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REFERENCE CRITERIA FOR ECOLOGICAL RIVER HEALTH ASSESSMENT IN ETHIOPIAN HIGHLANDS

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Abstract
The concept of a reference condition is increasingly used to describe benchmarks against which current condition is compared. This study defines criteria that represent reference sites for assessing highland streams in four major river basins of Ethiopia. Twenty a priori criteria were chosen as representative for Ethiopian highlands and they reflect some aspects of naturalness and address wide range of human disturbances. The most relevant criteria were those related to point and diffuse source of pollution, land use change, riparian vegetation, and river morphology. Of 104 study sites 22 were selected as reference based on established criteria and benthic macro-invertebrate (BMI) derived metrics were used for validation. BMI distribution in a scatter plot showed different level of degradation between reference and impaired sites. Moreover, biotic score established for Ethiopian highland streams revealed that 95% of a priori reference sites were within the range of 'high' ecological status and the scores showed high discrimination efficiency (above 93%) between reference and impaired sites. Reference criteria proposed in this study can be considered as valuable parameters to implement biomonitoring program in Ethiopian highland river ecosystems where true pristine areas are nonexistent as a result of different human activities and urbanization.

Key Words: *Reference sites, Biomonitoring, Highland streams, A Priori criteria*

Introduction

Implementation of biomonitoring programs aiming to assess the ecological status of streams and rivers requires the establishment of biological reference conditions with which monitoring sites can be compared. Ecological reference conditions represent the expected status of biological communities in least impaired or relatively undisturbed and homogeneous streams with respect to the morphological, hydrological, physicochemical and biological conditions. Several definitions of reference condition have been identified to account for the fact that all ecosystems experience some level of human disturbance, and truly pristine sites are virtually nonexistent. Many authors defined reference condition as the condition that is representative of a group of 'least impacted' sites organized by

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selected physical, chemical and biological characteristics (Davis and Simons, 1995; Hughes, 1995; Barbour *et al*., 1996; Reynoldson and Wright, 2000; Hering *et al*., 2003; Bailey *et al*., 2004; Stoddard *et al*., 2006). Most biological assessments are based on the concept of comparing the current condition to natural conditions in the absence of human disturbance or alterations (Stoddard *et al*., 2006). A number of methods have been used to establish reference condition (e.g. Hughes, 1995). Some of these methods include extensive spatial survey, predictive modelling, historical data, and expert judgment. Each method of determining the reference condition has its own strengths and weaknesses and each method relies on ecosystem classification to some degree (Hughes, 1995). Nevertheless, the extensive spatial survey is widely used because it allows achieving a sufficient number of minimally disturbed sites in a specific region and gives a chance to evaluate 'a priori' criteria established elsewhere but relevant for the region. An 'a priori criteria' were used by many authors to distinguish a reference site from impaired site (Hering *et al*., 2003). The criteria selected as an 'a priori' should define the least environmental disturbance caused by human activities (Stoddard *et al*., 2006) and most of these criteria should be fulfilled by selected reference sites to clearly define the reference or acceptable healthy ecosystem (Bailey *et al*., 2004). The criteria of appropriate reference sites may vary among regions, water bodies and habitat types. However, the most commonly used criteria include hydro-morphological characteristics, physicochemical parameters, land use pattern and riparian vegetation (Hughes, 1995; Barbour *et al*., 1996; Hering *et al*., 2003; Nijboer *et al*., 2004; Moog and Sharma, 2005). In developing countries where resources and historical knowledge are limiting factors, the use of abiotic and riparian vegetation criteria are often applied to describe the characteristics of sites in a region that are least and most exposed to stressors (Moog and Sharma, 2005).

For this study, the aim of setting criteria is to identify low levels of alteration in environmental variables supporting benthic macroinvertebrate communities. Setting criteria for some observed pressures may not be too difficult and can be approached from different perspectives. For example, the intensity of point source pollution and the magnitude of its impact can be determined by observing the distribution of the sources in a watershed or by direct measurements of the concentration of pollutants in the water. Similarly, land use patterns in the watershed can be obtained from local land use maps or geographic information system based land use maps. Nevertheless, expressing the pressures and their impact level in quantitative terms remains a challenge because it requires detailed analysis of appropriate data sets (current and historical data). Thus, to develop criteria for selecting reference sites, variables under different categories require analysis based on local conditions and available data.

Therefore this study was conducted with the objective of establishing criteria that explicitly define the reference or acceptable healthy streams and rivers ecosystem for biomonitoring programs in highlands of Ethiopia.

Materials and methods

Study Area

The present study was conducted in four major river basins of Ethiopia namely Awash, Wabe-shebele, Genale-Dawa and Rift Valley, lying between 6°57'N and 9°05'N latitude and 38°07'E and 40°06'E longitudes (Figure 1) in dry and light rainy seasons in 2012/2013. Of 104 sampling sites, 98 sites were located in the Ethiopian montane grassland–woodlands ecological region with altitude ranging from 1,900 to 2,500 m a.s.l, while the remaining 6 were distributed between 2,600 and 3,200 m a.s.l. in afro-alpine ecoregion of Bale highlands. Rainfall distribution in the study area is bimodal, with a short rainy season from February to April, and the main rainy season occurring from June to September (NMA, 2012).

The study area is characterized by scarce coverage of natural vegetation. Although there are attempts to cover mountain escarpments with forest, only state or community forests (e.g. Chilimo, Suba, Wondogenet) are covered by indigenous natural forest such as *Juniperus procera, Podocarpus falcatus, Prunus africanum, Olea europaea,* and *Hagenia abyssinica* in the upper Awash and central rift valley basins. The head waters of Genale and Wabe-shebele basins are dominated by *Erica aroborea, Erica trimera, Alchemilla haumannii* and *Alchemilla ellenbeckii* at higher altitudes, and *Juniperus procera*, *Hagenia abyssinica* and different grass species in the protected escarpments (e.g. Bale National Park and Adaba forest). In unprotected areas of all basins, the natural vegetation was cleared and replaced by farmland, grazing land and/or Eucalyptus plantation.

Sampling sites were distributed strategically in the national park, protected forest areas, rural–agricultural areas and urban-industrial sites to represent different stress gradients. The major threats in rural areas are removal of riparian vegetation, nutrient loading from farmlands, flushing of reservoirs with a consecutive siltation of the river bed, sand excavation, intensive livestock grazing and watering, water extraction and diverse in stream activities. In urban areas the major pollutants comprise diffuse and punctual loads of untreated domestic and industrial wastes.

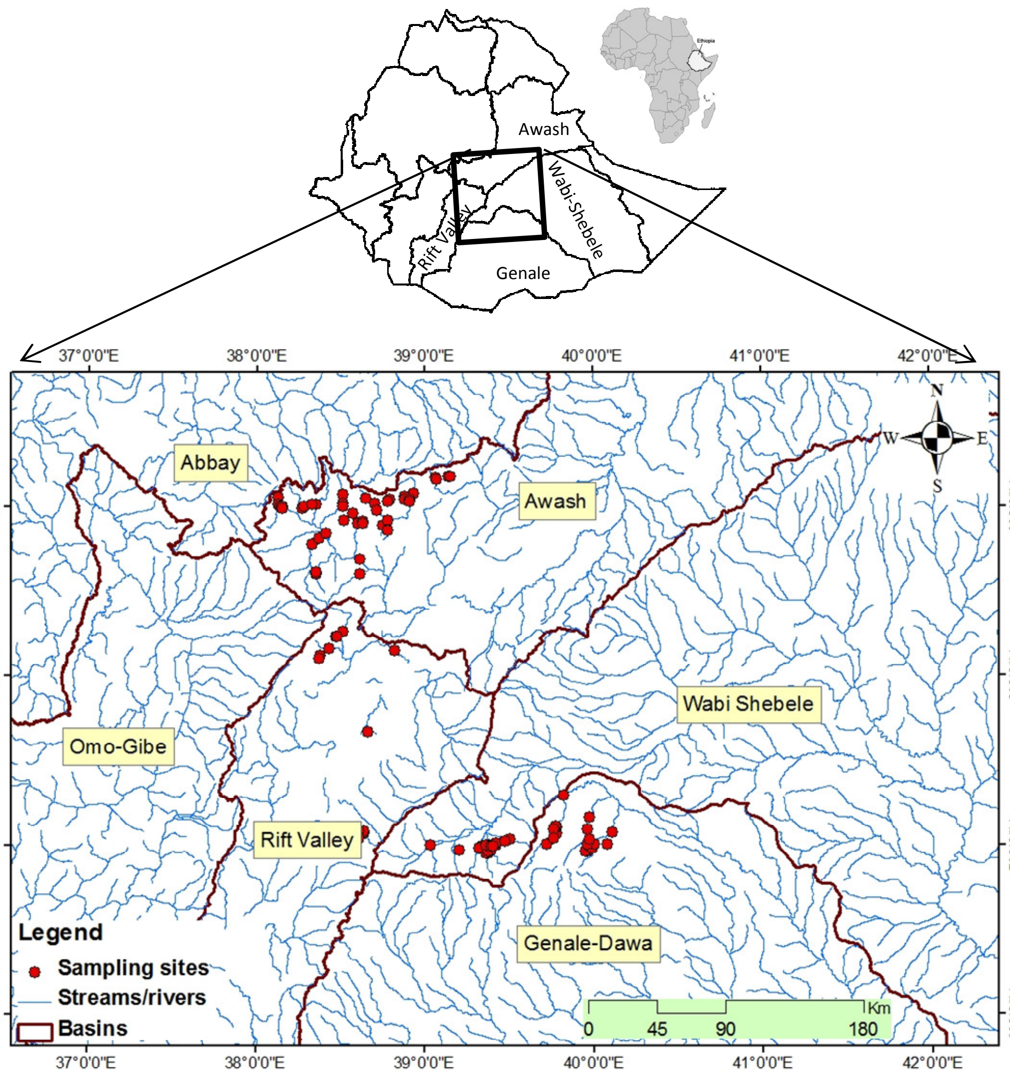

Figure 1: Spatial distribution of study sites in the upper section of four drainage basins.

Sample Collection and Laboratory Analysis

Water quality parameters including dissolved oxygen and conductivity were measured *in situ* using a portable WTW multi-parameter probe ((Model HQ40D, HACH Instruments). Two liters of water were collected from each investigation site and stored in ice box until return to JIJE laboratory P.L.C. and National Fishery Research center for laboratory analysis. In the laboratory, total phosphorus and biochemical oxygen demand were measured following the standard methodology described in APHA (1997). Macroinvertebrates were collected using standard square frame (side 25cm) hand net with mesh size of 500 µm following multi-habitat sampling approach (Moog, 2007). In the laboratory, each sample was passed through a set of sieves with different mesh size (5000, 3000, 1000 and 500µm) to separate size class of macroinvertebrate groups. Identification was performed to the lowest possible taxonomic level (genus-species) based on the available keys. Coleoptera, Hemiptera, Molluscs, Trichoptera and Ephemeroptera were identified by taxonomic specialists from Natural Histroy Museum and BOKU university in Vienna, Austria.

A priori criteria related to catchment land use (e.g. % farmland, % forest cover, % urbanization, population density) were obtained from local land use maps.

Revision of reference criteria

A priori criteria used to select the reference sites in various regions were reviewed not only to select the most frequently used, but also to determine the most appropriate criteria for highland streams in Ethiopia. The criteria reviewed were those defined by Hughes (1995) and Barbour *et al.* (1996) for the United States streams, Hering *et al.* (2003) and Nijboer *et al.* (2004) for European streams and Moog and Sharma (2005) for Hindu Kush Himalayan regions. Those criteria identified as common and determining for Ethiopian highlands streams and rivers such as cattle watering, washing and excavation were considered as compulsory for all sites. Criteria established elsewhere but not relevant or not common for Ethiopian highland streams and rivers were excluded based on expert judgement during the field study.

Validation of 'a priori' criteria

To evaluate the performance of selected 'a priori' criteria, a validation process was applied using the biological data. The validation method should be preferably an approach which does not depend on the method used to select the reference sites (Nijboer *et al.*, 2004). Macroinvertebrate metrics including richness, composition and sensitivity were selected as indicators of biological conditions to verify whether reference sites presented least impaired or near-natural conditions. For example ,the number of EPT taxa (number of families belonging to Ephemeroptera, Plecoptera and Trichoptera) were applied as single metrics. The mean values of macroinvertebrate metrics and indices of sites grouped as reference and impaired were used to perform box-plots to represent the range of variation of each metric between the two stress levels. In addition biotic score developed for assessing different level of stream degradation in Ethiopian highland streams and rivers (Aschalew and Moog, 2015) was used for validation. Discrimination efficiency was calculated as total number of impaired sites fall below 25 percentile of reference sites based on Ofenboeck *et al.* (2004).

Multivariate analysis was used to determine whether the sampled sites were placed into specific groups that will minimize variance within groups and maximize variance among groups. NMS (Non-metric Multi-dimensional Scaling) is an ordination technique that seeks to explain the variation in species community data (Kruskal, 1964). NMS was analyzed based on log (x+1) transformed benthic invertebrate data using PC-ORD version 5. Mean values of physicochemical parameters were calculated and compared between reference and impaired sites. One way ANOVA was used for significant test between two stress gradients.

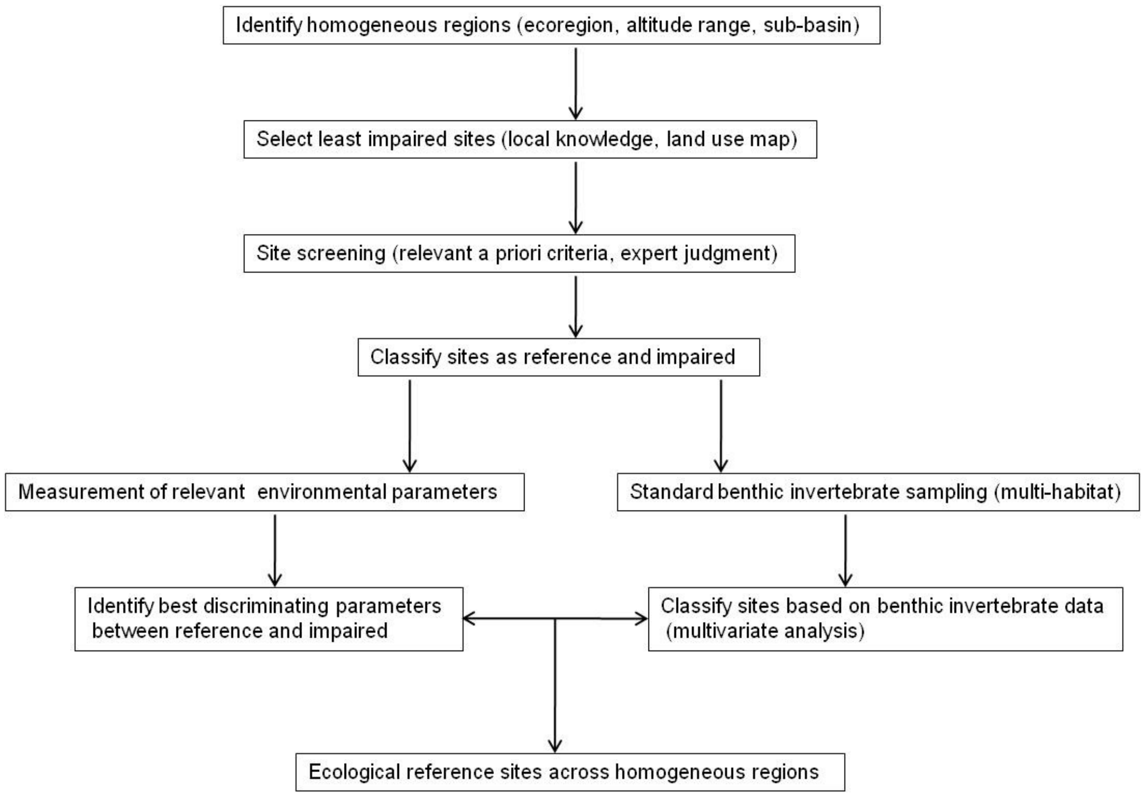

Figure 2: A conceptual model for establishing ecological reference sites for Ethiopian highland streams and rivers

Results and Discussion

The study identified and defined 20 a priori criteria representative of Ethiopian highlands and they reflect some general aspects of naturalness and address wide range of human disturbances (Table 1). Of the total 104 investigation sites, 22 sites fulfilled the minimum requirement for reference condition in Ethiopian highlands. To be considered as reference site, at least 16 criteria are required including the four compulsory 'a priori' criteria (* in Table 1) in the sense of the 'best attainable condition' (Stoddard *et al.*, 2006). With this method, sufficient numbers of reference sites were found from study river basins: 12 sites in Genale-Dawa basin, five sites in Wabe-shebele basin, three sites in the Rift Valley basin and two sites in the Awash basin. Similarly these sites have been distributed in to two study ecoregions (4 sites in Afro-alpine and 18 in the Montane grassland and woodlands).

Table 1 Criteria for selection of reference sites adapted for Ethiopian highland streams.

Group	Criteria	Reference
River morphology	1. Natural channel structure typical to the region (riffle, run, pool)	Hughes (1995) Barbour *et al.* (1996)
	2. No migration barrier	Hering *et al.* (2003)
Habitat composition	3. Representative diversity of substrate composition appropriate to the region	Hughes (1995) Barbour *et al.* (1996)
	4. Spawning habitats for the natural fish population	Moog and Sharma (2005)
Hydrological condition	5. No alteration of the natural hydrograph and discharge regime	Barbour *et al.* (1996) Hering *et al.* (2003)
	6. No water extraction, damming or diversion upstream	Nijboer *et al.* (2004)
Point source pollution	7. No point source pollution and eutrophication*	Hering *et al.* (2003)
	8. Natural color and odor*	Nijboer *et al.* (2004)
	9. Total phosphorus concentration < 0.035 mg/l	Moog and Sharma (2005)
	10. Oxygen concentration > 8mg/l and Saturation range between 95 – 110%	Moog and Sharma (2005)
	11. BOD_5< 2mg/l	Moog (1988)
	12. No known cattle watering points upstream*	Present study
	13. Minimal washing and bathing activities	Present study
Non-point source of pollution	14.Absence of known or expected diffusion input	Hering *et al.* (2003) Nijboer *et al.* (2004)
	15. Crop farming < 20% of the catchment area	Hering *et al.* (2003)
	16. Natural land use (forest, bushes, grassland)> 60% coverage	Present study
	17. Rural resident < 0.5% in the catchment area	Present study
	18. No sand or gravel excavation upstream*	Nijboer *et al.* (2004)
Riparian vegetation and wildlife	19. Having adjacent natural vegetation appropriate to the type and geographic location of the stream	Barbour *et al.* (1996)
	20. Presence of wild animals that are representative of the region and drive some support from aquatic ecosystems	Barbour *et al.* (1996)

BOD_5 Five days biological oxygen demand

The concept behind the reference site approach is to establish controls as a benchmark to represent least impaired conditions in water bodies and evaluate against monitoring sites. The extensive spatial survey suggests a sufficient number of minimally disturbed sites available in Ethiopian highland streams and rivers and gave a chance to evaluate a priori criteria established elsewhere (e.g. Hughes, 1995; Barbour *et al.,* 1996; Hering *et al.*, 2003; Nijboer *et al.,* 2004; Moog and Sharma, 2005). The criteria chosen in the present study defined the least amount of environmental disturbance caused by human activities for an acceptable ecological condition.

It was rarely possible to find sites which fulfil all a priori criteria. For instance, streams in central highland (tributaries of Awash and Rift Valley basins) could not fulfil the required criteria as proposed, thus the minimum number of criteria to define reference condition was set to 16 criteria (compulsory criteria included) in the sense of the 'beast attainable condition' (Stoddard *et al.*, 2006). In most highlands of Ethiopia, lack of proper soil and water conservation, high number of cattle, deforestation, siltation, shifting population density and expansion of farmland are still on-going problems, that makes it more difficult to find less impaired site in the region. Furthermore, early historical information on stream conditions or biotic diversity is quite scarce. Therefore, this study established an approach that includes compulsory and optional 'a priori' criteria that are representative for the region.

The most important criteria during selection procedure were those related to land use, riparian vegetation and point sources of pollution. The riparian vegetation cover in Ethiopian highland streams is highly variable and restricted to state protected areas, religious sites, steep gorges and national parks. Locally, riparian vegetation is frequently cleared for firewood, crop farming, construction and grazing. Even though vegetation along the stream channel varies widely in type, size, form, growth rate and longevity, riparian vegetation plays a crucial role in nutrient uptake, organic matter supply and soil stabilization. In highland mountains of Ethiopia (e.g. Bale Mountains) small bushes, shrubs and grasses are dominant, while trees and shrubs are common in the river escarpment. The riparian vegetation in the river channel are associated with the presence of wildlife which gets benefit from stream water (e.g. *Antelope sp.*, black and white colobus monkey and many bird species). In unprotected areas most indigenous riparian vegetation were cleared and converted to eucalyptus plantation or farm lands. Thus it is believed that inclusion of criterion in relation with presence of indigenous riparian vegetation and associated wildlife can represent reference condition in Ethiopian highlands.

Water quality parameters are primary factors that determine the nature of the biota in streams and rivers. In rural areas of Ethiopia numerous water quality problems have been associated with eutrophication caused by nutrient load from various sources (e.g. washing, agriculture and cattle waste) while domestic and industrial wastes are major factors in urban areas. The absence of urban and industrial discharges upstream of potential reference sites must be considered in the criteria because the effluents upstream of potential reference sites have a greater effect on water quality and then to the biota. For example high concentrations of phosphorus from effluent discharges can cause water quality problems by over stimulating the growth of algae that intern contribute to the loss of oxygen needed by aquatic animals (e.g. benthic invertebrates, fishes) (Hall *et al.*, 2001).

Specific but very frequent pressures that occur in Ethiopian highland including cattle watering, washing and rural residents are also important criteria. About 85% of the population of Ethiopia live in rural areas, and depends on agriculture (crop and livestock) such that streams and rivers are used intensively either for production (livestock watering and crop farming) or as a source for domestic use. Thus, criteria related to cattle watering and intensity of crop farming upstream of the reference sites is included. Furthermore, in-stream activities such as cloth washing using various detergents are common phenomena, where sites affected by intense washing activities cannot represent reference conditions.

The absence of point source of pollution from domestic sources and industries upstream of potential reference sites was a key criterion which should be fulfilled by all reference sites. The effluents upstream of reference sites have a greater effect on streams especially at low flow time, because a little effluent discharge results in high concentration of nutrients that may cause significant effect on stream biota.

On the other hand, criteria related to river morphology, habitat composition, hydrological conditions were fulfilled by all reference sites. In Ethiopian highlands the major human alterations on hydrology are caused by damming, diversion and water abstraction. These activities are common for irrigation, livestock watering and domestic consumption. Since the demands for irrigation are highest in the dry months where most streams are at their lowest flow discharge, the effect of diversion and water abstraction has significant effect on downstream environments. Stream bank degradation due to gravel and sand mining, and erosion and siltation are major sources of morphological alterations in most highland streams of Ethiopia. Since specific studies are lacking to define minimal hydro-morphological disturbances in the study area, relevant criteria from other sources were adapted to characterize reference sites for Ethiopian highland streams (e.g. Hughes, 1995; Barbour *et al.*, 1996).

Most environmental parameters measured in the field and laboratory were significantly different between reference and impaired sites (Table 2). Among the microhabitats, rocks (macrolithal) and fine sands were significantly different while the dominant cobbles (mesolithal) and pebbles (microlithal) were not significantly different between reference and impaired sites. The natural forest constitutes about 80 % of the land use in the reference sites unlike its small proportion in impaired sites. Agricultural activities were observed everywhere but the proportion was significantly high in impaired sites.

Table 2: Means, standard deviations and ranges (in bracket) for environmental parameters in 'a priori' reference and impaired sites

Parameters	'a priori' reference sites n=22	'a priori' impaired sites n=82	p-value
Conductivity(µS/cm)	146.1±66.5(45.4-265)	464±757 (60-6500)	.053
DO (mg/l)	8.2±0.7(6.8-9.4)	6.5±2.3 (0-11.7)	.001
DO %	107.8±6.5 (94.6-120)	89±32(0-149)	.009
BOD5 (mg/l)	1.9±0.5(1.4-2.8)	61±190 (0.6-1059)	.035
TP(mg/l)	0.033±0.004(0.03-0.05)	0.56±1.1(0.01-6.1)	.024
Catchment size (km^2)	112±190 (8-794)	663±1094 (11-4914)	.015
% Riffle	82.7±9.7(55-95)	73±18(10-100)	.024
Velocity(m/s)	0.5±0.2(0.2-0.85)	0.45±0.2(0.1-1.05)	.119
Substrate type			
% Megalithal	23.6±15.9(0-50)	8.4±10.6(0-40)	.001
%Macrolithal	37.8±10.8(20-55)	25±16.8(0-75)	.001
%Mesolithal	27±9.5(15-45)	26.5±16.8 (0-70)	.897
%Microlithal	10.7±11(0-35)	11.5±11.8(0-50)	.782
%Akal	1±2 (0-10)	10±23(0-100)	.061
%Psammal/fine sand	0	18.7±27.3(0-85)	.002
Land use			
% forest/bushes	82±25(55-100)	9±18(0-35)	.000
% Agriculture	17±23(5-35)	60±35(0-100)	.003
% Urbanization	0.4	24±37(0-100)	.003

Sites are significantly different at $p < 0.05$, TP total phosphorus, DO dissolved oxygen

Macroinvertebrate Communities

Lower abundances of macroinvertebrates were observed at all reference streams, which is in accordance with the concept that mild pollution has a tendency to increase total abundance of macroinvertebrates. Sensitive taxa richness (Aschalew and Moog, 2015) was generally high in the reference sites but constitute less than 10% in the impaired sites. For example, about 83 % of the reference sites constitute Perlidae but only 4 % of impaired sites have this taxon. Perlidae, Philopotamidae, Lepidostomatidae, Leptoceridae, Heptageniidae, Leptophlebiidae, Scirtidae and Psephenidae were common taxa recorded in the reference sites while these taxa were absent or rare in impaired sites. From sensitive taxa groups Baetidae and Hydropsychidae were the most diverse and abundant in both reference and impaired sites. In rural agricultural and siltation sites Caenidae, Coenagrionidae, Corixidae, Notonectidae, Naucoridae, Gyrinidae and Dytiscidae were fairly ubiquitous.

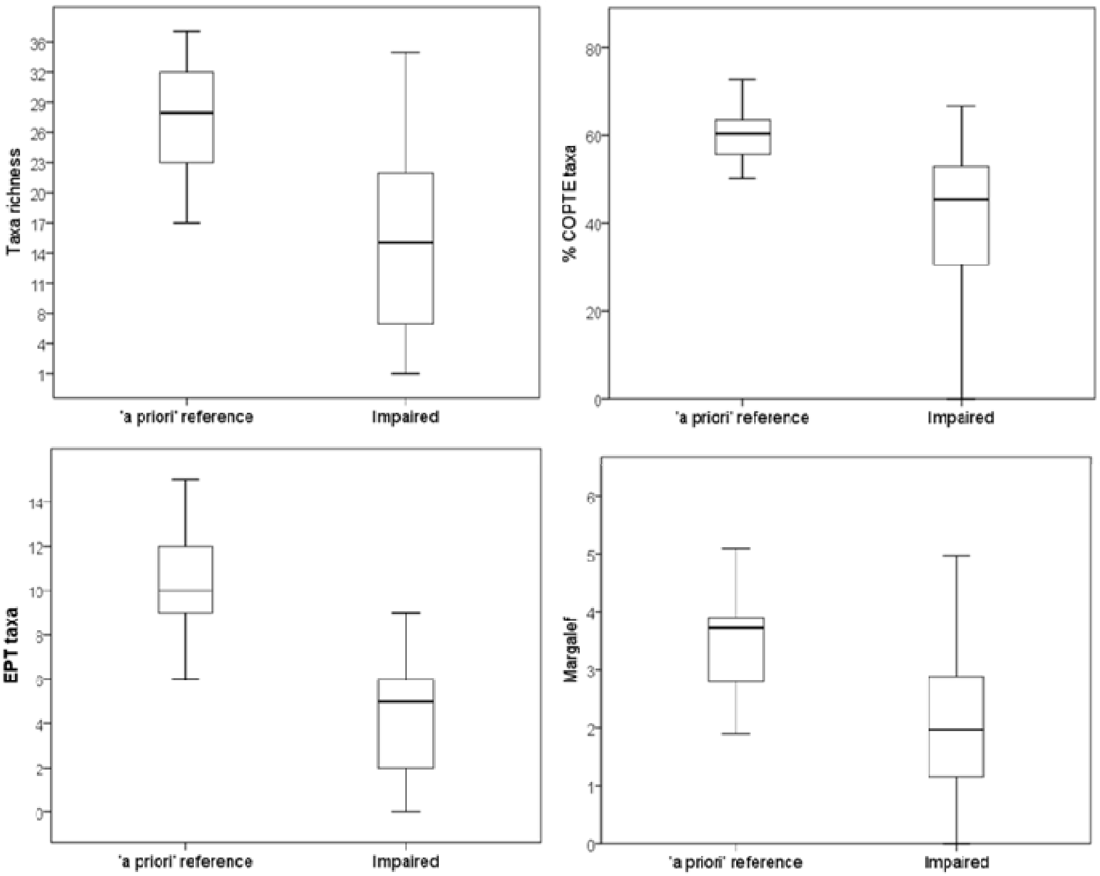

Figure 3: Box-and-whisker plots metrics/indices showing the median values in the a priori reference and impaired sites. Bar line within the box represents median number, boxes represent first- and third-quartile ranges (25th and 75th percentiles) and range bars show maximum and minimum of non-outlier numbers. EPT Ephemeroptera, Plecoptera and Trichoptera, COPTE Coleoptera, Odonata and EPT.

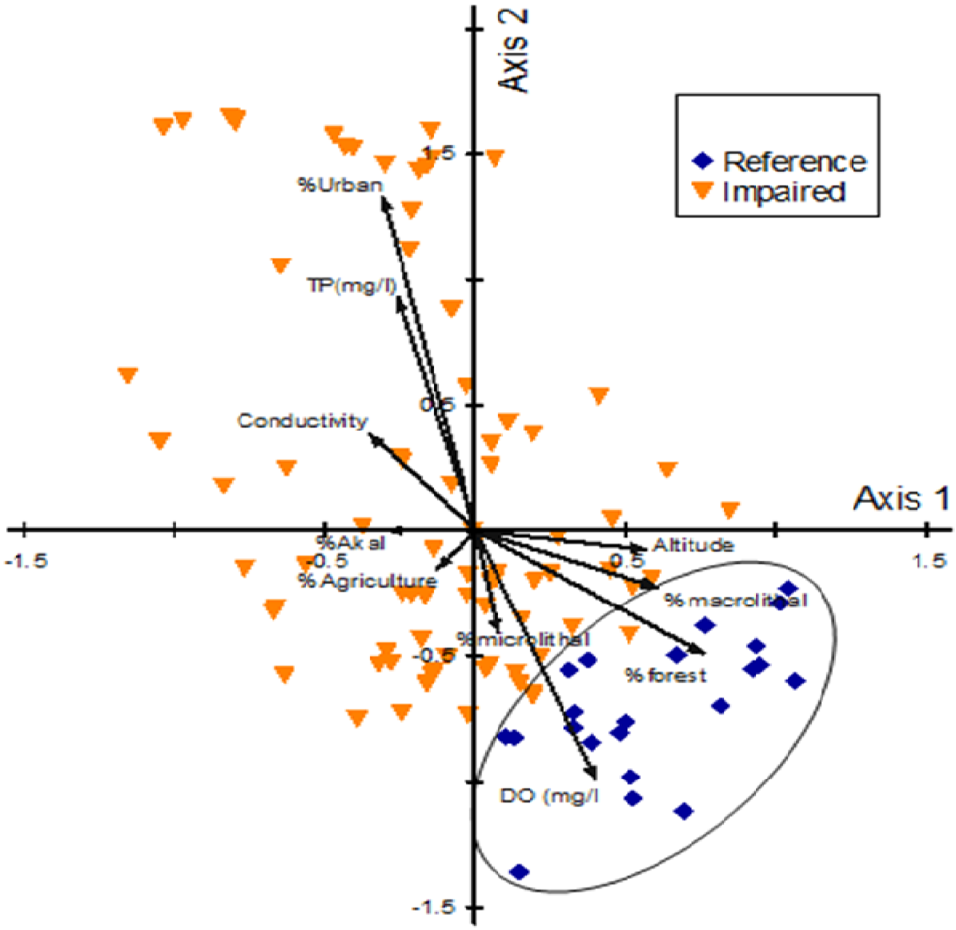

Figure 4: Non-metric multidimensional scaling (NMS) scatter plot for 102 taxa from 104 sites. Final stress for 2 dimensions = 14.3, number of iteration = 166, 'a priori' class overlay. The vector length and direction show dominant environmental parameters responsible for distribution of invertebrates.

NMS clearly showed differences in benthic invertebrate composition between reference and impaired sites (Fig. 4). The most important environmental variables influencing macroinvertebrate composition were TP, conductivity, % of urban which were directed to impaired sites . Likewise percent of forest cover and dissolved oxygen were pointing away from impaired sites as these parameters decrease with increase in pollution load and human interference.

Selecting the reference sites in areas that are highly affected by human activities is difficult because of the occurrence and interaction of natural and human generated stress to specific ecosystems which requires a validation step to confirm and refine the selected reference sites. Macroinvertebrate assemblages were used for validation purposes by applying community distribution in the NMS analysis and using ecological threshold values defined for Ethiopian highland river assessment (Aschalew and Moog, 2015). The use of macroinvertebrate assemblages to validate reference conditions has been recommended by many authors because they reflect the influence of all the stressors acting on the environment, and are capable of detecting the presence of certain types of disturbances that are difficult to recognise with the common screening methods used (Karr, 1999; Karr and Chu, 2000; Wallin *et al.*, 2003; Bailey *et al.*, 2004; Nijboer *et al.*,2004; Chaves *et al.*, 2006).

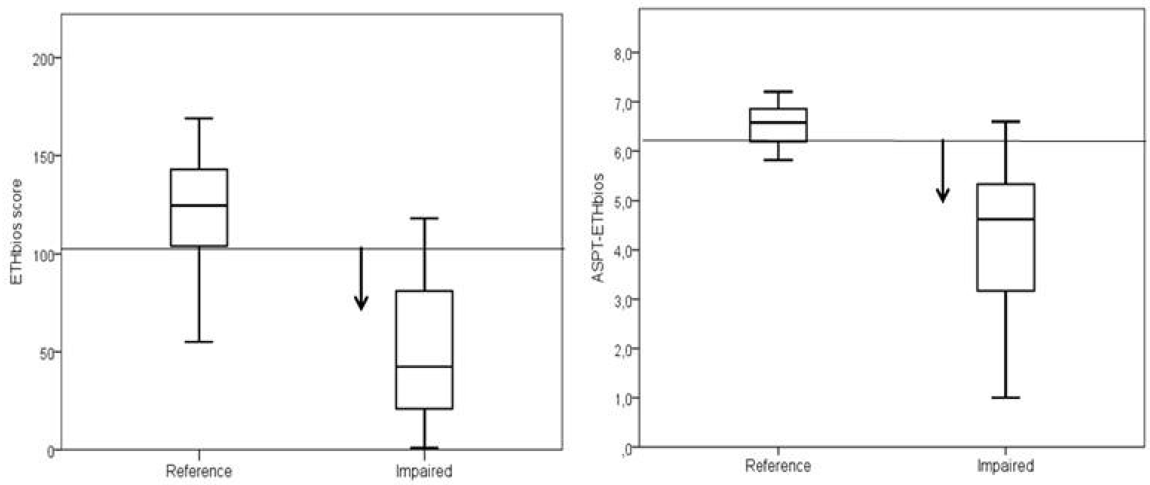

Figure 5: The discrimination efficiency is expressed as the percentage of stressed sites showing values lower than the 25th percentile of reference values for ETHbios total score and the derived Average score per taxa (ASPT).

ETHbios (total score and the corresponding ASPT) developed based on benthic macroinvertebrates for assessing Ethiopian highlands confirmed clear separation of reference and impaired sites designated independently from invertebrate data. ETHbios threshold value established for 'high ecological status' was considered as reference and ETHbios score and the corresponding ASPT value showed 93 % and 96 % discrimination efficiency between reference and impaired sites. This shows the proposed criteria can be used as a proper parameters to select references sites in Ethiopian highland streams. Although there was a clear stress gradient between the reference and impaired sites (a priori criteria), few impaired sites showed nearly similar taxonomic distribution with reference sites. Differences in macroinvertebrate metrics in the reference and impaired sites were reported by different authors (Armitage *et al.*, 1983; Chutter, 1998; Dallas, 2000; Dicken and Graham, 2002; Sporka *et al.*, 2006). In this study reference sites presented higher median values than disturbed sites at different exposure of stressors levels, proving that the analysis of macroinvertebrate communities independently confirm the appropriateness of selected criteria for reference site selection.

The criteria proposed in this study are relatively simple and quick to apply in order to select reference sites, and especially helpful in small river basins in the highlands of Ethiopia. It could be important to include additional criteria such as alien animal species or invasive aquatic weeds when considering specific study area or lake associated rivers in Ethiopian highlands (> 1800 m a.s.l.).

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