of Caspian Sea Basin

The general aim of an index of biotic integrity (IBI) is to offer policy makers, managers and stakeholders an overview of the ecosystem state of a site in one synthetic measure (Quataaert, 2011).

Legislative developments such as the US Clean Water Act (CWA), the European Water Framework Directive (WFD, European Commission, 2000) and similar initiatives in other countries have served to strengthen the recognition of aquatic biota and ecological values (Hermoso et al., 2010). Among phytoplankton, macrophytes and macro-invertebrates, which are important organisms for bio-assessment according to the WFD, fish have been regarded as a particularly effective biological indicator for aquatic environmental quality and anthropogenic stress. To our knowledge, among 48 countries in Asia with an extent of 4.43 million km², multi-metric fish indices were only developed in a few countries like Pakistan (Ganasan and Hughes, 1998), India (Qadir and Malik, 2009) and China (Liu et al., 2010; Jia et al., 2013).

Therefore, two model-based fish indices were developed in A2 and A3 to assess the ecological status of cold-water and cyprinid streams of the Caspian Sea Basin in Iran. This was done by (1) quantifying human pressures at different spatial scales, (2) identifying applicable fish metrics that are showing a response to human pressures and (3) integrating these metrics into a multi-metric fish index.

In this context, 88 sites (44 reference and 44 impacted) were considered for A2 and 102 sites (50 reference and 52 impacted) for A3. For both streams types, pressure variables were defined associated to different human pressure types at different spatial scales then all these variables were used to generate a regional pressure index that accounted for potential effects of multiple human pressures. In A2 and A3, the most dominating human pressure was land use, in particular urbanization and agriculture, followed by water quality in A2 and by hydrology in A3. In A2, most sites were affected by double and triple pressures whilst in A3 by multiple pressures, whereas only least sites were influenced by a single pressure (see Figure 2).

Figure 2: Distribution of the regional pressure index (RPI) for 190 sampling sites in A2 and A3
In **A2**, at reference sites brown trout occurred as the only species, while at impacted sites other taxa could be dominant such as *Alburnoides eichwaldii, Squalius cephalus, Capoeta capoeta, Barbus lacerta, Paracobitis malapterura, Neogobius pallasii* and *Oncorhynchus mykiss* which were classified as non-type specific species. Overall, nine candidate fish metrics were defined for **A2** in further steps, as fish species diversity of reference sites for these cold-water streams was extremely low.

In **A3**, 22 taxa were identified during the fish sampling in which 19 taxa were observed only in the reference sites. Consequently 63 candidate fish metrics were proposed for **A3** initially.

The principle of the site-specific indices in **A2** and **A3** was to measure the deviation of observed from predicted reference metrics, and then to compute the probability that the site represents reference conditions. Based on the degree of deviation, the final ecological status class was identified within a 5-tiered assessment scheme. Reference metrics were predicted as a function of natural environmental predictors which are relatively unaffected by human pressures (e.g. altitude; slope; mean, minimum and maximum air temperatures; catchment size). The final core metrics were identified out of a set of proposed or candidate metrics. As a matter of fact, in first step, all metrics were modelled as a function of the above-mentioned predictor variables, thus accounting for natural variability of fish metrics for the streams of **A2** and **A3**. In this context, only reference sites in the absence of human pressures were applied. After selecting the best fitted metrics, the response of the retained metrics to human pressures was tested by box-plots and Mann-Whitney U test in a further step. This was to select metrics that have a strong ability to discriminate between impacted and reference sites as well as to consider their sensitivity to a gradient of human pressure class. Then, redundant metrics were excluded by correlation tests and finally, two metrics for **A2** and seven metrics for **A3** were obtained to develop a multi-metric fish index.

In terms of functional aspects, in **A2**, two core fish metrics are related to density and population structure of brown trout, while in **A3**, seven core fish metrics cover the five structural and functional types of metrics: biodiversity (number of native species), habitat (biomass of intolerant species to habitat degradation, density of rheophilic species), reproduction (biomass of lithophilic species), trophic level (percentage biomass of insectivorous species) and water quality sensitivity (density of intolerant species to oxygen depletion, biomass of intolerant species to water quality degradation). All these core metrics in **A2** and **A3** showed a negative reaction under human pressures as well.

The final fish index for each, **A2** and **A3**, was computed based on the arithmetic mean of the standardised and transformed fish core metrics values ranging from 0 to 1. Then this fish index was divided into five categories (scores) based on the distribution of both, impacted and reference sites for representing the ecological status. Overall, among 44 impacted sites for **A2** and 52 for **A3**, 30 sites in **A2** and 45 sites in **A3** are in a moderate, poor or bad status, i.e. there is strong need for restoration actions. Therefore, this index can serve as a tool for decision makers for future management of river status.

The multi-metric fish indices were independent from the environmental conditions of any site. Also, the fish indices showed a strong response to human impacts. Figure 3 shows the workflow of the modelling process and multi-metric fish index development at a glance for **A2** and **A3**.
Figure 3: Graphical overview – procedure of multi-metric fish index in both A2 and A3 (after Logez and Pont, 2011)
2.4. Conclusions

Overall, out of the results of A 1-3, the following conclusions can be drawn:

In first article “for modelling brown trout absence/presence”:

- The potential distribution of brown trout for the whole Iran was explored by the models.
- Some areas outside the currently known distribution were identified by the models.
- Slope and mean air temperature as well as its range were the most important variables in predicting brown trout occurrence.

In second and third articles “for fish indices development”:

- The degradation of the streams in the Caspian Sea Basin is widespread.
- Land use pressure is the key pressure in these streams.
- Single pressures are not common, but many sites are affected by a combination of pressures in the Caspian Sea Basin streams.
- For cyprinid streams, fish metrics did not show pressure specific responses but reacted in similar way to multiple pressures.
- The multi-metric fish index for cold-water streams involved two metrics, while for cyprinid streams, it involved seven metrics.
- There were significant differences regarding the structural and functional types of fish metrics between cold water streams and cyprinid streams.
- The developed multi-metric fish indices provide an overall measure of the ecosystem condition of a site for policy makers, managers and stakeholders.

In conclusion, this dissertation provides the basic steps to establish a monitoring system in Iran that reflects the ecological status of rivers. In fact, this monitoring system can be further developed and then be used by river managers to apply more appropriate measures in order to maintain or to re-achieve a good ecological status in the future, as this is required for a sustainable use of aquatic resources. Although Iran is a very diverse country and there are some parts with plenty of water, many parts are very dry. Moreover, according to this dissertation results, different human pressures affecting Iranian running waters were observed. Single pressure occurrence is less common than multiple pressures. Consequently, the proper use of water is a critical issue for the development of Iran; therefore, such tools are required to balance the different needs in various riverine systems. In this context, the developed fish indices help to identify existing limitations, in terms of hydrology, morphology, water quality, connectivity and etc. Examples of questions that could be answered in future are: how much water has to be kept in the river, what has to be water quality, what kind of morphology has to be maintained, etc... As there are many questions which can be answered with such indices, it is strongly recommended to further improve the method developed in this dissertation.

Moreover, as there are many species (especially sensitive ones) with distribution patterns already changed or still unclear due to human pressures and other reasons, the applied modelling of presence/absence extends the knowledge about distribution patterns of species in Iran. Using such an approach, the potential fish species diversity of Iran can be further delineated and in addition, the effects of climate change should be investigated.

However, it is necessary to set the following steps beforehand:
Preparing a Catchment Characterization and Modelling database (CCM) for the whole Iran
Providing more appropriate climatic data with better resolution
Providing newest/updated land use/cover maps
Providing some new variables like geology, which was missing in this study but can be very useful for future studies
Providing appropriate, homogeneous and standardized fish data for the whole Iran
Defining fish zonation for Iranian rivers
Investigating the ecological status of big rivers of Iran in future

In conclusion, I hope this dissertation be a starting point for the further ecological studies in Iran.
2.5. References


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3. Research articles

3.1. Article 1 - Pages 29 - 36


3.2. Article 2 - Pages 37 - 64


3.3. Article 3 - Pages 65 – 102

Predicting presence and absence of trout (Salmo trutta) in Iran

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ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form 4 December 2013
Accepted 4 December 2013
Available online 12 December 2013

Keywords:
Brown trout
Species distribution modelling
Iran

ABSTRACT

Species distribution modelling, as a central issue in freshwater ecology, is an important tool for conservation and management of aquatic ecosystems. The brown trout (Salmo trutta) is a sensitive species which reacts to habitat changes induced by human impacts. Therefore, the identification of suitable habitats is essential. This study explores the potential distribution of brown trout by a species distribution modelling approach. Furthermore, modelling results are compared to the distribution described in the literature. Areas outside the currently known distribution which may offer potential habitats for brown trout are identified. The species distribution modelling was based on five different modelling techniques: Generalised Linear Model, Generalised Additive Model, Generalised Boosting Model, Classification Tree Analysis and Random Forests, which are finally summarised in an ensemble forecasting approach. We considered four environmental descriptors at the local scale (slope, bankfull width, wetted width, and elevation) and three climatic parameters (mean air temperature, range of air temperature and annual precipitation) which were extracted on three different spatial extents (15x15 km). The performance of all models was excellent (<0.08 according to the TSS-True Skill Statistic) criterion. Slope, mean and range of air temperature were the most important variables in predicting brown trout occurrence. Presented results deepen the knowledge about distribution patterns of brown trout in Iran. Moreover, this study gives a basic background for the future development of assessment methods for riverine ecosystems in Iran.

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Introduction

Iran is the second largest country in Southwest Asia (1,654,195 km²), and is larger than France, Germany and Spain together. The country lies in the Palearctic zoogeographical region bordering the Oriental and African ones (Coad and Videnkin, 2004), and features a great diversity of aquatic species. Overall, the ichthyofauna of Iran comprises a total of 203 species (180 native of which 40 are endemic and 23 exotic species) (Esmaili et al., 2010; Telmori et al., 2012). Freshwaters are already exposed to numerous anthropogenic stressors, and are naturally fragmented in stream networks or intermittent water bodies. One of the major human impacts on Iranian rivers is the poor water quality due to urbanization, agriculture and industrial activities (Coad, 1980; Kabir et al., 1999a,b; Esmaili et al., 2007). Other impacts are associated with changes in hydrology, restricted water recourses, increasing hydropower plant constructions and introduced species (Mostafavi, 2007; Abdoli and Naderi, 2009). All impacts collectively resulted in seven fish species categorized as endangered (EN), and five as vulnerable (VU). Most likely, many other fish species have not been included in this classification due to insufficient data (Esmaili et al., 2007). Therefore, modelling freshwater fish distributions seems particularly important to implement management and conservation strategies (Dauwalter and Ibel, 2008; Loge and Pont, 2011) especially for sensitive species like brown trout which have already declined in their original distribution.

This study aims to develop a framework for accurate predictive distribution models for brown trout (Salmo trutta) as a model species for further biological assessment activities in Iranian rivers which, to our knowledge, has not been done so far. Brown trout as an indicator species shows sensitivity to a variety of human
pressures (e.g., water pollution, habitat degradation). Normally, it inhabits headwaters with high oxygen saturation, steep slope, fast flow, suitable temperature regimes and adequate food (Elliott, 1994; Abdoli, 2000). Due to anthropogenic influences, the brown trout was eliminated from many original habitats in Iran (Goaf, 2013). Although a basic evaluation of the species’ distribution based on expert judgment exists, a quantitative evaluation based on a statistical approach is missing. Therefore, this study aims at building a species distribution model (SDM) to find the potential distribution of brown trout for Iran.

Brown trout shows a wide distribution and is recorded from all over Europe, northern Africa, and western Asia (i.e. from the British Isles to western Siberia, and from the Atlas Mountains in North Africa to the glacial streams of Iceland) (MacCrimmon and Marshall, 1968). Current occurrences of brown trout in Iran are reported from the Caspian Basin in the north, from the Urmia Basin in the north-west, and the endorheic Namak Basin in the north-central region of Iran (Abdoli, 2000; Abdoli and Naderi, 2006; Goaf, 2013). However, based on the different literature sources describing the historical zoogeography of the basins (Bernatchez, 2001), the distribution of brown trout (Heckel, 1843; Walzak, 1973) and the phylogenetic relationships between different populations (e.g. Bernatchez, 2001; Hachemzadeh et al, 2012), it might be reasonable that brown trout also occurs in other Iranian basins (e.g. Tigres Basin). Even if the species is absent, it is unclear whether natural physical barriers (e.g. geologic history), anthropogenic activities, or climatic changes triggered its absence in those regions. Furthermore, with the exception of its current known distribution, little information is available concerning the potential of other basins to be inhabited by brown trout populations. Therefore, this study investigated the potential distribution over the whole extent of Iran.

Species distribution modelling has been a central issue in ecology in recent years (Guisan and Thuiller, 2005). An increasing number of studies in ecology, biogeography, and conservation biology have tried to build predictive models of species distribution, aiming at a better protection and management of natural resources and ecosystems. In stream fish ecology, there have been studies assessing impacts of habitat alteration (Gomez and Pont, 2011), estimates on habitat suitability for species re-introductions (Lek et al., 1996), predicting the likelihood of species invasions (Pavoine et al., 2012), examining the influence of scale and geography or relationships between fishes and landscape variables (Pont et al., 2005), identifying areas of persistence for threatened or endangered species (Dannevitz and Kabel, 2008) and finally, demonstrating the utility of species distribution modelling to guide conservation management of stream fishes (Bilje et al., 2013).

Various statistical methods are used to model species distributions in the field of freshwater ecology (e.g. Lek and Guéguen, 1999; Pont et al., 2005; Buisson et al., 2008). All modelling techniques relate the observed distribution of a species to several environmental variables (Austin, 2007; Eilith and Leathwick, 2008). Nevertheless, some authors (e.g. Elith and Graham, 2005; Thuiller et al., 2006) have demonstrated large discrepancies between different techniques, thus making the choice of an appropriate approach even more difficult. The results of different models are not only dependent on the relationship between species occurrence and environmental conditions (linear or nonlinear) but also on the used dataset, i.e. information on presence and absence (Elith and Graham, 2008). Accordingly, summing different model types into an ensemble forecasting approach reduces uncertainty of individual techniques (Aranto and New, 2007). Both local and regional environmental variables can be useful for predicting species presence/absence. However, selection of environmental variables primarily depends on the ecological and biophysical processes influencing the biota. Practically, the availability of data as well as the purpose and requirements of the applied models (Austin, 2007) guide the variable selection. Hence, we test the suitability of available parameters characterising local and regional conditions to evaluate their ability to predict the distribution of brown trout in Iran.

The objectives of this study are: (1) development of a robust statistical framework to predict brown trout distribution in Iran, (2) comparison of model performances over the extent of Iran, and (3) characterisation of the environmental predictors and their importance in the models on the Iranian extent.

Materials and methods

Study area

The study area was the country of Iran which encompasses 19 river basins (Goaf, 1980) (Fig. 1). Iran’s climate is classified as arid to semi-arid and more than 80% of the country has less than 250 mm annual rainfall. Mountain ranges block off the interior of Iran, where conditions are extremely continental. The narrow plains on the Caspian shore and the Persian Gulf are more humid. Rain falls mainly from November to May, although the level is much higher in the Caspian littoral zone and much less in the interior plateau (Goaf, 2013).

Fish data

Occurrence data for brown trout covering several time periods were collected from two main data sources: (1) collated databases originating from previous field samplings, from several museums as well as from the literature containing actual and historical information (e.g. Berg, 1940; Sadati, 1977) and (2) our own field sampling data recorded in 2011 for validation. The primary database contained around 1700 sites which were reduced to 1090 sites after a detailed quality check concerning the reliability of the biological as well as the spatial information. All sites with an unclear position to the river network, outside the temporal period between 1950 and 2000, stocked with brown trout population and located in lakes and wetlands were excluded. In the dataset, positive occurrences of brown trout were limited to the Caspian, Urmia and Namak basins (Fig. 1).

We sampled 15 randomly and accessible trout absence sites plus 15 randomly and accessible sites with confirmed trout occurrence for the validation in early autumn of 2011, using single pass electric fishing (e.g. CEN, 2003). Length of the sampling site was calculated as 10–20 times the river width and overall at least a distance of 100 m was sampled to cover all available habitat types (i.e. riffles, runs, pools) (e.g. EFl+ Consortium, 2009). We established one stop net in the upstream reach and sampled the whole stream width with one (≤5 m wetted width) or two anodes (>5 m wetted width) followed by one or two hand-nets. The sampling effort moved slowly upstream to cover the habitat with a sweeping movement of the anodes, while attempting to draw fish out of hiding (EFl+ Consortium, 2009). The stunned fish were collected by two persons who accompanied the electric fishing team. Finally, after the identification the fish were released back into the stream.

Natural environmental variables

We calculated the following variables to describe environmental conditions at the sampling sites: elevation (EE), stream slope (SLO), wetted width (W,WID), bankfull width (B,WID), maximum air temperature (Max,TEM), minimum air temperature (Min,TEM), mean air temperature (A,TEM), range of air temperature (R,TEM) and annual precipitation (PRE). As a catchment layer similar to CCM2 (Catchment Characterisation and Modelling database;
Fig. 1. Distribution of study sites with occurrence data used in the modelling of brown trout in different freshwater basins plus the distribution of brown trout in Iran as described in the literature.

Voge et al., 2003, 2007; de Jager and Voge, 2010) is not available for Iran, we therefore extracted ELE, W, WID and B, WID from Google Earth (Google Inc., 2005, Version 5), as Iran has different climates the water level of rivers is considerably affected and therefore two types of width could be recognisable. B, WID was the potential maximum width of the main river channel, typically marked by a change in vegetation, topography, or texture of sediment. SLO was calculated in a 1 km stretch for each site. Climate variables were extracted from WorldClim data (Hijmans et al., 2005, 2007) to characterise annual climate trends based on records for 50 years of monthly means (1956–2000), and interpolated at 30 arc-seconds grid extent (approximately 1 km at the Equator). Climate variables were extracted in circular buffers around each sampling site in three different size classes (1, 5 and 10 km) which hereafter are called small, medium and large extent respectively in the text. Climate processes can act on multiple scales, and we used these different buffer sizes to test whether effects were strongest at the small, medium and large extent. The other variables were calculated only at the site scale. Variable redundancy within environmental variables was tested by Spearman’s rank correlation (r). If two variables were highly correlated (r > 0.75) (Filipe et al., 2013), one of them was excluded to avoid co-linearity.

Modelling techniques and ensemble forecasting

In this study the BIOMOD (BiDiversity MODelling) package (Thuiller, 2003) was used within the R software (R Development Core Team, 2011). These tools enabled the examination of methodological uncertainties and the maximization of predictive performance of the SDMs (Thuiller et al., 2009a). This study compared the following five modelling techniques: (1) Generalised linear model (GLM) (McCullagh and Nelder, 1989), performed with polynomial terms (Pont et al., 2005; Logez et al., 2012) using a stepwise procedure to select the most significant variables based on the Akaike information criterion (AIC) (Akaike, 1974). (2) Generalised additive model (GAM) (Hastie and Tibshirani, 1990), performed with automatically selected smooth splines as a nonparametric extension of GLM to capitalise on the strengths of GLM without requiring the problematic steps of postulating a specific parametric response function. As for GLM, a stepwise procedure using the AIC was used to select the most parsimonious model. (3) Classification tree analysis (CTA) (Breiman et al., 1984), used with an internal 10-fold cross-validation to select the best trade-off between the number of leaves of the tree and explained deviance (Thuiller, 2003). CTA provides a good alternative to regression techniques, because it does not rely on an a priori hypothesis on the relationship between independent and dependent variables. (4) Generalised boosting models (GBM) (or boosting regression trees, BRT) (Friedman et al., 2000; Friedman, 2001), performed with a maximum number of 3000 trees and internal 10-fold cross-validation (Marion et al., 2009). GBM are highly efficient at fitting data that are non-parametric (Ridgeway, 1999). (5) Random forests (RF) (Breiman, 2001) are a combination of tree predictors such that each tree depends on the values of a random vector sampled independently and with the same distribution for all trees in the forest. Random forests are actually a learning ensemble consisting of a bagging of un-pruned decision tree learners with a randomised selection of features at each split. Finally, all five modelling techniques were combined in an ensemble-forecasting framework as recommended by Araújo and New (2007).

Pseudo-absence method

This study is based on a heterogeneous data set containing information from several sources (see Section "Fish data"). Due to varying sampling methods and investigation targets of compiled original datasets, the absence of brown trout could not be verified in all sites where the species was not recorded. Accordingly, sites that had no records for brown trout were not directly considered as actual absence in the models but build the basis for a repeated pseudo-absence selection in the modelling procedure. False absences can decrease the reliability of prediction models (Cherfaoui and Lobo, 2008), and consequently, we used the "pseudo-absence" approach. The pseudo-absence dataset is created during the model calibration by a random selection of a given number of points with a potential absence, i.e. points where the species was not recorded. This random selection was repeated ten times to cover different gradients in the dataset of pseudo-absences (Thuiller et al., 2009a; Barbet-Massin et al., 2012).
The workflow of the modelling framework to predict brown trout occurrence.

Results

Brown trout was recorded at 63 sites out of the 1900 sites (Fig. 1). After correlation analyses (Table 1) seven environmental parameters (B, WID, W, WID, SLO, ELE, A, TEM, K, TEM, and PRE) remained as independent variables for the modelling. Their characteristics are described in Table 2.

Changing the extent of climate variables had no strong influence on the model performance (Table 3). The TSS, the sensitivity and the specificity of each single model among all extents as well as the average of the models within each extent were ‘excellent’ (i.e. >0.8 for TSS and >0.85 for sensitivity and specificity) (Table 3). The GLM had an inferior performance compared to the four other techniques (i.e. <0.81 in TSS), whereas RF had the highest performance values in all extents (i.e. >0.97 in TSS (Table 3)).

In total, most occurrences were predicted for the Caspian, Urmia and Namak basins. The spatial pattern of predicted brown trout presences was coherent with the described distribution area (based on the literature) and showed similar results for the different extents of the climatic variables. As a representative example, Fig. 3 shows the predictions from the ensemble model using the large extent of climate variables. However, the models also identified potential sites outside the known distribution area. Those sites were found in the Tigris Basin and in the eastern part of the Caspian Basin (Fig. 3).

The relative importance of the environmental predictors did not show significant differences between different extents in average (Table 4). The variables SLO, A, TEM and K, TEM at the highest importance values (>18%), whereas B, WID, W, WID, ELE and PRE showed the lowest values (<6%). Nonetheless, the relative importance of variables was different among the different models in each extent (see Table 4, data only for large extent are shown because it was the same for other extents). As the results of large extent show in Table 4, in GBM and RF models the variables SLO, A, TEM and K, TEM had highest values (≥10%), whereas SLO, A, TEM, K, TEM and PRE were more important (≥10%) in GAM and CTA models. Finally, A, TEM and K, TEM were most important (≥30%) for GLM. Therefore, each model was dominated by two to four environmental predictors and each extent in average was dominated by three variables. The variables B, WID, W, WID and ELE were of low importance (<6%) in all models (Table 4).

According to the results of predicted occurrences it was possible to define conditions suitable for brown trout. The range and the mean of the variables SLO, A, TEM and K, TEM had similar ranges in all extents (Table 5). The suitable range of SLO was between 0.3% and 28%, the suitable conditions of climate variables were found between 5.5 and 17°C for ATEM and between 7.5 and 15.7°C for K, TEM (Table 5).

Finally, the independent validation underlined a good model performance. Out of 15 sites with species absence and 15 sites with species presence, all were predicted correctly by the final model in all three extents.

Discussion

Brown trout response to environmental variables

For stream fish, temperature appears to be one of the main determinants of spatial distribution (e.g. Bussone et al., 2008; Lopez et al., 2012). Freshwater fish as ectothermic animals are particularly sensitive to temperature with effects on their metabolism,
### Table 1
Matrix of Spearman rank correlations of environmental variables (N = 1056). The upper numbers are Spearman correlation coefficients and the lower numbers are P-values. Correlations of r > 0.70, are shown in bold.

<table>
<thead>
<tr>
<th>W,Wd</th>
<th>SL</th>
<th>E</th>
<th>ELE</th>
<th>Max,TEM</th>
<th>Min,TEM</th>
<th>A,TEM</th>
<th>R,TEM</th>
<th>PRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small extent</td>
<td>W,Wd</td>
<td>0.74</td>
<td>-0.36</td>
<td>-0.25</td>
<td>0.44</td>
<td>0.43</td>
<td>0.44</td>
<td>-0.01</td>
</tr>
<tr>
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<td>-0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.77</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.66</td>
</tr>
<tr>
<td>Small extent</td>
<td>W,WD</td>
<td>-0.34</td>
<td>-0.46</td>
<td>0.39</td>
<td>0.45</td>
<td>0.43</td>
<td>0.43</td>
<td>-0.13</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Large extent</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.75</td>
</tr>
<tr>
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<td>-0.41</td>
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<td>0.05</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
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<td>Medium extent</td>
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<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.89</td>
</tr>
<tr>
<td>Large extent</td>
<td>-0.48</td>
<td>-0.44</td>
<td>-0.46</td>
<td>-0.46</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Small extent</td>
<td>ELE</td>
<td>-0.45</td>
<td>-0.47</td>
<td>-0.42</td>
<td>-0.42</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
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<tr>
<td>Medium extent</td>
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<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>-0.48</td>
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<td>-0.46</td>
<td>-0.46</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Small extent</td>
<td>Max,TEM</td>
<td>0.83</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
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<tr>
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<td>0.50</td>
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<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Large extent</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
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<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
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</tr>
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<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Small extent</td>
<td>A,TEM</td>
<td>-0.09</td>
<td>-0.09</td>
<td>-0.09</td>
<td>-0.09</td>
<td>-0.09</td>
<td>-0.09</td>
<td>-0.09</td>
</tr>
<tr>
<td>Medium extent</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14</td>
</tr>
<tr>
<td>Large extent</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Small extent</td>
<td>R,TEM</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
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</tr>
<tr>
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<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
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<td>-0.36</td>
<td>-0.36</td>
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<td>-0.36</td>
</tr>
<tr>
<td>Large extent</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.36</td>
</tr>
</tbody>
</table>

Abbreviations: W,Wd, bankfull width; W,WD, wetted width; SLO, stream slope; A,TEM, mean air temperature; PRE, annual precipitation; ELE, elevation; R,TEM, range of air temperature.

### Table 2
Mean and range (minimum–maximum) of environmental variables at different extents.

<table>
<thead>
<tr>
<th>Extent</th>
<th>W,Wd (m)</th>
<th>W,WD (m)</th>
<th>SLO (%)</th>
<th>ELE (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>92.9</td>
<td>32.5</td>
<td>1.7</td>
<td>762</td>
</tr>
<tr>
<td>Range</td>
<td>1.0–33.10</td>
<td>1.0–68.6</td>
<td>0.0–24.0</td>
<td>1–27–2208</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extent</th>
<th>A,TEM (°C)</th>
<th>R,TEM (°C)</th>
<th>PRE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>17.2</td>
<td>12.7</td>
<td>459</td>
</tr>
<tr>
<td>Range</td>
<td>5.5–27.5</td>
<td>6.9–16.6</td>
<td>53–1498</td>
</tr>
</tbody>
</table>

Abbreviations: W,Wd, bankfull width; W,WD, wetted width; SLO, stream slope; ELE, elevation; A,TEM, mean air temperature; R,TEM, range of air temperature; PRE, annual precipitation.
Table 3
Prediction accuracy measured using sensitivity, specificity, and TSS for all extents in pseudo-absence method.

<table>
<thead>
<tr>
<th>Model</th>
<th>Sensitivity [%]</th>
<th>Specificity [%]</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA</td>
<td>96.7</td>
<td>91.0</td>
<td>0.88</td>
</tr>
<tr>
<td>GAM</td>
<td>95.4</td>
<td>97.8</td>
<td>0.83</td>
</tr>
<tr>
<td>GBR</td>
<td>95.2</td>
<td>90.0</td>
<td>0.85</td>
</tr>
<tr>
<td>GLM</td>
<td>95.6</td>
<td>93.3</td>
<td>0.80</td>
</tr>
<tr>
<td>RF</td>
<td>96.0</td>
<td>90.0</td>
<td>0.88</td>
</tr>
<tr>
<td>Average</td>
<td>96.6</td>
<td>90.2</td>
<td>0.87</td>
</tr>
<tr>
<td>CTA</td>
<td>95.1</td>
<td>91.3</td>
<td>0.86</td>
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<tr>
<td>GAM</td>
<td>97.5</td>
<td>95.3</td>
<td>0.83</td>
</tr>
<tr>
<td>GBR</td>
<td>96.9</td>
<td>88.6</td>
<td>0.85</td>
</tr>
<tr>
<td>GLM</td>
<td>97.6</td>
<td>82.4</td>
<td>0.80</td>
</tr>
<tr>
<td>RF</td>
<td>98.0</td>
<td>96.9</td>
<td>0.98</td>
</tr>
<tr>
<td>Average</td>
<td>97.1</td>
<td>89.3</td>
<td>0.86</td>
</tr>
<tr>
<td>CTA</td>
<td>96.9</td>
<td>88.9</td>
<td>0.86</td>
</tr>
<tr>
<td>GAM</td>
<td>96.1</td>
<td>87.4</td>
<td>0.84</td>
</tr>
<tr>
<td>GBR</td>
<td>96.6</td>
<td>87.9</td>
<td>0.85</td>
</tr>
<tr>
<td>GLM</td>
<td>95.9</td>
<td>84.6</td>
<td>0.80</td>
</tr>
<tr>
<td>RF</td>
<td>99.0</td>
<td>98.5</td>
<td>0.98</td>
</tr>
<tr>
<td>Average</td>
<td>96.8</td>
<td>89.2</td>
<td>0.86</td>
</tr>
</tbody>
</table>

breeding, development and growth (Mann, 1996). Accordingly, mean air temperature has been widely shown as an important variable determining fish distributions (e.g. Pont et al., 2005; Buisson et al., 2008; Abdoli and Naderi (2009), which is in line with the results of this study. The results showed that the brown trout was clearly linked to areas with cold temperatures, indicating a cold-stenothermal behaviour highlighted by many authors such as: Elliott (1964), Pont et al. (2005) and Abdoli and Naderi (2009). Loege et al. (2012) reported eurythermal behavior of brown trout in their study. In contrast to previous studies (Pont et al., 2005; Buisson et al., 2008; Filipe et al., 2013) the importance of the thermal range (range of air temperature) is highlighted in our study. Probably, the range of air temperature was constrained according to restricted variability in other study areas. Loege et al. (2012) highlighted mean air temperature as a dominant parameter determining brown trout distribution but assigned a minor role to thermal range.

Furthermore, slope was of great importance in all extents which is in accordance with Mann (1996), Pont et al. (2005) and Filipe et al. (2013). At the reach scale, river slope is a surrogate for the hydraulics. High slope values are typical for suitable brown trout habitats. Loege et al. (2012) used slope in association with stream size and runoff as a surrogate of stream power which reflects the ability of a stream to move bed substrate and varies with both stream slope and discharge. Consistently, the presence of brown trout increased with increasing stream power in their study. In line with Pont et al. (2005), stream width (bankfull and wetted width) did not show considerable importance for brown trout distribution.

**Brown trout prediction**

Literature records the distribution of brown trout in three basins in Iran (Abdoli, 2000; Esmarull et al., 2010). The results of our modelling framework highlighted these basins as the major area of distribution as most occurrences were predicted there. However, all models also predicted suitable habitats for brown trout outside these areas. In contrast to the described distribution (e.g. Abdoli, 2000; Abdoli and Naderi, 2000) the models predicted brown trout presences in the eastern part of the Caspian Basin as well as in

![Fig. 3. Predicted distribution of brown trout according to the ensemble model based on "large extent" climate variables. (A) predicted sites inside of the described distribution area in the eastern part of Caspian Basin and (B) predicted sites outside of the described distribution area in Tigris Basin.](image)

Table 4
Relative importance (in percentage) of environmental variables for each extent and all models.

<table>
<thead>
<tr>
<th>Model type</th>
<th>IL.WID</th>
<th>W.WID</th>
<th>SLO</th>
<th>ELE</th>
<th>AT.E</th>
<th>K.TEM</th>
<th>PRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small extent</td>
<td>Ensemble</td>
<td>0.9</td>
<td>0.2</td>
<td>42.4</td>
<td>2.4</td>
<td>38.8</td>
<td>24.6</td>
</tr>
<tr>
<td>Medium extent</td>
<td>Ensemble</td>
<td>2.3</td>
<td>4.0</td>
<td>27.3</td>
<td>1.1</td>
<td>35.2</td>
<td>27.2</td>
</tr>
<tr>
<td>Large extent</td>
<td>Ensemble</td>
<td>1.9</td>
<td>0.3</td>
<td>27.4</td>
<td>0.6</td>
<td>44.6</td>
<td>19.7</td>
</tr>
<tr>
<td>CTA</td>
<td>5.9</td>
<td>0.0</td>
<td>35.4</td>
<td>0.0</td>
<td>23.0</td>
<td>22.3</td>
<td>13.5</td>
</tr>
<tr>
<td>GAM</td>
<td>0.0</td>
<td>0.0</td>
<td>12.8</td>
<td>0.0</td>
<td>53.0</td>
<td>23.2</td>
<td>11.8</td>
</tr>
<tr>
<td>GLM</td>
<td>0.0</td>
<td>0.0</td>
<td>48.4</td>
<td>0.4</td>
<td>46.8</td>
<td>17.0</td>
<td>0.5</td>
</tr>
<tr>
<td>RF</td>
<td>0.0</td>
<td>0.0</td>
<td>2.3</td>
<td>0.0</td>
<td>67.6</td>
<td>30.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Abbreviations: IL.WID, bankfull width; W.WID, wetted width; SLO, stream slope; AT.E, mean air temperature; PRI, annual precipitation; ELE, elevation; K.TEM, range of air temperature.
the Tigiris Basin (Fig. 3). Some fisheries scientists hypothesized that brown trout may occur in the eastern part of the Caspian Basin which is supported by the results of the models (Fig. 3) but proof is mixed to date. Moreover, the available sampling information goes back 20 years when the rainbow trout (Oncorhynchus mykiss), an exotic species, was already stocked (Kabi et al., 1996b) which may additionally impede the proof of former brown trout presence.

The models also identified areas in the Tigiris Basin as potential habitats for brown trout. This seems reasonable as brown trout occurs in the upstream parts of Tigiris in Turkey (Turan et al., 2011). Additionally, from a biogeographical point of view, the Tigiris Basin was the migration route of brown trout to the Namak Basin in palaeo-historical times before the mountains between the basins lifted up (Boedinger, 1896; Berg, 1948–1949, 1950). Sufficient sampling data for these regions is lacking, especially in upstream regions. This is important to mention, because almost every year new species are being discovered in remote and mountainous regions of Iran (e.g. Coad, 2008; Teimori et al., 2012).

Brown trout and human impacts

Human activities over recent decades have huge impacts on brown trout occurrences in Iran. Brown trout is currently considered as a vulnerable taxon in Iran (Kabi et al., 1996a; Mostafavi, 2007). Coad (2000) identified this species as one of the top four threatened freshwater fish species in Iran. Furthermore, Nezami et al. (2000) considered this taxon as endangered. As Akhani et al. (2010) indicated half of the forest in the Caspian Basin was eradicated in recent decades, i.e. from 3.6 million hectares to 1.8 million. In contrast, the extent of agriculture and development areas has increased over recent decades (Akhani et al., 2010). Besides land cover, the construction of dams represents another constraint to fish species occurrence. The number of dams in Iran has increased dramatically. Currently, there are 607 dams of which 595 were built between 1974 and 2012. Moreover, 559 dams are planned and 142 dams are under construction (http://damsinfo.wave.com/dam-states-fa.html). Additionally, water pollution and gravel mining have impacts on water quality, consequently affecting sensitive species (Coad, 1990; Kabi et al., 1996a,b; Abdoli, 2000; Ismaeilik et al., 2007; Mostafavi, 2007). A practical example is given for the LicoirChay River population in the Urmia Basin where trout is now confined to a single river. However, the majority of adequate habitats were destroyed through agriculture and domestication of sheep and goats (Anonymous, 1977). In the lar River, situated in the Caspian Basin and Karaj River in the Namak Basin, native populations suffered from overfishing due to nets, chemicals and explosives (Sarher, 1969). Hence, the native populations of brown trout have declined dramatically. Therefore, our results have important implications for conservation activities and management. The modelling framework has the potential to highlight areas of trout potential occurrence and to identify sites where trout is absent due to habitat degradation. Consequently, based on more detailed future studies effective conservation and restoration measures can be undertaken to maintain and (re)establish brown trout populations.

Conclusions

The presented modelling framework has proven its suitability to identify brown trout habitats on the Iranian scale. The developed model enables to improve management planning as well as conservation actions. Finally, our model shows, beside a user-friendly applicability, a good performance and prediction accuracy which offers opportunities for further use, e.g. integration into multitemetric IBL.

Acknowledgements

Part of this work was funded by the Austrian Science Fund (FWF, Predictive Index of Biotic Integrity for running waters of Iran, contract number P 28560-B17) as well as by the EU FP7 project BioFresh – Biodiversity of Freshwater Ecosystems: Status, Trends, Pressures, and Conservation Priorities (Contract 226874). The Ministry of Sciences, Technology of Iran awarded a scholarship to Hosein Mostafavi (first author). Special thanks are given to Wilfried Thullier for his helpful comments on methodology. We also thank Azad Teimori, Majid Bakhtiar, Gholamreza Amiri Chadi, Hamid Nikzad for supplying data on fish species of Iran. Anonymous reviewers supplied helpful comments on the paper. We also thank Darren Thornburgh for editing the text.

References

A New Fish Based Multi-Metric Assessment Index for Cold-Water Streams in the Iranian Caspian Sea Basin

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A New Fish Based Multi-Metric Assessment Index for Cold-Water Streams in the Iranian Caspian Sea Basin

Abstract

The aims of this study are to analyse human pressures and develop a multi-metric fish index for the cold-water streams dominated by brown trout in the Iranian Caspian Sea Basin. We sampled fish and environmental data in 67 medium sized streams in the Caspian mountains. In total, nine fish metrics were calculated. In addition, 27 criteria describing major anthropogenic pressures for the entire sampling area were used to generate a regional pressure index that accounted for potential effects of multiple human pressures.

For the index development we first defined reference sites and then quantified differences of fish metrics between reference and impacted sites. We used multiple linear regressions describing metric responses to natural environmental differences in the absence of any human pressures. By including impacted sites the residual distributions of these models described the response range of each metric to human pressures, independently of natural environmental influence.

Finally, by testing the ability of each metric for discrimination of impacted and reference sites as well as their sensitivity to a gradient of regional pressure index, the metrics density of brown trout and biomass of sub-adult brown trout explained human pressures best. Our multi-metric fish index performed well in discriminating between reference and impacted sites, giving a significant negative linear response to a gradient of regional pressure index. Overall, the development of such an index offers an opportunity to enhance national bio-monitoring programmes in Iran.

Keywords: multi-metric fish index; regional human pressure index; cold-water streams; Iran
1. Introduction

In Iran, similar to other regions in the world, rivers play a critical role in underpinning development of the country e.g. their waters supply industry, agriculture and domestic users, generate energy etc. However, the national river management has insufficiently understood the importance of ecosystems and their integrity as natural infrastructure. Consequently, many riverine ecosystems are now suffering from various human alterations. These human pressures are directly affecting the physico-chemical conditions of running waters and strongly influencing aquatic biota. Indeed, according to Coad (1980), Kiabi et al. (1999 a, b), Abdoll (2000), Esmaeili et al. (2007), Abdoli and Naderi (2009) and Mostafavi et al. (2014), nearly all Iranian river basins are heavily affected by human activities. Accordingly degradation of Iranian rivers and streams is widespread. In order to protect, enhance and restore rivers a strong tool for the ecological assessment of Iranian running waters is mandatory (Schmutz et al., 2007).

Biological indicators, i.e. fish, benthic invertebrates, phytoplankton and macrophytes are considered for the ecological assessment of rivers in Europe (Water Framework Directive - WFD, European Commission, 2000), North America (US Clean Water Act - CWA), and in many other regions of the world. Among the biological indicators fish have been regarded as particularly effective to highlight the quality of the aquatic environment and indicate anthropogenic pressures (e.g. Karr, 1981; Karr and Chu, 1999; Schmutz et al., 2000; Hering et al., 2010; Hermoso et al., 2010). However, Iran has not included the fish fauna in routine monitoring programs yet.

In this study, we chose the cold-water streams of the Caspian Sea Basin as a framework to develop the first fish based assessment method in Iran because this region represents a homogenous bio-geographical unit. In addition, different human pressures occur in the rivers and the availability of data characterising the rivers and their fish populations is better than in other regions of Iran.

Among the developed methods for the assessment of running waters, multi-metric predictive models have broad applicability. These models are able to reflect inter- and/or intra-regional variations in assemblage and population structure which are caused by variations in natural conditions (e.g. Oberdorff et al., 2001 and 2002; Pont et al., 2006 and 2009). Even though cold-water multi-metric indices have been already developed by some authors (e.g. Langdon, 2001; Hughes, et al. 2004; Lyons, 2006; Logez and Pont, 2011) they are not applicable to Iranian cold water streams because the North of Iran is considered as part of the Irano-Anatolian biodiversity hot spot, which contains many centres of local endemism – and consequently, its species diversity and composition as well as the population structure are different to other regions of the world (Majnonian et al., 2005; Abdoli and Naderi, 2009; Coad, 2014).

Moreover, up to now all studies in Iran exclusively described human pressures (except water quality) at local scale not quantifying different types of pressures (i.e. land use, morphology, connectivity) at different spatial scales (i.e. site, regional, catchment, global). For understanding
the response of biota to human activities it is essential to quantify different types of pressures at
different spatial scales.

Therefore, the objectives of this paper are (1) to assess human pressures and (2) to develop a
multi-metric fish index.

2. Material and methods

The methodology used for this study was mostly derived from Oberdorff et al. (2002), Pont et al.
(2006 and 2009), EFI+ consortium (2009), Logez and Pont (2011), Moya et al. (2011) and
Schinegger et al. (2012). However, major differences exist in terms of the amount and type of
human pressures included, the amount and type of fish metrics tested and types of
environmental descriptors used.

2.1. Study area, site selection and fish species composition

Our study area is located in the Caspian Sea Basin of north Iran (Fig. 1). The Caspian Sea
Basin is bordered by Caspian Sea in the north and Alborz mountains in the south. According to
Coad (2014) this basin encompasses 182,100 km² in Iran (excluding the sea). In the study area
a clear river zonation indicating the dominant species (similar to Aarts and Nienhuis (2003) and
Lasne et al. (2007)) is widely missing and only a few individual rivers have been empirically
classified so far (Kiabi et al., 1999b; Abdoli and Naderi, 2009). As a consequence, we selected
cold-water streams where dominating brown trout is confirmed historically and by local experts
(Fig. 1). For instance as shown in Fig. 1, in region I, II and III (Mazandaran, Guilan and Ardabil
provinces respectively) the occurrence of this species is referenced by e.g. Abdoli (2000),
Abbasi (2006), Sasansaraei (2007), Abdoli and Naderi (2009) and our experience. In total, we
selected 88 sampling sites in medium sized streams in those mentioned regions, whereas 44
sites are considered to be reference sites and the other 44 sites are impacted. All sites were
classified according to the criteria of Table 1. However, it is essential to indicate that finding a
site without continuity interruptions (e.g. ground sills) is almost impossible in this basin based on
our personal experience and other literature (e.g. Kiabi et al., 1999a; Mostafavi et al., 2004;
Abdoli and Naderi, 2009). Therefore, according to e.g. Hughes et al. (1986) and Stoddard et al.
(2006), when real reference sites are missing, least disturbed conditions are selected instead.

2.2. Fish data sampling and definition of a sampling site

Fish sampling was undertaken in autumn seasons because we intended to have consistent low
flow conditions as well as presence of different size classes of fish according to CEN standard
(CEN, 2003). However, sampling was interrupted by high flows in 2010. In order to guarantee
consistent sampling condition and more robust assessment, 44 sites were shifted to next
autumn season (2011). Length of the sampling site was calculated as 10 to 20 times the stream
width and at least a distance of 100 m was sampled (Langdon, 2001; EFI+ consortium, 2009).
We established one stop net in the upstream reach and sampled one pass the whole river width
with one anode for each 5 m wetted width followed by two or three hand-netters. The sampling
team moved slowly upstream to cover all typical habitats with a sweeping movement of the
anodes, while attempting to draw fish out of hiding with the electric shocker (EFI+ Consortium, 2009). The stunned fish were collected by two additional persons who accompanied the electric fishing team. After species identification according to Abdoli (2000), Abdoli and Naderi (2009) and Esmaeili et al. (2010) measurements were taken of total length and individual weight. We distinguished between adults (> 15 cm) and subadults (≤ 15 cm) according to Kheyrandish et al. (2010) and Vatandost (2010). All fish were released back into the stream afterwards.

2.3. Environmental data sampling

We first divided each sampling site to equally spaced transects with 1 m distance within transects, then twelve environmental parameters were collected once during the sampling (Table 2): average bankfull width (maximum width the stream attains, typically marked by a change in vegetation, topography, or texture of sediment), average wetted width, average flow velocity, average discharge, water temperature, dissolved oxygen (DO), pH, conductivity (EC), turbidity, NO₃, NO₂ and PO₄²⁻. Flow velocity was measured with a digital water velocity meter (Global Water Flow Probe, FP111). Discharge for each transect was calculated by multiplying the velocity for each subsection by the vertical area (water depth and wetted width) of the subsection. All subsection discharges were then summed to compute the total discharge for the each cross-section. Finally, the average discharge was computed as the mean of transects. In addition, water temperature, pH and EC were measured by Multi-parameter water analyser portable (HANNA HI 9828); DO by Oxygen meter portable (HACH HQ30D); turbidity by Turbidimeter portable (HACH 2100Qis); NO₃, NO₂ and PO₄²⁻ by Multi-parameter analyser portable (HACH DR/890). Furthermore, the sampling sites were divided based on their location in “forest region” or “grassland region” according to land use/cover map.

2.4. Selection and evaluation of environmental predictor variables

To develop models that enable site-specific predictions of reference metric values we selected a limited number of candidate predictor variables that are major descriptors of river habitat at the reach and regional scale and are assumed to be relatively unaffected by human pressures according to Pont et al. (2009). Consequently, elevation, drainage size, air temperature (i.e. annual mean air temperature (Tmean), July mean air temperature (Tmax), January mean air temperature (Tmin) plus the thermal range between January and July (Trange)), annual mean precipitation and average slope were chosen for the modelling purpose (Table 2).

The climatic variables (air temperature and precipitation) were obtained from the WorldClim predictors, which are often used to characterize current climatologic conditions and seasonality. The WorldClim data describe 50 years of monthly means collected at climate stations between 1950 and 2000 (Hijmans et al., 2005 and 2007) and interpolated at 30 arc-seconds grid extent (approximately 1 km at the Equator).

Other topographical variables (e.g. slope, drainage size) were extracted from CCM2 (Catchment Characterization and Modelling database) based on a 100 m resolution digital elevation model (Vogt et al., 2003 and 2007; de Jager and Vogt, 2010).
Initially, we observed a significant difference between reference sites located in “forest region” and “grassland region” in terms of density and biomass of brown trout based on the Mann-Whitney U test and box-plot graphs. Therefore, major land use/cover type was added as an additional co-variable in the modelling process. Moreover, since the sampling took place in two different years (2010 and 2011) it was also added as a co-variable in the modelling process. All predictor variables were examined for co-linearity by Spearman’s rank correlation (p). If two variables were highly correlated (p > 0.70) one of them was excluded.

2.5. Prediction of fish based metrics, standardization and rescaling

In the beginning, we used nine candidate metrics according to the literature (e.g. Lyons, 1996; Hughes et al., 2004): absence/presence of brown trout, number of species, density (n/ha) and biomass (kg/ha) of brown trout (three modifications: all, adults and sub-adults), and ratio of non-river type specific species (species naturally not belonging to salmonid zone). For six metrics, i.e. density and biomass of brown trout (adult, sub-adult and total) we used a modelling procedure, for other metrics an alternative procedure was defined (see below). Normal distribution of each selected predictor and dependent variable was tested by the Shapiro-wilk test.

Environmental predictor variables from 44 reference sites were used to build models. According to Pont et al. (2006 and 2009) and Moya et al. (2011), we used multiple linear regressions with stepwise selection method (Akaike information criteria according to Hastie and Pregibon, 1993). The square of each predictor variable was also included to account for potential non-linear relationships. Moreover, some data transformations were applied prior to analyses to satisfy statistical assumptions (see Table 4).

The model performance was mainly checked according to mentioned criteria in EFI+ consortium (2009) and other literature as follows: First, standardised residuals and leverage values were extracted. Afterwards, the normality of the residuals (Q-Q plot and histogram of the residuals), the heteroskedasticity of the residuals (graph of the standardized residuals versus standardized expected values), the influence of leverage values (graphs of residual values versus the leverage values), and the relationship between observed and expected values (a linear relation of the form $y = x$ was expected) were visually checked. Furthermore, the evaluation of the model was completed by internal-validation based on bootstrapping (Efron and Tibshirani, 1993). Error distribution of each model was estimated by 100 random samples with replacement. To check the results of the internal-validation, we used the histogram of residuals obtained by bootstrap (EFI+ consortium, 2009).

Once models were fitted, we computed residuals with the following equation according to EFI+ consortium (2009) and Logez and Pont (2011):

\[
R_i = \log(O_i + 1) - \log(E_i + 1)
\]
43

where;

\( R_i \) is residual; \( O_i \) is observed and \( E_i \) is expected value and the value of one was added both to observed and predicted values to handle sites presenting no fish belonging to the metric considered.

In the next step, the metric score \((M)\) of each metric was obtained by standardising the residuals of the model in the following way:

\[
(11) \quad M_i = \frac{(R_i - M)}{s_R}
\]

where;

\( R_i \): Residual value (difference between observed and expected metric) from sites i to n; \( M \): Median value of the residuals; i to n; \( s_R \): Standard deviation of the residuals in the whole undisturbed dataset.

Standardized residuals vary from \(-\infty\) to \(+\infty\). To guarantee that each metric varies within a finite interval and in addition from 0 to 1 two transformations were applied. All values over a maximum (percentile 95) and below a minimum (percentile 5) were replaced by this maximum \((\text{Max})\) and this minimum \((\text{Min})\). Then the following transformation was applied to each metric score:

\[
(12) \quad \text{Rescaled } M_i = \frac{(M_i - \text{Min})}{\text{Max} - \text{Min}}
\]

For other non-modeled metrics i.e. presence/absence of brown trout, number of species, ratio of non-type specific species we did not use modelling procedures as the values for the reference sites were constant within the reference data (i.e. “1” for “presence/absence of brown trout” and “number of species” and “0” for the “ratio of non-type specific species”). We directly used deviations between observed and reference values for further analyses.

2.6. Human pressures data collection

Several anthropogenic pressures were collected for each sampling site according to Degerman et al. (2007); EFI+ consortium (2009) and Schinegger et al. (2012) (Table 1).

The data set incorporated 27 pressure variables associated to the following seven pressure groups: (1) land use, (2) connectivity, (3) hydrology, (4) morphology, (5) water quality, (6) biological pressures and (7) other pressures (see Table 1). Human pressures were assessed at three spatial levels: drainage, segment and site. “Drainage” is the contributing area upstream of the site, “segment” considered 1 km because the size of all primary catchments (the smallest level of catchment) in our database was smaller than 100 km\(^2\) thus it was considered 500 m upstream and 500 m downstream of the sampling site and finally the site level was the area sampled by electric fishing.
Land use pressures were measured on drainage and site levels. Information on connectivity pressures was collected on the segment level. The remaining pressures were collected on site level (Table 1).

All pressure variables were classified along a five-step graded classification scheme, i.e. (1) high, (2) good, (3) moderate, (4) poor and (5) bad status according to Degerman et al. (2007); EFI+ consortium (2009), Schinegger et al. (2012) and Anonymous (2013). In fact, in cases of limited pressure information a reduced number of classes were used, whereby pressures with low evidence were classified as class 3 and pressures with high evidence as class 4 or 5. Then, we applied Spearman’s rank correlation test to identify redundant variables in order to exclude variables with high co-linearity ($p > 0.70$).

2.7. Calculation of the regional pressure index (RPI) from human pressures data

After excluding correlated variables, first, $M_{\text{morph_instr}}$ was computed according to Schinegger et al. (2012):

\[ M_{\text{morph_instr}} = \frac{M_{\text{channel}} + M_{\text{crosssec}} + M_{\text{instrhab}}}{3} \]

Second, we calculated an index for each of the seven dominating pressure groups similar to Schinegger et al. (2012), i.e. land use pressures (LUP), connectivity pressures (CP), morphological pressures (MP), hydrological pressures (HP), water quality pressures (WQP), biological pressures (BP) and other pressures (OP). These indices were computed by averaging the single pressure parameter values of classes 3, 4 and 5 to avoid values $< 3$ compensating for values $\geq 3$.

\[ LUP = \frac{LU_{\text{agri sit}} + LU_{\text{urb sit}}}{2} \]

\[ CP = \frac{C_{B_s,up} + C_{B_s,do}}{2} \]

\[ MP = \frac{M_{\text{Morph_instr}} + M_{\text{ripec}} + M_{\text{floodpr}} + M_{\text{sediment}}}{4} \]

\[ HP = \frac{H_{\text{imp}} + H_{\text{hydrop}} + H_{\text{waterabstr}}}{3} \]

\[ WQP = \frac{W_{\text{eutroph}} + W_{\text{aci}} + W_{\text{osilt}} + W_{\text{toxic}}}{4} \]

\[ BP = \frac{B_{\text{explo}} + B_{\text{intro}}}{2} \]
Third, we calculated the number of pressure groups affected ("affected group"). In our study, this value varied from one to four depending on how many of the seven pressure group indices (LUP, CP, MP, HP, WQP, BP, and OP) were higher than or equal to 3.

Afterwards, to indicate the degradation of a site by multiple pressures into one single index value, we calculated a regional pressure index (RPI) for each site as follows:

\[ RPI = \frac{\text{LUP} + \text{CP} + \text{MP} + \text{HP} + \text{WQP} + \text{BP} + \text{OP}}{7} \times \text{affected group} \]

The RPI varied from 0 to 20, because the maximum pressure was 4 out of 7 pressure groups. Therefore, they were rescaled into five classes according to the number of pressure groups involved (Table 3).

2.8. Index calculation, scoring and validation

After the modelling of fish metrics, we first tested the redundancy of the metrics using Spearman's rank correlation coefficient (\(p > 0.80\)). As a matter of fact, this cut-off was defined in order to keep more metrics. After that, the sensitivity of the candidate metrics to human pressures was evaluated using Mann-Whitney U and Chi-square tests between impacted and reference sites. Selected metrics were also tested regarding the ability to discriminate among RPI class by box-plot graphs.

The multi-metric fish index was calculated by arithmetic mean of the standardized and transformed metric scores from the entire dataset. In order to validate this fish index, its independency was examined against the natural environmental predictor variables by linear regression analysis. Moreover, we randomly split the original dataset into two subsets, i.e. 60% (26 reference and 26 impacted sites) and 40% (18 reference and 18 impacted sites). Hereafter, we tested successfully modelled fish metrics versus the human pressures according to the graphical visualization and Mann-Whitney U test to find the best metrics for the recognition of reference and impacted sites. Afterwards, among remaining fish metrics, the redundant ones were excluded by Spearman's rank correlation test (\(p > 0.80\)). Finally, the fish index developed by remaining metrics in 60% of dataset was validated by 40% dataset versus human pressure class (graphical visualisation) as well as regression test against the regional pressure index. This now represents another validation besides the internal validation of the reference models based on bootstrapping.

We then divided this multi-metric fish index into five categories (scores) regarding the distribution of both impacted and reference sites as implemented in similar studies (e.g. Schmutz et al., 2007; EFI+ Consortium, 2009; Marzin et al., 2014). Based on this classification,
class 1 is between the upper value of the first quartile of reference sites as lower boundary and
the maximum value of reference sites as upper boundary, class 2 between quartile 3 of
impacted sites and quartile 1 of reference sites, class 3 between quartile 2 and quartile 3 of
impacted sites, class 4 between quartile 1 and quartile 2 of impacted sites and class 5 between
minimum value and quartile 1 of impacted sites.

3. Results

3.1. Fish community and environmental characteristics

At reference sites brown trout occurred as sole species, while at impacted sites other species
could be dominant such as Alburnoides eichwaldii, Squalius cephalus, Capoeta capoeta, Barbus
lacerta, Paracobitis malapterura, Neogobius pallasi and Onchorhynchus mykiss which we
named non-type specific species.

Environmental characteristics of investigated rivers are described in Table 2. According to
Spearman’s rank correlation test, there was co-linearity among all types of temperature
variables and elevation (p > 0.70)). We therefore selected maximum air temperature for further
analysis. Finally, drainage size, slope, maximum air temperature, type of major land use/cover
(forest/grassland) and year (2010/2011) were retained as predictors for the modelling process.

According to box-plot visualization (Fig. 2 a, b) as well as Mann-Whitney U test (P < 0.05), the
density and biomass of brown trout is significantly lower in forest region in comparison to
grassland region. This is due to the fact that in grassland streams balanced trout populations
are found. In forest region the length distribution of brown trout is dominated by young of the
year fish which grow better compared to the grassland, however older life stages hardly
occurring in forest streams and therefore the brown trout is not able to form healthy populations
(see Fig. 3 a and b). In consequence although higher growth rates in forest streams, the
biomass is lower than in grassland streams. According to Fig. 4, there is trend of sinking
biomass values as the elevation increases in grassland.

3.2. Dominating pressure groups, multiple pressures and regional pressure index

The most dominating human pressure was land use (LUP), in particular urbanization and
agriculture, followed by water quality (WQP) (Fig. 5).

After accounting for redundancy, 20 pressure variables remained for further analysis out of the
total 27. As Fig. 5 illustrates, in comparison to other pressures the number of sites affected by
LUP was highest in total (36) and in both regions (20 in forest region and 16 in grassland
region) followed by WQP and morphological pressure (MP) respectively. Overall, the observed
pressure groups in both regions were almost the same, except “other pressure” (OP) occurred
only in forest region. Fig. 6 shows the results for the RPI in classes C0 to C4 (i.e. from no/slight
pressure to quadruple pressures). Indeed, the frequencies of impacted sites by double and
triple pressures were the same in both regions (eight in grassland and six in forest) as well as
had highest frequencies in total (14).
3.3. Fish metric selection, index calculation and validation

After our metric screening process, among nine metrics (density and biomass of brown trout, density and biomass of adult and sub-adult brown trout, number of species and ratio of non-type specific species) only two metrics (the density of brown trout and biomass of sub-adult brown trout) remained for the fish index development.

Among all six modelled metrics, only three metrics (density of brown trout, density and biomass of sub-adult brown trout) fulfilled the criteria related to modelling (e.g. Sharpino-wilk test with p > 0.05, normality of the residuals, linear relationship between observed and expected values, leverage plots).

According to Mann-Whitney U and Chi square tests, all metrics except presence/absence of brown trout metric showed significant difference between reference and impacted sites (P < 0.05). Moreover, on the basis of correlation coefficient, high redundancy was observed between density of brown trout and density of sub-adult brown trout metrics as well as between number of species and ratio of non-type specific species metrics (p > 0.80). Finally, density of brown trout and biomass of sub-adult brown trout were selected as core metrics for the development of the multi-metric fish index (Fig. 7). It is essential to indicate that the number of species or the ratio of non-type specific species were not selected for further analysis because the two metrics are less suitable for discrimination of RPI classes according to Fig. 7.

After splitting the original dataset into two subsets for the validation of fish index, the same fish metrics actually remained again for the calculation of the fish index. Moreover, the responses of the developed fish indices (MMI1 and MMI2) from 60% and 40% datasets versus human pressure class and regional pressure index (RPI) were consistent (Figs. 8 (a, b) and 9). Furthermore, stepwise linear regression between the multi-metric fish index (developed from the entire dataset) and environmental predictor variables (drainage size, slope and maximum air temperature) showed none of environmental variables was retained and the part of the multi-metric fish index variability explained by these environmental variables was not significant (R² = 0.04). Therefore, index variation is just related to the human pressures and not to the natural variation of the environment which confirms the robustness of our multi-metric fish index for the assessment.

Table 4 shows the models of core metrics explained by their coefficients. In model 1, the density of brown trout is predicted by type of region and slope and model 2 the biomass of sub-adult brown trout by type of region, drainage size and maximum air temperature. In addition, based on our database “R” of both models was > 0.800.

The multi-metric fish index which was calculated based on the arithmetic mean of the standardized and rescaled core metrics divided into five categories (scores) regarding the distribution of both impacted and reference sites (Fig. 10). Based on this classification, class 1
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covers values between 0.97-1, class 2 between 0.91-0.96, class 3 between 0.78-0.90, class 4 between 0.51-0.77 and class 5 between 0-0.5.

4. Discussion

Despite of a fish fauna with extremely low species richness, it was possible to develop an appropriate and reasonable fish index for the cold-water streams of the Caspian Sea Basin in Iran. Headwater streams actually provide a variety of ecosystem services which are important for local and regional sustainable development. Failure to provide proper environmental protection for small headwater streams will ultimately compromise efforts to sustain healthy river systems (Lyons, 2006). Therefore, conservation of cold-water streams is important.

A very important metric for fish index in our study was the density of brown trout. A similar metric was also used by Hughes et al. (1998). Brown trout represents an intolerant species, which is likely to be the first to disappear following human pressures and the last to recover after restoration (McCormick et al., 2001). Density of brown trout in comparison with biomass of sub-adult brown trout metric showed statistically slightly better discrimination between RPI classes. Nevertheless, both single or two-metric fish index showed a similar performance. A fish index containing two metrics instead of one seems to be more robust, especially for future applications in rivers where additional stressors not tested in our dataset may occur.

Compared to most warm-water streams, cold-water streams represent depauperate faunas mainly composed of intolerant species (Lyons, 1996; Mebane et al., 2003; Hughes et al., 2004). The low species richness commonly observed in cold-water streams limits the amount of available metrics and their variability (Lyons, 1996). In our study, we observed extreme low species diversity especially in reference sites; we had only one species (brown trout). However, in reference sites of other studies more than one cold-water species were recorded (Lyons et al., 1996; Mundahl and Simon., 1999; Mebane et al., 2003; Wang et al., 2003; Hughes et al., 2004; Logez and Pont, 2011). Consequently, the number of candidate metrics available for our study was even lower than other studies. We initially identified nine candidate metrics and selected only two core metrics. In contrast, Langdon (2001) examined 14 candidate metrics and finally selected six core metrics; Dauwalter et al. (2003) examined 39 and selected seven; Hughes et al. (2004) examined 109 metrics and selected eight; Logez and Pont (2011) examined 96 metrics and selected four. In contrast to common IBIs which reflect important components of community ecology, i.e. species richness, trophic, habitat, tolerance and reproductive guilds, our study could not investigate all other mentioned attributes due to low species diversity.

One of the well-known patterns in cold-water fish assemblage degradation is replacement of cold-water species by warm-water species (e.g. Kanno and MacMillan, 2004; Kanno et al., 2010). This is consistent with our study as under human pressures, non-type specific species such as A. eichwaldii, S. cephalus, C. capoeta, B. lacerta, P. malapterura, N. pallasi and O. mykiss were observed which all except O. mykiss (rainbow trout, an exotic and more tolerant
species) are considered as warm-water species. However, in grassland region, warm-water species were not observed in all impacted sites and were generally occupied by rainbow trout. Non-type specific species seem to be favoured in forest region probably as a result of different environmental conditions: the mean altitude in forest region compared to grassland region is lower (1732 m versus 2311 m) and mean air temperature is higher (13.6 °C versus 8.8 °C).

In our study, we sampled only streams where native populations without stocking were present. Fisheries department in Iran recently started stocking brown trout to support populations for recreational purposes. The input of stocked fish possibly will change the size class distribution of fish populations and the number of individuals present in a given site. Therefore, according to Logez and Pont (2011) fish stocking may change the observed metric responses to human pressures and we do not recommend using information from such stocked places for the assessment.

Abundance of individual species may vary seriously over time (Lyons, 2006). Therefore, this fish index is only applicable for the data collected during autumn. In our study we used data of two different years and year was included as an independent variable in the modelling process. This co-variable was not selected by the models indicating that the annual variation in population density and biomass does not interfere with our index results. However, long-term data should be tested when is available to verify applicability of this method.

The negative linear relation of density of brown trout with slope indicated that our fish index is recommended for high gradient streams only (slope bigger than 2.8 °/km) as it is expected the density of brown trout decreases in streams with lower slope. The index is also not appropriate for perennial streams that have mean air temperature more than 17 °C or maximum air temperature more than 22 °C because those streams may have a different fish fauna. Indeed, it is developed only for salmonid river types occupied only by brown trout.

Method of sampling is particularly important because sampling efficiency and sampling effort strongly influence the fish index scores (e.g. Simon and Sanders 1999). As currently in Iran the method of fish sampling is not standard for such a work we therefore, strongly recommend to use our method for the ecological assessment of streams.

There are also some main human pressures influencing the rivers in the Caspian Sea Basin and the inhabiting brown trout populations. According to our findings, the most frequent pressure is land use change in this basin. This can be explained by the fact that half of the forest in the Caspian Sea Basin was eradicated in the recent decades from 3.6 million hectares to 1.8 according to Akhani et al. (2010). Water quality as a pressure is dedicated to effluents of agriculture, livestock, slaughter houses, or restaurants, which are directly released into rivers without any treatment. This was underlined by DO, NO3- and PO4-3 values of 5.5, 17.33 and 3.227 mg/l measured in some impacted sites.
Morphological degradation is mainly linked to channelization. This pressure originates from farmland acquisition, flood prevention, river bed and bank erosion control as well as gravel mining and sand extraction. Hydrological changes are mostly related to water abstraction due to agricultural irrigation. The flow of some streams for some months of the year is reduced to only a fraction of their original magnitude. It was also observed that the habitat quality (water depth, wetted width) and connectivity (including lateral connectivity and drying up of side arms) were influenced by reduction of flow velocity. All these findings are in agreement with other studies (e.g. Bernardo et al., 2003; Meador and Carlisle, 2007).

Moreover, to our knowledge, almost all rivers of the Caspian Sea Basin are disconnected from the sea due to ground sills (with drops up to 1.5 m for the establishment of bridges) or/and dams. Due to the mentioned connectivity barriers, no long-distance migratory species (e.g. Acipenser sp., C. wagneri; R. caspicus; R. rutilus) were observed in our study sites, consequently, our fish index was not designed for long-distance migratory fish species and therefore it is not applicable for this purpose. Future IBIs should incorporate the loss of long-distance migratory species, e.g. based on historical data in order to fully reflect the pressure of connectivity disruptions at the catchment level.

Overexploitation and unusual methods of fishing such as using toxics and dynamite are the other known threats (Abdoli, 2000; Esmaeili et al., 2007). Furthermore, species invasions and aquaculture are another pressure in Iran and in Caspian Sea Basin rivers. Esmaeili et al. (2007) mentioned potential effects of exotics on the local fishes including predation, habitat alteration, hybridization, and introduction of disease or parasites. Therefore, changes in habitat use by native fauna may take place even if species extinction does not occur. In our study we observed that in some sites occupied by rainbow trout, the abundance of natural population of brown trout was severely reduced. However, we recommend further studies to survey not only the effects of exotic species on local species but also the effects of all mentioned pressures separately.

Our index performed well in discriminating between reference and impacted sites, showing a significant negative linear response along a gradient of human pressures. However, this index can be tested and even improved in future by larger datasets and more details in order to serve as a tool for decision makers for future management of river status.

Acknowledgements

Part of this work was funded by the Austrian Science Fund (FWF, Predictive Index of Biotic Integrity for running waters of Iran, contract number P 23650-B17). The Ministry of Sciences, Technology of Iran awarded a scholarship to Hossein Mostafavi (first author). Special thanks are given to Houman Liaghati, Bahram Kiabi, Asghar Abdoli, Saber Vatandost, Ebrahim Fataei, Darush Moghadas, Keyvan Abbasi, Gholamreza Amini Ghadi and Hamid Reza Bagherpour for their support during our field sampling in Iran. We also thank Carina Mielach and Tim Cassidy for editing the text.
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Captions to tables

Table 1. Human pressure classification into seven groups and their definitions.

Table 2. Environmental characteristics of sampling sites in total, forest and grassland regions.

Abbreviation: Tmean (Annual mean air temperature), Tmin (January mean air temperature), Tmax (July mean air temperature), Trange (the thermal amplitude between January and July), DO (dissolved oxygen), N (number of sites), SD (mean standard deviation), max (maximum), min (minimum).

Table 3. Regional pressure index (RPI) classification according to the number of pressure groups involved.

Table 4. Coefficients of fitted regression models for the prediction of core fish metrics.
<table>
<thead>
<tr>
<th>Human pressure variable</th>
<th>Type</th>
<th>Code</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agriculture</td>
<td>LUP</td>
<td>LU_agri, sit</td>
<td>Range: 50m from stream; 1 = none, 3 = along one bank, 5 = along both sides</td>
</tr>
<tr>
<td>Urbanisation</td>
<td>LUP</td>
<td>LU Urb, sit</td>
<td>Range: 100m from stream; 1 = &lt;5%, 3 = 5% &amp; &lt;10%, 5 = 10%</td>
</tr>
<tr>
<td>&quot;Agriculture&quot;</td>
<td>LUP</td>
<td>LU agri, dr</td>
<td>Extent and impact of agriculture and silviculture; 1 = &lt;10%, 3 = 10% &amp; &lt;40%, 5 = 40%</td>
</tr>
<tr>
<td>&quot;Urbanisation&quot;</td>
<td>LUP</td>
<td>LU urb, dr</td>
<td>Extent and impact of urban areas; 1 = &lt;1%, 3 = 1% &amp; &lt;15%, 5 = 15%</td>
</tr>
<tr>
<td>Migration barrier upstream</td>
<td>CP</td>
<td>C B, s up</td>
<td>Barriers on the segment level upstream; 1 = no, 3 = partial, 4 = yes</td>
</tr>
<tr>
<td>Migration barrier downstream</td>
<td>CP</td>
<td>C B, s do</td>
<td>Barriers on the segment level downstream; 1 = no, 4 = partial, 4 = yes</td>
</tr>
<tr>
<td>Channelisation</td>
<td>MP</td>
<td>M channel</td>
<td>Alteration of natural morphological channel plan form; 1 = no, 3 = intermediate, 5 = straightened</td>
</tr>
<tr>
<td>Channelisation</td>
<td>MP</td>
<td>M crosssec</td>
<td>Alteration of cross-section; 1 = no, 3 = intermediate, 5 = technical cross-section/U profile</td>
</tr>
<tr>
<td>Channelisation</td>
<td>MP</td>
<td>M instrob</td>
<td>Alteration of in-stream habitat condition; 1 = no, 3 = intermediate, 5 = high</td>
</tr>
<tr>
<td>&quot;Channelisation&quot;</td>
<td>MP</td>
<td>M ripveg</td>
<td>Alteration of riparian vegetation close to shoreline; 1 = no, 2 = slight, 3 = intermediate, 5 = high</td>
</tr>
<tr>
<td>Flood protection</td>
<td>MP</td>
<td>M floodpr</td>
<td>Presence of dykes for flood protection; 1 = no, 3 = yes</td>
</tr>
<tr>
<td>&quot;Flood protection&quot;</td>
<td>MP</td>
<td>M ripflod</td>
<td>If the river has a former floodplain, proportion of connected floodplain still remaining, Floodplain = area connected during the flood; 1 = &gt;50%, 2 = 10-50%, 3 = less than 10%, 5 = some water bodies remaining or no</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>MP</td>
<td>M sediment</td>
<td>Input of fine sediment (mainly mineral input; bank erosion, erosion from agricultural land); 1 = no, 3 = yes</td>
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<tr>
<td>&quot;Flow velocity increase&quot;</td>
<td>HP</td>
<td>H veloincr</td>
<td>Is there an impact on flow conditions (mean velocity) due to channelisation, flood protection, etc.; 1 = no, 3 = yes</td>
</tr>
<tr>
<td>Impoundment</td>
<td>HP</td>
<td>H imp</td>
<td>Natural flow velocity reduction on site because of impoundment; 1 = no (no impoundment), 3 = intermediate, 5 = strong</td>
</tr>
<tr>
<td>Hydropointing</td>
<td>HP</td>
<td>H hydrop</td>
<td>Site affected by hydropointing; 1 = no (no hydropointing), 3 = partial, 3 = yes</td>
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<tr>
<td>Water abstraction</td>
<td>HP</td>
<td>H waterabstr</td>
<td>Site affected by water flow alteration/minimum flow; 1 = no/no water Abstraction, 3 = intermediate (less than half of the mean annual flow), 5 = strong (more than half of mean annual flow)</td>
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<tr>
<td>&quot;Reservoir flushing&quot;</td>
<td>HP</td>
<td>H refushi</td>
<td>Fish fauna affected by flushing of reservoir upstream of site; 1 = no, 3 = yes</td>
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<td>Eutrophication</td>
<td>WQP</td>
<td>W eutrophi</td>
<td>Artificial eutrophication, 1 = no, 3 = low, 4 = intermediate (occurrence of green algae), 5 = extreme (oxygen depletion)</td>
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<tr>
<td>Acidification</td>
<td>WQP</td>
<td>W asi</td>
<td>Acidification; 1 = no, 3 = yes</td>
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<tr>
<td>Organic pollution</td>
<td>WQP</td>
<td>W osclit</td>
<td>Siltation; 1 = no, 3 = yes</td>
</tr>
<tr>
<td>Toxicity</td>
<td>WQP</td>
<td>W opoll</td>
<td>Is organic pollution observed; 1 = no, 3 = intermediate, 5 = strong Toxic priority substances (organic and nutrient appearance); 1 = no or very minor, 3 = weak (important risk, link to particulate substance) 5 = high concentration (a clearly known input)</td>
</tr>
<tr>
<td>Impact of exploitation</td>
<td>BP</td>
<td>B explo</td>
<td>Fishing, at site affecting fauna, information based on local fishermen; 1 = no, 3 = intermediate, 5 = strong</td>
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<tr>
<td>Introduction of fish</td>
<td>BP</td>
<td>B intro</td>
<td>New fish species to river basin; 1 = no introduction, 2 = introduction, but no reproduction and low density, 3 = no reproduction, high density, 4 = reproducing, low density, 5 = reproducing, high density</td>
</tr>
<tr>
<td>Other pressures</td>
<td>OP</td>
<td>O imp</td>
<td>e.g. explosion of oil pipe; 1 = no, 3 = weak, 5 = strong (expert judgment )</td>
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*a* excluded variables after correlation test

*"*

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<th></th>
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<th>Grassland region</th>
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<td>SD</td>
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<td>PO₄³⁻ (mg/l)</td>
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<td>Class</td>
<td>Definition</td>
<td>Range of each class</td>
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<td>---------------------</td>
<td>-------------------------------------------------</td>
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<td>class 0 (C 0)</td>
<td>Un-impacted/slightly impacted sites</td>
<td>values ranging &lt; 3</td>
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<td>class 1 (C 1)</td>
<td>single pressure from respectively one groups</td>
<td>values ranging from 3 to 5</td>
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<td>class 2 (C 2)</td>
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<td>triple pressures from respectively three groups</td>
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<tr>
<td>class 4 (C 4)</td>
<td>quadruple pressures from respectively four groups</td>
<td>values ranging from 12 to 20</td>
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Table 4

<table>
<thead>
<tr>
<th>Name of the metrics</th>
<th>Intercept</th>
<th>Type of region</th>
<th>Slope</th>
<th>Drainage size</th>
<th>Maximum air temperature</th>
<th>R</th>
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<tr>
<td>Density of brown trout (log+1)</td>
<td>2.893</td>
<td>0.287</td>
<td>-0.004</td>
<td></td>
<td></td>
<td>0.835</td>
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<tr>
<td>Biomass of sub-adult brown trout (log+1)</td>
<td>1.563</td>
<td>0.766</td>
<td>0.002</td>
<td>0.091</td>
<td></td>
<td>0.802</td>
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</tbody>
</table>
Captions to figures

Fig. 1. Map of fish sampling sites in Caspian Sea Basin of Iran and natural distribution of brown trout in this basin according to literature (I, II and III).

Fig. 2. Comparison of the density and biomass of brown trout between forest and grassland regions at reference sites (a=forest and b=grassland).

Fig. 3. Comparison of the total length distribution of brown trout between forest and grassland regions at reference sites (a=forest and b=grassland).

Fig. 4. Relation between biomass of brown trout and elevation at reference sites of grassland.

Fig. 5. Number of sites regarding no/slight pressure, affected by land use pressures (LUP), connectivity pressures (CP), morphological pressures (MP), hydrological pressures (HP), water quality pressures (WQP), biological pressures (BP) and other pressures (OP) per region and entire.

Fig. 6. Number of sites with no, single, double, triple and quadruple pressures in forest and grassland regions and entire.

Fig. 7. Box-plot graph regarding density of brown trout, density and biomass of sub-adult, number of species and ratio of non-type specific species metrics versus regional pressure index class (C 0= Class 0, C 1: Class 1, C 2: Class 2, C 3: Class 3: C 4: Class 4).

Fig. 8. Box-plot graphs regarding multi-metric fish indices (MMI1: 60% dataset, a; MMI2: 40% dataset, b) versus human pressure class.

Fig. 9. Regression of multi-metric fish index of 60 and 40% dataset versus regional pressure index (RPI).

Fig. 10. The quartiles of both reference and impacted sites by classification into 5 categories.
Fig. 1
Fig. 2

(a)

(b)

Forest region
Grassland region

Density of brown trout (per ha)

Biomass of brown trout (g/ha)
Fig. 3

(a)

(b)

Fig. 4

Elevation (m)

Biomass (gr)
A new fish-based multi-metric assessment index for cyprinid streams in the Iranian Caspian Sea Basin

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Abstract

A major issue for water resource management is the assessment of environmental degradation of lotic ecosystems. The overall aim of this study is to develop a multi-metric fish index for the cyprinid streams of the Caspian Sea Basin (MMICS) in Iran. As species diversity and composition as well as population structure in the studied streams are different to other regions, there is a substantial need to develop a new fish index. We sampled fish and environmental data of 102 sites in medium sized streams. We analysed human pressures at different spatial scales and determined applicable fish metrics showing a response to human pressures. In total, five structural and functional types of metrics (i.e. biodiversity, habitat, reproduction, trophic level and water quality sensitivity) were considered. In addition, we used 29 criteria describing major anthropogenic human pressures at sampling sites and generated a regional pressure index (RPI) that accounted for potential effects of multiple human pressures.

For the MMICS development, we first defined reference sites (without human pressures) and secondly quantified differences of fish metrics between reference and impacted sites. We used a Generalized Linear Model (GLM) to describe metric responses to natural environmental differences in the absence of any human pressures. By including impacted sites, the residual distributions of these models described the response range of each metric to human pressures, independently of natural environmental influence.

Finally, seven fish metrics showed the best ability to discriminate between impacted and reference sites. The multi-metric fish index performed well in discriminating human pressure classes, giving a significant negative linear response to a gradient of the RPI. These methods can be used for further development of a standardised monitoring tool to assess the ecological status and trends in biological condition for streams of the whole country, considering its complex and diverse geology and climate.

Keywords: multi-metric fish index, regional pressure index, cyprinid rivers, Iran
1. Introduction

The maintenance and restoration of aquatic ecosystems has become a common goal for sustainable river basin management. The ultimate effect of human activities in river catchments leads to pressures on the biota and biological processes (Karr and Chu, 1999). Fish among other organisms (i.e., phytoplankton, macrophytes, macro-invertebrates) have been regarded as a particularly effective biological indicator of aquatic environmental quality and anthropogenic stress, based on their sensitivity and advantages regarding e.g., taxonomy, trophic levels, economic and aesthetic values etc. (Karr and Chu, 1999; Schmutz et al., 2000 and 2007; Hering et al., 2010).

The first fish-based assessment as IBI (Index of Biotic Integrity) was developed by Karr (1981). Then, several environmental assessment methods, which were mostly inspired by this seminal work, were developed in different regions, especially in America and Europe over the last decades (e.g., Hugueny et al., 1996; Angermeier et al., 2000; Pont et al., 2006; Schmutz et al., 2007; Meador et al., 2008). To our knowledge, among 48 countries in Asia with an extent of 4.43 million km², multi-metric fish indices were only developed in a few countries like Pakistan (Ganasan and Hughes, 1998), India (Qadir and Malik, 2009) and China (Liu et al., 2010; Jia et al., 2013).

Iran’s area is 1,629,807 square kilometres. It is located in the Palearctic zoogeographical realm bordering the Oriental and African ones (Coad and Vilienkin, 2004) and thus, wide ranges of geographical and geological conditions coupled with climatologically diverse environments provide specific and enormous species diversity in Iran. In this context, a new index, based on the specific biotic and environmental conditions of Asian/Iranian rivers, is required to reflect regional differences in fish distribution and assemblage structure.

Furthermore, water-quality monitoring programs in Iran have been mainly based on the determination of physical and chemical parameters; in contrast, the biological assessment of rivers especially by fish is very limited, but should be implemented in future for several reasons.

In Iran, so far, ecological monitoring by fish is typically based on species presence/absence data, however it is not used to evaluate ecological conditions and to inform decision makers.

Nevertheless, a first fish-based multi-metric assessment index for cold-water streams for the Caspian Sea Basin in Iran was developed recently by Mostafavi et al. (in review). However, the fish species diversity of these cold-water streams was very low (i.e., reference rivers are mostly occupied by brown trout (Salmo trutta) only), which resulted in only two fish metrics (related to density and population structure of brown trout) that were proposed for this index. For multi-species rivers, an IBI is understood to be a multi-metric index that integrates structure, composition, trophic ecology, and reproductive attributes of fish assemblages at multiple levels of ecological organisation (Karr and Chu, 1999). As these objectives were not principally examined for Iran to date, the recent study aims to develop a multi-metric index for cyprinid streams (i.e., streams dominated by cyprinid species) in the Iranian Caspian Sea Basin adjusted...
to the regional fish fauna. This is especially important, as northern and western Iran is considered as part of the Irano-Anatolian biodiversity hot spot, which contains many centres of local endemism — and consequently, its species diversity and composition as well as the population structure can be different to other regions in Europe and Asia (Majnonian et al., 2005; Abdoli and Naderi, 2009; Coad, 2014). For example, this basin supports species such as *Barbus lacerta*, *Barbus mursa*, and *Capeota capoeta* which have not been analysed in IBI studies so far.

IBIs are based on the assumption that various human pressures, e.g. hydrological and morphological alterations, connectivity disruptions, water quality problems and biological pressures (e.g. due to invasive species) as well as various land uses affect riverine fish assemblages (e.g. Kiabi et al., 1999; Abdoli, 2000; Mostafavi, 2007; Abdoli and Naderi, 2009; Mostafavi et al., 2014). These pressures have not been quantified for the cyprinid streams of the Caspian Sea Basin so far. Moreover, these pressures led some species of this basin to be categorized in the Red List of IUCN (International Union for Conservation of Nature, http://www.iucnredlist.org/) (e.g. *Stenodus leucichthys*: Extinct in the wild; *Acipenser persicus*, *Acipenser stellatus*, *Acipenser nudaentris*, *Acipenser gueldenstaedtii*, *Huso huso*: Critically Endangered; *Caspioomyzon wagneri*: Near Threatened; *Luciobarbus brachycephalus*, *Acipenser ruthenus*: Vulnerable). To our knowledge, all existing related studies in Iran (e.g. Qaneh et al., 2006; Kamali and Esmaeili, 2009; Sharifinia et al., 2012) exclusively described human pressures (except water quality) at local scale and did not quantify all types of pressures in different spatial scales. However, it is of great importance to quantify different types of pressures at various spatial scales, in order to better understand the response of biota to human activities (Schinagge, 2012 and 2013; Trautwein et al., 2012). Furthermore, in this study we examine appropriate fish metrics for showing a response to specific human pressure types for cyprinid rivers since this theory was not even tested for the first IBI attempt in Iran by Mostafavi et al. (in review).

IBIs are among the most appropriate methods to evaluate running waters based on predictive models (e.g. Pont et al., 2006 and 2009). These models incorporate numerous possible sources of inter- and/or intra-regional variations in assemblage and population structure caused by variations in natural environmental conditions (e.g. Oberdorff et al., 2001 and 2002; Pont et al., 2006 and 2009). These models enable site-specific estimation of metric values expected when the human pressures are absent in accordance with environmental characteristics of the measured site, while alternative procedures require development of a classification system (Oberdorff et al., 2001). As species diversity and composition as well as the population structure of reference sites of the cyprinid rivers of the Caspian Sea Basin are completely different to other regions of the world, these methods have to be tested for the environmental conditions of this region. We selected the Caspian Sea Basin because this basin represents a homogenous bio-geographical and ecological unit and availability of environmental and fish assemblage data is better here than in other regions in Iran.
Therefore, the objective of this paper is to develop a model-based fish index to assess the ecological status of cyprinid streams of the Caspian Sea Basin in Iran. This method will integrate the following steps: (1) quantifying human pressures at different spatial scales, (2) identifying applicable fish metrics showing a response to human pressures and (3) integrating these metrics into a multi-metric fish index.

2. Material and methods

Our methods are generally based on the methods developed by EFI+ consortium (2009) and relevant publications deriving from this project (e.g. Pont et al., 2006; Logez and Pont, 2011; Schinegger et al., 2012 and 2013). However, major methodological differences exist regarding the amount and type of human pressures included and the amount and type of fish metrics tested. Moreover, we included different types of environmental descriptors as well as some different statistical distributions and link functions for the modelling of fish metrics. Finally, some dissimilar criteria for the selection of the core fish metrics for the development of the fish index and some different tests for further analysis were applied.

2.1. Study area

The Caspian Sea Basin with an area of 182,100 km² encompasses three ecoregions on Iranian territory (Kura-South Caspian Drainages, Caspian Highlands and Turan Plain) (Abell et al., 2008). This basin is inhabited by 116 fish taxa in total (101 native plus 15 alien) (Esmaeili et al., in preparation). We selected cyprinid streams of two ecoregions (Kura-South Caspian Drainages, Caspian Highlands) (Fig. 1 A, B) for this study.

2.2. Fish data sampling and definition of sampling sites

First according to the land use/cover map and dam distribution layer, subcatchments delineated from the CCM2 map (River and Catchments Database for Europe, version 2.1 provided by Vogt et al. (2003 and 2007) and de Jager and Vogt (2010)) were pre-classified as reference (class 1 according to Table 1) or impacted subcatchments (class > 1 according to Table 1) using ArcGIS Desktop 9.3 (ESRI® 1999–2008). Afterwards, 75 sites of small to medium-sized rivers (width ≤ 20m) were randomly selected in the reference subcatchments and 75 in the impacted subcatchments. Other pressure variables i.e. morphology, hydrology, water quality and biology were measured according to Table 1 in the field during the sampling and used together with the pre-classification for the final classification of sites, i.e. reference sites class 1 and impacted sites class > 1 according to Table 1.

However, it is important to state that finding a site without continuity interruptions (e.g. ground sills) is almost impossible in this basin, based on our personal experience and other literature (e.g. Kiabi et al., 1999; Mostafavi et al., 2004; Abdoli and Naderi, 2009). Therefore, according to e.g. Hughes et al. (1986) and Stoddard et al. (2006), when real reference sites are missing, least disturbed conditions are selected instead.
During field work, 48 sites were rejected due to one of the following reasons: not accessible, dry, river size and depth not suitable for sampling, turbidity of water or flow velocity too high. Finally, 50 sites remained as reference and 52 as impacted sites.

Fish sampling was undertaken in autumn (2012) due to low flow conditions and presence of different size classes of fish according to the CEN standard (CEN, 2003). The length of sampling sites was calculated as 10 to 20 times the stream width and at least a distance of 100 m was sampled (Langdon, 2001; EFI+ Consortium, 2009). In addition, all sampled sites had more than 50 caught individuals to minimise the risk of false absences.

We established one stop net in the upstream reach and sampled one pass the whole river width with one anode for each 5 m wetted width followed by two or three hand-netters. The sampling team moved slowly upstream to cover all typical habitats with a sweeping movement of the anodes, while attempting to draw fish out of hiding with the electric shocker (EFI+ Consortium, 2009). The stunned fish were collected by two additional persons who accompanied the electric fishing team. After species identification according to Abdoli (2000), Abdoli and Naderi (2009) and Esmaeili et al. (2010 and in preparation) abundance and weight of each species were measured. All fish were released back into the stream afterwards.

2.3. Environmental data sampling

For each sampling site, eleven environmental parameters were measured once during the sampling (Table 2): average bankfull width (maximum width the stream attains, typically marked by a change in vegetation, topography, or texture of sediment), average wetted width, flow velocity, water temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), turbidity, NO$_3^-$, NO$_2^-$ and PO$_4^{3-}$.

In order to calculate the flow velocity, first the time that an object (e.g. small sticks) needs to pass through a defined segment was measured three times, and then the mean time value divided by the segment length was used as an estimate for flow velocity. In addition, water temperature, pH and EC were measured by Multi-parameter Water Analyser Portable (HANNA HI 9828); DO by Oxygen Meter Portable (HACH HQ30D); turbidity by Turbidimeter Portable (HACH 2100Qis); NO$_3^-$, NO$_2^-$ and PO$_4^{3-}$ by Multi-parameter Analyser Portable (HACH DR/890).

2.4. Human pressures data collection

Various human pressures were collected for each sampling site according to Degerman et al. (2007); EFI+ consortium (2009) and Schinegger et al. (2012) (Table 1).

The data set incorporated 29 pressure variables associated with the following six pressure types: (1) land use, (2) connectivity, (3) morphology, (4) hydrology, (5) water quality and (6) biology (Table 1). Human pressures were assessed at up to four spatial scales: drainage, primary catchment, segment and site. “Drainage” is the contributing area upstream of the site, “primary catchment” is the smallest level of catchment classification in the CCM2 database.
(Vogt et al., 2003 and 2007; de Jager and Vogt, 2010), “segment” is considered as a 1 km long stretch for small rivers (catchment < 100 km²) and 5 km for medium-sized rivers (catchment ≤ 500 km²). Finally, the site level is the area sampled by electric fishing.

Land use pressures were measured on drainage, primary catchment and site levels. Information on connectivity pressure was collected on segment and catchment level but in our study both scales had the same amount of this pressure, therefore, only the segment level is indicated in Table 1. Finally, the remaining pressures refer to the site level (Table 1).

All pressure variables were classified along a five-step graded classification scheme, i.e. (1) high, (2) good, (3) moderate, (4) poor and (5) bad status. In fact, in cases of limited pressure information a reduced number of classes was used, whereby pressures with low evidence were classified as class 3 and pressures with high evidence as class 4 or 5. We applied Spearman’s rank correlation test to identify redundant variables in order to exclude variables with high co-linearity (p>0.70).

2.5. Data management and software

Data regarding climatic and topographical variables (e.g. annual mean air temperature, precipitation, slope, drainage size) as well as land use and connectivity information were extracted from related layers in the software ArcGIS Desktop 9.3 (ESRI© 1999–2006). All table-structured data from GIS as well as parameters recorded in the field were managed in MS Excel © (Microsoft, 2010). Further analyses on pressure types, regional pressure index (RPI) calculation and modelling were processed in IBM SPSS Statistics 21.

Fig.2 shows the workflow of the modelling process and multi-metric fish index development at a glance. The assessment and index method development comprises in general five steps as follows:

2.5.1. Method development step 1: Calculation of the regional pressure index (RPI)

To evaluate the pressure status of cyprinid rivers in terms of different human pressures, first, after excluding correlated variables, the instream morphology pressure index (M_morph_instr) was computed according to Schindegger et al. (2012):

\[ M_{\text{morph\_instr}} = \frac{M_{\text{channel}} + M_{\text{crosssec}} + M_{\text{instr\_hab}}}{3} \]

Subsequently, an own index for each of the six dominating pressure types, i.e. land use (LUP), connectivity (CP), morphology (MP), hydrology (HP), water quality (WQP), and biology (BP) was calculated by averaging the single pressure parameter values of classes 3, 4 and 5 - to avoid values < 3 compensating for values ≥ 3 (Schindegger et al., 2012).
\[ LUP = \frac{LUP_{agri\_sit} + LUP_{urb\_sit}}{2} \]

\[ CP = \frac{C_{B\_s\_up} + C_{B\_s\_do}}{2} \]

\[ MP = \frac{M_{morph\_instr} + M_{ripreg} + M_{floodpr} + M_{sediment}}{4} \]

\[ HP = \frac{H_{imp} + H_{hydrop} + H_{waterabstr} + H_{tempimp}}{4} \]

\[ WQP = \frac{W_{eutroph} + W_{toxic}}{2} \]

\[ BP = \frac{B_{explo} + B_{intro}}{2} \]

Afterwards, we calculated the number of pressure types affected ("affected types"). In our study, this value varied from one to five depending on how many of the six pressure type indices (LUP, CP, MP, HP, WQP and BP) were higher than or equal to 3 (according to Schinegger et al., 2012).

Finally, to indicate the degradation of a site by multiple pressures into one single index value, we further calculated a regional pressure index (RPI) for each site as follows:

\[ RPI = \frac{LUP + CP + MP + HP + WQP + BP}{6} \times \text{affected type} \]

The RPI varied from 0 to 25, because the maximum pressure types occurred for a site was 5 out of 6. Finally, RPI was rescaled into five classes according to the number of pressure types involved, hereafter it was named human pressure class: class 0 - containing values less than 3 (unimpacted/slightly impacted sites (reference sites are also included in this class)); class 1 - values ranging from 3 to 5 (single pressure from respectively one type); class 2 - values ranging from 6 to 8 (double pressures from respectively two types); class 3 - values ranging from 9 to 11 (triple pressures from respectively three types); class 4 - values greater than 11 (multiple pressures from respectively four and five types).

2.5.2. Method development step 2: Selection and evaluation of environmental predictor variables

In this step, a limited number of candidate predictor variables that are major descriptors of river habitat at the reach and regional scale were actually selected for site-specific predictions of reference metric values according to e.g. Oberdorff et al. (2001 and 2002), Buisson et al. (2008), Pont et al. (2009), Logez et al. (2012) and Filipe et al. (2013). It was assumed that they are relatively unaffected by human pressures. For instance, drainage size is used for potential
habitat capacity as a substitute for river size (as a direct measure of local stream size may be
affected by flow and channel alteration). For stream fish, temperature also appears to be one of
the main determinant factors of spatial distribution. Air temperatures (i.e. annual mean air
temperature (Tmean), July mean air temperature (Tmax), January mean air temperature (Tmin)
plus the thermal range between January and July (Trange)) are highly correlated with water
temperatures but less affected by local human pressures. Annual mean precipitation
characterizes the local runoff, and average slope is a surrogate for substrate size and water
velocity. All these predictors are fully characterized in Table 3.

Forest and grassland regions (according to land use/cover map) were also included in the
modelling process because these regions can significantly influence the density and biomass of
some species (e.g. brown trout, Mostafavi et al., in review). The two freshwater ecoregions plus
forest and grassland regions were coded and entered in the models as nominal (categorical)
variables.

The climatic variables (air temperature and precipitation) were obtained from the WorldClim
predictors, which are often used to characterize current climatologic conditions and seasonality.
The WorldClim data describe 50 years of monthly means collected at climate stations between
1950 and 2000 (Hijmans et al., 2005 and 2007) and are interpolated at 30 arc-seconds grid
extent (approximately 1 km at the equator). Other topographical variables (i.e. slope, drainage
size) were extracted from CCM2, which is based on a 100 m resolution digital elevation model
(Vogt et al., 2003 and 2007; de Jager and Vogt, 2010).

All predictor variables (except freshwater ecoregions plus forest and grassland regions) were
examined for co-linearity by Spearman’s rank correlation (ρ), if two variables were highly
correlated (ρ >0.70) one of them was excluded.

2.5.3. Method development step 3: Fish metric description, selection, modelling,
standardisation and rescaling

In this step, models were used to predict values for each fish metric and for a given site in the
absence of human pressures (i.e. a value corresponding to “a reference condition”). These
predicted metric values were computed from environmental predictor variables using
Generalised Linear Models. The methodology used for metric selection and modelling was
mostly derived from Oberdorff et al. (2002), Pont et al. (2006 and 2009), EFI+ consortium
(2009), Logez and Pont (2011), Moya et al. (2011), Marzin et al. (2012) and Schinegger et al.
(2013) as follows:

2.5.3.1. Fish metrics description

Similar to other studies (e.g. Hughes et al., 2004; EFI+ Consortium, 2009; Schinegger et al.,
2013), each collected species was assigned to five structural and functional types of metrics:
biodiversity, habitat, reproduction, trophic level and water quality sensitivity. These attributes
were extracted from literature (e.g. Abdoli, 2000; Abdoli and Naderi, 2009; EFI+ Consortium,
2009; Esmaeili et al., 2010; Coad, 2014) and fish experts in Iran (Table 4). In fact, 12 fish metric types of different variants (absolute and relative number of species, density (n/ha) and biomass (kg/ha)) were pre-selected for further analyses (Table 5). These variants reflect most of the important ecological aspects of fish assemblages according to Noble et al. (2007) and Schneegger et al. (2013). Fish metrics like tolerant and alien ones were "0" in the reference sites (i.e. not present) or were highly variable or unresponsive to human pressures (like intermediate metrics). Therefore, these metrics were initially excluded according to Hughes et al. 1998 and Angermeier and Davideanu 2004. Finally, 69 fish metrics were used as candidate metrics for the modelling procedure (see Table 5).

2.5.3.2. Prediction of fish metrics

We applied a Generalized Linear Model (GLM) for the modelling. For “contentious” metrics (e.g. biomass (kg/ha), density (n/ha) and proportion (percentage)) based on the type of their distributions (e.g. Gaussian or inverse Gaussian) an appropriate link function (e.g. identity, logarithm, power) was selected. Moreover, Poisson distribution and logarithmic link were used to model count data (richness metrics). In addition, an offset was defined by total species number for richness metrics (e.g. EFI+ consortium, 2009) and if these count metrics were over-dispersed, we preferred to choose negative binomial distribution that is a classical alternative to control the over-dispersion in regression analysis of count data (e.g. McCullagh and Nelder, 1989; Loge and Pont, 2011). The square of each explanatory variable was also included to account for potential non-linear relationships (e.g. Moya et al., 2011). The coefficients of the models were estimated at the maximum of likelihood. For each metric, environmental predictor variables were selected using a stepwise procedure based on Akaike’s information criterion (AIC; Pont et al., 2006; Loge and Pont, 2011). This procedure selects the combination of variables that minimize the model’s AIC.

To evaluate the model performance, R square, standardised residuals and leverage values were extracted. Afterwards, the normality of residuals (using Q-Q plot and histogram), the heteroskedasticity of residuals (graph of standardized residuals versus standardized expected values), the influence of leverage values (graph of residual values versus leverage values), and the relationship between observed and expected values (a linear relation of the form $y = x$ was expected) were visually evaluated. Furthermore, this process was completed by internal-validation based on bootstrapping (Efron and Tibshirani, 1993). Error distribution of each model was estimated by 100 random samples with replacement. The results of internal-validation were observed using histograms of residuals obtained by bootstrap (EFI+ consortium, 2009). Metrics generally matching these criteria were used in the next step.

2.5.3.3. Standardisation and rescaling of fish metrics

Once the models were fitted, we computed residuals according to Pont et al. (2009) and Loge and Pont (2011) by using the following equation:

\[ R_i = \log(O_i + 1) - \log(E_i + 1) \]
where:

- \( R_i \) is the residual; \( O_i \) is the observed and \( E_i \) is the expected value. The value of 1 was added to both observed and predicted values, to handle sites presenting no fish belonging to the metric considered.

Then, the score of each metric (\( M \)) was obtained by standardising the residuals of the model in the following way:

\[
(10) \quad M_i = \frac{(R_i - M)}{S_{R_i}}
\]

where:

- \( R_i \): Is the residual value (difference between observed and expected metric) from sites i to n.
- \( M \): Is the median value of the residuals; i to n.
- \( S_{R_i} \): Is the standard deviation of the residuals in the whole undisturbed dataset.

As standardised residuals vary from \(-\infty\) to \(\infty\) and in order to guarantee that each metric varies within a finite interval from 0 to 1, two transformations were applied. All values over a maximum (percentile 95) and below a minimum (percentile 5) were replaced by this maximum (\( \text{Max} \)) and this minimum (\( \text{Min} \)). Then the following transformation was applied to each metric score:

\[
(11) \quad \text{Rescaled } M_i = \frac{(M_i - \text{Min})}{\text{Max} - \text{Min}}
\]

2.5.4. Method development step 4: Fish metric sensitivity to human pressures

In this step, the sensitivity of the candidate metrics to human pressures was evaluated using the Mann-Whitney U test (Bonferroni-correction; \( p < 0.05 \) / number of tests) between impacted and reference sites. In addition, reaction of selected metrics to human pressure class was tested by box-plot graphs. Moreover, metrics with a median of reference sites less than 0.80 were rejected as they have lower potential to discriminate between reference and impacted conditions. Afterwards, metrics with high co-linearity were excluded using Spearman’s rank correlation test (\( p > 0.80 \)). In fact, this cut-off was defined in order to keep more variants.

2.5.5. Method development step 5: Index calculation, scoring and validation

After selection of final fish metrics, the multi-metric fish index of cyprinid streams (MMICS) was calculated using the arithmetic mean of the standardised and transformed metric scores. We then for the management purposes divided MMICS into five categories (scores) based on the distribution of both impacted and reference sites as done in similar studies (e.g. Schmutz et al., 2007; EFIs Consortium, 2009; Marzin et al., 2014). Class 1 (high) covers lower values of quartile 3 of reference sites as lower boundary and maximum value of reference sites as upper boundary, class 2 (good) lies between the minimum values of reference sites and the upper values of quartile 2 of reference sites, class 3 (moderate) between lower values of quartile 3 of impacted sites and minimum values of reference sites, class 4 (poor) between 0.41 and upper
values of quartile 2 of impacted sites and class 5 (bad) between minimum values of impacted sites and 0.40.

In order to detect which specific human pressure types and RPI have the strongest relation with the multi-metric fish index (MMICS), a regression tree test was applied with the multi metric fish index as dependent variable and the pressure variables (land use, connectivity, morphological, hydrological, water quality and biological pressures plus regional pressure index) as independent variables using the CHAID method in IBM SPSS Statistics 21. The method settings were as follows: 10 times cross validation, maximum tree depth = 3, minimum cases in parent node = 5, minimum cases in child node = 3, adjust significant p-values (p<0.05 / number of tests) and R-square calculated as follows: 1-(risk estimate value / squared standard deviation displayed at the root node).

The difference between grassland and forest regions was examined by box plot graph and Mann-Whitney U test. Moreover, Spearman’s rank correlation test was used to compare fish metrics with specific human pressure types based on the adjusted significant p-value (p<0.05 / number of tests). Finally, for validation of the fish index (MMICS), its independency was examined versus natural environmental predictor variables by linear regression analysis. Moreover, we randomly split the original dataset into two subsets, i.e. 60% (30 reference and 31 impacted sites) and 40% (20 reference and 21 impacted sites). Hereafter, we tested successfully modelled fish metrics versus the human pressures according to the graphical visualization and Mann-Whitney U test (with Bonferroni-correction) to find the best metrics for the recognition of reference and impacted sites. Afterwards, among remaining fish metrics, the redundant ones were excluded by Spearman’s rank correlation test (p>|0.80)). Finally, the fish index developed by remaining metrics in 60% of dataset was validated by 40% dataset versus human pressure class (graphical visualisation) as well as regression test against the regional pressure index.

3. Results

3.1. Human pressure analysis

After accounting for redundancy, 20 pressure variables out of 29 remained for further analysis (see Table 1). In our study, the most frequent human pressure was land use (LUP), occurring at 43 sites, in particular urbanization and agriculture. This was followed by hydrological pressure (HP) at 35 sites, in particular water abstraction (Fig. 3 A). In total, 26 sites were affected by multiple pressures, whereas only two sites were influenced by a single pressure. The frequencies of impacted sites by double and triple pressures were the same (12) (Fig. 3 B).

3.2. Environmental characteristics and fish community

Environmental characteristics of investigated streams are described in Table 2 and 3. Using Spearman correlation tests, a co-linearity among some environmental predictor variables (p>|0.70|) was found, whereby seven variables, i.e. drainage size, slope, minimum air
temperature, range temperature, precipitation, type of major land use/cover (forest/grassland) 
and ecoregion were finally retained for the modelling process.

22 taxa from six families were identified during the fish sampling, in which Cyprinidae with 14 
taxa showed the highest diversity, while Poeciliidae and Salmonidae showed the lowest 
diversity with one taxon each. Moreover, 19 taxa are native and three are alien. Overall, the 
studied streams were dominated by cyprinid species more than 70 percent (Table 4).

3.3. Fish metric selection, MMICS calculation and validation

In total, only 39 fish metrics out of 69 fulfilled the selection criteria for modelling (Table 5), of 
which 14 fish metrics were excluded as they did not show a response to human pressures (Fig. 
4 and Table 5). Among those 14 fish metrics, five fish metrics did not show significant 
differences between impacted and reference sites according to the Mann-Whitney U test 
(p>0.001), and the median of reference sites for seven fish metrics was also less than 0.80 
(Fig. 4, see a and b).

In the next step, the correlation test indicated a high redundancy among the 25 remaining fish 
metrics (p>0.80) which is why 18 fish metrics were excluded due to high correlation. Finally, 
seven fish metrics (number of native species, density of intolerant species to oxygen depletion, 
biomass of intolerant species to water quality degradation, biomass of intolerant species to 
habitat degradation, density of rheophilic species, biomass of lithophilic species and percentage 
biomass of insectivorous species) were chosen as core metrics for the calculation of multi-
metric fish index of cyprinid streams (MMICS) (Fig. 5 and Table 5).

The coefficients of the core metric models are shown in Table 6. In general, only a few 
environmental predictor variables were included in the modelling process of each metric. In fact, 
six metrics with two predictor variables and one metrics with three predictor variables were 
modelled. Slope was the most important variable for five out of seven models (Table 6). In 
addition, the R² of these models were higher than 0.35.

In general, fish metrics did not show pressure specific responses but reacted in a similar way to 
multiple pressures. Strong reaction is documented for land use, water quality and 
hydromorphology but not for other pressures (i.e. connectivity and biology) at p<0.001 level (see 
Table 7).

After splitting the original dataset into two subsets for the validation of fish index (MMICS), it 
was observed that the same fish metrics remained again for the calculation of the fish index. 
Moreover, the reactions of the developed fish indices (MMI1 and MMI2) from 60% and 40% 
datasets versus human pressure class and regional pressure index (RPI) were consistent (Figs. 
6 (A, B) and 7).
Stepwise linear regression between the multi-metric fish index of original dataset (MMICS) and environmental predictor variables (drainage size, slope, minimum air temperature, range temperature and precipitation) showed that none of the environmental variables was retained and the variability in this index (MMICS) explained by these environmental variables was not significant (p>0.05) (see also Fig. 8).

The regression tree showed the best relation between the regional pressure index (RPI) and the multi-metric fish index (MMICS) in comparison to the other human pressure types ($R^2 = 0.75$, $P = 0.000$).

Further, the MMICS was divided into five categories (scores), based on the distribution of both impacted and reference sites (Fig. 9 A): Class 1 (high) covers values between 0.90-1.00, class 2 (good) values between 0.78-0.89, class 3 (moderate) values between 0.61-0.77, class 4 (poor) values between 0.41-0.60 and class 5 (bad) values between 0.00-0.40. Overall, among 52 impacted sites, 35 sites are in a moderate, poor or bad status, i.e. there is strong need for restoration actions.

The comparison of the MMICS in grassland and forest regions also displayed that the index in the impacted grassland sites is significantly lower than in the forest region (Fig. 9 B, C).

4. Discussion

In Iran, similar to Europe and elsewhere, there are numerous human alterations and pressures directly affecting the physicochemical conditions of running waters and strongly influencing aquatic biota (e.g. Kiabi et al., 1999; Abdoli, 2000; Abdoli and Naderi, 2009; Esmaeili et al., 2007; Mostafavi et al., 2014). Multi-metric fish indices, like the one we developed, are powerful tools for the ecological assessment of streams (e.g. Pont et al., 2006 and 2009; Schmutz et al., 2007; Meador et al., 2008; Ruaro and Gubiani, 2013).

4.1. Human pressures

The intensity of pressures in our study is higher compared to studies from Europe (Schinegger et al. 2012) because most sites are affected by multiple pressures indicating high potential of stress for fish.

4.1.1. Land use pressures

Akhani et al. (2010) indicated that half of the forest in the Caspian Sea Basin was eradicated in recent decades (from 3.6 million to 1.8 million hectares). In contrast, the extent of agriculture and build-up areas increased. This finding is mirrored by our data as most sites were affected by land use pressures (43 out of 52 impacted sites). Moreover, an increase of land use pressure is often accompanied by an increase of hydrology, morphology and water quality pressures (Osborne and Wiley, 1988), which is in line with our results, as 35 out of 52 sampling sites were impacted by hydrological alteration, 31 by morphological alteration and 28 by water quality problems.
4.1.2. Hydrological pressures

Water abstraction is one of the most important hydrological pressures according to our findings. Water is abstracted for the purpose of agricultural irrigation via establishment of dams as well as direct water abstraction from streams by artificial channels and pumps. Some rivers and streams have no flowing water for some months of the year, or flows are reduced to only a fraction of their original magnitude (often observed during our monitoring and even the flow velocity of some sampling sites was zero). It was also observed that water abstraction influenced water quality of rivers via reduction of flow velocity, the habitat quality (water depth, wetted width) and connectivity (including lateral connectivity and drying up of side arms). All these findings are in agreement with other studies (e.g. Bernardo et al., 2003; Meador and Carlisle, 2007).

In this regard, almost all core metrics except density of intolerant species to oxygen depletion showed a negative response to hydrological pressures (dominated by water abstraction pressure). This is more or less in accordance with a study of Benejam et al. (2009), where number of intolerant species, proportion of intolerant individuals, number of benthic species, number of families, number of native species and number of insectivore species showed significant response to water abstraction. Although tolerant species are not included in our core metrics, we observed tolerant species (e.g. Pseudorasbora parva, Carassius carassius, Gambusia holbrooki) in the affected sites.

4.1.3. Connectivity pressures

To our knowledge, almost all rivers of the Caspian Sea Basin are disconnected from the sea due to ground sills (with drops up to 1.5 m for the establishment of bridges) or/dam dams. Due to the mentioned connectivity barriers, no long-distance migratory species (e.g. Aciplens sp., C. wagneri; R. caspicus; R. nutilus) were observed in our study sites, while these species have been reported in many of the sampled rivers in the past (e.g. Berg, 1948; Rostami, 1961; Holčík and Oláh, 1992; Abdoli, 2000; Mostafavi, 2007; Abdoli and Naderi, 2009; Coad 2014). Consequently, our fish index was not designed for long-distance migratory fish species and therefore it is not applicable for this purpose. Future IBIs should incorporate the loss of long-distance migratory species, e.g. based on historical data in order to fully reflect the pressure of continuity disruptions at the catchment level.

In our study, connectivity pressures were not correlated with any metrics while Schmutz et al. (2007) indicated that insectivorous, omnivorous, intolerant and lithophilic metrics showed positive response (increase) to connectivity disruptions in the European rivers. This difference might be related to the fact that European rivers are affected by a different combination of pressures, e.g. a higher proportion of dammed rivers, however, this should be studied in more detail in future studies in Iran.

4.1.4. Water quality pressures
According to our study, observed water quality pressures are mostly related to untreated sewage of cities and agriculture for the recent decades. Water pollution was not a major problem before 1960 because of the underdeveloped state of cities, industry and agriculture (Coad, 1980).

We observed that the “jube” system, i.e. a series of channels carrying water along the streets of most towns and cities, is also a source of pollution. It functions to irrigate roadside trees but also serves to carry away detergents and other pollutants, which may be poured into the nearest river or stream (indicated also by Coad, 1980 and Mostafavi, 2007). In addition, we observed that the effluent of agriculture and some livestock, factories, slaughter houses, hospitals, restaurants and etc. is directly discharged into rivers without any treatment. Agricultural effluents also contain high levels of phosphate, nitrogen, potash and pesticides which is in line with our study as measured parameters like NO$_3^-$, NO$_2^-$ and PO$_4^{3-}$ reached up to 0.17, 9.60 and 5.70 mg/l respectively in some impacted sites. Consequently, the richness and abundance of sensitive or intolerant species like Barbus lacerta, Luciobarbus mursa, Salmo trutta were severely reduced or these species even disappeared entirely in some sites. Conversely, the abundance of some tolerant species like P. parva, C. carassius, G. holbrooki increased. Moreover, all core metrics in our study showed very significant and negative correlation with this type of pressure. This is consistent with Schmutz et al. (2007), who indicated that insectivorous, intolerant and lithophilic species exclusively responded (decreased) to water quality pressures. Furthermore, Schinegger et al. (2013) pointed out that intolerant, rheoparous, tolerant and omnivorous species showed significant reaction to this pressure type. In comparison with them, richness and intolerant metrics are in line with our study, but other metrics were excluded in the modelling process and correlation test. However, omnivorous species (e.g. P. parva, C. carassius, G. holbrooki) were observed in sites with this type of pressure and rheoparous species also showed negative reaction but was left out due to correlation with other metrics.

### 4.1.5. Morphological pressures

Based on our observation, channelisation is one of the main morphological pressures in this area which is generally linked to farmland acquisition, construction of bridges or roads, flood prevention as well as river bed and bank erosion control.

Moreover, gravel mining and sand extraction are other main drivers for morphological pressures, changing the stream’s physical habitat characteristics and leading to e.g. siltation, clogging of the riverbed, turbidity and degradation of the riparian vegetation (Lau et al., 2006; Padmalal et al., 2008; Paukert et al., 2011). The intensity of this pressure in some rivers we sampled is very high and the associated fine sediment inputs result in high turbidity (values up to 1185 NTU were observed). Based on our results, almost all core metrics except intolerance of species (density) to oxygen depletion showed significant correlation to this pressure type. Schmutz et al. (2007) and Schinegger et al. (2013) combined morphology, hydrology and connectivity pressure types into one type named hydro-morphology (HMC), which we also considered. In Schmutz et al. (2007) only insectivorous, intolerant and lithophilic metrics
exclusively responded to the hydromorphological pressures, in Schinegger et al. (2013) only
richness, intolerant, tolerant and rheoporous metrics while in our study any metric responded to
these combined pressures at the p<0.001 level.

4.1.6. Other Biological pressures
Actually, overexploitation and unusual methods of fishing such as using cast net, electricity,
toxics and dynamite are the other known threats based on our study and others (Abdoli, 2000;
Esmaeili et al., 2007). Overall, out of our seven core metrics, only four were correlated to this
pressure type. Most likely, this variation is due to the fact that biological pressures are rare in
our data set. Therefore, this aspect should be investigated in further studies.

4.2. Applicable fish metrics as well as showing a response to specific human
pressures
Our index involved five (out of six) structural and functional types of metrics related to
biodiversity, habitat, reproduction. trophic level and water quality. These should foster the
robustness of our index, as such traits are sensitive to human pressures and are comparable
among assemblages even across ecoregions that differ in their taxonomic composition (e.g.
Statzner et al., 2001; Moya et al., 2011).

Moreover, according to regression tree result, RPI had the strongest relation with MMICS in
comparison with other pressure types. It can be true because most sites were affected by
different pressure types and RPI actually evaluated both pressure intensity and multiple
pressure effects on sampling sites.

The fish index (MMICS) of impacted sites in the grassland region was significantly lower than in
the forest region. Most likely, the reason is that the severity of water abstraction pressure was
higher in the grassland region. Consequently, the effects of other pressures are intensified in
that region.

According to our dataset, all core metrics showed significant reaction to most pressure types,
but specific metrics for specific pressure types were not generally found, as e.g. described by
Schinegger et al. (2013). However, this hypothesis should be tested with a larger dataset in
future.

4.3. Uncertainty in the application of a multi-metric fish index (MMICS)
Our fish index is recommended for wadeable streams in the cyprinid zone with slope between 3
to 25 percent, wetted width less than 20 m and drainage size less than 500 km². To date, 116
species have been recorded from the southern part of the Caspian Sea Basin, belonging to
Iranian territory (Esmaeili et al., in preparation). In our study, 22 taxa were recorded indeed.
This difference is due to several reasons. First, 27 of 119 species are occurring only in the
marine section of the Caspian Sea Basin, but not in the rivers. Second, 18 species are
migratory ones and due to connectivity pressures they are not able to reach the spawning
habitats generally (Abdoli and Naderi, 2009 and Kiabi et al., 1999). Third, our sampling sites were limited to certain areas (as stated before, e.g. regarding slope, catchment size, river width and being wadeable), therefore, some species like *Pelecus cultratus*, *Aspius aspius*, *Tinca tinca*, *Vimba persa*, *Liza saliens*, *Esox lucius* and etc. were not included in our study. Finally we did not cover the whole basin, one ecoregion (Turan Plain) was not considered in our study. Nevertheless, our findings are defensible, as our study covers the most common species (e.g. Abdoli and Naderi, 2009; Esmaeili et al., 2010 and in preparation; Coad, 2014).

Abundance of individual species may vary seriously over time (Lyons, 2006). Therefore, this fish index is only applicable for data collected during autumn. In addition, the sampling method is particularly important because sampling efficiency and sampling effort strongly influence the fish index scores (e.g. Simon and Sanders 1999). As currently, the method for fish sampling in Iran is not standardised, we strongly recommend using European standards, as e.g. CEN (2003).

5. Conclusion

Our index performed well in discriminating between reference and impacted sites, showing a significant negative linear response along a gradient of human pressures independent of natural environmental variability. Overall, the development of such an index proposes an opportunity to enhance national bio-monitoring programmes in Iran.

Acknowledgements

Part of this work was funded by the Austrian Science Fund (FWF, Predictive Index of Biotic Integrity for running waters of Iran, contract number P 23650-B17). The Ministry of Sciences, Technology of Iran awarded a scholarship to Hossein Mostafavi (first author). Special thanks are given to Majid Bakhtiyari, Houman Liaghati, Bahram Kiabi, Asghar Abdoli, Hamid Reza Esmaeili, Ahmad Reza Mehrabian, Hossein Kermanian, Florian Pletterbauer, Clemens Trautwein, Saber Vatandost, Azad Teimori, Mehrgan Ebrahimi, Ebrahim Fataei, Keyvan Abbasi, Gholamreza Amir Ghadi, Hamid Reza Bagherpour, Arash Arsham for their support in the process of this study. We are very thankful to the Environment department of Iran and its affiliated institutions in Tehran, Mazandaran, Gilan, Ardabil and East Azerbaijan and their employees Ahmad Reza Lahiyanzadeh, Dariush Moghadas, Amir Abdoos, Hossein Deghani, Ebrahim Rahimi, Mohamad Mirzaei, Hossein Alinejad, Behrouz Alkhani and many others. We also thank Erika Thaler for editing the text.
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Captions to figures

Fig.1. Distribution of fish sampling sites in cyprinid streams of two Freshwater Ecoregions of the Caspian Sea Basin (A and B).

Fig.2. Flow chart describing the procedure of multi-metric fish index (MMICS) development.

Fig.3. A: Number of sites regarding no/slight pressure, affected by land use pressure type (LUP), connectivity pressure type (CP), morphological pressure type (MP), hydrological pressure type (HP), water quality pressure type (WQP) and biological pressure type (BP). B: Number of sites with no, single, double, triple and multiple pressures.

Fig.4. Box-plot graphs regarding excluded metrics versus human pressure class. “a” shows metrics which didn’t show significant difference between impacted and reference sites according to Mann-Whitney U test (P>0.05), “b” shows metrics where the median of reference sites is less than 0.80.

Fig.5. Box-plot graphs regarding core metrics versus human pressure class.

Fig.6. Box-plot graphs regarding multi-metric fish indices (MMI1: 60% dataset, A; MMI2: 40% dataset, B) versus human pressure class.

Fig.7. Regression of multi-metric fish index of 60 and 40% dataset versus regional pressure index (RPI).

Fig.8. Multi-metric fish index of original dataset (MMICS) versus environmental predictor variables.

Fig.9. Classification of the multi-metric fish index of original dataset (MMICS) into five categories on the basis of observed scores at reference and impacted sites (A). Multi-metric fish index of original dataset (MMICS) regarding reference (Ref) and impacted (Imp) sites in forest (B) and grassland (C) regions.
Fig. 1

Legend
- Fish sampling site
- Ecoregion boundary
Fig. 8

Fig. 9

(A)  (B)  (C)

1  2  3  4  5

Ref  Imp  Ref  Imp  Ref  Imp
Captions to tables

Table 1. Human pressure classification into six human pressure types (LUP: Land Use; Pressure, CP: Connectivity Pressure, MP: Morphological Pressure, HP: Hydrological Pressure, WQP: Water Quality Pressure, BP: Biological Pressure) and their definitions.

Table 2. Environmental variables measured at the sampling sites.
Abbreviation: N (number of sites), SD (mean standard deviation), min (minimum), max (maximum).

Table 3. Predictor variable characteristics of sampling sites.
Abbreviation: Tmean (Annual mean air temperature), Tmax (July mean air temperature), Tmin (January mean air temperature), Trange (the thermal amplitude between January and July), N (number of sites), SD (mean standard deviation), min (minimum), max (maximum).


Table 5. Name and definition of candidate metrics as well as metrics fitted well in the modelling process (Modelling metrics), metrics excluded in reaction to human pressures (Excluded metrics), core metrics selected for multi-metrics fish index after correlation test (Core metrics).
Type: biodiv = biodiversity, hab = habitat, repro = reproduction, troph = trophic level, wq = water quality; Variants: nsp = number of species, dens = density [Ind/ha], biom = biomass [kg/ha], perc-nsp: number of species of guild in relation to all species, perc-dens = density of guild in relation to all guilds, perc-biom = biomass of guild in relation to all guilds, all = all six variants are included; Direction: decr = metric decreases with increasing human pressure; Reaction according to our database.

Table 6. Regression coefficients and the criteria selected for the seven models used to model fish assemblages.

Table 7. Matrix of Spearman rank correlations of specific human pressure types and core metrics. The upper numbers are Spearman correlation coefficients and the lower numbers are P values (Number of sites = 102).
| Table 1 |
|----------------|----------------|----------------|
| **Human pressure variable** | **Type** | **Classification** |
| Agriculture | LUP | LU_agri sit | Range: 50m from stream; 1 = none, 3 = along one side, 5 = along both sides |
| Urbanisation | LUP | LUUrban sit | Range: 100m from stream; 1 = <5%, 3 = >15%, 5 = >10% |
| *Agriculture | LUP | LU_agri_pc | Extent and pressure of agriculture and silviculture; 1 = <1%, 3 = >10% & <40%, 5 = >40% |
| *Urbanisation | LUP | LU_agri_pc | Extent and pressure of urban areas; 1 = <1%, 3 = >1% & <15%, 5 = >15% |
| *Agriculture | LUP | LU_agri_dr | Extent and pressure of agriculture and silviculture; 1 = <10%, 3 = >10% & <40%, 5 = >40% |
| *Urbanisation | LUP | LU_agri_dr | Extent and pressure of urban areas; 1 = <1%, 3 = >1% & <15%, 5 = >15% |
| Migration barrier upstream | CP | C_B_s_up | Barriers on the segment level upstream; 1 = no, 3 = partial, 5 = yes |
| Migration barrier downstream | CP | C_B_s_do | Barriers on the segment level downstream; 1 = no, 4 = partial, 5 = yes |
| Channelisation | MP | M_channel | Alteration of natural morphological channel plan form; 1 = no, 3 = intermediate, 5 = straightened |
| Channelisation | MP | M_crosssec | Alteration of cross-section; 1 = no, 3 = intermediate, 5 = technical cross-section (U-profile) |
| Channelisation | MP | M_instream | Alteration of in-stream habitat condition; 1 = no, 3 = intermediate, 5 = high |
| *Channelisation | MP | M_embankm | Artificial embankment; 1 = no (natural status), 2 = slight (local presence of artificial material for embankment), 3 = intermediate (continuous embankment but permeable), 5 = high (continuous, no permeability) |
| Channelisation | MP | M_riparian | Alteration of riparian vegetation close to shoreline; 1 = no, 2 = slight, 3 = intermediate, 5 = high (no vegetation) |
| Flood protection | MP | M_floodpr | Presence of dykes for flood protection; 1 = no, 3 = yes |
| *Flood protection | MP | M_floodimp | If the river has a former floodplain: Proportion of connected floodplain still remaining; Floodplain = area connected during the flood; 1 = >50%, 2 = 10-50%, 3 = >10%, 5 = some water bodies remaining or no |
| Sedimentation | MP | M_sediment | input of fine sediment (mainly mineral input; bank erosion, erosion from agricultural land); 1 = no, 3 = yes |
| *Flow velocity increase | HP | H_velocon | Pressure on flow conditions (mean velocity due to channelisation, flood protection, etc.); 1 = no, 3 = yes |
| Impoundment | HP | H_imp | Natural flow velocity reduction on site because of impoundment; 1 = no (no impoundment), 3 = intermediate, 5 = strong |
| Hydropeaking | HP | H_hydro | Site affected by hydropeaking; 1 = no (no hydropeaking), 3 = partial, 5 = yes |
| Water abstraction | HP | H_waterabstr | Site affected by water flow alteration/minimum flow; 1 = no (no water abstraction), 3 = intermediate (less than half of the mean annual flow), 5 = strong (more than half of mean annual flow) |
| *Reservoir flushing | HP | H_flush | Fish fauna affected by flushing of reservoir upstream of site; 1 = no, 3 = yes |
| *Temperature pressure | HP | H_tempimp | Water temperature pressure; 1 = no, 3 = yes |
| *Eutrophication | WQP | W_eutrop | Artificial eutrophication; 1 = no, 3 = low, 4 = intermediate (occurrence of green algae), 5 = extreme (oxygen depletion) |
| *Acidification | WQP | W_aci | Acidification; 1 = no, 3 = yes |
| *Organic siltation | WQP | W_silt | Siltation; 1 = no, 3 = yes |
| *Organic pollution | WQP | W_poll | Is organic pollution observed; 1 = no, 3 = intermediate, 5 = strong |
| *Toxicity | WQP | W_toxic | Toxic priority substances (organic and nutrient appearance); 1 = no or very minor, 3 = weak (important risk, link to particular substance), 5 = high concentration (a clearly known input) |
| Pressure of exploitation | BP | B_explo | Fishing, at site affecting fauna, information based on local fishermen; 1 = no, 3 = intermediate, 5 = strong |
| Introduction of fish | BP | B_intro | New fish species to river basin; 1 = no introduction, 2 = introduction, but no reproduction and low density, 3 = reproduction, low density, 4 = reproducing, low density, 5 = reproducing, high density |

* excluded variables after correlation test
* according to Iranian water quality standard (Sazman Hezfat Mohaj Zist Iran, 2014)
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<th>Average wetted width (m)</th>
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<th>DO (mg/l)</th>
<th>pH</th>
<th>EC (μS/cm)</th>
<th>Turbidity (NTU)</th>
<th>NO$_3^-$ (mg/l)</th>
<th>NO$_2^-$ (mg/l)</th>
<th>PO$_4^{3-}$ (mg/l)</th>
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### Table 3

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<td>HTOL</td>
<td>Hab</td>
<td>Atroph</td>
<td>Repro</td>
<td>HabitSp</td>
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<td>O2M</td>
<td>LIM</td>
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<td>INSV</td>
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<td>Alburnus elclamationi</td>
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<td>O2M</td>
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<td>RH</td>
<td>INSV</td>
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<td>RHPAR</td>
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<td>INSV</td>
<td>LITH</td>
<td>LIPAR</td>
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<td>Alburnus helenae</td>
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<td>O2M</td>
<td>HINTOL</td>
<td>EURY</td>
<td>PLAN</td>
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<td>B. capoeta</td>
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<td>IM</td>
<td>O2M</td>
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<td>RH</td>
<td>HERB</td>
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<td>RHPAR</td>
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<td>Carassius gibelio</td>
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<td>O2M</td>
<td>HINTOL</td>
<td>EURY</td>
<td>OMNI</td>
<td>PHYT</td>
<td>LIPAR</td>
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| Hemiculter leuciscus  | Cyprinidae   | TOL   | O2M  | HINTOL | EURY | OMNI  | PELA   | EUPAR    | A
| Luciobarbus capito    | Cyprinidae   | INTOL | O2M  | HINTOL | RH   | INSV  | LITH   | RHPAR    | Na            |
| Luciobarbus maurusia  | Cyprinidae   | INTOL | O2M  | HINTOL | RH   | INSV  | LITH   | RHPAR    | Na            |
| Pseudohabia parva     | Cyprinidae   | TOL   | O2M  | HINTOL | EURY | OMNI  | PHIL   | EUPAR    | A
| Rhodeus amarus        | Cyprinidae   | INTOL | O2M  | HINTOL | LIMNO | OMNI  | OSTRAT | LIPAR    | Na            |
| Squilla cephalus      | Cyprinidae   | TOL   | O2M  | HINTOL | RH   | OMNI  | LITH   | RHPAR    | Na            |
| Cobitis sp.           | Cobitalidae  | IM    | O2M  | HIM  | RH   | INSV  | PHYT   | EUPAR    | Na            |
| S. aurata             | Cobitalidae  | IM    | O2M  | HIM  | RH   | INSV  | PHYT   | EUPAR    | Na            |
| Neogobius pallasi     | Gobiidae     | TOL   | O2M  | HINTOL | EURY | INSV  | SPEL   | EUPAR    | Na            |
| Neogobius melanostomus| Gobiidae     | TOL   | O2M  | HINTOL | EURY | INSV  | LITH   | EUPAR    | Na            |
| Paracantholepsis vulpes| Nemacheilidae| INTOL | O2M  | HINTOL | RH   | INSV  | LITH   | EUPAR    | Na            |
| Oxyorocheilus sp      | Nemacheilidae| IM    | O2M  | HINTOL | RH   | INSV  | LITH   | EUPAR    | Na            |
| Gambusia holbrooki    | Poecilidae   | TOL   | O2M  | HINTOL | LIMNO | INSV  | VIVI   | LIPAR    | A
<p>| Salmo trutta          | Salmonidae   | INTOL | O2M  | HINTOL | RH   | INSV  | LITH   | RHPAR    | Na            |</p>
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<tr>
<th>Trait</th>
<th>Definition</th>
<th>Type</th>
<th>Modelled metrics</th>
<th>Excluded metrics</th>
<th>Core metrics</th>
<th>Direction</th>
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<td>Nsp-all</td>
<td>Total number of fish species, including native and alien species.</td>
<td>biodiv</td>
<td>dens, biom</td>
<td>dens</td>
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<tr>
<td>Nsp-native</td>
<td>Number of native species.</td>
<td>biodiv</td>
<td>nsp, dens, biom</td>
<td>nsp</td>
<td>decr</td>
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<tr>
<td>Nsp-alien</td>
<td>Number of alien species</td>
<td>biodiv</td>
<td></td>
<td></td>
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<tr>
<td>Wogen-INTOL</td>
<td>In general intolerant to usual (national) water quality parameters.</td>
<td>wq</td>
<td>all except percms, dens</td>
<td>nsp</td>
<td>biom</td>
<td>decr</td>
</tr>
<tr>
<td>WOCO2-OZINTOL</td>
<td>Tolerant to low Oxygen concentration (O₂), more than 6 mg/l in water.</td>
<td>wq</td>
<td>biom percms dens</td>
<td>percms percms nsp dens</td>
<td>nsp</td>
<td>biom</td>
</tr>
<tr>
<td>HTOL-HINTOL</td>
<td>Habitat degradation intolerance.</td>
<td>hab</td>
<td>all except percms</td>
<td>nsp percms biom</td>
<td></td>
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<tr>
<td>Hab-RH</td>
<td>Degree of rheophily (habitat): Fish prefer to live in a habitat with high flow conditions and clear water.</td>
<td>hab</td>
<td>all except percms</td>
<td>nsp percms percms biom</td>
<td></td>
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<tr>
<td>Hab-EURY</td>
<td>Degree of rheophily (habitat): Fish that exhibit a wide tolerance of flow conditions, although generally not considered to be rheophilic.</td>
<td>hab</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HabSp-RHPAR</td>
<td>Preference to spawn in running waters.</td>
<td>hab</td>
<td>all except nsp, percms</td>
<td></td>
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<tr>
<td>Repro-LITH</td>
<td>Fish spawn exclusively on gravel, rocks, or pebbles.</td>
<td>repro</td>
<td>all</td>
<td>nsp percms percms nsp, percms dens, biom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atroph-OMNI</td>
<td>Adult consists of more than 25% plant material and more than 25% animal material. Generalist.</td>
<td>troph</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atroph-INSV</td>
<td>Insectivorous species.</td>
<td>troph</td>
<td>all</td>
<td>nsp percms biom</td>
<td></td>
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36
### Table 6

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<th>Fish metrics</th>
<th>Intercept</th>
<th>Drainage size</th>
<th>Slope</th>
<th>Tmin</th>
<th>Trange</th>
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<tr>
<td>Number of native species</td>
<td>1.263</td>
<td>0.048</td>
<td>0.136</td>
<td>0.213</td>
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<td>Density of intolerant species to oxygen depletion</td>
<td>5.63</td>
<td>0.084</td>
<td>-0.925</td>
<td>1.039</td>
<td>0.899</td>
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<td>Biomass of intolerant species to water quality degradation</td>
<td>7.001</td>
<td>-0.281</td>
<td>0.65</td>
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<td>Biomass of intolerant species to habitat degradation</td>
<td>5.45</td>
<td>-0.074</td>
<td>0.765</td>
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<tr>
<td>Density of rheophilic species</td>
<td>3.028</td>
<td>-0.032</td>
<td>1.317</td>
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<td>Biomass of lithophilic species</td>
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<td>-0.023</td>
<td>0.65</td>
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<td>Percentage biomass of insectivorous species</td>
<td>4.287</td>
<td>0.055</td>
<td>-0.001</td>
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### Table 7

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<th>Land use</th>
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<th>Water quality</th>
<th>Hydrology</th>
<th>Morphology</th>
<th>Hydro-morphology</th>
<th>Biological pressure</th>
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<td>Number of native species</td>
<td>-0.463**</td>
<td>-0.174</td>
<td>-0.422**</td>
<td>-0.377**</td>
<td>-0.418**</td>
<td>-0.303*</td>
<td>-0.276*</td>
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<td>Density of intolerant species to oxygen depletion</td>
<td>-0.335*</td>
<td>-0.227*</td>
<td>-0.421**</td>
<td>-0.249*</td>
<td>-0.294*</td>
<td>-0.257*</td>
<td>-0.018</td>
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<tr>
<td>Biomass of intolerant species to water quality degradation</td>
<td>-0.641**</td>
<td>-0.147</td>
<td>-0.546**</td>
<td>-0.496**</td>
<td>-0.567**</td>
<td>-0.404*</td>
<td>-0.298*</td>
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<tr>
<td>Biomass of intolerant species to habitat degradation</td>
<td>-0.640**</td>
<td>-0.145*</td>
<td>-0.545**</td>
<td>-0.496**</td>
<td>-0.565**</td>
<td>-0.402*</td>
<td>-0.295*</td>
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<tr>
<td>Density of rheophilic species</td>
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<td>-0.275*</td>
<td>-0.471**</td>
<td>-0.398**</td>
<td>-0.403**</td>
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<tr>
<td>Biomass of lithophilic species</td>
<td>-0.650**</td>
<td>-0.054</td>
<td>-0.523**</td>
<td>-0.444**</td>
<td>-0.492**</td>
<td>-0.330*</td>
<td>-0.338*</td>
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<tr>
<td>Percentage biomass of insectivorous species</td>
<td>-0.463**</td>
<td>-0.308*</td>
<td>-0.391**</td>
<td>-0.423**</td>
<td>-0.390**</td>
<td>-0.374*</td>
<td>-0.149</td>
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** Correlation is significant at the 0.001 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Acknowledgements

Though only my name appears on the cover of this dissertation, a great many people have contributed to its production. I owe my gratitude to all those people who have made this dissertation possible and because of whom my graduate experience has been one that I will cherish forever.

There are no proper words to convey my deep gratitude and respect for my thesis and research advisor, Ao. Univ. Prof. DI Dr. Stefan Schmutz, the head of the Institute of Hydrobiology and Aquatic Ecosystem Management, “for his continuous kind fatherly support and sympathy regarding my PhD study and the unpleasant events that have unfortunately happened to me in my private life during my study in Vienna” also “for his patience when I was not knowledgeable enough at the beginning of this field study as it was very new for my country”, and “for his motivation, enthusiasm, and immense knowledge”. To be his PhD student was a dream that came true.

I would also like to express my sincere gratitude to Dr. Rafaela Schinegger and DI. Carina Mielach who were as kind sisters to me and DI. Kurt Pinter who was as a brother to me and all the time they were following up the progress of the project and my situation. They were also giving me effective consultations; assisting me and warmly guiding me regarding the scientific and non-scientific issues all the time.

My sincere thanks should go to exam committee: Ao. Univ. Prof. Dr. phil. Herwig Waidbacher, Prof. Robert Hughes, Prof. Dana Infante and Prof. Hamid Reza Esmaeili for the generously of dedicating their time in order to provide me valuable comments toward improving my work.

I am very grateful to Dr. Andreas Melcher, DI. Florian Pletterbauer, Dr. Clemens Trautwein, Dr. Günther Unfer for sacrificing their time and revising my articles; commenting on my views and helping me understand and enrich my ideas.

I would like to deeply acknowledge Prof. Brian W. Coad from Natural Museum in Canada and Prof. Abdolrassoul Salman Mahini from Agricultural Sciences Natural Resources University of Gorgan; our international project collaborators who kindly and freely delivered their valuable information and data regarding Iran, to me.

I would like to acknowledge O.Univ.Prof. Dr. phil. Mathias Jungwirth, Ao.Univ.Prof. Dipl.-Ing. Dr. nat. techn. Susanne Muhar, Prof. Dr. Dr. Erwin Lautsch (from Berlin), DI. Bernhard Zeiringer, DI. Helga Kremser, and other scientific staff in our institute in Vienna for their supports and helps.

My very sincere thanks to Prof. Liaghati, the Dean of the Research, Sciences and Environmental Institute of Shahid Beheshti University in Iran who is very intellectual and supportive professor. He kindly provides opportunity for young researchers to show their ability and being successful. He is as an older brother to me.

My special thanks to Prof. Ghobadian, Prof. Shahbazi, Prof. Kiabi and Prof Abdoli, DI. Henric Majnonian who kindly supported and encouraged me to find my scientific way.

My special thanks to my Iranian Scientific Counsellor in France; Prof. Qorbani and former counsellors Prof. Qorbani, Prof. Abdollahi, Prof. Meshkatoddini, as well as dear Mr. Nazemi, our Responsible Administrative-Financier in France. As well as Mrs. Khodami, Mrs. Farokhnejad and the rest staff working in the Ministry of Sciences, Research and Technology in Iran. In addition, my sincere thanks to Dr. Malekotifar and Mrs. Hashemi our person in
charge at the Iranian embassy, Vienna.

I am also indebted to my friends who supported me during my fish sampling in Iran and collecting data there: Dr. Majid Bakhtiyari, Dr. Azad Teimori, Dr. Ahmad Reza Mehrabian, Dr. Saber Vatandost, Dr. Ebrahim Fataei, Dr. Keyvan Abbasi, Mr. Gholam Reza Amiri Ghadi, Mr. Hamid Reza Bagherpour, Mr. Arash Arsham; as well as the whole environmental office directors and staff who issued the permission for the fish sampling, kindly accompanied me in the field work and provide accommodation for us e.g. Dr. Lahijanzadeh, Dr. Dariush Moghadas, Dr. Valinejad, Dr. Afkhami as many others. My special thanks also to my very supportive and cooperative flatmate Dr. Meysam Ebrahimi.

I am also grateful and very thankful to Prof. Blum, Dr. Ali Moghadam and Dr. Zahra Ghelichipour who helped me to adjust myself to the new environment; my second homeland: Austria.

I am also thankful to the system staff who maintained all things so efficiently that I never had to worry about them. I never forget their favor to me. My dears: Christian Dorninger, Martin Seebacher, Franziska Schmuttermeier and Erika Thalerto.

I am also grateful to the following former or current staff in the Research, Sciences and Environmental Institute of Shahid Beheshti University, Tehran-Iran, for their support. Prof. Khoshbakht, Prof. Majid Mahdavi Damghani, Majid Zohari, Mohammad Abdi and Maryam Foomezhi.

I also deeply acknowledge the financial support of FWF (Austrian Science Fund) in Austria that funded the entire research as well as the Ministry of Science, Research and Technology of Iran that awarded me the scholarship to provide my living costs in Vienna. Certainly without their financial support this research could not be achieved its objective to the end.

Last, but not least, I thank my beloved family to whom this dissertation is dedicated to, who has been a constant source of love, concern, support and strength all these years. I would like to express my heart-felt gratitude to my family. My family has aided and encouraged me throughout this procedure. I have to give a special mention for the support given by father, my mother, my brothers Abdolhakim and Ali, my sister in laws Parastoo and Ehsaneh and my lovely nephews and niece: Amir Ahmad, Mohamad Mehdi and Fatemeh. One more time, I warmly appreciate the generosity and understanding of my extended family.

Vienna, 01. November 2014
Hossein Mostafavi