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PRIMARY RESEARCH PAPER

Assessment of an index of biological integrity (IBI) to quantify the quality of two tributaries of river Chenab, Sialkot, Pakistan

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Abstract Nullah Aik and Nullah Palkhu, two tributaries of river Chenab, were investigated for the assessment of fish habitat degradation as indicator of stream health. Fish abundance data were collected from 18 sites from September 2004 to April 2006 to develop multimetric indices for fish assemblage integrity and to detect the intensity of habitat degradation. A total of 12 metrics were calculated on the basis of taxonomic richness, habitat preference, trophic guild, stress tolerance and origin of species to develop stepped and continuous index of biological integrity (IBI) criteria. Cluster analysis (CA) classified sites based on species composition into three groups, viz., reference, moderately impaired and impaired groups. Non-metric multidimensional scaling (NMDS) was applied to identify underlying ecological gradient to highlight the habitat degradation. NMDS segregated two sites as less impaired, five sites as moderately impaired and eleven sites as impaired groups. Axes 1 and 2 explained a total variation of 53.3%. First axis explained the level of habitat impairment, whereas axis 2 indicated species richness along longitudinal gradient of streams. Sites located upstream of Nullah Aik showed higher IBI scores which dropped to its lowest in downstream sites near Sialkot city. Lowest values of IBI of sites in close proximity of city indicated the role of anthropogenic activities in catchment areas. The results indicated that variability in water chemistry can be related as a function of stream sites impairments (i.e., unimpaired, moderately impaired, and severely impaired). Water quality parameters showed strong correlation with IBI scores. Significantly negative correlation of IBI scores with COD, TDS, turbidity, Fe, Cr, Zn and positive correlation with DO and pH was found. The results can be used for restoration and future management of small streams passing through urban areas of Pakistan.

Keywords Fish communities · Heavy metals · Industrial and municipal waste · Stream health · Sialkot · Tanneries

Introduction

Streams and rivers are facing number of environmental problems throughout the world largely associated with anthropogenic activities in their catchment areas (Young et al., 2004). The adverse effects of human

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activities have resulted in degradation of stream and riverine ecosystem (Schleiger, 2000) which ultimately alter the structure and function of stream biota (Stoddard et al., 2006). This is more critical in developing countries (Bozzetti & Schulz, 2004) and in particular, in those areas where streams are passing through urban areas with large industrial activities (Qadir et al., 2008). Number of factors has been reported which have direct link with stream and river degradation. Physical and chemical factors such as flow, depth and width of stream, light, turbidity, temperature, organic and inorganic carbon, oxygen, nutrients, and metals may be related directly or indirectly to biological changes (such as absence of native species, intrusion of non-native species, decline in diversity and productivity, loss of integrity in fish assemblage, etc.) and may contribute in complexity and integrity of streams and rivers (Mullins, 1999). The biological response and sensitivity of different organisms/communities to such physical or chemical changes can be used as an indicator for the assessment of habitat quality (Karr, 1991). Biological indicators have significant importance in evaluation of environmental degradation due to anthropogenic activities and have long history of their use (Simon & Lyons, 1995). The use of biological indicators in freshwater ecosystem is becoming popular (Davis & Simon, 1995; Joy & Death, 2004). Biological indicators may reflect the intensity of anthropogenic stress and have been used as a tool in risk assessment and evaluation of human induced changes in freshwater ecosystem (Toham & Teugels, 1999).

Assemblages of freshwater organisms such as fish, macro-invertebrates and periphytons which respond to both environmental contaminants and habitat changes are commonly used to assess the biotic condition of streams, lakes and wetlands because the integrity of these assemblages provides a direct measure of ecological conditions of these water resources (Ode et al., 2005). Fish have been regarded as an effective biological indicator of environmental quality and anthropogenic stress in aquatic ecosystems (Fausch et al., 1990; Simon, 1991) not only because of its iconic value, but also because of sensitivity to subtle environmental changes (Karr, 1981) and represents a wide range of tolerance at community level (Plafkin et al., 1989). Fish is sensitive to changes in water chemistry due to different anthropogenic activities from their catchment. Fish responses to environmental disturbances, including hydro-morphological factors are different in time and space in comparison to simpler organisms, as they tend to be integrated over larger intervals. Fish has been identified as suitable for biological assessment due to its easy identification and economic value (Simon & Lyons, 1995; Smith et al., 1999; Siligato & Böhmer, 2001). Fish assemblages have widely been used as ecological indicators to assess and evaluate the level of degradation and health of rivers and streams at various spatial scales (Zampella et al., 2006). Plafkin et al. (1989) observed that there are many advantages of using fish assemblage as biological indicator. Most of the streams exhibit variations in different sections especially from upstream to downstream and these variations become more remarkable in streams facing problem of habitat degradation particularly from direct discharge from untreated industrial and municipal effluents (Qadir et al., 2008). Upstream sections are generally less degraded with relatively less changed physical, chemical and biological conditions of habitats (Drake, 2004). Sites located in upstream sections have generally been considered as reference sites and can be compared with rest of sites for assessment of stream health (Joy & Death, 2004). Fish assemblages may differ on longitudinal gradient in streams according to various biological aspects such as species diversity, stress tolerance, habitat preferences, feeding behaviours and origin of species (Ganasan & Hughes, 1998). These variations depict the level and severity of degradation in stream health.

Both multimetric and multivariate methods have been used to characterize biotic conditions and to establish thresh holds of ecological impairment (Ode et al., 2005). The index of biological integrity (IBI) is most commonly used to assess stream health based on biological criteria (Mebane et al., 2003). It is an index for quantitative assessment of the biological quality/health of streams (Ganasan & Hughes, 1998) and was formulated to integrate information from various aspects of an ecosystem into numerical value that indicate the quality rating for streams, rivers, lakes, etc. (Ganasan & Hughes, 1998). This criterion has been widely used to assess and categorize the condition of streams and rivers by studying the structure and composition of fish assemblage (Simon & Lyons, 1995; Bozzetti & Schulz, 2004). The concept of IBI was developed by Karr (1981) to assess the ecological status of streams and rivers in mid western United States and has been widely applied in other parts of the world with modification, either addition or deletion of biological metrics, depending upon biological conditions of an ecosystem (Ganasan & Hughes, 1998). Ganasan & Hughes (1998) applied IBI to determine level of degradation in two rivers such as Khan and Kshipra in India. Fausch et al. (1990) and Simon & Lyons (1995) applied IBI criteria to study lakes, streams and rivers with some modification based on regional, geographic and climatic variation in fish distribution and assemblage structure. Toham & Teugels (1999) used IBI to quantify the impact of industrial deforestation on freshwater biodiversity in Central African tropical streams. Kleynhans (1999) developed IBI in the form of fish assemblage integrity index (FAII) using metrics to explain the habitat quality of fish assemblages in different segments of Crocodile River, South Africa. Araújo (1998) used IBI to recognize the impacts of the effluents (heavy metals, pesticides and organic solvents) on fish assemblage from industrial areas into *Paraıba do Sul* River. Bozzetti & Schulz (2004) highlighted the ecological conditions of subtropical streams using IBI in southern parts of Brazil. Fish-based IBI was used to assess upstream brooks in Flanders, Belgium (Breine et al., 2004). River conditions at varying spatial scales were also evaluated in New Zealand (Joy & Death, 2004). In central India, Ganasan & Hughes (1998) studied the impact of municipal and industrial effluents on fish assemblage based on IBI criterion.

This study is the first effort where IBI was developed and applied to assess the biological health of streams in alluvial plains of Pakistan. To our information there is no such study exist in literature where IBI was used to assess the ecological integrity of urban streams of highly industrial city: the Sialkot known for its tanning industry. Conservation of freshwater streams of Sialkot district present a daunting challenge for environmental managers due to its rapidly increasing human population, urbanization, agricultural and industrial activities (Qadir et al., 2008). The IBI criteria used in this study is a combination of 12 metrics of fish assemblage which are grouped into four categories, viz., species diversity and composition; trophic composition; and fish abundance and health (Ganasan & Hughes, 1998). IBI metrics were derived from previous studies conducted by various workers in different regions of the world on streams and rivers. IBI was developed for two tributaries of river Chenab, Nullah Aik and Nullah Palkhu, which are facing environmental degradation due to indiscriminant industrial, urban and agricultural pollution. These streams are located in densely populated and industrialized region of Punjab, Pakistan. Major activities in upstream region are characterized by agriculture sector, whereas industrial and municipal activities highly impacted the middle and downstream regions.

Main objective of the present study was to develop IBI for alluvial streams of river Chenab to assess the impact of anthropogenic activities on fish assemblage structure and to investigate the spatial variations of IBI in relation to biological conditions of streams and water quality parameters. The results will highlight the need for better future protection of fish fauna in small streams from anthropogenic activities. The initiative will be helpful to measure fish assemblage characteristics that are responsive to human-induced environmental changes. The present study is the first such attempt to develop IBI for tributaries of river Chenab, Pakistan for assessment of habitat quality of stream in terms of fish assemblage and will be a useful document for future studies in the same eco-region.

Methodology

Study area

The present study was conducted on Nullah Aik and Nullah Palkhu, two tributaries of Chenab River, Pakistan. These tributaries (streams) originate from Pir Punjal range in Jammu and Kashmir, entering into alluvial plain of Punjab covering a total distance of about 131.86 and 99.5 km before falling into the river Chenab (Fig. 1). Climate of the region is mainly subtropical with well defined rainy (July to September), autumn (October and November), winter (December and February), short spring (March and April), and a relatively dry pre-monsoon season (summer between May and June). The catchment area (1,762 km^2) of Nullah Aik and Palkhu receives annual rainfall of about 950 mm. Major share of rains is received during the monsoon season (July and September) causing frequent floods. During monsoon season, maximum discharge of inflowing water at site 2, 5

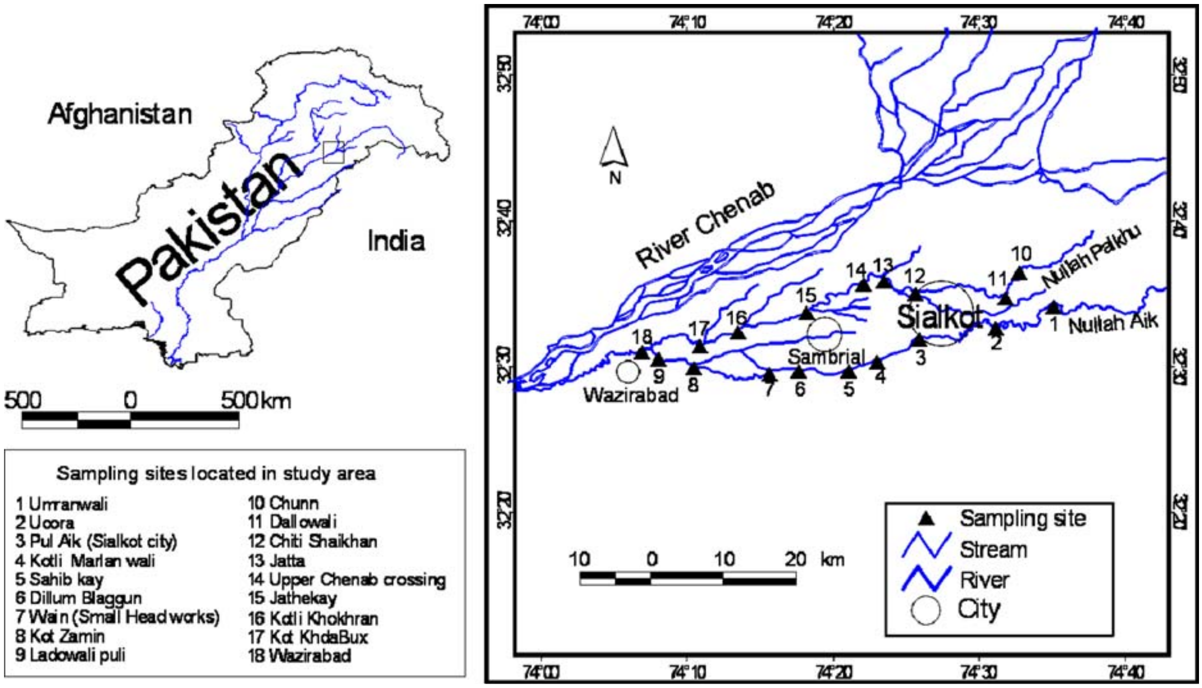

Fig. 1 Map of study area showing sampling sites located on Nullah Aik and Nullah Palkhu, tributaries of river Chenab, Pakistan

and 9 of Nullah Aik was 35,000, 5,000 and 1,500 Cs, respectively (Anonymous, 2000). Maximum discharge of Nullah Palkhu is 240, 388 and 1,346 (cusec/s) at sites 14, 15 and 18, respectively (Anonymous, 2000). Discharge capacity of Nullah Aik decreases from upstream to downstream. During monsoon season, downstream cannot support the huge volume of water resulting flood in downstream catchment. The elevation of the streams varied between 217 and 241 m. Both stream flowed permanently with mean stream width and depth of 5.10–30.64 (m) and 0.76–3.89 (m), respectively. Upstream region was relatively wider as compared to downstream. Highest average stream width was recorded in upstream region for sites 1 and 2 located on Nullah Aik. Average stream velocity varied between 0.37 and 1.1 (m/s). Most of sediments were loamy; however, sandy soils were more prevalent in upstream sites of Nullah Aik. Macro-habitat types (such as pool, riffle and run) were identified at different sampling sites (Appendix 1—supplementary material).

Agriculture is the predominant land use but varies along the upstream and downstream catchment. Most of the land in upstream and downstream is under intensive cultivation and major crops grown are wheat and rice. However, fodder crops and vegetables are also cultivated to fulfil the local demands (Qadir et al., 2008). Apart from rain feeding, three kinds of irrigation practices such as tube well irrigation (water from aquifer), stream water irrigation by pumping (hundreds of pumps have been established along the sides of streams) and diversion of water into small distributaries (this type of irrigation is restricted to the surroundings of Begowala town) are common. Stream banks were dominated by tall tufted grasses along upstream region. Woody riparian vegetation of deciduous trees typical of sub-tropical zone (Malik & Husain, 2006) was found downstream of Nullah Aik. In contrast, vegetation in downstream region of the Nullak Palkhu comprised short and tall grasses with scattered scrubs.

Upstream of Nullah Aik and Nullah Palkhu from Sialkot city in general, exhibit good water quality throughout the year. According to Qadir et al. (2008) source of surface water contamination in upstream region are mainly related to agricultural activities in the catchment and deterioration of stream water quality in middle segment of the streams is due to large input of direct discharge of untreated industrial effluent and municipal waste from Sialkot city. Industrial and municipal waste contains high quantity of pollutants such as Chemical Oxygen Demand (COD), soluble salts, nutrients and heavy metals. Inflow of toxic effluents result in low level of dissolved oxygen (DO) which makes water unsuitable for fish fauna. No fish was reported during field studies from middle segment of these streams. Water quality improves in downstream region due to joining of small freshwater streams to these tributaries. Improvement in water quality and fish community was observed in Nullah Palkhu as compared to Aik. Small channels which bring water from water logged areas located around irrigation canals play vital role in re-establishment of fish fauna in downstream segments of Nullah Palkhu. In case of Nullah Aik, a small head work is constructed to divert water for irrigation purposes which causes reduction in downstream water.

Major anthropogenic activities in the catchment that influence the physical, chemical and biological characteristics of the streams are industrial, municipal and agricultural activities. Point and non-point contaminants are major sources of pollution to these river tributaries. Point sources are tannery effluents, industrial effluents and municipal waste, whereas atmospheric deposition, urban and agricultural runoff are non-point sources. These streams are disproportionately degraded by water pollution, agricultural land use and irrigation farming practices, head work construction and over-fishing. These anthropogenic activities have been recognized as major contributors which effect freshwater resources of Sialkot district (Qadir et al., 2008). Sialkot is an important foreign exchange earner city of Pakistan known for its leather garments, surgical instruments and sports good production worldwide (Kazmi, 1995) is situated in catchment of these streams. Major industrial activities are concentrated in and around the urban area and along the major roads/highways. The industry of Sialkot mainly consists of small and medium industrial units that are scattered throughout the city. A total of 3,229 different industrial units had been established in Sialkot (http://en.wikipedia.org). Among these more than 264 tanneries are the most important and more tannery units have been expected to be established in near future (Mehdi, 2005). Leather sector is contributing a major share in exports of Sialkot. Tanning is a basic and essential step in the production of finished leather product that takes place in tanneries. Unfortunately, no industrial waste treatment plant has been set up in this region. All tanneries and other industrial units in Sialkot and nearby cities such as Sambrial and Wazirabad indiscriminately discharge untreated industrial effluents along with domestic sewage into streams which ultimately drain into river Chenab. The indiscriminate discharge of untreated industrial and municipal effluents has recognized a continuous threat for freshwater ecosystems of district Sialkot (Qadir et al., 2008).

According to population and census organization, Pakistan (2000), estimated population density of catchment area of Nullah Aik and Palkhu is 903 persons/km^2, making it one of the populous areas of Pakistan. According to an estimate, there are ~1,000,000 domestic animals including cattle, buffaloes, horses, donkeys, sheep and goats in rural areas.

Fish and water sampling

A total of 18 sampling sites were located in Nullah Aik and Nullah Palkhu, which were selected on the basis of existing information about the point source of pollution, human habitation, agricultural and drainage pattern. The study reach of each stream was about 65 km and the distance between the sites varied between ~4 and 12 km (Appendix 1—supplementary material). A total of nine sites were selected on each stream. Out of these nine sites two sites were located in upstream of Sialkot city, while seven sites in downstream side (Fig. 1). Sites facing least disturbances from anthropogenic activities were treated as reference sites after preliminary survey during June 2004. These sites were only under stress from agricultural runoff as no other activity was identified in their catchment area. During preliminary survey, every sampling site was marked with a Global Positioning System (GPS) for re-identification at the time of next sampling.

Fish sampling was carried out during pre- and post-monsoon season from September 2004 to April 2006. The sampling activities were started early in the morning usually commencing from 5:00 am to 12:00 pm. Three sites were sampled per day and whole sampling schedule lasted for 1 week. Before going in field for fish sampling, a detailed weather forecast for study area was studied to avoid the rainy days. Sampling was conducted during sunny days when stream flow was at normal level. All fish samples were collected using variety of fishing nets of varying mesh sizes as suggested by Bhat (2003); seine net (8 m × 1.5 m with mesh size of 1 cm), cast nets (circumference 15 m with 1.5 cm mesh size) and dragnets (7 m × 1 m with mesh size of 1.5 cm) within 100 m range from each sampling site. Seine net was used to sample fish in water with depth less than 1.5 m, whereas cast and dragnets were used where depth of water was more than 1.5 m. The intensity of sampling for each site was standardized as 20 efforts for cast net, 3 h duration for gill net and 10 trials for drag net Bhat (2003). All sampling sites of the streams were intensively sampled in an effort to capture as many fish species as possible in proportion to their abundance (Matthews, 1985). After capturing fish, specimens were immediately transferred into tubs filled with stream water to reduce chances of fish mortality. Sampling crew consisted of minimum of four persons who handle the netting process. The whole process of sampling was done very carefully with drag net traversing the distance from downstream to upstream. Few specimens of fish that could not be identified in the field were preserved for purpose of further identification and processing in the laboratory, while rest of specimens were released back into the stream water (Ganasan & Hughes, 1998). Released fishes were only native, whereas exotic fishes were not returned back to the stream. The juvenile fishes, which have tail length less than 20 mm, were excluded because of inadequacy in their capturing (Helms and Feminella 2005). Taxonomic identification and classification was done on the basis of morphometric characteristics up to the species level. All fishes were identified to species, counted and examined for external anomalies. Fish species were identified following regional keys (Talwar & Jhingran, 1991; Mirza, 2003; http://www.fishbase.org).

The information about various ecological aspects of a fish species was extracted from existing local literature (Talwar & Jhingran, 1991; Jayaram, 1999; Mirza, 2003; http://www.fishbase.org). Fish species were classified into different ecological groups on the basis of taxonomic diversity, habitat preference, stress tolerance (intolerant, moderately intolerant and tolerant species), feeding habits and origin of species (native and exotic species). The column and bottom feeder were included into the category of habitat preference Fish feeding habits were determined by the following into four trophic groups (herbivore, omnivore, invertivore and carnivore) on the basis of their feeding preferences (Matthews, 1998). Another important parameter related to the biological health streams, i.e. infestation of exotic species was incorporated on the basis of origin of species. Higher number of the exotic individual and species indicates the ecological degradation of stream ecosystem (Moyle et al., 2003).

Sub-tropical and temperate streams are highly variables in fish diversity in different seasons throughout the year (Ganasan & Hughes, 1998). It was highlighted that fish composition of a single season gives inadequate information to evaluate the scores for IBI metrics. Usually seasonal changes in fish assemblages are not considered (Bozzetti & Schulz, 2004), since Karr et al. (1986) in his first IBI study (1981) did not find temporal dependence. Bozzetti & Schulz (2004) highlighted that seasonal differences in species composition do not reflect to influence general IBI results. Other studies of temporal variability in IBI score have also indicated minimal effects from among years and within season fluctuations in species composition (Hughes et al., 1998; McCormick et al., 2001). Pyron et al. (2008) addressed within temporal variation of IBI scores for two sampling event and indicated non-significant differences in IBI scores. Therefore, all fish individuals captured during different seasons from same site were used for analysis (Ganasan & Hughes, 1998). Similarly, Kushlan (1976) also supported pooling up seasonal species composition collected from the same site for further analysis and suggested that information related to species composition collected during one season may lead to inaccurate assessment of stream health. Each individual site should be sampled for fish species abundance many times in year, especially when the life history of different fish species is less known (Lowe Mc Connell, 1987). Long & Walker (2005) also combined the data collected from a site to avoid seasonal and inter-annual variations for further analysis.

From each site that fish was captured surface water samples were also collected. A composite water sample was made by combining a series of samples taken 30 cm below the water surface, within 100 m range of a sampling site. Water sampling container was drained into sampling bottles up to the mouth without trapping any bubble. The water samples were preserved by adding 1 ml of nitric acid (Analytical grade) at $pH < 2$ and stored at 4°C (to minimize deterioration prior to chemical analysis) for the analyses of metal ion concentrations. All the analyses were carried out according to the standards of APHA (1998). Chemicals and standard solutions used in the study were obtained from Sigma/Fluka and were of analytical grade.

For each water sample quality parameters such as Temperature, pH, Dissolved Oxygen (DO), COD, Total Dissolved Solids (TDS), Turbidity, Nitrate (NO_3^-), orthophosphate (PO_4^{3-}) and heavy metals, viz. Fe, Pb, Cd, Cr, Ni, Cu and Zn were analysed. DO, pH and TDS of the water samples were measured at the spot. DO was measured with DO meter (970 DO_2 Meter IP65 Jenway Essex, UK), pH with portable pH meter (Thermo Orien 240A) and TDS by conductivity meter (Hi 8033 Hanna Hungry). Water quality parameters, viz. COD, turbidity, nitrate (NO_3^-), orthophosphate (PO_4^{3-}) and heavy metals were determined in the laboratory. COD (mg/l) was determined by using COD kits (HACH spectrometric methodology). Turbidity (NTU) of water samples was calculated using Turbidity meter (Model HACH 2100 A). PO_4^{3-} and NO_3^- were determined spectrophotometrically using the Ammonium molybdenum method and phenol-disulphonic acid method (Allen et al., 1974). The water samples for total metal analysis were digested on hot plate in Environmental Biology Laboratory (EBL) at Quaid-i-Azam University, Islamabad, using mixture of nitric and perchloric acids (USEPA, 1990). Trace metal analysis was carried out using a Fast Sequential Atomic Absorption Spectrometer (Varian FS 240AA). The metal standards prepared were checked with standard reference material obtained from Fluka and the deviation found was insignificant. Average values of three replicates were taken for each determination. The precision of analytical procedures, expressed as the relative standard deviation ranged from 5 to 10%.

Selection of reference sites

Reference condition of aquatic water resources such as streams and rivers generally reflects natural conditions with minimal disturbance from anthropogenic activities. Reference condition explains the undisturbed biological, chemical and physical components of a site or group of sites. In ecological assessment of freshwater resources, identification of reference sites is vital to highlight, the impact of human activities on aquatic ecosystem (Zhu & Chang, 2008). Identification of reference sites helps in understanding the relationship between human disturbance and biological condition of a stream (Fritz, 2004) and to scale bio-metrics appropriately. Reference sites are the segments of water bodies that reflect the natural conditions and are least affected by anthropogenic activities (Reynoldson et al., 1997). The choice of an appropriate reference condition is a major problem in all ecological assessments (Hughes, 1994). The knowledge of metric responses to human impact is also appropriate for the selection of reference conditions. The reconstitution of a reference fish assemblage based on historical data (Hughes, 1994) is impossible in the study area. Identification and selection of reference sites in present study was difficult as the catchment area of Nullah Aik and Nullah Palkhu is densely populated and its fish fauna is poorly known because ecological and historical information about fish assemblage is extremely scarce/absent. Quantitative physical, chemical as well as biological standards are needed against which streams/rivers in the region can be compared to determine the influence of cumulative stressor (Radwell & Kwak, 2005). Selection of reference sites was done based on evaluation of the biological, physical habitat and water quality data of streams as suggested by USEPA (2006) and Springe et al. (2006). Similarly, Zhu & Chang (2008) also used cumulative data set based on physical, chemical and biological conditions of upper Yangtze River to define reference sites for calculation of biological integrity. There exists no information with regard to reference site identification in Pakistan and there is need to develop reference condition for streams and rivers. In present study, sites with minimal disturbance or least impairment or best conditions in terms of fish communities in Nullah Aik and Nullah Palkhu were treated as reference sites. Reference sites possess a maximum IBI score (Bozzetti & Schulz, 2004) which was further compared with other sites located on the same stream or sites located on other streams of the same ecoregions (areas of homogenous ecological systems or areas that have the potential for similar biological communities) to quantify the anthropogenic stress on biological integrity of the ecosystem. Selection of least-disturbed/impairment sites was further confirmed by the application of biological (fish) data to cluster analysis (CA).

Statistical analysis

Assessment of habitat degradation and identification of reference sites in relation to underlying ecological gradients

Descriptive statistics such as means and standard deviation of physiochemical parameters of water samples of all sites was calculated using Statistica 5.5 (Softstat Inc., 1999). Cluster analysis was used to group the sites on the basis of similarities within fish assemblage and for identification of reference sites. Ward's method was selected as a linkage method and Euclidean matrix as dissimilarity distance measure. Same method has been used to characterize fish abundance data by McCune & Grace (2002) and Bozzetti & Schulz (2004) into classification of sites into groups with different level of habitat degradation. Non-metric multidimensional scaling (NMDS) ordination was calculated to highlight the intensity of stress on fish abundance along longitudinal gradient and to confirm the sampling sites identified as reference sites using CA. Pair-wise similarity metrics was used in ordination analysis to calculate the stress level between the similarity matrix and ordination axes (Clarke & Warwick, 1994; Casatti et al., 2006). CA and NMDS ordination analysis was calculated using Statistica 5.5 (Softstat Inc., 1999) and PC ORD ver. 4.0 (McCune & Mefford, 1999) respectively.

Calculation of index of biological integrity (IBI)

Biological integrity which encompasses compositional, structural and functional levels of organization refers to the ability of a biological system to function and maintain itself and ultimately to evolve in the face of changes in environmental conditions (Angermeier & Karr, 1994). Biological integrity has played an important role in an assessment of environmental health and in bio-monitoring of environmental degradation. IBI is a multimetric approach which uses combination of metrics (Mebane et al., 2003) for biotic health of streams suffering with anthropogenic activities and was developed to assess the health condition of studied streams. Biological indices based on fish have become common tool in ecological monitoring (Scardi et al., 2008). While (multi-metric) biotic indices are commonly used, even the most successful ones have been criticized due to lack of their diagnostic capacity, unpredictable interactions between different metrics and the inherent circularity of selecting indicators that are supposed to respond to anthropogenic stressors (Scardi et al., 2008). In the present study, different metrics were calculated using biological information of fish species either with help of literature and personal experiences. Twelve different metrics relevant to assessment of anthropogenic impacts on fish communities were calculated based on structure, composition and assemblage pattern of fish species. IBI scoring criteria were modified as proposed by Ganasan & Hughes (1998). IBI metrics were categorized into five main groups, viz. fish species diversity, habitat preference, stress tolerance, trophic composition and origin of fish species (Table 1).

Species diversity metrics were calculated to express fish species diversity which includes number of native fish species, total number of fish families and total number of individuals of species. Metrics for number of native species were calculated as introduced by Steedman (1988) by replacing the total number of fish species used by Karr (1981). Metrics of number of native species and families have been used as an indicator of anthropogenic stress on fish assemblage by different authors. Sometimes long-term exposure to anthropogenic activities in the catchment may cause complete elimination of a fish family or families from particular stream/river (Oberdorff & Hughes, 1992; Ganasan & Hughes, 1998). Therefore, matrix of number of native families was included as described by Ganasan & Hughes (1998). Matrix of total number of individuals of a species in the site which indicates species richness level and ecological conditions of an aquatic ecosystem was included without any modification as described by Ganasan & Hughes (1998) and Karr (1981).

Habitat preference metrics which relate habitat quality of the sampling sites in terms of water quality were calculated using number of benthic and water column species. Originally this matrix was used to incorporate number of darter species and sunfish species for mid-western streams of United States by Karr (1981). Later on Oberdorff & Hughes (1992) replaced these two metrics with number of benthic dweller species and number of column dweller

Table 1 Scheme for rating of index of biological integrity (IBI) metrics for Nullah Aik and Palkhu tributaries of river Chenab

S. no.	Major categories of metrics	Scoring criteria	Continuous IBI metrics score[a]		Stepped IBI metrics scores		
		Individual metrics	10 (best)	0 (worst)	5 (best)	3 (fair)	1 (worst)
1	Species diversity	Number of native species	15	0	>12	12–6	<6
2		Number of native families	8	0	>7	4–7	<4
3		Total number of individuals	306	0	>250	100–250	<100
4	Habitat preference	Number of bottom feeder species	4	0	>4	2–4	<2
5		Number of column feeder species	11	0	>9	4–9	<4
6	Stress tolerance	Number of intolerant species	4	0	>4	2–4	<4
7		Proportion of individuals as tolerant species	0	100	<40	40–70	>70
8	Trophic composition (%)[b]	Proportion of individuals as herbivore species	0	45	>40	20–40	<20
9		Proportion of individuals as omnivore species	0	28	>15	15–25	<25
10		Proportion of individuals as insectivore species	0	71	>40	20–40	<20
11		Proportion of individuals as carnivore species	100	20	<50	25–50	>25
12	Origin of species (%)[b]	Proportion of individuals as exotic species	0	18	<5	5–10	>10
13	Health of fish	Proportion of individual of with abnormalities	NA	NA	NA	NA	NA
	IBI score		80	0	>43	43–23	<23
	Integrity class		Less impaired	Highly impaired	Less impaired	Impaired	Highly impaired

NA Not applied in present research paper

[a] The range of continuous IBI method is between 0 and 10. The values for each metrics were obtained from ($n \times 10$). $n =$ individual IBI score for each category obtained through Eqs. 1 and 2

[b] Information extracted from Karr, (1986), Minns et al. (1994), Ganasan & Hughes (1998), and www.fishbase.com

species. Higher number of benthic and column dwellers in habitats is associated with good habitat quality and showed a decreasing trend with an increase in turbidity, pollutants, reduced oxygen content and organic matter (Hughes et al., 1998).

Fishes were grouped on the basis of stress tolerance into tolerant, moderately tolerant and intolerant by following Oberdorff et al. (2002). Intolerant species were present at stream segment exhibiting good water quality and absent at degraded segment of stream. Tolerant species were present at most of the sites ranging from least disturbed to moderately degraded sites dominant at moderately degraded sites. Moderately tolerant species were those species which were found at least degraded sites as well as moderately degraded sites but have preferences for least degraded sites. Two metrics related to stress tolerance were calculated to assess the sensitivity of different species, viz. number of intolerant species and proportion of individual as tolerant species. Number of intolerant species which provides information regarding the sensitivity of fish species to environmental degradation (Shinn, 2000) was calculated as described by Karr (1981).

Trophic composition related with trophic status of fish species was calculated based on literature and information extracted from www.fishbase.com. Four metrics such as proportion of individuals as herbivorous species, proportion of individuals as omnivorous species, proportion of individuals as insectivorous species and proportion of individuals as carnivorous species were included as measure of trophic composition of a fish assemblage based on their feeding patterns. Three metrics such as proportion of individuals as omnivorous, invertivorous and carnivorous species were calculated as described by Karr (1981). Presence of introduced species in a natural ecosystem is always problematic for the equilibrium of ecosystem (Howard, 2002) and metric for proportion of individuals as non-native species was calculated based on presence of introduced species to assess the infestation intensity of non-native species in study area.

Application of IBI

Two methods such as stepped and continuous scoring metrics were used to assess biological health of stream at 18 monitoring sites. The score values of different metrics calculated for the evaluation of stream conditions for studied streams are given in Table 1. Continuous IBI was calculated as proposed by Minns et al. (1994) and Ganasan & Hughes (1998) and ranged from 0 to 10 for metric score and 0–100 for IBI score. For all metric scores upper threshold value of 10 was used for least disturbed habitat (reference condition) and lower value of 0 was used for highly degraded habitat. Each metric score was calculated by a ratio of observed metric site score (O) and score at reference site (R) and multiplied with 10 (Bozzetti & Schulz, 2004). For the tolerant, carnivore and non-native metrics scoring criteria were reciprocal to all other metrics and calculated using Eq. 1.

$$\text{Individual IBI score (tolerant, carnivore and non-native species)} = \left\lfloor 10 - \left({}^{O}\!/_{R} \times 10 \right) \right\rfloor \qquad (1)$$

In case of these metric scores, highest score (10) was assigned to lowest proportions and lowest score (0) to highest proportions of tolerant, carnivore and non-native species. Overall continuous metric score for each site was computed by a ratio of total of metric scores and total number of metrics and was multiplied by 100. Stepped IBI scoring criteria was also used along with continuous IBI and derived from Ganasan & Hughes (1998). All metric scores were treated the same as discussed in continuous IBI. Each metric score was scored as 1 (impaired), 3 (moderately impaired) and 5 (less impaired) representing conditions of habitat that deviates from threshold level of reference sites (Fausch et al., 1990; Kleynhans, 1999). For the carnivores, tolerant and non-natives metrics scoring criteria were also reciprocal to all other metrics. Stepped IBI score for sites ranged between 0 and 50 and were calculated by the following Eq. 2:

$$\left\lfloor \text{Stepped Individual IBI Score} = {}^{a}\!/_{b} \right\rfloor \times 50 \qquad (2)$$

where a = stepped IBI score of individual metrics and b = total number of metrics

Classification of integrity classes

Many authors classified the integrity classes into three to five groups. For example Karr et al. (1986) grouped the IBI classes into five groups. These classes were categorized as very poor, poor, fair, good and excellent. Plafkin et al. (1989) classified the IBI qualitative classes into four groups, viz. non-impaired, slightly impaired, moderately impaired and severely impaired. In the present study for the qualitative assessment of sites in terms of IBI were categorized into three classes. As indicated by Ganasan and Hughes (1998) and Lyons et al. (2000), large number of classes may cause problem during restoration process for managers to decide for the management actions. Therefore, on the basis of overall IBI score all sites were categorized into three qualitative classes such as less impaired, moderately impaired and impaired as described by Ganasan & Hughes (1998). Overall IBI scores were ranged between 0–80 and 0–45 for continuous and stepped IBI classes, respectively. IBI scores for continuous integrity classes were characterized as impaired (<43), moderately impaired (43–63) and less impaired (>63). Overall IBI scores for three stepped integrity classes were ranged as impaired (<25), moderately impaired (25–35) and less impaired (>35).

Results

Classification of sites and species in clusters based on habitat degradation and identification of reference sites

CA identified three groups/clusters of sites based on spatial difference of fish abundance data (Fig. 2). Cluster 1 consisted of two sites (1 and 2) situated in

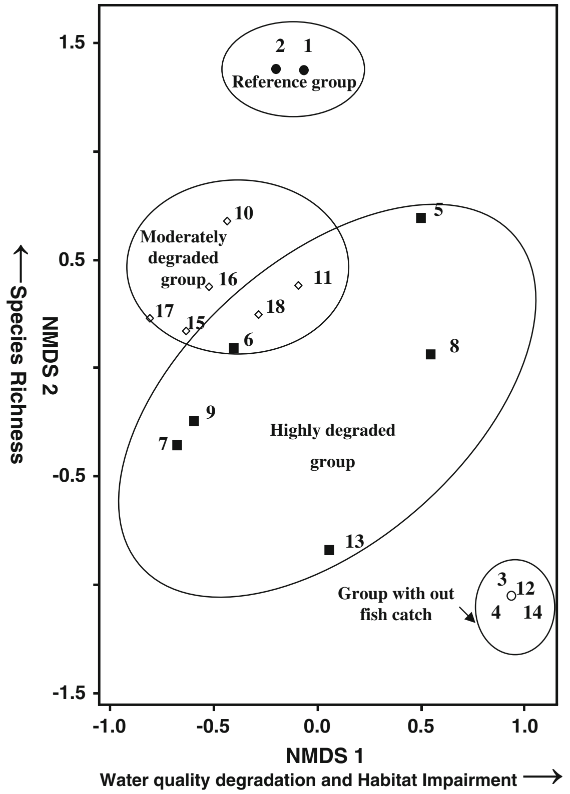

Fig. 2 Two-dimensional plot of sampling sites located on Nullah Aik and Palkhu showing the four groups based on ordination resulting from the NMDS based on a similarity Bray-Curtis coefficient

the upstream of Nullah Aik. This cluster was distinguished from other clusters on the basis of species diversity. Out of the total of 24 species recorded, a total of 58 and 63% species were recorded at sites 1 and 2 and constituted 39.7% of the total fish abundance recorded from all studied sites. Among water quality parameters highest average concentration of DO contents, lowest COD and dissolved metal content were recorded from these sites. No point sources of pollution such effluents from municipal and industrial waste was identified in their catchment and was relatively least disturbed with good water quality. Only agricultural activities were found in the surrounding area. These sites were comparatively more diverse, exhibited complex fish assemblage and reflected low level of habitat degradation. Highest stream width of 28 m was recorded to those of other clusters. These sites were also considered as reference sites because of least disturbance from anthropogenic activities. Cluster 2 comprised seven sites which includes 3, 4, 5, 8, 10, 12 and 14 with highly degraded habitat condition with minimum species diversity. These sites receive contamination from industrial and urban effluents and surface runoff from urban area. Water quality of these sites was found highly contaminated (Qadir et al., 2008). Lowest DO, highest values of COD, salts and heavy metals were recorded in this cluster. These parameters make the habitat unsuitable for fish species. Species richness ranged between zero catch and five species. Most of the sites in third cluster represented the zero fish catch from four sites situated in close proximity of the Sialkot city. These sites receive huge volume of industrial effluents (especially from tanneries) and domestic sewages from different point sources. Cluster 3 consisted of nine sites (6, 7, 9, 10, 11, 15, 16, 17 and 18) located in far downstream sites of Sialkot city and showed moderate degradation of habitat conditions. After traversing distance, water quality gradually become improved due to sedimentation of suspended solid and improvement of DO contents in stream water. The major human activity in the catchment area of these sites was agricultural practices along with scanty contribution of domestic sewage coming from small villages. These showed an improved ichtheo-fauna ranging from the four to 12 species. More number of fish species was recorded from sites located on Nullah Palkhu due to improvement of water quality through dilution process, whereas site located on Nullah Aik did not show improvement in species diversity due to presence of small head works.

Identification of ecological gradients

Figure 3 represented site ordination of NMDS which explained 73% of the total variance in relation to fish abundance at sites. First axis explained 39.8% of total variation whereas second and third axes accounted 13.5 and 10.8%, respectively. Two ecological gradients were defined along first and second axes. First axis provided information related to water quality degradation and habitat impairment. Degradation of water quality and habitat impairment increases from lower to upper side along the axis of the ordination plot. Highly impaired sites which were located in close vicinity of Sialkot city were found on the lower side and least impaired sites on the upper side of the

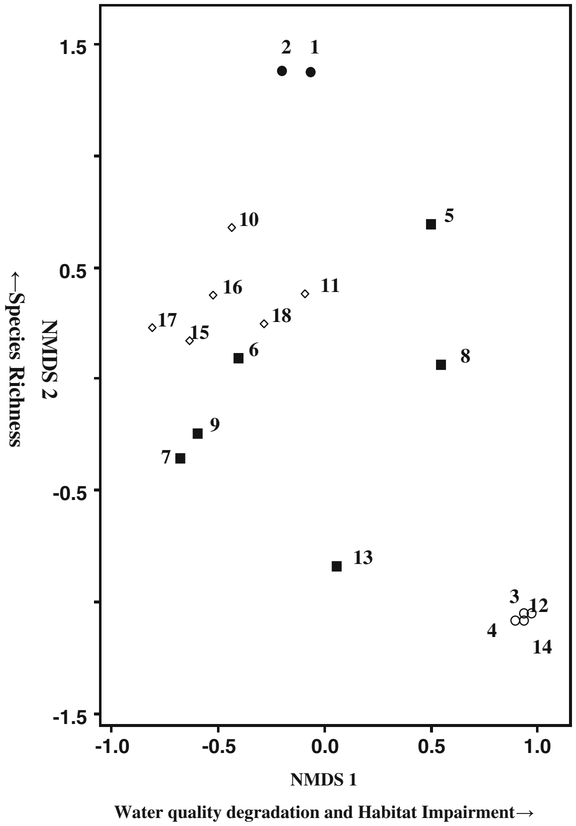

Fig. 3 Two-dimensional plot of sampling sites located on Nullah Aik and Palkhu showing the four groups based on ordination resulting from the NMDS based on a similarity Bray-Curtis coefficient

ordination plot. Second axis provided information about species richness along longitudinal gradient of streams (Fig. 3). NMDS grouped all sites into four groups. First group consisted of less impaired such as sites 1 and 2 with high species diversity. Second group was separated with 6–14 fish species richness sites (7, 9, 10, 11, 15, 16 and 17) and regarded as moderately impaired group. Third group consisted of site (5, 6, 8, 9, 13 and 18) which contributed less than six species and considered as impaired group. Fourth group was made up of sites (3, 4, 12 and 14) with zero catch of fish specimen and ranked as highly impaired group.

Species ordination plot explained distribution and association of different species dwelling in different habitat. Three groups of fish species (intolerant, moderately tolerant and tolerant) based on their ability to tolerate stress can be recognized (Fig. 4). First group consisted of intolerant species (*Labeo rohita*, *L. calbasu*, *L. dero*, *Osteobrama cotio*, *Sperata seenghala* and *Parambassis ranga*) inhabiting at upstream sites. Second group consisted of species (*Mystus cavasius*, *Wallago attu*, *Cyprinus reba*, *Xenentodon cancila*, *Cirrhinus cirrhosus*, *Puntius sophore*, *P. ticto* and *Oreochromis niloticus*) which were distributed in both upstream and downstream sites. *Channa punctatus*, *Heteropneustes fossilis*, *Notopterus notopterus* and *Polyacanthus fasciatus* were resistant/tolerant species residing at downstream sites and formed third group. Along first axis tolerant species group was clearly segregated on right side whereas other two groups showed overlap. Second axis explained the spatial gradient of species distribution. Two groups of species which had the preferences in upstream and downstream can be recognized along this axis. Intolerant species group was identified on the upper side of the ordination diagram, whereas other two groups showed overlap.

Fish abundance and assemblage in relation to its spatial distribution

A total of 24 fish species belonging to 12 families were identified from 18 sampling sites belonging to different habitats and trophic levels (Appendix 2—supplementary material). Five sites (3, 4, 12, 13 and 14) were recognized as the most polluted sites which do not support the fish fauna. Two sites (1 and 2) upstream of Nullah Aik were identified as the least polluted sites which were treated as reference sites. The results showed that at most of the sampling sites, native fish species dominated both studied streams. Reference sites (1 and 2) shared about 39.7% of the total relative fish abundance from all sites, whereas 61.3% individuals were collected from other 16 sites. Out of 24 fish species 22 species were native which contributed 93.4% of the total fish species. Maximum number of 15 native species was recorded from site 2. Similarly maximum number of native families was also recorded from site 2. Two non-native species such as *Oreochromis niloticus* and *Cyprinus carpio* were also recorded from different sites which contributed 6.3% of total fish species. Among these, *O. niloticus* was found in upstream and downstream sites of Nullah Palkhu, whereas *Cyprinus carpio* was recorded only from site 17 located at downstream of Nullah Palkhu. Among all fish species *Channa*

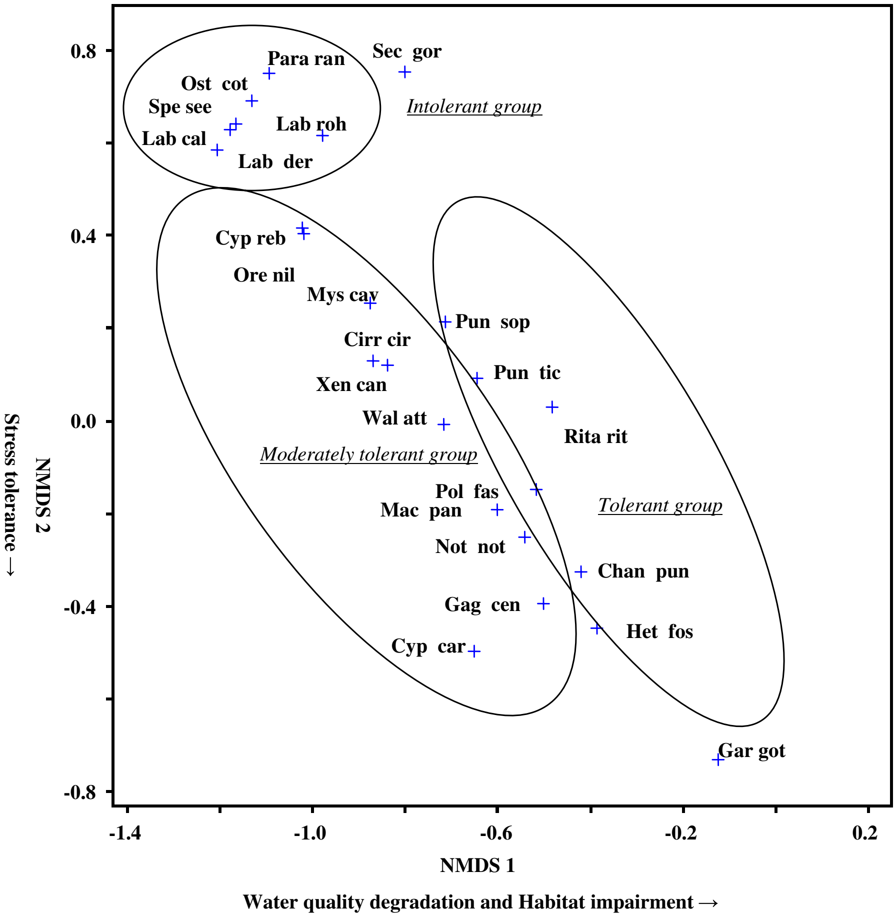

Fig. 4 Two-dimensional plot showing distribution of different fish species in Nullah Aik and Palkhu showing the four groups based on ordination resulting from the NMDS based on a similarity Bray-Curtis coefficient

punctatus was the most abundant species with relative abundance of 19.6% of the total fish diversity.

Five bottom dweller, 16 column dweller and two surface feeder species were recorded which contributed 30.1, 68.8 and 1.1% of total fish abundance data of both streams (Appendix 2—supplementary material). Majority of column feeder and bottom feeder species were confined to upstream of Nullah Aik and downstream of Nullah Palkhu. Four resistant species such as *Channa punctatus*, *Puntius sophore*, *P. ticto* and *Heteropneustes fossilis* were recorded which showed wide distribution from less impaired to impaired sites. These species shared about 54.1% of the total fish abundance data. Fish species which were restricted to upstream were categorized as intolerant/sensitive species. These species included *Labeo rohita*, *L. calbasu*, *L. dero*, *Osteobrama cotio*, *Gagata cenia*, *Sperata seenghala* and *Parambassis ranga* and contributed about 13.5% of the total fish abundance data. Intolerant species were confined to less impaired part of streams and absent in moderately and highly impaired section of the streams. Moderately tolerant species such as *Mystus cavasius*, *Wallago attu*, *Cyprinus reba*, *C. carpio*, *Rita rita*, *Polyacanthus fasciatus*, *Securicula gora*, *Garra gotyla*, *Xenentodon cancila*, *Macrognathus pancalus*, *Cirrhinus cirrhosus* and *Oreochromis niloticus* contributed 32.4% of the total abundance data and dominated at less impaired sites but least abundant at moderately impaired sites. Herbivore species such as *L. rohita*, *L. calbasu*, *Cyprinus reba*, *C. carpio*, *Cirrhinus cirrhosus*, *Osteobrama cotio* and *Securicula gora* showed narrow/restricted restricted distribution and were recorded at reference sites. Among three omnivorous species, *Oreochromis niloticus*, and *Garra gotyla* were found in upstream sites, whereas *Heteropneustes fossilis* was restricted at moderately impaired sites in downstream. Among four invertivore species, two species such as *Puntius sophore* and *P. ticto* showed even distribution at less impaired and moderately impaired sites. Nine carnivorous species were identified in downstream sites. These were *Mystus cavasius*, *Sperata seenghala*, *Rita rita*, *Xenentodon cancila*, *Macrognathus pancalus*, *Notopterus notopterus*, *Wallago attu*, *Gagata cenia* and *Channa punctata*.

Metric scores and IBI scores

The results of 12 individual metrics for continuous and stepped IBI criteria are given in Tables 1 and 2. Species diversity metrics (such as number of native species, number of native families and total number of species individuals) were used as a measure of species diversity. In terms of metric scores, different trends were observed. Metric score for number of native species, number of native families and abundance of species were highest in "less impaired" integrity class, and lowest in "impaired" integrity class. In case of these three metrics of species diversity, minimum overlap was observed for three integrity classes (Fig. 5a–c). Higher metric scores related to habitat preferences (number of column and bottom dweller species) were recorded in "less impaired" integrity class which gradually decreased in "moderately impaired" and "impaired" integrity classes (Fig. 5d, e). This stresses that as the level of pollution increases, the number of column and bottom dweller species decreases.

Intolerant species (sensitive native species) colonized at reference sites and were rare at impaired sites. Metric score for stress intolerant species in "less impaired" integrity class was highest as compared to "moderately impaired" and "impaired" integrity classes (Fig. 5f). Absence of intolerant species from impaired sites reflected biological degradation of habitat and poor water quality of the sites. Proportion of tolerant species was highest in "moderately impaired" integrity class and lowest in "less impaired" integrity class (Fig. 5g). High proportion of tolerant species in "moderately impaired" and "impaired" integrity classes and absence of intolerant species highlighted significant change in structure of fish assemblage in study area.

IBI score for proportion of herbivorous species was highest in "less impaired" integrity class which indicated that most of herbivorous species had preferences for the less impaired sites. Herbivorous species contributed about 45% of the total fish abundance in "less impaired" integrity class (Fig. 5h). Highest metric score for proportion of omnivores and insectivore species was observed in "moderately impaired" and "less impaired" integrity classes, whereas lowest metric score in "impaired" integrity class (Fig. 5i, j). No clear distribution pattern of omnivore and insectivore in different integrity classes was observed. Higher proportion of carnivore was recorded in "moderately impaired" integrity class in comparison to other classes. About 50% of total abundance was shared by carnivore in "moderately impaired" integrity class (Fig. 5k). "Moderately impaired" integrity class had lowest metric score for proportion of carnivore and highest in "less impaired" integrity class. Box and whisker plots indicated that carnivore species were abundant in downstream sites rather than upstream sites.

Maximum proportion of non-native species was recorded from less impaired site included in "less impaired" integrity class. Higher proportion of non-native species was assigned lowest metric score because presence of alien species represents biological disturbed habitat (Fig. 5l). Minimum metric score was measured in "less impaired" integrity class due to infestation by introduced species.

Three qualitative integrity classes, viz. less impaired, moderately impaired, impaired were obtained from sum of stepped and continuous IBI scores. The range of scores for each class is given in Table 2. Out of 18 sites two sites (reference sites 1 and 2) depicted maximum score and were placed into less impaired integrity class. Five sites (8, 10, 15, 16 and 17) fall into moderately impaired integrity class, whereas eleven sites (3, 4, 5, 6, 7, 9, 11, 12, 13, 14 and 18) were categorized in impaired integrity class (Table 2).

Assessment of IBI along longitudinal gradient of Nullah Aik and Nullah Palkhu and relationship with water quality parameters

IBI scores for stepped and continuous integrity classes were highest in upstream sites of Nullah Aik (1 and 2) which decreased to its ebb in the city (site 3 and 4) and recovered in downstream sites such as 5, 6 and 7 (Fig. 6a, b). Maximum IBI scores for Nullah Palkhu were observed in downstream sites 15, 16 and 17, respectively. Sites 10 and 11 of upstream

Table 2 Stepped and continuous IBI metrics values and qualitative classification evaluated on the basis of fish fauna recorded during study period from Nullah Aik and Palkhu: tributaries of river Chenab

Stream	Nullah Aik									Nullah Palkhu								
Region	Upstream		Downstream							Upstream		Downstream						
Metrics/sampling sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Number of native species	14	15	0	0	4	4	7	4	3	6	5	0	1	0	11	10	10	5
A	**9.3**	**10**	**0**	**0**	**3.3**	**2.7**	**4.7**	**2.7**	**2**	**4**	**3.3**	**0**	**0.7**	**0**	**7.3**	**6.7**	**8**	**3.3**
B	*5*	*5*	*0*	*0*	*1*	*1*	*3*	*1*	*1*	*3*	*1*	*0*	*1*	*0*	*3*	*3*	*5*	*1*
Number of native families	7	8	0	0	4	3	5	3	3	4	4	0	1	0	7	8	7	4
A	**8.8**	**10**	**0**	**0**	**5**	**3.8**	**6.3**	**3.8**	**3.8**	**5**	**5**	**0**	**1.3**	**0**	**8.8**	**10**	**8.8**	**5**
B	*5*	*5*	*0*	*0*	*3*	*1*	*3*	*1*	*1*	*3*	*3*	*0*	*1*	*0*	*5*	*5*	*5*	*3*
Number of bottom feeder species	3	4	0	0	1	1	3	2	2	1	1	0	1	0	4	2	3	2
A	**7.5**	**10**	**0**	**0**	**2.5**	**2.5**	**7.5**	**5**	**5**	**2.5**	**2.5**	**0**	**2.5**	**0**	**10**	**5**	**7.5**	**5**
B	*3*	*5*	*0*	*0*	*1*	*1*	*3*	*1*	*1*	*1*	*1*	*0*	*1*	*0*	*5*	*1*	*3*	*1*
Number of column feeder species	11	10	0	0	2	3	3	2	2	6	4	0	0	0	9	8	8	3
A	**10**	**9.1**	**0**	**0**	**1.8**	**2.7**	**2.7**	**1.8**	**1.8**	**5.5**	**3.6**	**0**	**0**	**0**	**8.2**	**7.3**	**7.3**	**2.7**
B	*5*	*5*	*0*	*0*	*1*	*1*	*1*	*1*	*1*	*3*	*3*	*0*	*1*	*0*	*5*	*5*	*5*	*1*
Number of intolerant species	6	5	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0
A	**10**	**8.3**	**0**	**0**	**0**	**0**	**0**	**1.7**	**0**	**0**	**0**	**0**	**0**	**0**	**3.3**	**0**	**0**	**0**
B	*5*	*5*	*0*	*0*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*0*	*1*	*0*	*1*	*1*	*1*	*1*
% Individual of tolerant species	46	30	0	0	68	94	86	57	100	56	89	0	100	0	74	71	58	83
A	**5.4**	**7**	**0**	**0**	**3.2**	**0.6**	**1.4**	**4.3**	**0**	**4.4**	**1.2**	**0**	**0**	**0**	**2.6**	**2.9**	**4.2**	**1.7**
B	*5*	*5*	*0*	*0*	*3*	*1*	*1*	*3*	*1*	*3*	*1*	*0*	*1*	*0*	*1*	*1*	*1*	*1*
% Individual of Carnivore	12.3	15.4	0	0	16.1	55.3	55.1	9.5	64.4	45.9	29.1	0	100	0	48.8	46	44	50
A	**8.7**	**8.5**	**0**	**0**	**8.4**	**4.5**	**4.5**	**9.1**	**3.6**	**5.4**	**7.1**	**0**	**0**	**0**	**5.1**	**5.4**	**5.6**	**5**
B	*5*	*5*	*0*	*0*	*5*	*1*	*1*	*5*	*1*	*3*	*3*	*0*	*0*	*0*	*3*	*3*	*3*	*1*
% Individual of herbivore species	42.5	45.1	0	0	16.1	0	3.7	42.9	0	16.3	0	0	0	0	4.4	1.5	23.1	0
A	**9.4**	**10**	**0**	**0**	**3.6**	**0**	**0.8**	**9.5**	**0**	**3.6**	**0**	**0**	**0**	**0**	**1**	**0.3**	**5.1**	**0**
B	*5*	*5*	*0*	*0*	*1*	*1*	*1*	*5*	*0*	*1*	*0*	*0*	*0*	*0*	*1*	*1*	*3*	*0*
% Individual of invertivorous species	27	33	0	0	68	45	13	33	11	35	71	0	0	0	28	40	18	48
A	**3.8**	**4.6**	**0**	**0**	**9.6**	**6.3**	**1.8**	**4.6**	**1.5**	**4.9**	**10**	**0**	**0**	**0**	**3.9**	**5.6**	**2.5**	**6.8**
B	*3*	*3*	*0*	*0*	*5*	*3*	*1*	*3*	*1*	*3*	*5*	*0*	*0*	*0*	*3*	*3*	*1*	*3*
% Individual of omnivorous species	18	6	0	0	0	0	28	14	24	3	0	0	0	0	19	12	15	2
A	**4.1**	**8.5**	**0**	**0**	**0**	**0**	**0**	**5.4**	**1.6**	**9.6**	**0**	**0**	**0**	**0**	**3.6**	**5.8**	**5**	**10**
B	*3*	*5*	*0*	*0*	*1*	*0*	*1*	*5*	*3*	*5*	*1*	*0*	*1*	*0*	*5*	*5*	*3*	*5*
Total number of individuals	292	306	0	0	31	47	107	21	45	98	79	0	1	0	205	137	91	46
A	**9.5**	**10**	**0**	**0**	**1.0**	**1.5**	**3.5**	**0.7**	**1.5**	**3.2**	**2.6**	**0**	**0.0**	**0**	**6.7**	**4.5**	**2.8**	**1.5**
B	*5*	*5*	*0*	*0*	*1*	*1*	*3*	*1*	*1*	*1*	*1*	*0*	*1*	*0*	*3*	*3*	*1*	*1*
% Individual of non native species	8	16	0	0	0	0	0	0	7	3	0	0	0	0	7	0	3	0
A	**5**	**0**	**0**	**0**	**10**	**10**	**10**	**10**	**5.6**	**8.1**	**10**	**0**	**0**	**0**	**5.6**	**10**	**8.1**	**10**
B	*3*	*1*	*0*	*0*	*5*	*5*	*5*	*5*	*3*	*5*	*5*	*0*	*5*	*0*	*3*	*5*	*5*	*5*
Cont. IBI score A	76	80	0	0	40	29	36	49	22	47	38	0	4	0	55	53	54	43
Stepped IBI score B	43	45	0	0	23	14	20	27	13	27	21	0	11	0	32	30	30	19
Integrity Class A	**LI**	**LI**	**HI**	**HI**	**HI**	**HI**	**HI**	**I**	**HI**	**I**	**HI**	**HI**	**HI**	**HI**	**I**	**I**	**I**	**HI**
Integrity Class B	*LI*	*LI*	*HI*	*HI*	*HI*	*HI*	*HI*	*I*	*HI*	*I*	*HI*	*HI*	*HI*	*HI*	*I*	*I*	*I*	*HI*

A individual continuous metrics and *B* individual stepped metrics, continuous integrity classes: highly impaired = below 46, impaired = 46–66 and less impaired = above 66, stepped integrity classes: highly impaired = below 25, impaired = 25–35 and less impaired = above 35

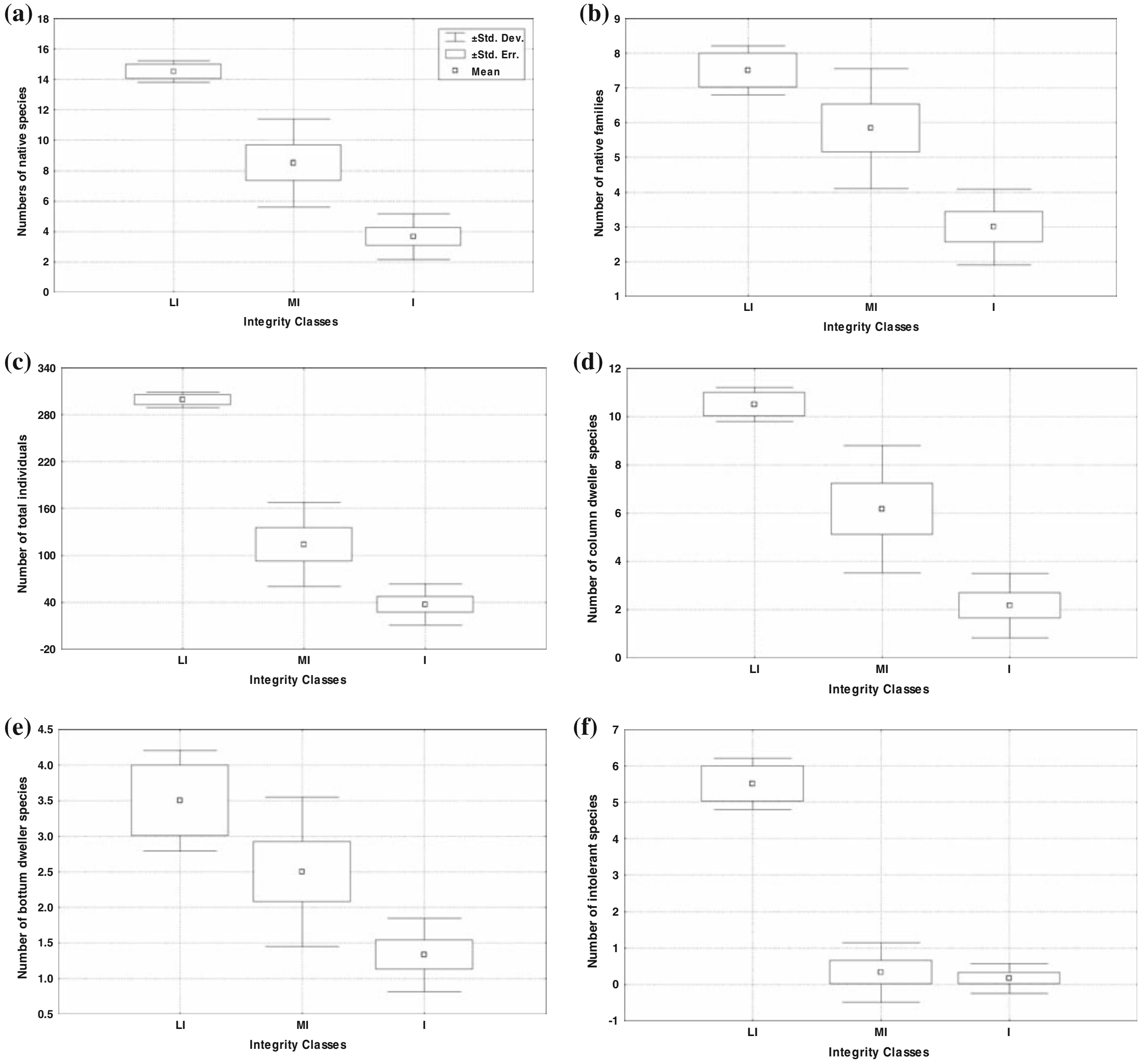

Fig. 5 Box and wisker plot showing scores individual IBI metrics in three integrity (**a** number of native species, **b** number of native families, **c** total number of individuals, **d** number of column dweller species, **e** number of bottom dweller species, **f** number of intolerant species, **g** proportion of individuals as tolerant species, **h** proportion of individuals as herbivore species, **i** proportion of individuals as omnivore species, **j** proportion of individuals as invertivore species **k** proportion of individuals as carnivore species and **l** proportion individuals as of exotic species)

were moderately rich with fish fauna. Minimum values were observed at sites 12, 13 and 14 near the city. Upstream sites of Nullah Aik were the least disturbed sites, while sites close to the city towards downstream were highly polluted with untreated discharge of industrial effluents as well as domestic sewage discharged from city. The IBI score at downstream sites of Nullah Palkhu increases as these sites receive different unpolluted water channels and gradually decrease till Wazirabad city. Contrary to Nullah Aik, IBI score for downstream of Nullah Palkhu was greater (55). IBI scores varied along longitudinal gradient in both streams representing the intensity of anthropogenic stress. The results of both stepped and continuous IBI scores showed along longitudinal gradient for Nullah Aik and Palkhu (Fig. 6a, b). In case of Nullah Aik maximum IBI scores (80 and 76) was recorded at upstream sites

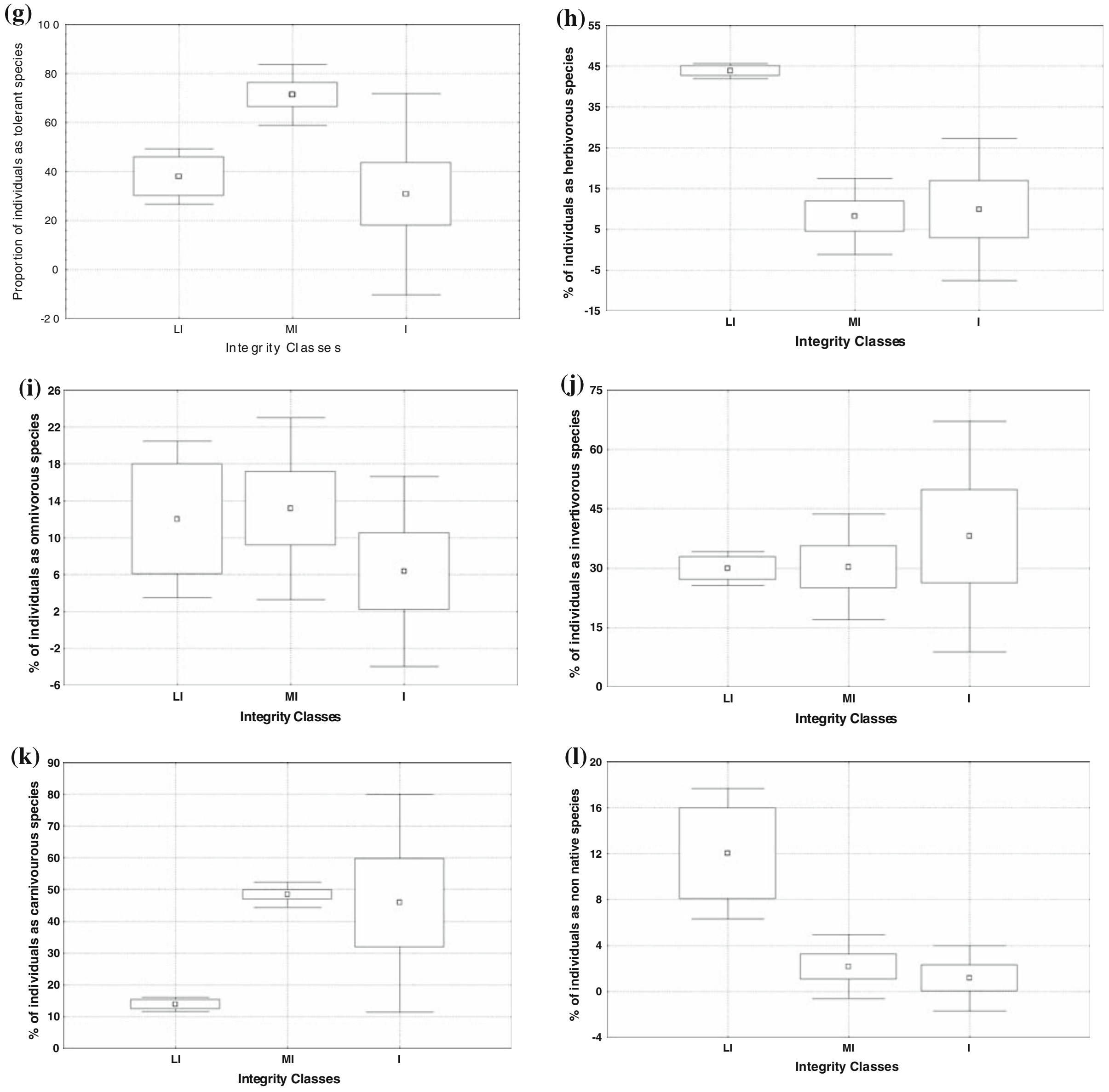

Fig. 5 continued

(1 and 2) which decreased in mid-stream sites and regain in downstream sites.

Appendix 4 (supplementary material) and Table 3 showed that water quality parameters, viz. COD, TDS, turbidity and metals had negative correlation with IBI scores. IBI score was significantly positively correlated with DO and pH. Significantly negative correlation was found between DO, COD and TDS. Metals such as Cr, Zn and Fe were negatively associated with IBI scores. COD, TDS and turbidity showed positive correlation with temperature.

The results of physiochemical parameters (such as temperature, pH, DO, COD, EC, TDS, turbidity, NO_3^-, PO_4^{3-} and toxic metals such as Fe, Pb, Cd, Cr, Ni, Cu and Zn) of water quality of different sampling sites are illustrated in Appendix 3 (supplementary material) and indicated that variability in water chemistry was related as a function of stream sites

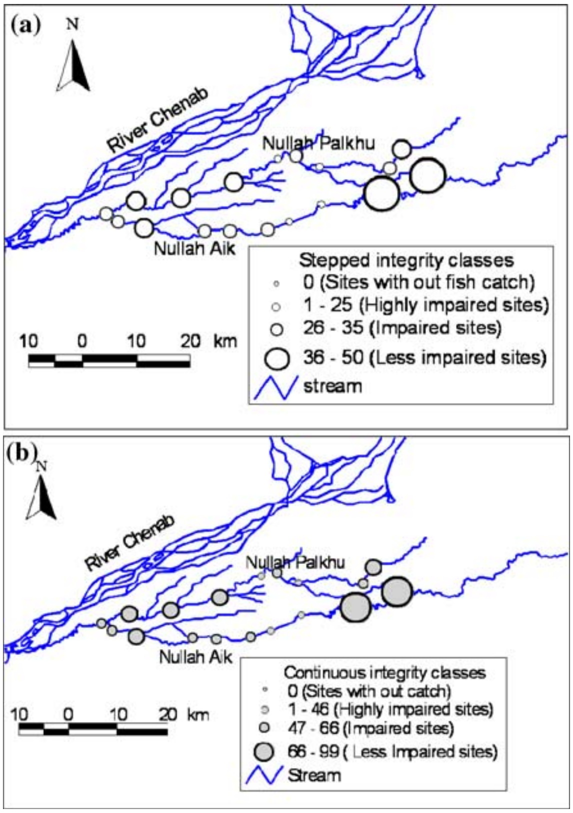

Fig. 6 Showing trend of stepped (**a**) and continuous (**b**) IBI scores along the longitudinal distance from upstream to down stream of Nullah Aik and Nullah Palkhu

(i.e. unimpaired, moderately impaired and severely impaired) and depended on the chemical properties.

Small variations in water temperature, COD and DO were recorded and mean values varied from 23.13 to 25.54°C. In contrast, large variations were found in COD and DO that varied between 26.74–494.75 and 0.99–7.99 (mg/l), respectively.

TDS and pH ranged from 7.55 to 8.26 and 328.66–883.78 (mg/l). All water samples were highly turbid in nature with turbidity values ranging from 2849 to 255.12 (NTU).

Nitrates which are end products of the aerobic decomposition of organic nitrogenous matter and are most highly oxidized form of nitrogen compounds ranged from 0.51 to 2.72 (mg/l), whereas PO_4^{3-} contents ranged from 0.54 to 5.66 (mg/l).

Significant positive correlation was found between different metals, viz. Pb, Cu, Cr, Ni, Zn and Fe indicated their common origin or possible sources of origin. Average concentration of Fe was highest followed by Pb, Cr, Zn, Ni, Cu and Cd. Cr showed positive association with Fe, Pb and Cd. Similarly, Cu was positively correlation with Fe, Pb and Cd, whereas Zn showed negative association with Cu and Cd, respectively.

Concentration of metals such as Fe, Cr and Zn was highest in highly impaired group followed by impaired, moderately impaired and lowest in less impaired group. Similar trend was observed for COD, TDS, salinity and turbidity values. The results also showed DO and pH was highest in the least impaired and moderately impaired streams sites and lowest in highly impaired streams sites. Variation in NO_3^-, and PO_4^{3-} contents were observed between different impaired groups.

Discussion

Variations in individual IBI metrics on spatial scale and effect of environmental factors on fish assemblages

Impacts of anthropogenic activities related with industrialization, population expansion, urbanization and agriculture on biological integrity of water resources are very complex and long lasting (Hu et al., 2007). Human influences such as changes in water chemistry or physical habitat modifications could alter fish assemblage and diversity by disrupting their structures and functions. Variations in fish assemblage could be detected through changes in components of the community, functional groups, species diversity and relative abundance. The results showed high fish abundance at reference sites (less disturbed) in comparison to disturbed sites. These results are analogous to the results obtained by Karr et al. (1986), Oberdorff & Hughes (1992) and Toham & Teugels (1999). The results also illustrated that species diversity decreases as human impact increases (Belpaire et al., 2000; Breine et al., 2004). Sites located on both streams were affected by various anthropogenic stresses such as industrial effluents, municipal sewage, atmospheric deposition and surface runoff from agricultural and urban areas. These factors negatively influence the structure and composition of fish assemblage in streams passing through cities and can adversely affect their habitat

Table 3 Correlation between physiochemical variables and IBI scores measured at 18 sampling sites located on Nullah Aik and Nullah Palkhu

	IBI score	Temp. (°C)	pH	DO (mg/l)	COD (mg/l)	TDS (mg/l)	Turbidity (NTU)	NO_3^- (mg/l)	PO_4^{3-} (mg/l)	Fe (mg/l)	Pb (mg/l)	Cd (mg/l)	Cr (mg/l)	Ni (mg/l)	Cu (mg/l)	Zn (mg/l)
IBI score	1															
Temp. (°C)	−0.29	1.00														
pH	**0.56**	−0.02	1.00													
DO (mg/l)	**0.80**	**−0.48**	**0.71**	1.00												
COD (mg/l)	**−0.70**	0.45	−0.45	**−0.68**	1.00											
TDS (mg/l)	**−0.58**	**0.50**	−0.29	**−0.64**	**0.62**	1.00										
Turbidity (NTU)	−0.36	**0.54**	−0.14	−0.42	0.43	**0.58**	1.00									
NO_3^- (mg/l)	0.02	0.03	0.07	0.26	−0.11	0.02	−0.12	1.00								
PO_4^{3-} (mg/l)	−0.41	0.03	−0.39	−0.46	0.45	**0.55**	0.27	−0.18	1.00							
Fe (mg/l)	**−0.56**	0.35	**−0.53**	−0.70	**0.50**	**0.64**	0.29	0.18	0.26	1.00						
Pb (mg/l)	−0.12	0.38	−0.43	**−0.61**	0.19	0.32	0.32	−0.22	0.11	0.40	1.00					
Cd (mg/l)	0.09	0.07	−0.34	−0.28	−0.10	0.01	−0.15	0.08	−0.27	**0.58**	**0.57**	1.00				
Cr (mg/l)	**−0.60**	**0.47**	−0.44	**−0.81**	0.51	**0.60**	0.32	−0.11	0.29	**0.85**	**0.51**	**0.48**	1.00			
Ni (mg/l)	0.23	0.02	−0.19	−0.09	−0.09	−0.16	0.23	−0.35	−0.14	−0.25	**0.55**	−0.02	−0.12	1.00		
Cu (mg/l)	0.08	−0.02	−0.38	−0.27	−0.16	0.03	−0.10	0.12	−0.32	**0.48**	**0.68**	**0.92**	0.37	0.24	1.00	
Zn (mg/l)	**−0.55**	**0.47**	−0.13	−0.45	0.51	0.34	**0.47**	−0.34	0.46	0.07	0.02	**−0.53**	0.35	0.10	**−0.57**	1.00

Correlations highlighted as bold are significant at $P < 0.05$

which becomes vulnerable for fish survival. These results were in accordance with Wichert & Rapport (1998) and Casatti et al. (2006). Large number of bottom and column dweller species was recorded from least impaired sites and decreasing trend was observed towards downstream sites. Column dwellers are generally sensitive to bottom depletion of oxygen and sedimentation which affect their feeding; Oberdorff & Hughes (1992). Organic and inorganic chemicals from industrial and domestic sewage and reproduction (Kestemont et al., 2000) are indiscriminately discharged into streams which consume DO during oxidation process resulting in depletion of oxygen (Eklov et al., 1998). Heavy metals and Persistent Organic Pollutants (POPs) in freshwater have been mainly associated with industrial and agricultural sources. Accumulation of these pollutants in body tissues of fish species may result in reduction of growth and cause disturbance in physiological processes (Lwanga et al., 2003). Upstream sites were less disturbed from anthropogenic activities with large number of intolerant species which declined towards downstream. Six intolerant species were recorded from reference sites which were absent from degraded sites. Kennard et al. (2005) and Habit et al. (2006) also recorded intolerant species from reference sites which gradually disappear from the sites with high level of contamination. *Channa punctatus*, *Puntius sophore*, *P. ticto* and *Heteropneustes fossilis* can tolerate pollution stress and were encountered at moderately degraded sites. Presence of intolerant fish species generally indicates species richness of sites which are relatively less disturbed by anthropogenic activities (Karr et al., 1986). In contrast higher proportion of tolerant individuals reflects habitat degradation. Tolerant species are the last to disappear in disturbance conditions and reappear first in comparison to other species as habitat become favourable (Ganasan & Hughes, 1998). The percentage of carnivorous individuals was higher at moderately degraded sites in downstream, whereas minimum proportion was recorded at least disturbed sites. High percentage of herbivore species was recorded at reference sites. Similar results were also reported by Bhat (2003). However Ganasan & Hughes (1998) found higher proportion of carnivorous species at reference sites. Stream width has been identified as important factor which influence richness of herbivore species, i.e. more is the stream width more will be the proportion of herbivorous species. The results showed that stream width was higher at reference sites with low flow rate. This in turn allows suspended sediment to settle down resulting in clear water which favours the establishment of phytoplankton which forms the food of herbivorous fishes (Borges et al., 2003). Most herbivores species were found upstream and carnivores in the downstream sites. These results highlighted that more detailed studies on the feeding habits and preferences of these species are required for better understanding of spatial pattern in feeding guilds. The relative abundance of insectivorous species found during current study was almost the same at reference and degraded sites. These results are consistent with the findings of Bhat (2004). Among three omnivore species, *O. niloticus* was found in the upstream, whereas other two species such as *Garra gotyla* and *Heteropneustes fossilis* were found in downstream sites. Oberdorff & Hughes (1992) showed that omnivorous species had a wide range of food selection and can switch from one food to another. Relatively higher proportion of omnivorous species at reference site may be due to more availability of food as compared to other sites. Toham & Teugels (1999) described that omnivorous species are comparatively more successful at reference sites and degraded sites than any other trophic groups. Among non-native species *O. niloticus* showed wide distribution upstream as well as downstream of Nullah Palkhu. It is considered as a potential threat to diversity of native fish fauna of the studied streams. It was introduced to freshwater resources of Pakistan from River Nile basin in 1985 and now has been reported in most of the rivers, streams, lakes and small ponds (Mirza, 2003). In contrast to other non-native species, *Cyprinus carpio* was captured only from site 17 located downstream of Nullah Palkhu. This species is intensively cultured in ponds and fish farms to fulfil the local demand of white meat. During monsoon season due to heavy rains fish farms overflow and this species makes its way to streams. According to Kasulo (2000) non-native species have the ability to spread from one ecosystems to another through inter-connected water ways and have significant impact on local fish species by predation, resource competition and interference with reproduction. These species have been reported as a carrier of parasites of different diseases from one ecosystem to another and used as an ecological indicator of biological health of stream and river (Kennard et al., 2005). High richness of non-native species can be correlated with the level of biological degradation. Intrusion of non-native species into freshwater ecosystem disrupt the food webs making the aquatic ecosystem more vulnerable for native fauna (Scrimgeour & Chambers, 2000; Wolter et al., 2000; Habit et al., 2006) and their presence is considered problematic for management and conservation of water resources (Kennard et al., 2005).

The results highlighted that fish diversity increases as streams move downstream. Vila-Gispert et al. (2002) stated that fish diversity increases from upstream to downstream of undisturbed streams. Pollutants from point and non-point source and other factors such as introduction of alien species, exploitation of water for irrigation purpose and habitat alteration by construction of dams and canals has been recognized as significant threats for loss of species diversity and changes in spatial trends of species distribution (Moyle & Leidy, 1992; Lyons et al., 2000; Kouamélan et al., 2003; Mebane et al., 2003). Fish diversity of Nullah Aik and Palkhu was highly fragmented. Discharge of untreated pollutants from industries and urban waste, pumping of stream water for irrigation purposes and presence of small head works at site (7) are main causes of fragmentation of fish species. These threats may increases the risk of local extinction of fish species (Cetra & Petrere, 2006). Surface water contamination could result in reduction in fish diversity and abundance as only few tolerant species can thrive in highly polluted water due to their adaptability and resistivity to water pollution (Pinto et al., 2006). The results highlighted that upstream of Aik and Palkhu streams were mainly affected by agricultural activities, whereas downstream were heavily polluted from discharge of untreated industrial effluents and domestic wastes. Walton et al. (2007) concluded that streams which passed through human settlements generally receive pollutants from point sources (industrial and municipal sewage) and non-point sources including agricultural and urban runoff and atmospheric deposition. These pollution sources inexorably affect directly or indirectly the biota of the streams, in particular fish fauna. Heavy load of industrial and municipal effluents have been recognized to have negative effect on habitat conditions which become unfavourable for native/sensitive species and favourable for the opportunist/tolerant species (Pinto et al., 2006). Tolerant species generally have the ability to survive in organically enriched environment from industrial and municipal discharges (Ganasan & Hughes, 1998). The results indicated that upstream sites were dominated by intolerant species as relatively undisturbed habitat conditions prevail. The fish diversity decreases as study streams pass through Sialkot metropolitan which lies in midstream region generates large volume of untreated industrial and municipal wastes. Impairment of sites also increases as study streams move from upstream towards downstream. Impairment of study sites can be related to high level of COD, TDS, turbidity and toxic metals, viz. Cr, Zn, Cu, Pb, Ni, Fe and Cd (Qadir et al., 2008). The results found negative correlation of DO with turbidity, COD, TDS and toxic metals. Less impaired groups showed better water quality in terms of high level of DO and low level of COD, turbidity, TDS and toxic metals.

Validation of reference sites and variations in overall IBI scores

Defining a reference condition against which other streams can be compared present a unique challenge because most of aquatic ecosystems have been altered to some extent by changes in physical, chemical or biological structure (e.g. impoundment, pollution or introduction of exotic species). The selection of reference sites was based on findings of Sudaryanti et al. (2001), Hart et al. (2001) and Tejerina-Garro et al. (2005). Sudaryanti et al. (2001) assessed the impact of different human activities on riverine ecology of river Brantas, Indonesia, and indicated that it is difficult to find any un-disturbed reference site in developing countries due to anthropogenic activities in catchment area of aquatic resources. They suggested sites with relatively better ecological characteristics can provide baseline for ecological change in streams and can be considered as reference sites because the choice of an appropriate reference condition is a major problem in most of the ecological assessments. The decision about the health of the stream is an essential part of biological assessment (Hughes, 1994). In order to study the health status of any stream, it should be compared with stream segments having best ecological conditions; such stream segments are called reference sites. These sites represent the minimal disturbed conditions indicating the good physio-chemical and biological quality of the stream (Qadir et al., 2008). In evaluation of ecological health of streams, it is essential to study various characteristics of the reference site. No completely undisturbed reference sites were identified in the catchment of Nullah Aik and Palkhu because of intensive utilization of land resources for urbanization, industrial activities and agriculture processes (Qadir et al., 2008). Sites 1 and 2 were represented as reference sites were located at 247 and 245 m height from the sea level with no apparent source of pollution in catchment. However, some agricultural activities were found. Thus, runoff from agricultural field and atmospheric deposition of pollutants can be considered the only source of contamination. Both sites showed relatively similar physical, chemical and biological characteristics. Maximum average width (25.46 and 30.64 m) was recorded from these sites with an average depth (1.10 and 1.00 m). Stream bank vegetation of the two sites was very similar, dominated by tall grasses mainly *Saccharum* spp.; stream bed sediment were pre-dominantly sandy with least organic substances. From these sites freshwater bivalves were also recorded which were absent from other sites and water was clear with no unpleasant odour which is produced during decomposition process, thus representing habitat condition suitable for flouring of Fish fauna. The results also indicated that highest fish abundance was recorded from these sites and depicted relatively better ecological conditions with least impact from anthropogenic processes. The selection of reference sites was also validated by multivariate techniques such as CA and NMDS. Among three clusters of sites from both streams (viz. less degraded, moderately degraded and degraded sites) classified using CA less degraded sites were considered as reference sites. Similarly, NMDS also separated sites into four groups based on the intensity of habitat impairment due to anthropogenic stress. Thus, least impacted sites selected as reference sites were treated as threshold level of biological conditions of habitat in terms of quantitative values of IBI scores.

IBI provides multiple indicator evaluation system to describe biological conditions of streams (Groom et al., 2005) and has been used to assess the level of impairment at different spatial scales. The results of metric scores and IBI scores indicated that degradation of the studied streams can strongly be related to anthropogenic activities. The results revealed upstream sites with better water quality had higher IBI scores than sites located midstream and downstream regions. Low IBI scores were related with environmental degradation and decline in site quality with downstream distance primarily to increasing impacts from direct discharge of industrial and municipal effluents in the streams. There is no treatment plant for industrial and sewage treatment in Sialkot city (Qadir et al., 2008). The effluents are directly discharged either into nearby streams, drains, ponds or/and agricultural land. Gammon (1998) also attributed agriculture and urban discharge as major factor responsible for habitat degradation in downstream to those of upstream region. Anthropogenic disturbances have been recognized as a major contributor for changing chemical composition of stream water (Qadir et al., 2008) which ultimately disturbs the ecological integrity of an ecosystem. The results showed significantly negative correlation of IBI scores with COD, TDS, turbidity, Fe, Cr, Zn and positive correlation with DO and pH (Appendix 4—supplementary material and Table 3). The results stress the role of industrial and municipal effluents in deterioration of water quality which negatively influence ecological health of streams.

Factors such as stream discharge, width, depth and substrate also influence integrity of aquatic resources and have shown correlation with IBI scores (Casatti et al., 2006). Regular stream discharge rate and large width of streams generally favour higher IBI score due to presence of high diversity of intolerant and column dweller species (Oberdorff & Porchcr, 1992; Tejerina-Garro et al., 2005). Stream discharge, width, depth and substrate influence the distribution of fish species (Oberdorff & Porchcr, 1992; Casatti et al., 2006). Similarly, Radwell & Kwak (2005) also highlighted that physical attributes of river system are highly influential and play major role in discriminating among rivers to assess their spatial integrity. Width of Nullah Palkhu increases towards downstream and sites located downstreams showed higher IBI scores. Depth of stream and rivers also influence the overall IBI scores and is difficult to portray the exact picture of fish assemblage in deep water due to inadequacy in sampling efforts as cited by Ganasan &

Hughes (1998). Thus, large sized streams with good water quality should have significantly more species than a small, poor quality stream. Sites located upstream of Nullah Aik (reference sites) had higher IBI score and had maximum width which decreases towards downstream sites. Sites with high IBI scores were least disturbed which were characterized by complex and sensitive fish assemblages sensitive to changes in habitat quality. Sensitive species generally avoid polluted sites by relocating their niche to the sites which are relatively less disturbed (Mebane et al., 2003). Based on quantitative overall IBI score, three qualitative classes were developed (impaired, moderately impaired and less impaired). Ganasan & Hughes (1998) also adopted classification system for assessment of ecological integrity of aquatic habitats which comprised of three qualitative classes (poor, fair and good). The use of less number of classes has been proven more beneficial for restoration of aquatic ecosystem in comparison to use of large number of classes (Ganasan & Hughes, 1998; Lyons et al., 2000). Most of sites in two tributaries of Chenab river were impacted expect sites (1 and 2) located upstream of Nullah Aik. Sites located upstream were classified as "less impaired" integrity class on the basis of their high IBI score. These sites were also characterized with high level of DO and pH in comparison to other integrity classes. Sites with lowest IBI score placed in impaired integrity classes were located downstream close to Sialkot city a major source of industrial and municipal pollution. These sites also had high contents of toxic metals related with anthropogenic origin such as Cr, Zn, Fe, Ni, Cd, Pb and Cu and lower level of DO and high level of COD, TDS and turbidity. Ganasan & Hughes (1998) also found minimum IBI scores near sources of pollution and highest in extreme downstream sites. Water quality of Nullah Palkhu improves as it moves away from the midstream towards downstream to the Sialkot city in comparison to Nullah Aik. IBI scores for downstream of Nullah Aik did not improve as it moved downstream probably due to pumping of water from streams for irrigation purposes (Qadir et al., 2008). Pumping of stream Water for irrigation is a known practice in Pakistan because it is a cheap source for crops irrigation. Large number of water pumps was found during sampling. Upstream sites of Nullah Palkhu experience low flow rate throughout the year especially in early summer and IBI scores for these sites were in medium range (25–35 for stepped IBI and 45–65 continuous IBI) and classified into "moderately impaired" integrity class. As the streams move away from Sialkot city towards downstream on the west water quality gradually improves as small water channels add in before these streams fall into river Chenab. Improvement of IBI scores for sites located downstream far from Sialkot city on Nullah Palkhu was observed which could be related to better water quality and habitat conditions. Many small channels collecting water from seepage of canals join Nullah Palkhu resulting improvement of surface water which becomes favourable for re-establishment of fish assemblages.

During present study stepped and continuous IBI criteria depicted the similar results. Stepped and continues IBI criteria were found useful for the quantification of fish biological integrity of studied streams. Stepped IBI scoring method was developed by Karr (1986) and later adopted by different authors (Oberdorff & Hughes, 1992; Minns et al., 1994; Hughes et al., 1998; Toham & Teugels, 1999; McCormick et al., 2001) with some modification. Dauwalter & Jackson (2004) and Bozzetti & Schulz (2004) applied the continuous scoring method for the calculation of IBI scores. Mebane et al. (2003) used continuous IBI scoring method and indicated that it is good for graphical representation. Ganasan & Hughes (1998) applied both the traditional (stepped) IBI scoring method as developed by Karr et al. (1986) and continuous IBI scoring methods. The results of this study supported the use of IBI for the assessment of fish assemblage and habitat condition. IBI is more practicable index for the assessment and monitoring of fish assemblage in small streams rather than big rivers and lakes due to sampling inadequacies (Lyons et al., 2000). These inadequacies are due to more depth of water in which incomplete fish sampling might have resulted. Other comprehensive and precise sampling methods such as electro-fishing should be adopted to minimize the sampling errors (Ganasan & Hughes, 1998). Before application of IBI, ecological managers must consider methods of their sampling effort, selection of reference site and richness of native and non-native species to minimize the errors to extract more reliable results (Mebane et al., 2003). Habitat quality of study of streams is essential to understand the overall impact of human activities (Spence et al., 1999). This approach is an important step towards assessment at ecosystem level considering human and environmental factors (Joy & Death, 2004). Urbanization, ill-planed industrialization in catchment and discharge of effluent without any treatment into Nullah Aik and Nullah Palkhu is putting fish assemblage under stress and are constant threat to local streams. This study highlights the importance of an integrated effort for management and restoration of such ecosystems using an integrated approach of IBI and multivariate techniques.

Conclusions

IBI can be used for assessment and monitoring of fish assemblage in small streams of Pakistan. The results highlighted that habitat quality study of streams is essential to understand the overall impact of human activities (Spence et al., 1999). The results of this study support the use of IBI as an important tool for ecological assessment at ecosystem level with consideration of human related environmental factors and IBI can be used as an indicator for fish assemblage and habitat condition. IBI was successfully developed and applied for Nullah Aik and Palkhu, tributaries of river Chenab, to assess the stream health of streams at 18 selected sites to portray a wide range of anthropogenic impacts on fish assemblage. Assessment of stream health using IBI provided an appropriate approach about habitat condition and its relationships to the human activities in catchment area and can be used as indicator of fresh water ecosystem degradation. Comparative assessment of biotic conditions in streams allows along spatial gradient. Stepped and continuous IBI criteria evaluated the sites from quantitative information of IBI into qualitative integrity classes (less impaired, moderately impaired and impaired) with least error. Most of the sampling sites were included in impaired integrity class which indicated that fish assemblage of both streams has been altered by human activities. Finally, it is concluded that IBI based on fish assemblages is most useful criteria for the assessment of habitat quality and streams health. Urbanization, ill-planed industrialization in catchment of study area and discharge of effluent without any treatment into Nullah Aik and Nullah Palkhu had negative impact on fish assemblage and diversity. These factors are considered important contributor to wipe out the local aquatic fauna and constant threat to local streams. The study highlights the importance of an integrated effort for management and restoration of stream ecosystems. This study stressed the importance to initiate concrete steps to control the indiscriminate discharge of industrial and municipal waste into the streams from further degradation.

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