# DEVELOPING MACROINVERTEBRATE TRAIT- AND TAXONOMICALLY-BASED APPROACHES FOR BIOMONITORING WADEABLE RIVERINE SYSTEMS IN THE NIGER DELTA, NIGERIA

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

OVIE AUGUSTINE EDEGBENE

ORCid Number: 0000-0003-4032-3923

March 2020

**ABSTRACT**

Riverine systems are increasingly subjected to pollution due to rapid urbanisation, industrialisation, and agricultural activities. Increasing pollution in freshwater systems impairs water quality, causes biodiversity loss and impairs aquatic ecosystem functionality and supply of ecosystem services. Rivers in the Niger Delta region of Nigeria are particularly vulnerable to urban pollution and agricultural activities as natural forests are increasingly replaced by urbanisation and agriculture. The differential effects of these pressures on the ecological processes of these river systems are poorly explored, as is the development of appropriate biomonitoring tools for routine monitoring of river health. In this study, a physico-chemically-based approach and macroinvertebrate trait- and taxonomic- approaches were developed to better understand the effects of multiple pressures on riverine systems, while developing multimetric indices to enable sustainable management of rivers within the region. Sixty-six stations in 20 river systems within the Edo and Delta States of the Niger Delta ecoregion were monitored seasonally for a period of five (2008–2012) years.

The physico-chemically based approach makes apparent the extent of degradation of rivers and streams in the Niger Delta. For each dominant land use type, river stations were classified into least impacted stations (LIS), moderately impacted stations (MIS) or heavily impacted stations (HIS). Of 11 stations within urban catchments, only two were considered least impacted, suggesting that urgent measures are necessary to revise the current trajectories of urban rivers within the region. Most of the stations designated as MIS and HIS in the urban and urban-agriculture catchments were found to be significantly correlated with increased nutrients, EC and $BOD_5$. Characteristics of most of the MIS and HIS within rivers in urban catchments evidenced the so-called urban stream syndrome, a state of persistent degradation of urban streams.

The results of the traits and ecological preferences approach showed traits sensitive to urban and urban-agriculture pollution. Traits and ecological preferences that were associated with the LIS

include the possession of hardshell, moderate and high sensitivities to oxygen depletion, very large body sized individuals (>20-40mm), swimmers, flattened body shape, a preference for temporary attachment, crawling, respiration with aerial/vegetation, possession of breathing tubes, possession of strap or other apparatus for respiration, streamlined body, and a high sensitivity to oxygen depletion. Permanent attachment as an ecological preference associated with LIS was also positively correlated with increasing dissolved oxygen (DO) and was deemed a pollution sensitive ecological preference. The possession of very small body size (<5mm), associated with HIS, was deemed a pollution-tolerant trait and was negatively correlated with DO, confirming the deteriorating state of the urban and urban-agricultural rivers. The impact of urban-forestry pollution on the distribution pattern of macroinvertebrate traits and ecological preferences was also explored in the selected rivers. Traits and ecological preferences such as possession of hard-shell, large body size, and grazing as a feeding preference which were significantly positively associated with the LIS, were also either significantly positively correlated with DO, or significantly negatively correlated with increasing any two of flow velocity, water temperature, $BOD_5$ and nutrient. These traits and ecological preferences were deemed sensitive in forested rivers receiving urban pollution. Further, burrowing, the pupa aquatic stage, and predation which were significantly positively associated with HIS on the RLQ ordination, were also significantly negatively associated with DO. These traits were deemed tolerant of forested systems receiving urban pollution.

Multimetric indices (MMI) were developed, validated and applied for urban, urban-agriculture and urban-forested (MMI-urban, MMI-urban-agric and urban-forest) areas. Of the 26 metrics that satisfactorily discriminated between the LIS, the MIS, and the HIS for MMI-urban, only five metric were retained for integration into MMI-urban, they are log VeL, Hemiptera abundance, % Coleoptera + Hemiptera, % Chironomidae + Oligochaeta and Evenness index. Further, of the 18 metrics that satisfactorily discriminated between the LIS, the MIS, and the HIS for MMI-urban-

agric, only 12 metrics were retained and nine proved to be redundant. The nine metrics represent different measures; two of them were retained in addition to Chironomidae/Diptera abundance, % Odonata and Oligochaeta richness. The two metrics selected in addition to the Chironomidae/Diptera abundance, % Odonata and Oligochaeta richness were the Margalef index and the logarithm of relative abundance of sprawler. For the MMI-urban-forest, 14 metrics satisfactorily discriminated between the LIS, the MIS, and the HIS, and 12 metrics were retained and 11 proved to be redundant. The non-redundant metric was Trichoptera abundance. Three metrics were further selected in addition to the Trichoptera abundance which include % Chironomidae + Oligochaeta, Coleoptera + Hemiptera richness and Shannon diversity. The MMI-urban and MMI-urban-agric indices performed better for LIS designated stations compared to the MIS and HIS deignated stations. The developed indices proved effective as biomonitoring tools for assessing the ecological health of rivers in the urban and urban-agriculture catchments within the Niger Delta.

Overall, the results of the macroinvertebrate traits and ecological preferences, and taxonomic approaches showed the strength in the complementarity of both approaches in developing biomonitoring tools for assessing levels of deterioration in riverine systems. The study contributes significantly to understanding the ecology of riverine systems in the Niger Delta, particularly those subject to urban stresses, agricultural activities and urban pollution in forested systems, and thus makes an important contribution to the science and practice of biomonitoring in Nigeria where such studies are sparse.

## TABLE OF CONTENTS

**LIST OF TABLES**

## LIST OF FIGURES

## LIST OF APPENDICES

**ABBREVIATIONS AND ACRONYMS**

| | |
|---|---|
| ANOVA | Analysis of Variance |
| APHA | American Public Health Association |
| BOD | Biochemical Oxygen Demand |
| CCA | Canonical Correspondence Analysis |
| COA | Correspondence Ordination Analysis |
| CPOM | Coarse Particulate Organic Matter |
| DCA | Detrended Correspondence Analysis |
| DO | Dissolved Oxygen |
| EC | Electrical Conductivity |
| EIA | Environmental Impact Assessment |
| EPIA | Environmental Post Impact Assessment |
| EPT | Ephemeroptera, Plecoptera and Trichoptera |
| ETOC | Ephemeroptera, Trichoptera, Odonata and Coleoptera |
| FFG | Functional Feeding Group |
| FMEV | Federal Ministry of Environment |
| FMWR | Federal Ministry of Water Resources |
| FPOM | Fine particulate Organic Matter |
| HIS | Heavily Impacted Stations |
| HTC | Habitat Template Concept |
| IBI | Index of Biological Integrity |
| IQR | Inter-quartile Range |
| LIS | Least Impacted Stations |
| MIS | Moderately Impacted Stations |

| | |
|---|---|
| MMI | Multimetric Index |
| NESREA | National Environmental Standard and Regulations Enforcement Agency |
| NOSDRA | National Oil Spill Detection and Response Agency |
| PAST | Palaeontological Statistical Package |
| PCA | Principal Component Analysis |
| RDA | Redundancy Analysis |
| SPEAR | Species at Risk |
| TBA | Trait-Based Approach |
| WWTW | Wastewater Treatment Works |

**ACKNOWLEDGEMENTS**

I wish to express my gratitude to the South Africa National Research Foundation (NRF) and the World Academy of Sciences (TWAS) for funding this research work.

I acknowledge and appreciate the effort of Professor Francis Ofurum Arimoro and Dr Oghenekaro Nelson Odume in supervising this PhD project. Professor Arimoro was more than a father figure in encouraging me, even when all hope was lost. I deeply appreciate your help, and pray God may reward you greatly in all your endeavours. Thank you, Dr Odume for all your support in ways too numerous to mention. God bless you and your family for your assistance.

I thank Miss Winnie Malhaba and Miss Pindiwe Ntokolo of the Institute for Water Research, Rhodes University, Grahamstown, South Africa, for their support during the PhD programme. Miss Winnie Malhaba, thank you for helping with the study area map. God bless you abundantly.

Special thanks to Mr. Frank Chukwuzuoke Akamagwuna for his encouragement when all hope seemed lost during data and statistical analyses. God bless and reward you greatly for your contribution in making this PhD thesis a success. Your invaluable advice helped in shaping this work.

Thanks to all staff and students of the Institute for Water Research, Rhodes University, Grahamstown, South Africa, with special thanks to the Director of the Institute for Water Research, Professor CG (Tally) Palmer, for her motherly support and encouragement during my stay at the Institute.

Thanks to Dr Ferdy de Moor and Dr Helen Barber-James of the Albany Museum, Grahamstown, South Africa for helping in identifying some of the macroinvertebrate taxa.

I thank Mr. Joseph Ovwemuose for supporting me spiritually and morally; may God reward you and shower blessings upon you.

Special thanks to Mrs. Ejiro Odume, for your support during my stay with you and your family in Grahamstown, South Africa. Thank you for your hospitality and good will towards me and my family.

I am grateful to Pastor Kure Obed of the Living Faith Church, Hadejia, Jigawa State, Nigeria for his prayers and positive words of encouragement during my stay in South Africa for this PhD programme.

I appreciate the support, both physical and spiritual, of my wife Mrs. Tega Treasure Edegbene Ovie during our stay in Grahamstown, South Africa. Thank you for your moral support and your understanding during the late-night writing of this thesis. Thanks, too, to my children, Honour Orodena Ovie and Above Oghenefejiro Efemena, for your support and understanding during the PhD journey.

Special thanks to my mother, Mrs. Adeyemi Judith Edegbene, who was both a mother and father figure throughout my academic journey. God bless you and grant that you may eat the fruit of your labour.

**Above all, if it were not for the mercy, favour and love of God Almighty, my PhD dream would have vanished into thin air. Gracious God, to You alone is all glory, honour and adoration. May Your Name be glorified forever.**

## DEDICATION

This PhD thesis is dedicated to all those who thought they were pulling me down, but God Almighty used their deeds to elevate me to heights I never envisaged.

# CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

Population growth and urbanisation, together with a growing demand for food, have led to increasing pollution of water resources globally (Bringezur *et al.*, 2014; Tchakonte *et al.*, 2015). The consequences of increasing pollution in freshwater systems are impaired water quality, discharge of contaminants of emerging concerns in water bodies, biodiversity loss, and impaired aquatic ecosystem functionality and supply of ecosystem services (Allan, 2004; Mereta *et al.*, 2013; Krynak & Yates, 2018; Gieswein *et al.*, 2019). Urbanisation alters the functionality and ecological health of freshwater systems through the effects of urban syndrome which include nutrient enrichment, depletion of dissolved oxygen, and elevated concentrations of metal and dissolved solids (Walsh *et al.*, 2005; Kuzmanovic *et al.*, 2017; Desrosiers *et al.*, 2019).

Urban stream syndrome negatively affects the community structures of aquatic biota of freshwater systems in urban catchments (Poff *et al.*, 2006; Kuzmanonic *et al.*, 2017), as does agriculture and forestry. Agricultural activities contribute to land alteration, fragmentation of landscapes, and the influx of fertilisers, nutrients and sediments into freshwater systems (Allan, 2004; Walsh *et al.*, 2005; Krynak & Yates, 2018). Freshwater systems stressed by agricultural activites suffer from increased nutrient concentrations, dissolved oxygen depletion, accumulation of dissolved solids, and reduced biodiversity (Pallottini *et al.*, 2017; Krynak & Yates, 2018).

Apart from the effects of urbanisation, forestry, particularly man-made forestry, can also impact water resources. Although forestry constitutes an important component of river catchments, particularly in the Niger Delta, Nigeria, where they are a source of allochthonous food (Sedell *et al.*, 1990), man-induced forestry can impact the taxonomic and functional diversity of instream biota through changes in functional feeding groups (Fierro *et al.*, 2017). The impact of forestry is increased freshwater acidification through leaf decay and the release of acid-rich organic materials, alteration of flow velocity through water abstraction by alien vegetation, and of temperature regulation through shading (Wilby *et al.*, 2010; Lagrue *et al.*, 2011). Given that the major large-scale stressors of freshwater ecosystems in the Niger Delta are urbanisation, agriculture and forestry, this study focuses on these catchment-based activities and their effects on the ecological conditions of water resources, while at the same time, developing biomonitoring tools for managing riverine ecosystems.

In most parts of sub-Saharan Africa, most especially Nigeria, managing and monitoring water quality is still within the domain of physico-chemical analysis alone, and to some extent, basic analysis of in-stream biota (Adakole & Anunne, 2003; Odume *et al.*, 2011; Arimoro *et al.*, 2015). However, physico-chemical analysis alone cannot provide a comprehensive picture of the deteriorating states of freshwater systems because physico-chemical variation can be distorted by sampling locations and seasons, and may fail to address critical ecological activities in the ecosystems being studied (Bonada *et al.*, 2006). Therefore, the development of appropriate biomonitoring tools is pertinent to complement the physico-chemical variable analysis in order to support sustainable freshwater ecosystem management in Nigeria.

Nigerian freshwater ecosystems are divided into five ecoregions: the Bight Coastal, Lake Chad Catchment, Lower Niger-Benue, Northern West Coastal Equatorial and the Niger Delta (Kamdem-Toham *et al.*, 2006). The present study was conducted in the Niger Delta ecoregion situated in the rainforest belt, home to numerous creeks, streams and rivers. Rivers in the Niger Delta are surrounded by various land uses which include urban, agriculture and forestry (Arimoro & Ikomi, 2008). Presently, there are little or no biomonitoring tool for monitoring and assessing the impacts of urbanisation, agricultural activities and forestry on the riverine systems in Niger Delta, and more widely in Nigeria. An appropriate biomonitoring tool is necessary to assess the impacts of these activities on the Niger Delta riverine systems, the development of which is the primary focus of the present study. Such a tool is particularly important to support relevant government agencies in advancing and implementing their imperatives for sustainable development, and in managing freshwater ecosystems in the Niger Delta.

To develop appropriate biomonitoring tools, different groups of aquatic biota have been employed: macrophytes, phytoplankton, macroinvertebrates, fishes and birds (Aguiar *et al.,* 2011; Wu *et al.,* 2012; Pallottini *et al.,* 2017; Petriki *et al.,* 2017; Gieswein *et al.,* 2019). Of the biota used to develop biomonitoring tools, macroinvertebrates are the most frequently exploited because they can be collected easily, they are highly diverse, and they respond predictably to a variety of stressors and pollutants (Bonada *et al.,* 2006; Odume *et al.,* 2012). Macroinvertebrate traits and taxonomic constituents are now being explored in developing biomonitoring tools and protocols (Desrosiers *et al.,* 2019; Shull *et al.,* 2019).

The macroinvertebrate trait-based approach explores the functional characteristics of macroinverbrates to develop biomonitoring tools, while the macroinvertebrate taxonomic approach takes into account the structural diversity, composition and abundance of macroinvertebrates in an ecosystem to develop biomonitoring tools.

The macroinvertebrate trait-based approach in biomonitoring is expanding rapidly because of the mechanistic response of macroinvertebrate to stressors (Verberk *et al.,* 2013). The approach is an improvement on the usual descriptive studies of macroinvertebrate community structure (composition, abundance and diversity), as different macroinvertebrate taxa exhibit traits that enable them either to become resilient or vulnerable to perturbation (Townsend & Hildrew, 1994; Odume *et al.,* 2014). On the other hand, even though the trait-based approach is hailed as predictive and mechanistic in the science of biomonitoring, the macroinvertebrate taxonomically-based (e.g.

multimetric) approach is still useful, enabling the conservation of biota and an understanding of stressor effects on taxonomic diversity and composition; an important conservation goal. Further, the macroinvertebrate multimetric approach is useful because it combines multiple taxonomic measures into a multimetric index (Bonada *et al.,* 2006). Bonada *et al.* (2006) argue that, of the 12 different criteria they use to assess the biomonitoring powers of different approaches used in ecological assessment, macroinvertebrate trait- based and multimetric indices approaches proved more effective because they met ten of the 12 criteria they set, compared with other approaches such as single biotic index and functional feeding group (Bonada *et al.,* 2006). The macroinvertebrate multimetric index is preferred over other approaches because it integrates multiple metrics and indices to assess the quality of aquatic ecosystems (Baptista *et al.*, 2007; Odume *et al.*, 2012; Mereta *et al.*, 2013; Edegbene *et al.,* 2019; Shull *et al.,* 2019).

However, the macroinvertebrate trait-based approach alone may be insufficient to ascertain the level of perturbation a riverine system is undergoing; the trait-based approach takes into account the functional aspect of aquatic biota while the macroinvertebrate taxonomic approach accounts for the structural aspect of aquatic biota. The complementary use of both trait- and taxonomic-based approaches would give a clear picture of the prevailing ecological conditions of riverine systems. Hence, the selection of these two approaches: traits and ecological preferences, and taxonomic approaches to assess the ecological status of selected rivers in the Niger Delta, Nigeria. A complementary use of both macroinvertebrate traits and taxa was also employed in developing and applying multimetric indices in three landuse types namely: urban, urban-agriculture and urban-forested river catchments in the Niger Delta.

Taxonomic- and trait-based approaches at the species levels have proved to be more accurate in reflecting degree of perturbation that those at the generic and family level. However, in this study, a family level taxonomic level was used to develop the trait-based approach and taxonomic indices because identification guides were unavailable, and expertise in macroinvertebrate taxonomy in the Afrotropical region is scarce. This study, then, developed family level macroinvertebrate taxonomic- and trait-based biomonitoring tools for wadeable riverine systems in the Niger Delta.

The remaining part of this chapter reviews water resource management in Nigeria, describes approaches to monitoring surface water quality, locally and globally, and provides the rationale and aim of the study. The chapter ends with the thesis structure.

### 1.2 Water resource management in Nigeria

The 1999 constitution of the Federal Republic of Nigeria is the primary legal framework that provides for managing, conserving and protecting Nigeria's surface water resources (FEPA, 1991). Section 20 of the constitution empowers the State to protect and safeguard the water, air, land, forest and wildlife of Nigeria. In order to implement the provisions of the constitution, a number of laws have been enacted, among them, the Environmental Impact Asessmment Act of 1992, the National Environmental Standard and Regulations Enforcement Agency Act of 2007, and the National Oil Spill Detection and Response Agency Act of 2006.

The Environmental Impact Assessment Act (EIA Act of 1992) empowers the Federal Minsitry of Environment (FMEV) to carry out an environmental impact assessment (EIA) and compel private and public entitities to undertake an EIA before embaking on development projects likely to negatively impact on the environment. This requirement includes water resources. The intention of the Act is to identify and minimise any source of potential impact in the Nigerian environment, including surface waters. The EIA study is followed by an environmental post-impact assessment (EPIA) and finally, by mitigation measures. The EPIA regulates the operations of already existing private and public companies or firms to curtail the level of impacts their activities pose to the Nigeria environment, including surface water. If the activities of companies have impacted the environment negatively, mitigation measures are implemented to remedy the damage caused by the activities of the companies.

The National Environmental Standards and Regulations Enforcement Agency (NESREA) was established in 2007 by the Federal Government of Nigeria in recognition of the vacuum existing for effective enforcement of environmental laws, standards and regulations in Nige. The NESREA has developed 33 national environmental regulations for several aspects of the environment (including aquatic ecosystems) since its establishment. A number of regulations with a bearing on water resources in Nigeria have been promulgated. The first regulation in 2009 relates to the national environment (watershed, mountainous, hilly and catchments areas), and provide for the protection of watersheds and catchment areas by controlling activities which are inconsistent with good land management practices in vulnerable areas such as the Niger Delta. The second regulation in 2009 concerns the the national environmental (wetlands, river banks and lakes shores), and provides for the protection of wetland habitats, fauna and flora, and for pollution control. The focus of the third regulation in 2009 is the national environment (chemical, pharmaceuticals, soap and detergent manufacturing industries) and requires industries producing pollutants from the above-listed products and consumables to minimise their activities to ensure that the environment is protected. A fourth regulation is the national environmental (surface and ground water) quality control regulation in 2011 which provides for the restoration, enhancement and preservation of the the physical, chemical and biological integrity of the nation's surface waters.

Protection against oil pollution is provided via the National Oil Spill Detection and Response Agency (NOSDRA), established by Act of 2006 (NOSDRA Act, 2006), which is responsible for detecting and responding to oil spillages in Nigeria, with particular reference to the Niger Delta. Other functions of the NOSDRA are: (i) to ensure the co-ordination and implementation of the plan within Nigeria's waters, including 200 nautical miles from the basement from which the breadth of

Nigeria's territorial waters are measured; (ii) to undertake surveillance, report and alert relevant parties, and carry out other response activities related to oil spillages; (iii) to encourage regional co-operation among member states of the West African sub-region and in the Gulf of Guinea to combat oil spillage and pollution in Nigeria's contiguous waters; (iv) to strengthen national capacity and regional action to prevent, control, combat and mitigate marine pollution, and (v) to promote technical co-operation between Nigeria and member states of the West African sub-region.

The Federal Ministry of Environment (FMEV) is the umbrella body of NESREA and NOSDRA. The other body concerned with water issues in Nigeria is the Federal Ministry of Water Resources, whose main focus is on Nigeria's dams and irrigation activities. These water bodies are not the focus of this present study which is centred on rivers on the Niger Delta area.

One of the biggest challenges Nigeria faces is the lack of routine surface water quality monitoring by the regulatory agencies. Apart from occasional physico-chemical montoring in response to specific events, for example; environmental impact assessment (EIA) and mitigation measures no other forms of monitoring, such as biological, morphological or habitat-based, are implemented because no tools or standardised protocols exist. One of the primary motivations for this study is to fill this gap by developing a biological monitoring tool for rivers in the Niger Delta, realising the value of the chemical, physical and biological quality of water resources as practised elsewhere, such as in Europe (Hering *et al.,* 2006; Ofenbock *et al.,* 2004), USA (Shull *et al.,* 2019), and South Africa (Dickens & Graham, 2002; Bird, 2010).

### 1.3 Physico-chemical variables

Water physico-chemical variables describe the physical and chemical characteristics of aquatic ecosystems. Anthropogenic activities such urbanisation, industrialisation and agricultural activities can influence the water physico-chemical variables of riverine ecosystems

(Olomukoro & Ezemonye, 2007; Ojutiku & Kolo, 2011; Arimoro *et al.*, 2015). Depending on the extent and frequency of perturbations, changes in the physico-chemical characteristics of aquatic ecosystems can have adverse effects on the biotic community structure and function of the impacted ecosystem (Odume, 2014). Therefore, monitoring using physico-chemical variables remains a fundamental pillar of assessing and managing the ecological conditions of freshwater systems globally. Examples of commonly used physico-chemical variables include dissolved oxygen, five-day biochemical oxygen demand, chemical oxygen demand, nitrate, phosphate, sulphate, electrical conductivity, pH, turbidity, flow velocity and heavy metals (Edokpayi *et al.,* 2000; Jonnalagadda & Mhere 2001; Odume *et al.* 2011; Arimoro *et al.*, 2015; Edegbene *et al.*, 2015). It is, however, important to note that the selection of specific variables to be monitored is guided by a number of factors, including i) the nature of pollution, ii) the fate of the pollutant in the environment, and iii) available resources, (e.g.analytical machines).

Although physico-chemical monitoring is fundamental to ecological assessment, the approach has a number of limitations. First, it can be prohibitively expensive if a multitude of variables, which require expensive analytical techniques, are to be monitored. Second, physico-chemical monitoring only reflects the condition of the time and space when samples are collected and fails to integrate information over time. Third, the approach is not useful in terms of providing deeper insights into ecological complexities within riverine systems. For these reasons, biological monitoring (biomonitoring) is often used to complement physico-chemical monitoring in protecting and conserving riverine systems. Biomonitoring is the systematic use of aquatic biota and their attributes to reflect the ecological conditions of the aquatic system such as rivers, lakes and ponds (Odume, 2014) and it provides information needed to manage, conserve and sustain biodiversity, as provided, for example, in the Indices of Biotic Integrity (IBIs) (Bird, 2010; Mereta *et al.*, 2013) and the South Africa Scoring System version 5 (Dickens & Graham, 2002).

### 1.4 Biomonitoring

Biomonitoring is the systematic and careful assessment of the ecological conditions of freshwater ecosystems using biota such as plankton, macroinvertebrates, and fish (Bonada *et al.*, 2006; Friberg *et al.*, 2011). Biota that repond predictably along a gradient of environmental disturbances are referred to as biological indicators (bioindicators) (Bonada *et al.*, 2006). The presence, diversity, abundance, composition, distribution of bioindicators and their traits and ecological preferences are analysed to reflect the prevailing environmental conditions in freshwater biomonitoring (Ogbogu & Oladije, 2002; Olomukoro & Ezemonye, 2007; Bonada *et al.*, 2006; Monaghan & Soares, 2012; Mereta *et al.*, 2013; Desrosiers *et al.*, 2019; Odume, 2020).

Among the bioindicators used in freshwater biomonitoring, macroinvertebrates are the most widely explored (Rosenberg & Resh 1993; Bonada *et al.*, 2006; Odountan *et al.*, 2019). Macroinvertebrates' wide acceptability as bioindicators is predicated on their ubiquitous nature, long life spans, diversity, life-history characteristics, sedentary nature, and their varied response to perturbations in aquatic ecosystems (Odume, 2014; Odountan *et al.*, 2019). In addition, sampling and identification of macroinvertebrates to at least the family level is easy, and sampling equipment is affordable. Furthermore, macroinvertebrates occupy an important position in the aquatic ecosystem food web, serving mostly as a bridge between the producer and secondary consumers. Thus, macroinvertebrate trait- and taxonomic-based biomonitoring approaches and tools have been developed.

#### 1.4.1 The trait-based approach (TBA)

Traits are inherent characteristics possessed by organisms at individual biological organisation level (McGill *et al.*, 2006). Traits can be morphological (e.g. body form), reproductive (e.g. number of offspring per reproductive event), biochemical (e.g. DNA make-up) (Kuzmanovic *et al.*, 2017; Krynak & Yates, 2018; Desrosiers *et al.*, 2019). For clarity, this study adopts the definition of traits by Violle *et al.* (2007) who define a trait as “any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole organism, without reference to the environment or any other level of organisation”. Based on this definition, the product of the direct interaction of the organism with its external environment cannot be referred to as traits but as ecological preferences (Odume *et al.,* 2018a). Sensitivity to pH, flow velocity, and preference for specific habitat and food are examples of ecological preferences (Odume *et al.,* 2018a). Based on the Violle *et al.* (2007) definition, a trait is confined to the individual level of biological organisation alone, excluding features measurable at higher levels of organisations, such as population and community. Thus, in the present study, a distinction is made between traits and ecological preferences.

Individual traits may take different forms between species, or in time and space. For example, a particular trait such as a respiratory trait gill, may take the form of operculate gills, filametous gills, or leaf-like gills. These forms may differ according to the organism’s life stage. Odume *et al.* (2018a) argue that “the value, form or modality taken by a trait at any given time and space is referred to as a trait attribute”. Trait attributes can be moderated by biological and environmental factors which make trait-based approach applicable in biomonitoring of freshwater ecosystem.

The application of traits in freshwater biomonitoring is rooted in the habitat template concept (HTC) which postulates that organisms survive and thrive in an environment for which they have the appropriate trait combination to cope (Townsend & Hildrew, 1994). Thus, a correspondence is expected between the external environmental characteristics and the trait combination of the organism (Townsend & Hildrew, 1994; Odume, 2020). For example, a heavily impacted urban river is expected to be dominated by small-bodied organisms with rapid reproductive turnover, often producing many eggs and offspring per reproductive event (Doledec & Statzner, 2008). The HTC is a concept on which the assumption of TBA rests.

Studies using the TBA have followed two distinct lines of reasoning and analysis. The first approach is analysing multiple trait responses to the stressor of interest, for example, as in Akamagwuna *et al.* (2019) who analysed the responses of individual traits to fine sediment effects. This approach is the most common and is based on the recognitiion that individual traits within an organism are differentially stressed by anthropogenic activities. If carefully used, this approach can lead to the identification of signature traits for specific stressors, which in turn, can lead to the development of trait-based tools. The present study uses the analysis of multiple individual traits in order to identify traits that are sensitive to and tolerant of the main stressor types in the Niger Delta region.

The second approach is to analyse combinations and intereactions of traits as underlying processes responsible for the responses of individual species to a particular environmental stressor (Verberk *et al.*, 2008; 2013). The assumption here is that traits do not occur as a single individual entity, but in combination, interracting in an individual organism, collectively determining and influencing species adaptation, resilience and sensitivity. Verberk *et al.* (2013) developed a framework for this approach. Trait-based tools such as the pesticide-based species at risk (SPEAR) model can be seen as example of the latter approach. The SPEAR model was developed to assess the effect of agricultural pesticide, using traits that are mechanistically linked to pesticide effects (Liess *et al.*, 2008). Traits within the SPEAR model are life cycle, sensitivity, dispersal, and generation time, that is, voltinism (Liess *et al.*, 2008). The SPEAR model has been used widely in Europe and was recently tested in South Africa (Liess & Von der Ohe, 2005; Berger *et al.*, 2018; Malherbe *et al.*, 2018).

Apart from pesticide application, the TBA has also found utility in assessing the effects of other stressors such as urbanisation, industrialisation, and sedimentation (Mondy & Usseglio-Polatera, 2014; Ding *et al.*, 2017; Pallottini *et al.*, 2017; Akamaguana *et al.*, 2019). For example, Mondy & Usseglio-Polatera (2014) used life-history traits to assess specific risks of streams undergoing multiple pressures such as urbanisation and sedimentation. They found that shredders, which feed on leaves, were highly sensitive to sediment accumulation in their studied streams. Ding *et al.* (2017) assessed different responses of traits to environmental and spatial variables and found that environmental variables affect the functional and trait diversity of macroinvertebrates, while spatial variables shape the ecological traits of macroinvertebrates. Pallottini *et al.* (2017) assessed functional trait responses to agricultural and industrial pollution and concluded that functional traits are relevant for biomonitoring stressed freshwater systems.

Spatially, the TBA is gaining popularity and has been used in different regions of the world for monitoring freshwater ecosystem conditions, for example, in Europe (Bonada & Doledec, 2011; Kuzmanovic *et al.*, 2017; Murphy *et al.*, 2017; Berger *et al.*, 2018; Desrosiers *et al.*, 2019), in North America (Poff *et al.*, 2006; Poff *et al.*, 2010; Herbst *et al.*, 2018; Krynak & Yates, 2018), in South America (Tomanova *et al.*, 2008), in Asia (Aazami *et al.*, 2015; Ding *et al.*, 2017; Forio *et al.*, 2018) and in Africa (Akamagwuna *et al.*, 2019; Odume, 2020). For instance, Berger *et al*. (2018) identified taxa and traits responses to specific stressors, such as industrialisation and urbanisation. The study by Berger *et al.* (2018) reveals that traits respond predictably to different pollution gradients such as wastewater, chemical pollution, and sedimentation. Forio *et al.* (2018) model the relationship of macroinvertebrate traits and environmental conditions and assert that macroinvertebrate traits show distinct clustering according to the gradient of pollution in the studied river systems. A study by Desrosiers *et al.* (2019) reveals that anthropogenic pressure on traits is linked to the ecological quality of the environment. These studies point out the usefulness of macroinvertebrate traits in discriminating between polluted and non-polluted river sites.

Although the TBA in freshwater biomonitoring is a promising approach, it comes with its challenges. The main challenges include i) heterogeneity in biological traits at the different taxonomic levels; ii) trade-offs, spin-offs and body-plan constraints; iii) complexity in distilling the complex relationship between different attributes and the external environment; iv) difficulty in linking macroinvertebrate species traits to the whole community population in a given ecosystem; v) a lack of expertise in statistical prowess to analyse trait data; vi) a lack of regional literature, particularly in Africa, where information on traits is still evolving, and vii) an appropriate taxonomic level at which the trait analysis should be pitched (Verberk *et al.*, 2008; 2013; Akamagwuna, 2018; Odume, 2020).

With regard to the taxonomic level at which a trait should be analysed, the literature is inconsistent. For example, Schmera *et al.* (2017) argue that family level trait-based analysis is sufficient for freshwater biomonitoring. Consistent with Schmera *et al.* (2017), no significant difference was observed between results of a SPEAR family and that of species. In Afrotropical regions, where taxonomic expertise and life-history studies are sparse, a trait-based analysis at the species level is almost impossible. For these reasons, the trait-based analysis in the present study was taken to the family level. Because traits show significant variation between species of the same family, Chevenet *et al.* (1994) developed a fuzzy coding system to take account of inherent trait variability and plasticity between members of the same family. A fuzzy coding approach was thus used in this study. Apart from the use of the trait-based approach in biomonitoring freshwater systems, the taxonomically based approach is also important, because, while the trait-based approach considers the functional ecological, the taxonomically based approach accounts for the structural ecology of freshwater systems.

### 1.4.2 Taxonomically-based approach

The taxonomically based approach considers the abundance, composition, diversity and richness of family, genus, or species levels of biota in biomonitoring aquatic ecosystems (Karr, 1981; Lenat & Resh, 2001; Baptista *et al.*, 2007). Taxonomically based approaches using macroinvertebrate abundance, distribution, composition, diversity and their relationships with physico-chemical variables have been assessed for most Nigerian rivers and streams (Ogbeibu & Oribhabor 2002;

Egborge *et al.* 2003; Emere & Nasiru 2007; Olomukoro & Ezemonye 2007). For example, Edokpayi *et al.* (2000) assessed the influence of human activities on macroinvertebrates abundance and diversity in a southern Nigeria stream. They reported increased abundance and diversity of chironomids in heavily impacted stations. Ogbeibu & Oribhabor (2002) assesed the ecological effect of impoundment in a stream macroinvertebrates community structure. They recorded high abundance and diversity of macroinvertebrates in the control stations compared to the impounded stations of the stream. It was asserted by the authors that elevated nutrients and dissolved solids affected the taxonomic composition of macroinvertebrates in the impounded stations of the streams.

Apart from the application of general indices such as Shannon, Margalef and Simpson indices in freshwater biomonitoring in Nigeria, much attempts have not been made to developbiomonitoring indices specific for Nigerian streams such as single biotic, multimetric and multivariate indices as is already done in other countries in Africa like South Africa, Ethiopia and Kenya (Odume *et al.*, 2012; Mereta *et al.*, 2013; Lakew & Moog, 2015; Moges *et al.*, 2016; Aura *et al.*, 2017). Biomonitoring approaches using the taxonomically-based approach include the single biotic index, and multivariate and multimetric indices approaches (Bonada *et al.*, 2006). The single biotic index awards sensistivity scores to each taxon, for example, the Biological Monitoring Working Party (BMWP), the Tanzania River Scoring System (TARISS) and the Zambian Invertebrate Scoring System (ZISS) (Walley & Hawkes, 1996; Kaaya *et al.*, 2015; Dallas *et al.*, 2018). The multivariate approach uses a test site, that is, a control site, to compare the effect of human disturbance in a given ecosystem (Bonada *et al.*, 2006) and is a predictive model which correlates what is observed in a control site with impaired sites (Bonada *et al.*, 2006). The multimetric approach focuses on calculating the measures of structural and functional assemblage of biota in an ecosystem (Bonada *et al.*, 2006; Baptista *et al.*, 2007). In this study, the multimetric approach was employed as it combines different metrics into a multimetric index, making it more robust than the single biotic index.

To develop multimetric indices, the multimetric approach combines several metric measures, for example, abundance, composition, richness, diversity, functional feeding groups, and more recently, traits and ecological preferences (Bonada *et al.*, 2006; Baptista *et al.,* 2007; Monaghan & Soares, 2012; Odume *et al.,* 2012; Mereta *et al.,* 2013; Aazami *et al.,* 2015; Huang *et al.,* 2015; Gieswein *et al.,* 2019; Shull *et al.,* 2019). The strength of the multimetric approach is that it takes into account a combination of metrics, reflecting effects of anthropogenic stressors on multiple biological and ecological characteristics such as diversity, function, composition, and abundance (Bonada *et al.*, 2006; Odume *et al.*, 2012; Edegbene *et al.,* 2019). The selection and integration of metrics for developing multimetric indices needs careful consideration to avoid redundancy, and to avoid responses caused by the natural properties of the ecosystems (Odume *et al.*, 2012; Edegbene *et al.*, 2019).

The multimetric index was first developed in the United States of America for biomonitoring streams using fish asemblages (Karr, 1981). Thereafter, Karr (1991) also developed Indices of Biological Integrity (IBI) to assess ecological conditions of streams. Macroinvertebrate-based multimetric indices are now being developed to assess water quality and the ecological integrity of rivers and streams globally (Baptista *et al.*, 2007; Odume *et al.*, 2012; Aazami *et al.*, 2015; Camargo, 2017; Shi *et al.*, 2017; Shull *et al.*, 2019). For example, Carmago (2017) has developed a macroinvertebrate-based multimetric index for assessing the ecological conditions of polluted rivers in Spain. Carmargo (2017) concluded that the developed multimetric index proved effective in assessing the responses of macroinvertebrates to pollution and it was therefore recommended for mitigation measures in polluted rivers in Spain. More recently, Shull *et al.* (2019) developed a benthic macroinvertebrate multimetric index for large Semi-wadeable Rivers in the Mid-Atlantic Region (SWMMI) of the United States of America. The authors found that the two SWMMI developed proved useful and could be applied independently in assessing ecological conditions of rivers in the USA (Shull *et al.*, 2019). Similar multimetric indices have been developed for evaluating the ecological conditions of lakes, wetlands, streams and rivers in Chile (Fierro *et al.*, 2018), in China (Lu *et al.*, 2019), in South Africa (Odume *et al.*, 2012), in East Africa, for example, Ethiopia and Kenya (Mereta *et al.*, 2013; Lakew & Moog, 2015; Aura *et al.*, 2017), and in West Africa, for example, Nigeria (Edegbene *et al.*, 2019).

In developing macroinvertebrate-based multimetric indices, the following criteria need to be considered carefully to ensure indices are useful to ecosystem managers and policy makers: (i) the index must be sensitive to anthropogenic activities; (ii) it should measure disparity in metrics and perturbation at a scale that is useful for management, and (iii) it should include interpretable metrics (Schoolmaster *et al.*, 2013). These criteria are pertinent because individual metrics are typically pooled into a “multimetric” index (MMI), which provides an overall score of integrity for a given system (Schoolmaster *et al.*, 2013). Although the multimetric approach has been widely developed and used elsewhere, application of the approach is still rare in Nigeria (Edegbene *et al.*, 2019); therefore, developing and applying suitable specific regional multimetric indices for assessing selected rivers in the Niger Delta region would provide robust and integrated biomonitoring tools for assessing water quality conditions of the riverine systems in the Niger Delta. The approach employed in this study of combining both taxonomically and trait-based approaches to develop and apply stressor-specific multimetric indices in the Niger Delta region is the first of its kind in sub-Saharan Africa. The approach is an important step forward in the field of biomonitoring in sub-Saharan Africa, and Nigeria in particular, as water resources managers and decision makers can apply the developed multimetric indices for specific human disturbance within the region for which the indices are developed.

### 1.5 Rationale and significance of the study

As argued above, water resources in the Niger Delta region are deteriorating at an alarming rate, yet no integrating monitoring tool or approach exists. To fill this gap, the development of such a tool must be rooted in sound ecological theories and concepts. The trait-based approach is premised on the habitat templete concept, which sheds light on how the physical habitat template shapes, selects, and filters specific trait combinations, in ways such that only organisms with the appropriate combination of traits survive a particular environment and those without the appropriate trait combination give way. Even though the trait-based approach has found traction elsewhere, it has not been widely applied in Nigeria. Further, studies that commonly analyse multiple trait responses to a particular stressor usually do not pay attention to identifying signature traits of such stressors. Identifying a signature trait is useful in enabling the rapid development of trait-based tools. This study, then, contributes to the trait literature by exploring how specific complex stressors dominant in the Niger Delta, such as urbanisation, agriculture, forestry and their combinations, influence the distribution of traits, and in the process, identifies the trait signatures of these main stressors. The study also develops novel multimetric indices for monitoring and managing riverine systems in the Niger Delta, so representing an important step in the drive to achieve sustainable management of water resources in Nigeria.

### 1.6 Aim and objectives of the study

#### 1.6.1 Aim of the study

The aim of the study is to develop macroinvertebrate trait- and taxonomically-based biomonitoring indices and approaches for wadeable riverine systems in the Niger Delta, Nigeria.

### 1.6.2 Specific objectives of the study

(i) To develop a physico-chemically based approach for characterising and grading selected riverine systems in the Niger Delta that has been influenced by urbanisation, urban-agriculture and urban-forestry.

(ii) To explore the pattern and distribution of macroinvertebrate traits and ecological preferences in relation to the influences of urbanisation, urban-agriculture and urban-forestry in the Niger Delta.

iii) To develop and apply macroinvertebrate-based multimetric indices for assessing and monitoring the influences of urbanisation, urban-agriculture and urban-forestry in the Niger Delta.

## 1.7 Thesis structure

**Chapter 1** provides a general introduction to the research, an in-depth literature review, the rationale and significance of the study. It concludes with the research aim and specific objectives.

**Chapter 2** outlines the materials and methods and describes the area of study, the sampling location (rivers), sampling techniques and approaches used in the study and statistical analyses.

**Chapter 3** is the first results chapter in which physico-chemical characteristics were used to delineate rivers in the Niger Delta into impact categories based on land use types. Chapter 3 addressed objective 1.

**Chapter 4** is the second results chapter and explores the distribution pattern of traits and ecological preferences in urban, urban-agriculture and urban-forestry impacted rivers in the Niger Delta. Chapter 4 addressed objective 2.

**Chapter 5** explains the development of macroinvertebrate-based multimetric indices for urban, urban-agriculture and urban-forestry impacted rivers in the Niger Delta. Chapter 5 addressed objective 3.

**Chapter 6** provides a succinct review of all the results discussed in the earlier chapters, and explains the management application and implications of the results in monitoring the ecological health of rivers in Nigeria. The chapter addressed all objectives by way of synthesis.

# CHAPTER 2: DESCRIPTION OF STUDY AREA AND GENERAL MATERIALS AND METHODS

## 2.1 Introduction

This chapter provides the general description of the study area, and the rivers and stations studied. It also covers the description of the selected traits and ecological preferences, as well as the rationale for their selection. Candidate metrics selected for the development of the multimetric indices and the methods followed in their development are described. The chapter ends with a detailed description of the statistical methods applied in the study.

## 2.2. Description of study area

Nigeria is divided into five freshwater ecoregions: Bight Coastal, Lake Chad Catchment, Lower Niger-Benue, Niger Delta and Northern West Coastal Equatorial (Kamdem-Toham *et al.*, 2006). The present study was undertaken within the Niger Delta Ecoregion. The Niger Delta occupies an area approximately 70 000 $km^2$ at the southern tip of Nigeria. The region, which is reported to be the third largest wetland system in the world (Uluocha & Okeke, 2004; Umoh, 2008; Adekola & Mitchell, 2011), is characterised by diverse mangrove swamps, wetlands, inland waters, brackish waters, vegetation, and an extensive tropical rain forest (Umoh, 2008). Biodiversity within the region is high (Adekola & Mitchell, 2011).

### *Vegetation*

Some of the river reaches of the Niger Delta are surrounded by rainforest, but vegetation around river catchments in the area is largely influenced by anthropogenic activities which include urbanisation and agriculture.

Riparian vegetation in most of the rivers within the region consists of *Bambusa* spp., *Elaeis guineensis*, *Pandanus* spp., *Mitragyna ciliata*, *Nymphaea* spp., *Panicum repens*, *Pistia stratiotes*, and *Musanga* sp., *Musa* sp., while shrubs include *Acrosticuum aureum*, *Ficus* spp., *Alchornea* spp. (Arimoro *et al.*, 2014). *Rhizophora* spp. *Azolla* spp., *Nymphae* spp., *Ecchornia crassipes, Ceratophyllum* spp. and *Ultricularia* spp. are examples of instream macrophytes in rivers of the region (Arimoro *et al.*, 2014; Jonathan *et al.*, 2016).

### *Climate*

The Niger Delta area is characterised by a tropical climate of wet and dry seasons (Edegbene & Arimoro, 2012, Arimoro *et al.*, 2015). The wet season is characterised by extensive and intensive rainfall, which begins in April and ends in September. The dry season is characterised by high air temperatures, usually between 25$^{o}$C to 35$^{o}$C, starting in October and ending in March. The mean annual temperature is 28$^{o}$C, mean annual rainfall is 2000–3500 mm, and the relative humidity is 85% (Arimoro *et al.*, 2015; Jonathan *et al.*, 2016).

### *Geology and soils*

The Niger Delta consists mainly of quaternary sediments of structural foundation with a unique geomorphologic formation (Allen, 1965; Abam, 2016). Six major geomorphic units exist in the area, namely, Warri-Sambreiro deltaic plain, mangrove swamp forests, beaches and barrier islands, coastal plain sands, the Lower Niger flood plain and the Niger flood zone (Abam, 2016).

Three tertiary lithostratigraphic units form the geologic sequence of the Niger Delta (Short & Stauble, 1967). These three lithostratigraphic units are overlaid by quaternary deposits of various types: the Benin, Akata and Agbada formations (Youdeowei & Nwankwoala, 2012).

The Niger Delta soil types include gravel, sand, clay, silt, medium-fine silt, and a mixture of sand (Akpokodje, 1989). These soil types are embedded in four lithologic ages: the Quaternary, Miocene, Eocene and Paleocene (Akpokodje, 1989; Youdeowei & Nwankwoala, 2012).

***Anthropogenic activities around the studied river catchments***

Anthropogenic impacts in the catchments of the studied rivers are mainly urbanisation, industrialisation and agricultural activities. The region is known for its oil exploration and exploitation activities, which have resulted in increased rural-urban migration. Drainage systems in urban cities within the region are poor, and rivers are often impacted by untreated wastewater, storm water return flow, and run-offs from informal settlements (Adekola & Mitchell, 2011; Jonathan *et al.*, 2016). Fishing and subsistence crop farming are among the major agricultural activities within the region. There is evidence of nutrient enrichment in the rivers of the region as the result of the influx of fertilisers and organic input (Adekola & Mitchell, 2011; Jonathan *et al.*, 2016). Sedimentation and erosion, particularly in the far eastern part of the region is common, presenting itself as a critical stressor of riverine ecosystems.

## 2.3 Studied rivers and stations

Twenty rivers (Table 2.1; Figure 2.1) were selected for this study. Most of the rivers are situated in the western Niger Delta sub-region comprising the Edo and the Delta States (Jonathan *et al*., 2016). The studied rivers are mainly between first-order ($1^{o}$) and fifth-order ($5^{o}$), with lengths ranging from 42 km to 320 km (Arimoro *et al.*, 2015). River width ranged from 2.5 m – 20.4 m and depths between 0.17 m to 1.36 m). The flow velocity ranges between 0.09 $ms^{-1}$ – 1.3 $ms^{-1}$. Biotopes of the rivers studied are dominated mainly by silt, mud, fine sand, coarse sand, stones, pebbles and decaying macrophytes (Arimoro *et al.*, 2014). Shade and leaf materials are common features of rivers in forested catchments.

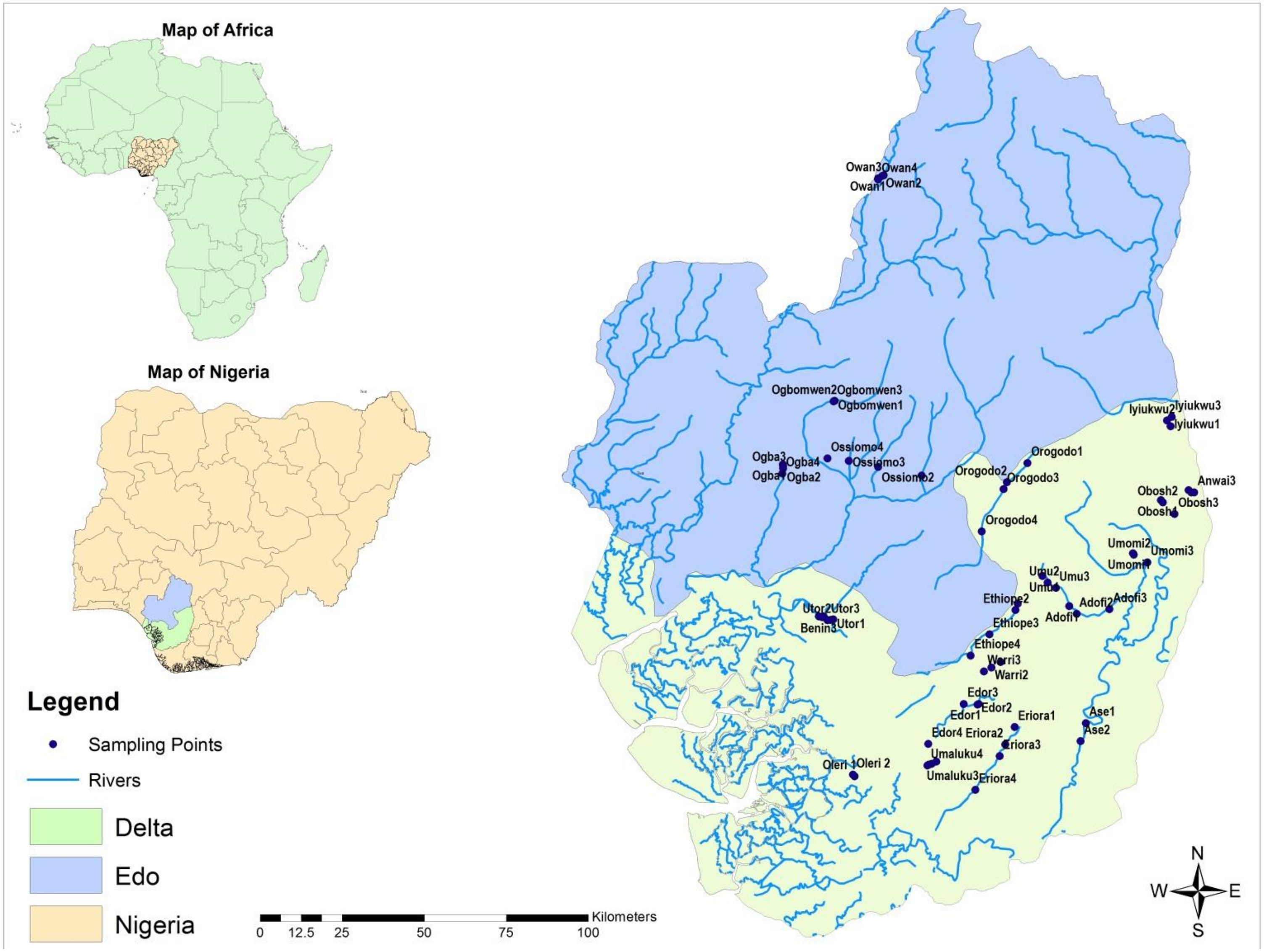



Figure 2. 1: Map of the study area showing location of the 20 rivers and 66 sampling stations in the Edo and Delta States of the Niger Delta. Inserts show maps of Africa and Nigeria.

Table 2. 1: Geographical location and catchment sizes of the stations within the selected 20 rivers in the Niger Delta area of Nigeria.

| S/N | River name | Station No. | River/Station codes | Latitude | Longitude | Catchment area ($km^2$) |
|---|---|---|---|---|---|---|
| 1 | Adofi | 1 | Ad1 | 5.93085 | 6.36853 | 294 |
| 2 | Adofi | 2 | Ad2 | 5.90942 | 6.38843 | 339 |
| 3 | Adofi | 3 | Ad3 | 5.92231 | 6.47865 | 450 |
| 4 | Anwai | 1 | An1 | 6.24838 | 6.69511 | 245 |
| 5 | Anwai | 2 | An2 | 6.24247 | 6.70266 | 247 |
| 6 | Anwai | 3 | An3 | 6.23900 | 6.71100 | 256 |
| 7 | Ase | 1 | As1 | 5.61100 | 6.41900 | 2687 |
| 8 | Ase | 2 | As2 | 5.56000 | 6.40200 | 2845 |
| 9 | Benin | 1 | Be1 | 5.89800 | 5.69300 | 4519 |
| 10 | Benin | 2 | Be2 | 5.90400 | 5.68500 | 4526 |
| 11 | Benin | 3 | Be3 | 5.90600 | 5.67800 | 4526 |
| 12 | Edor | 1 | Ed1 | 5.65800 | 6.17400 | 77 |
| 13 | Edor | 2 | Ed2 | 5.61900 | 6.14400 | 106 |
| 14 | Edor | 3 | Ed3 | 5.58700 | 6.12000 | 530 |
| 15 | Edor | 4 | Ed4 | 5.53500 | 6.06000 | 655 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16 | Eriora | 1 | Er1 | 5.59100 | 6.23200 | 42 |
| 17 | Eriora | 2 | Er2 | 5.55500 | 6.20400 | 61 |
| 18 | Eriora | 3 | Er3 | 5.50200 | 6.18500 | 164 |
| 19 | Eriora | 4 | Er4 | 5.43800 | 6.11000 | 288 |
| 20 | Ethiope | 1 | Et1 | 5.95804 | 6.20804 | 15 |
| 21 | Ethiope | 2 | Et2 | 5.91981 | 6.22073 | 127 |
| 22 | Ethiope | 3 | Et3 | 5.85318 | 6.14970 | 339 |
| 23 | Ethiope | 4 | Et4 | 5.80300 | 6.09400 | 2107 |
| 24 | Iyiukwu | 1 | Iy1 | 6.41600 | 6.62300 | 85 |
| 25 | Iyiukwu | 2 | Iy2 | 6.44000 | 6.63600 | 95 |
| 26 | Iyiukwu | 3 | Iy3 | 6.45600 | 6.64700 | 147 |
| 27 | Obosh | 1 | Ob1 | 6.22200 | 6.62000 | 102 |
| 28 | Obosh | 2 | Ob2 | 6.21300 | 6.62900 | 104 |
| 29 | Obosh | 3 | Ob3 | 6.18400 | 6.65800 | 127 |
| 30 | Ogba | 1 | Og1 | 6.31915 | 5.58750 | 40 |
| 31 | Ogba | 2 | Og2 | 6.31464 | 5.58750 | 45 |
| 32 | Ogba | 3 | Og3 | 6.30873 | 5.58750 | 45 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 33 | Ogba | 4 | Og4 | 6.29583 | 5.57917 | 53 |
| 34 | Ogbomwen | 1 | Ow1 | 6.49353 | 5.72520 | 525 |
| 35 | Ogbomwen | 2 | Ow2 | 6.49312 | 5.72346 | 531 |
| 36 | Ogbomwen | 3 | Ow3 | 6.49228 | 5.72218 | 531 |
| 37 | Oleri | 1 | Ol1 | 5.49100 | 5.77400 | 431 |
| 38 | Oleri | 2 | Ol2 | 5.49400 | 5.75600 | 445 |
| 39 | Orogodo | 1 | Or1 | 6.32333 | 6.25399 | 298 |
| 40 | Orogodo | 2 | Or2 | 6.27054 | 6.19773 | 505 |
| 41 | Orogodo | 3 | Or3 | 6.25140 | 6.18810 | 527 |
| 42 | Orogodo | 4 | Or4 | 6.13530 | 6.12861 | 681 |
| 43 | Ossiomo | 1 | Os1 | 6.28911 | 5.96374 | 338 |
| 44 | Ossiomo | 2 | Os2 | 6.31228 | 5.84456 | 215 |
| 45 | Ossiomo | 3 | Os3 | 6.32945 | 5.76458 | 140 |
| 46 | Ossiomo | 4 | Os4 | 6.33000 | 5.72100 | 67 |
| 47 | Owan | 1 | Oa1 | 7.118000 | 5.86000 | 6184 |
| 48 | Owan | 2 | Oa2 | 7.11070 | 5.85236 | 6213 |
| 49 | Owan | 3 | Oa3 | 7.10205 | 5.84795 | 6221 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50 | Owan | 4 | Oa4 | 7.09034 | 5.85417 | 6221 |
| 51 | Umaluku | 1 | Um1 | 5.51200 | 5.99600 | 778 |
| 52 | Umaluku | 2 | Um2 | 5.50400 | 5.97100 | 804 |
| 53 | Umaluku | 3 | Um3 | 5.49500 | 5.95300 | 839 |
| 54 | Umaluku | 4 | Um4 | 5.47900 | 5.93700 | 860 |
| 55 | Umomi | 1 | Ui1 | 6.06700 | 6.55800 | 44 |
| 56 | Umomi | 2 | Ui2 | 6.05500 | 6.56200 | 55 |
| 57 | Umomi | 3 | Ui3 | 6.04300 | 6.56200 | 57 |
| 58 | Umu | 1 | Uu1 | 6.01316 | 6.29481 | 104 |
| 59 | Umu | 2 | Uu2 | 5.99300 | 6.30700 | 115 |
| 60 | Umu | 3 | Uu3 | 5.98098 | 6.332070 | 131 |
| 61 | Utor | 1 | Ut1 | 5.89472 | 5.72168 | 3598 |
| 62 | Utor | 2 | Ut2 | 5.89583 | 5.70984 | 4480 |
| 63 | Utor | 3 | Ut3 | 5.89583 | 5.70407 | 4483 |
| 64 | Warri | 1 | Wa1 | 5.77000 | 6.18700 | 135 |
| 65 | Warri | 2 | Wa2 | 5.73700 | 6.17100 | 168 |
| 66 | Warri | 3 | Wa3 | 5.69600 | 6.15400 | 242 |

**River/Station codes:** Ad1=Adofi station 1, Ad2=Adofi station 2, Ad3=Adofi station 3, An1=Anwai station 1, An2=Anwai station 2, An3=Anwai station 3, As1=Ase station 1, As2=Ase station 2, Be1=Benin station 2, Be2=Benin station 2, Be3=Benin station 3, Ed1=Edor station 1, Ed2=Edor station 2, Ed3=Edor station 3, Ed4=Edor station 4, Er1=Eriora station 1, Er2=Eriora station 2, Er3=Eriora station 3, Er4=Eriora station 4, Et1=Ethiope station 1, Et2=Ethiope station 2, Et3=Ethiope station 3, Et4=Ethiope station 4, Iy1=Iyiukwu station 1, Iy2=Iyiukwu station 2, Iy3=Iyiukwu station 3, Ob1=Obosh station 1, Ob2 =Obosh station 2, Ob3=Obosh station 1, Og1=Ogba station 1, Og2=Ogba station 2, Og3=Ogba station 1, Og4=Ogba station 1, Ow1=Ogbomwen station 1, Ow2=Ogbomwen station 2, Ow3=Ogbomwen station 3, Ol1=Oleri station 1, Ol2=Oleri station 2, Or1=Orogodo station 1, Or2=Orogodo station 2, Or3=Orogodo station 3, Or4=Orogodo station 4, Os1=Ossiomo station 1, Os2=Ossiomo station 2, Os3=Ossiomo station 3, Os4=Ossiomo station 4, Oa1=Owan station 1, Oa2=Owan station 2, Oa3=Owan station 3, Oa4=Owan station 4, Um1=Umaluku station 1, Um2=Umaluku station 2, Um3=Umaluku station 3, Um4=Umaluku station 4, Ui1=Umomi station 1, Ui2=Umomi station 2, Ui3=Umomi station 3, Uu1=Umu station 1, Uu2=Umu station 2, Uu3=Umu station 3, Ut1=Utor station 1, Ut2=Utor station 2, Ut3=Utor station 3, Wa1=Warri station 1, Wa2=Warri station 2, Wa3=Warri station 3.

## 2.4 Dominant land use types in relation to the studied river ecosystems

The dominant land use type per sampling station was determined using Google earth satellite imagery. A particular land use type was considered dominant if it covered more than 70% of the adjacent catchment area within the sampling station as per the station catchment sizes indicated in Table 2.1 above. A similar method has been used by Pena-Cortes *et al.* (2011) and Fierro *et al.* (2017) to characterise land use types. Four land use types were initially defined in the 20 river catchments studied: urban, urban-agriculture, forestry and urban-forestry (Table 2.2). For the purpose of this study, three major land use types that have been reported to impact negatively on aquatic ecosystems were selected for the development of biomonitoring tools and protocols (Mereta *et al.,* 2013; Pallottini *et al.,* 2017; Ding *et al.,* 2018). The land use types are urban, urban-agriculture and urban-forestry as defined below:

### 2.4.1 Land use features

***Urban:*** The Urban features in the study area included unplanned development with poor drainage systems resulting in storm water return flow; untreated wastewater from nearby households; run-off from roads. Organic pollution is thus a principal feature of urban rivers in the Niger Delta.

***Urban-agriculture:*** Agricultural activities around the classified rivers in urban-agriculture land are fishing and crop farming. Fertilisers and other chemicals used in the farming and fishing activities have a major impact on the ecological state of rivers surrounded by urban-agriculture catchments.

***Urban-forestry:*** The Niger Delta lies within the tropical rainforest region of Nigeria. Two types of rainforest catchment occur: freshwater swamps, and saline/brackish mangrove swamps. The freshwater water swamps lie mainly in the Edo State axis of the Niger Delta, which harbours vegetation such as *Bambusa bambusa*, *Elaeis guineensis*, *Pandanus* spp., *Mitragyna ciliata*. The commonest features of forested rivers in the area adjacent to the urban activities are shade and leaf litter.

Table 2. 2: Dominant land use types in relation to the studied rivers and sampling stations

| **Land use type** | **Rivers** | **River/Station codes** | **No. of rivers** | **No. of stations** |
|---|---|---|---|---|
| Urban | Adofi | Ad3 | 8 | 11 |
| | Anwai | An1, An3 | | |
| | Ethiope | Et1, Et2 | | |
| | Obosh | Ob3 | | |
| | Ogba | Og1, Og2 | | |
| | Oleri | Ol2 | | |
| | Orogodo | Or3 | | |
| | Warri | Wa1 | | |
| Urban- agriculture | Anwai | An2 | 11 | 17 |
| | Edor | Ed2, Ed4 | | |
| | Eriora | Er3 | | |
| | Ethiope | Et3, Et4 | | |
| | Obosh | Ob1, Ob2 | | |
| | Ogba | Og3, Og4 | | |
| | Orogodo | Or2 | | |
| | Ossiomo | Os3, Os4 | | |
| | Owan | Oa3 | | |
| | Umaluku | Um1, Um2 | | |
| | Umu | Uu2 | | |
| Forestry | Adofi | Ad2 | 11 | 18 |
| | Edor | Ed1, Ed3 | | |
| | Eriora | Er1, Er2 | | |

| | | | | |
|---|---|---|---|---|
| | Oleri | Ol1 | | |
| | Ogbomwen | Ow1, Ow2, Ow3 | | |
| | Orogodo | Or4 | | |
| | Owan | Oa1, Oa4 | | |
| | Umaluku | Um3 | | |
| | Umomi | Ui3 | | |
| | Umu | Uu1 | | |
| | Utor | Ut1, Ut2, Ut3 | | |
| Urban-forestry | Adofi | Ad1 | 11 | 20 |
| | Ase | As1, As2 | | |
| | Benin | Be1, Be2, Be3 | | |
| | Eriora | Er4 | | |
| | Iyiukwu | Iy1, Iy2, Iy3 | | |
| | Orogodo | Or1 | | |
| | Ossiomo | Os1, Os2 | | |
| | Owan | Oa2 | | |
| | Umaluku | Um3, Um4 | | |
| | Umomi | Ui1, Ui2 | | |
| | Warri | Wa2, Wa3 | | |

### 2.5 Physico-chemical and macroinvertebrates data

#### 2.5.1 Physico-chemical

Physico-chemical variables and macroinvertebrate data were retrieved from Arimoro (2017), an unpublished database archived at the Department of Animal Biology, Federal University of Technology, Minna, Nigeria. Data in the database were collected seasonally on a monthly basis from 2008 to 2010 as indicated:2008: January - December;2009: February - July and November - December; 2010: January - April and July – December. The Arimoro (2017) database is almost the only database containing information on macroinvertebrates in Nigeria and, because of its extensive coverage of the Niger Delta, it was used for this study. Additional field data were collected seasonally on a monthly basis between 2011 and 2012 (2011: January - August and October – December; 2012: January - September) to supplement data retrieved from the database. The data collected in 2011–2012 were mainly from rivers not covered by the databases; these included Ethiope, Iyiukwu, Ogba, Obosh, Owan, Umu and Utor. Data for physico-chemical variables retrieved from the database and analysed for samples collected in 2011–2012 include water temperature, depth, flow velocity, electrical conductivity (EC), dissolved oxygen (DO), five-day biochemical oxygen demand ($BOD_5$), pH, nitrate and phosphate. Water depth was measured in metres using a calibrated rod. Flow velocity was measured using a timed float in the mid-channel over a distance of 10 metres (Gordon *et al.*, 1994). Dissolved oxygen, temperature, pH, EC were determined using a portable HANNA HI9829 multi-probe meter manufactured by HANNA instruments. The portable multi-probe meter was dipped into the water of each sampled station and the corresponding readings for each of the physico-chemical variables were measured immediately. Water samples for $BOD_5$ analysis were collected in 500 ml glass bottles at each sampled station and thereafter fixed with Winklers solution A (Manganese (II) sulphate) and B (Potassium iodide). The fixed samples were tied in black polythene bags for a period of five days to avoid algae growth (APHA, 1995). Five-day $BOD_5$ was analysed using a portable HANNA HI9829 multi-probe meter and the actual $BOD_5$ was calculated by subtracting the DO value of the first day from the DO of the fifth day (APHA, 1995).

Nitrate and phosphate were determined in the laboratory using spectrophotometric methods (APHA, 1995). Nitrate solution of 0 to 350 μg was prepared and used to plot a standard curve. A 4ml aluminium hydroxide suspension was added to 100 ml of water sample to declorise the water sample. Thereafter 1 ml of 0.1 M hydrochloric acid was added to 50 mls of the clear 100 ml water sample. A 220 nm was used in reading the optical density of the water sample, and then a standard curve measured in $mgl^{-1}$. Nitrate equivalent measured by converting the optical density using the reading from the standard curve and measured $mgl^{-1}$. Sulphate was determined by adding 50 ml of the water sample to 10 ml of glycerol-alcohol solution (APHA, 1995). A wavelength of between 360-440 nm was used to measure the absorbance of the sulphate solution. Thereafter about 0.2 g of $BaCl_2$- crystals was added to the sulphate solution resulting from the absorbance after shaking for about 20 minutes and the absorbance was taken after 30 minutes and measured in $mgl^{-1}$.

### 2.5.2 Macroinvertebrate sampling

Macroinvertebrate samples were collected in a D-frame kick-net (500 µm mesh size) (Lazorchak *et al.*, 1998) at each sampling station for a period of three minutes per biotope. On each sampling occasion, per station, samples were collected from all representative biotopes, which included vegetation, sand, silt, mud, and stones. Samples of macroinvertebrates collected from vegetation, sand, silt, mud and stones were grouped as composite samples and thereafter preserved in 70% alcohol for onward transfer to the laboratory for sorting, identification, and enumeration. Macroinvertebrates were identified to the family level under a stereoscopic microscope at X10 magnification using relevant taxonomic guides by Merritt & Cummins (1996); Day *et al.* (2003); de Moor *et al.* (2003).

### 2.6 Selected macroinvertebrate traits, ecological preferences and fuzzy coding

A total of 12 traits and ecological preferences categories resolved into 53 traits and ecological preference attributes selected for the present study. Details of the 12 traits and ecological preferences categories; the corresponding 53 traits and ecological preferences are presented in Table 2.3. Selected traits and ecological preferences presented in Table 2.3 were employed in Chapter 4

Table 2. 3: Traits and ecological preferences categories and attributes selected for the analysis in rivers draining selected four land use types catchment in the current study.

| **Trait category** | **Trait attribute** | **Trait code** |
|---|---|---|
| Respiration | Gills | A1 |
| | Tegument/cutaneous | A2 |
| | Aerial: spiracle | A3 |
| | Aerial/vegetation: breathing tube, strap/other apparatus | A4 |
| Body armouring | Hardshell | B1 |
| | Completely sclerotized | B2 |
| | Partly sclerotized | B3 |
| | Soft and exposed | B4 |
| | Cased/tubed | B5 |
| Turbidity preference | Clear and transparent waters | C1 |
| | Silty | C2 |
| | Turbid waters | C3 |
| | No preference | C4 |
| Voltinism (number of generations per year) | 1 year (Univoltine) | D1 |

| | | |
|---|---|---|
| | 2 years (Bivoltine) | D2 |
| | > 2years (Multivoltine) | D3 |
| | Longer than one year (Semivoltine) | D4 |
| Attachment mechanism | Free-living | E1 |
| | Temporary attachment | E2 |
| | Permanent attachment | E3 |
| Mobility | Climber | F1 |
| | Crawler | F2 |
| | Sprawler | F3 |
| | Swimmer | F4 |
| | Skater | F5 |
| | Burrower | F6 |
| Body shape | Streamlined | G1 |
| | Flattened | G2 |
| | Spherical | G3 |
| | Cylindrical/tubular | G4 |
| Food preference | Detritus (FPOM) | H1 |
| | Detritus (CPOM) | H2 |
| | Macrophytes/algae | H3 |
| | Animal materials | H4 |
| Response to oxygen depletion | Highly sensitive to oxygen depletion | I1 |
| | Moderately sensitive to oxygen depletion | I2 |
| | Moderately tolerant of oxygen depletion | I3 |
| | Highly tolerant of oxygen depletion | I4 |
| Body size | Very small (<5 mm) | J1 |
| | Small (>5-10 mm) | J2 |
| | Medium (>10-20 mm) | J3 |

| | | |
|---|---|---|
| | Large (>20-40 mm) | J4 |
| | Very large (>40-80 mm) | J5 |
| Aquatic stages | Egg | K1 |
| | Larva | K2 |
| | Nymph | K3 |
| | Pupa | K4 |
| Feeding habit | Predator | L1 |
| | Scraper | L2 |
| | Grazer | L3 |
| | Filter feeder | L4 |
| | Deposit feeder | L5 |
| | Shredder | L6 |

Traits and ecological preferences were selected primarily on how the dominant land use type was likely to stress the rivers draining it (Odume *et al.*, 2018a). For example, urban, urban-agriculture and urban-forestry land uses are likely to generate organic pollution, which may deplete dissolved oxygen and increase instream concentration of nutrients and metals. For forested land use, allochthonous input from vegetation around the river catchments may come into the river as wood debris, leading to retention of coarse particulate organic matter (CPOM), modification of channel morphology, and reduced flow if such vegation is alien (Ogren & King, 2008). Key stressors of urban, urban-agriculture, urban-forestry, and forestry land uses will, in turn, influence the distribution of macroinvertebrates mediated by traits and ecological preferences. A summary of the four land uses, the main mode of stress on aquatic macroinvertebrates traits, and ecological preferences in the studied rivers is presented in Table 2.4.

Information on macroinvertebrate traits and ecological preferences was retrieved mainly from the trait database for South African macroinvertebrates (Odume *et al.*, 2018b) and was supplemented with data from the literature (Lee & Bang, 2000; Hatt *et al.*, 2004; Roy *et al.*, 2005; Walsh *et al.*, 2005; Doledec & Statzner, 2008; Jones *et al.*, 2012; Heino, 2013; Kuzmanovic *et al.*, 2017; Krynak & Yates 2018). Where uncertainty remained, invertebrate experts at the Albany Museum, Grahamstown, South Africa were consulted. Because species level information is sparse in the Afrotropical region, trait information for analysis was retrieved at the family level, as is common practice (e.g. Ding *et al.*, 2017; Forio *et al.*, 2018; Odume, 2020).

To account for trait variability and plasticity between members of the same family, as well as different life stages of an organism, a fuzzy coding system was used (Chevenet *et al.*, 1994). Fuzzy coding is a method that organises trait information qualitatively and quantitatively (Chevenet *et al.*, 1994), and describes the relationship between taxon and traits and ecological preferences with regard to the amplitude of the taxon affinity to a given trait and ecological preference (Chevenet *et al.*, 1994; Forio *et al.*, 2018). A fuzzy coding system of 0-3 was used to assign affinity to macroinvertebrates in relation to trait and ecological preferences. Fuzzy coding values of 0, 1, 2, and 3 corresponding to no affinity, low affinity, moderate affinity and high affinity, respectively, were adopted (Chevenet *et al.*, 1994).

Table 2. 4: A summary of the land uses, mode of stress on aquatic macroinvertebrates traits and ecological preferences

| **Land use type** | **Key stressor** | **Main mode of stress** |
|---|---|---|
| Urban | Storm water return flow and sedimentation. | Urban storm water return flow often carries high levels of organic and inorganic sediments, and is rich in nutrients. High fine sediments can clog respiratory and filter feeding organs and abrade soft tissues (Larsen *et al.*, 2011). High fine sediment loads can also modify the physical habitat template, differentially affecting macroinvertebrates with a preference for stable microhabitats. Fine sediment, rich in organic material, may stimulate microbial activities, which in turn may lead to depletion of dissolved oxygen, with severe impacts on oxygen-sensitive macroinvertebrates. This stress is similar to nutrient enrichment, except that nutrient enrichment may stimulate selective macrophyte growth, providing habitats for macroinvertebrtates with a preference for macrophytes as habitat. A direct effect of storm water return flow rich in high fine sediment load is increased turbidity and thus low light penetration and algae production, which may, in turn, affect grazers (Sedell *et al.*, 1990; Guilpart *et al.*, 2012). |
| | Potential metal pollution | Elevated concentrations of metals are often reported from urban rivers in industralised catchments. Metals often lead to toxicity of an aquatic ecosystem to its resident biota. Eggs of organisms may be vulnerable to a highly toxic riverine environment occasioned by metals, which may result in a high risk for egg mortality (Kuzmanovic *et al.*, 2017). |
| Agriculture | Organic pollution | Organic pollution is a common feature of agricultural pollution and is usually associated with increases in nutrients such as nitrogen. Increases in the concentration of these nutrients may result in excessive algae growth, potentially favouring the grazers' biomass (Lee & Bang, 2000; Hatt *et al.*, 2004; Allan, 2004; Heino, 2013; Krynak & Yates, 2018). Organic pollution in an agricultural river catchment mainly comes from pesticides and fertiliser used in farm lands surrounding aquatic ecosystems. |
| Forestry | Allochthonous input | Allochthonous input from the riparian vegetation of rivers constitutes a food source for macroinvertebrates. Allochthonous inputs may be in the form of wood debris and leaf litter, which may lead to increases in CPOM. CPOM may in turn lead to the diversity and abundance of shredders relative to non-shredders. Allochtonous input may contribute to habitat complexity and diversity (Ogren & King, 2008). |

## 2.7 Candidate metrics selected for multimetric indices development

Multimetric indices are based on a combined range of candidate metrics in different metric measures. The approach is widely used on account of its combination of multiple metrics which form a multimetric index, as against the single biotic index which considers information of individual organisms only (Bonada *et al.*, 2006; Edegbene *et al.*, 2019).

A total of 77 candidate metrics were selected for the development of urban and 67 for urban-agriculture multimetric indices (MMIs) (Section 5.2.1, Table 5.1) while a total of 59 candidate metrics were selected for the development of urban-forest MMI (Section 5.2.1, Table 5.1). The selected candidate metrics fall into four measures of taxonomic measurements: abundance, composition, richness and diversity, together with measures of trait and ecological preferences (Table 5.1). Taxonomic metrics compare macroinvertebrate assemblages at various taxonomic levels, for example, abundance, composition, richness and diversity (Culp *et al.*, 2011). The trait-based metrics account for morphological, behavioural, physiological, and life-history patterns of macroinvertebrates (Violle *et al.*, 2007). Taxonomic features, trait, and ecological preferences were selected based mainly on the observed pattern of macroinvertebrate distribution in the studied river systems, and on the literature (Baptista *et al.*, 2007; Odume *et al.*, 2012; Mereta *et al.*, 2013; Fierro *et al.*, 2018; Edegbene *et al.*, 2019).

Measures of abundance tests for all components of macroinvertebrate community structures; composition measures take into account the relative abundance of macroinvertebrates in an entire sample (Bonada *et al.*, 2006). Richness measures were selected as they take into account the number of macroinvertebrate taxa in the entire sample (Baptista *et al.*, 2007), while diversity measures were selected because of their high discriminatory potential (Ntislidou *et al.*, 2018; Edegbene *et al.*, 2019). Trait and ecological preferences were selected for their resistance and resilience to environmental disturbance in river systems (Statzner *et al.*, 1994). Traits are also known to distinguish anthropogenic impacts that result in variations in morphological, behavioural and physiological characteristics (Charvet *et al.*, 1998). Details of selected taxonomic, trait and ecological preferences and their definition are presented in Chapter 5 section 5.2.1, Table 5.1.

## 2.8 Description of the statistical analyses employed in the study

Datasets used in this study were subjected to various statistical analyses. Data used in different statistical software were first arranged as data matrices in Microsoft Excel (2010 version) and thereafter exported or copied, depending on the statistical packages used. Chapters 3 to 5 explain in detail how relevant statistics were used to analyse the data; this section provides an overview of the main statistical methods used for the study.

### 2.8.1 Ordinations

***Principal component analysis (PCA)***

Principal component analysis (PCA) is an ordination analysis that is mainly used to elucidate the relationship between environmental variables in given stations (ter Braak & Verdonschot, 1995). It was used to elucidate the correlation between physico-chemical variables and the sampling stations for each main domninant land use category (Chapter 3). Principal component analysis was computed using Vegan package version 2.5.4 in R-statistics (Oksanen *et al.*, 2019 - R-core team, 2019). The co-variation function was used for the PCA ordination plots because the first PCA axis loadings were extracted for delineation of the studied rivers in terms of physico-chemical variables (Chapter 3). Co-variation placed Axis 1 higher in terms of percentage variance compared to the correlation function. The co-variation function was preferred to the correlation function, as the correlation is more appropriate for data that are far apart.

***Detrended correspondence analysis (DCA)***

A detrended correspondence analysis (DCA) is a test for unimodality and linearity assumption of canonical correspondence analysis (CCA) (ter Braak, 1995; Xu *et al.*, 2012). Detrended correspondence analysis is pertinent for choosing one of the the two ordination tests (CCA or redundancy analysis). When a gradient length of <3 in the first DCA axis is returned, an RDA is deemed appropriate, and when the gradient length is >3, a CCA is more appropriate (ter Braak & Verdonschot, 1995). A DCA was computed for macroinvertebrate metrics selected in Chapter 5 to determine the gradient length of macroinvertebrate datasets prior to selecting either a CCA or a RDA. Detrended correspondence analysis was plotted using Vegan package version 2.5.4 in R-statistics (Oksanen *et al.*, 2019 - R-Core Team, 2019).

***Redundancy analysis (RDA)***

A redundancy analysis (RDA) is an ordination analysis that elucidates the relationship between biological community structure and environmental variables for linear datasets (ter Braak & Verdonschot, 1995; Legendre & Legendre, 2012). Redundancy analysis was used in Chapter 5 in relating the selected metrics with the physico-chemical variables. It was employed because the metric dataset was linear, as indicated by the DCA gradient length of <3. Physico-chemical variables exhibiting multi-co-lineraity ($r \geq 0.8$) were removed from the RDA analysis. Redundancy analysis was computed using Vegan package version 2.5.4 within the R-programming environment (Legendre & Legendre, 2012).

***RLQ***

RLQ is an ordination analysis developed by Doledec *et al.* (1996) that performs multiple interactive ordinations and permutations on three matrices, environmental variables, taxa abundance, and traits. In this study, RLQ ordination analysis was performed to relate environmental variables (physico-chemical variables) (R) to macroinvertebrate taxa (L) and the traits and ecological preferences (Q) in Chapter 4. Correspondence ordination analysis (dudi.COA) was applied to the macroinvertebrate taxa table (Dray & Dufour, 2007) and a principal component analysis (dudi.PCA) applied to the trait table, while the Hillsmith (dudi.Hillsmith) function was applied to the environmental (physico-chemical) matrix (Dray & Dufour, 2007).

### 2.8.2 Fourth-corner analysis

The fourth-corner test is a multivariate permutational analysis that indicates concurrent correlations between multiple trait and ecological preferences, and environmental variables such as physico-chemical variables (Dray *et al.,* 2014; Akamagwuna *et al.,* 2019, Odume, 2020). The fourth-corner test elucidates a global picture of trait-environment relationship. The fourth-corner test reveals the traits and ecological preference attributes that either negatively or positively relate to given physico-chemical variables. Fourth-corner analysis was used in Chapter 4 to test the associations between the individual traits and ecological preferences, and the physico-chemical variables (Dray, 2013). The Monte Carlo permutation test at 999 permutations was used to compute the relationships between the physico-chemical variables and the macroinvertebrate trait and ecological preference data (Dray *et al.*, 2014).

### 2.8.3 Box plots

Box plots are graphical representations of statistics summaries such as inter-quartile ranges, median, outlier values, non-outlier values and extreme values (Barbour *et al.,* 1996). In Chapter 5 box plots were used to test for sensitivity and seasonality of the selected candidate metrics in relation to water quality impact gradient across the sampling stations. Box plots were computed using Statistica version 13.4.14 (TIBCO Software Inc., 2018).

### 2.8.4 Kruskal-Wallis test

The Kruskal-Wallis multiple comparison test is a non-parametric test that is used to test for statistical significance between ranks of multiple variables and was used to test for seasonal variability of the selected metrics in Chapter 5. The Kruskal-Wallis test was computed using Statistica version 13.4.14 (TIBCO Software Inc., 2018).

### 2.8.5 Mann-Whitney (U) test

The Mann-Whitney U test is a non-parametric test used to compare two sample ranks in the same population. It was used in Chapter 5 to confirm the sensitivity of selected metrics that had proved sensitive using the box plot (Baptista *et al.*, 2007).

Prior to using the Mann-Whitney (U) test, a Kolmogorov-Smirnov test was used to test for normality in distribution of the selected metrics in Chapter 5. The Kolmogorov-Smirnov test indicated a non-normally distributed metrics dataset, hence the choice of the Mann-Whitney test.

### 2.8.6 Simple correlation

Spearman's rank correlation is the non-parametric counterpart of the Pearson correlation coefficient that ranks and makes no assumption of normality (Ogbeibu, 2005). In Chapter 5, it was used to test for metric redundancy. When two or more metrics were highly correlated ($r \geq 0.78$), only one of such metrics was retained for integration into the multimetric index developed. The Spearman's rank correlation was performed using paleontological statistical package (PAST) (Hammer *et al.*, 2001).

### 2.8.7 Bar charts

A bar chart is a graph or chart that presents categorical data in a rectangular bar with lengths and heights proportional to the values being represented. In Chapter 5, bar charts were used to compare the performance (validity) of the developed multimetric indices (MMIs) in terms of water quality classes for least impacted (LIS), moderately impacted stations (MIS), and heavily impacted stations (HIS). It was also used in Chapter 5 to compare the water quality of the developed MMIs between wet and dry seasons.

### 2.8.8 Analysis of variance (ANOVA)

Analysis of variance is a statistical method that tests for significance in means of two or more samples. Two-way ANOVA was used to test for significant differences between stations and seasons in relation to selected physico-chemical variables across the main land use types. ANOVA was computed using Paleontological Statistical Package, PAST (Hammer *et al.*, 2001).

## CHAPTER 3: PHYSICO-CHEMICAL CHARACTERISATION OF THE SELECTED RIVERS, NIGER DELTA, NIGERIA

### 3.1 Introduction

Physico-chemical monitoring remains a critical approach to managing pollution in riverine ecosystems. It remains one of the most widely used approaches because standards and baseline limits can easily be set for physico-chemical variables, against which the effect of pollution on riverine systems can be benchmarked. The approach has been used to set effluent quality standards in Nigeria and other jurisdictions such as the European Union and the USA (FEPA, 1991; European Council, 2000; USEPA, 2016). However, different stresssors occasioned by land use types may influence physico-chemical conditions of riverine ecosystems differently. For example, rivers draining urban and agricultural catchments may be characterised by consistent and persistent water quality impairments such as elevated EC, turbidity, suspended solids, and metals because of a range of activities often associated with urban centres, such as urban storm water return flow, and discharges of effluents from municipal wastewater treatment works (WWTWs) (Paul & Meyer, 2001; Odume *et al.*, 2011; Kuzmanovic *et al.*, 2017).

The main land use types within the catchments of the studied river systems include urban development, agriculture, forestry and a combination of urban and agriculture, and urban and forestry activities. Urban and agricultural activities are known to impair the functionality of riverine systems through nutrient enrichment, pesticide inputs, dissolved substances resulting in increased EC, elevated sediment accumulation, and hydrological alteration (Elbrecht *et al.*, 2016; Kuzmanovic *et al.*, 2017). By contrast, forestry activities can increase water temperature, flow velocity and acidic content of the riverine systems (Wilby *et al.*, 2010; Lagrue *et al.*, 2011). The combined effect of urbanisation and agricultural activities can reduce the diversity of sensitive species while favouring that of tolerant species (Davies *et al.*, 2010; Wang *et al.*, 2012; Tchakonte *et al.*, 2015). Forestry impact on rivers can increase refugia for aquatic biota that have a high affinity for vegetation, particularly those with a preference for leaf litter and woody material (Sedell *et al.,* 1990; Odume, 2020). The different impacts of urbanisation, agricultural and forestry on physico-chemical water quality and the ecological integrity of riverine systems make it important to characterise river conditions based on physico-chemical variables.

The characteriastion of rivers along a pollution gradient can aid in determining the degree to which human-induced disturbances affect the functionality of the rivers. For instance, urbanisation and agricultural activities can have a debilitating effect on an aquatic system's morphological and biological stability (Paul & Meyer, 2001). Morphological instability can alter the river channel and modify the micro-habitat and overall habitat complexity and heterogeneity (Allan, 2004). Overall, physico-chemical monitoring in water quality management and physico-chemical conditions are important characteristics with a significant bearing on the distribution, abundance and diversity of both structural and functional components of the ecosystem. With such significance in view, this chapter aims to characterise the physico-chemical quality of the selected riverine system in relation to the dominant land use types, and to address objective one of this study: "To develop a physico-chemically-based approach for characterising and grading selected riverine systems in the Niger Delta, influenced by urbanisation, agriculture, forestry and their combinations".

## 3.2 Materials and Methods

### 3.2.1 Physico-chemical sampling

Physico-chemical sampling was undertaken seasonally on a monthly basis over a period of five years from 2008–2012 as described in Chapter 2, section 2.5.1. Samples were analysed for water temperature, depth, flow velocity, electrical conductivity (EC), dissolved oxygen (DO), five-day biochemical oxygen demand ($BOD_5$), pH, nitrate and phosphate.

### 3.2.2 Characterising the sampling stations along a gradient of dominant land use type

The stations within three dominant land use types that have been reported to negatively impact freshwater system (that is, urban catchment: 11 stations, eight rivers; urban-agriculture-catchment: 17 stations, 11 rivers; urban-forestry catchment: 20 stations, 11 rivers) were delineated along an impact gradient into three impact categories, namely, least impacted stations (LIS), moderately impacted stations (MIS) and highly impacted stations (HIS), by correlating the physico-chemical data with the selected river stations using PCA. Stations strongly correlated with physico-chemical indicators of pollution such as high nutrients, $BOD_5$, and high EC, were deemed heavily impacted, and those positively correlated with indicators of good water quality such as high DO, were deemed least impacted. The exact categorisation was undertaken by extracting the station coordinates on the first axis of the respective PCAs, and then the interstation distances were calculated by subtracting the least scoring station from the highest scoring station. Scores of subsequent stations were then subtracted from the highest scoring station. The interstation distances were converted to percent distances, after which a percentile distribution was used to delineate stations into one of three impact categories: LIS, MIS and HIS. The percentile distributions for each of the impact categories per land use type were 100-90$^{th}$ (LIS)' <90$^{th}$–50$^{th}$ (MIS) and <50$^{th,}$ (HIS). A similar method has been used by Murphy *et al.* (2013) and Odume *et al.*, (2016) to calculate species distances along the first axis of a canonical correspondence ordination plane (CCA). Principal component analysis ordination was performed using Vegan package version 2.5.4 in R-statistics (Oksanen *et al.*, 2019 - R-Core Team, 2019).

## 3.3 Results

### 3.3.1 Delineating sampling stations within the urban catchments along an impact gradient

Physico-chemical variables analysed for rivers draining urban catchments were correlated with the sampling stations using PCA. On Axis 1, which explained a total variance of 93.3%, with an

Eigen value of 172.9, Anwai River station 1 (An1) and Warri River station 1 (Wa1) were positively correlated with DO and pH, although the influence of DO in structuring the two stations was slightly higher than that of the pH (Figure 3.1). Still on Axis 1, stations such as Ogba River stations 1 and 2, (Og1 and Og2), Ethiope River station 2 (Et2) were positively correlated with EC, nitrate, $BOD_5$, phosphate, depth and flow velocity. Axis 2 of the PCA explained a total variance of 4.4% with an Eigen value of 8.2. Oleri River station 2 (Ol2) was positively correlated with water temperature on Axis 2 (Figure 3.1), while Adofi River station 3 (Ad3), Anwai River station 3 (An3) and Ethiope River station 1(Et1) were negatively correlated with water temperature on Axis 2 (Figure 3.1).

Based on the method developed for delineating the stations into impact categories, the results indicate that, of the 11 stations within urban rivers, two were designated LIS, four MIS, and five HIS (Table 3.1).

Table 3. 1: PCA coordinates for Axis 1, inter-station distances, percent interstation distances and categorisation of sampling stations within urban catchments during the study period (2008–2012). Note: LIS=least impacted stations, MIS=moderately impacted stations, HIS=heavily impacted stations.

| **Land use type** | **River/ Station codes** | **Station coordinates on PCA Axis 1** | **Inter-station distance** | **% Inter-station distance** | **Station impact category** |
|---|---|---|---|---|---|
| Urban | Wa1 | -19.81 | 42.72 | 100 | LIS |
| | An1 | -11.59 | 34.50 | 80.76 | LIS |
| | An3 | -9.49 | 32.40 | 75.84 | MIS |
| | Ad3 | -8.36 | 31.27 | 73.21 | MIS |
| | Ol2 | -5.78 | 28.69 | 67.15 | MIS |
| | Et1 | -2.12 | 25.03 | 58.59 | MIS |
| | Or3 | -1.07 | 23.98 | 56.12 | HIS |
| | Ob3 | 7.06 | 15.85 | 37.11 | HIS |

| | | | | | |
|---|---|---|---|---|---|
| | Et2 | 10.29 | 12.62 | 29.55 | HIS |
| | Og2 | 17.97 | 4.94 | 11.56 | HIS |
| | Og1 | 22.91 | 0.00 | 0.00 | HIS |

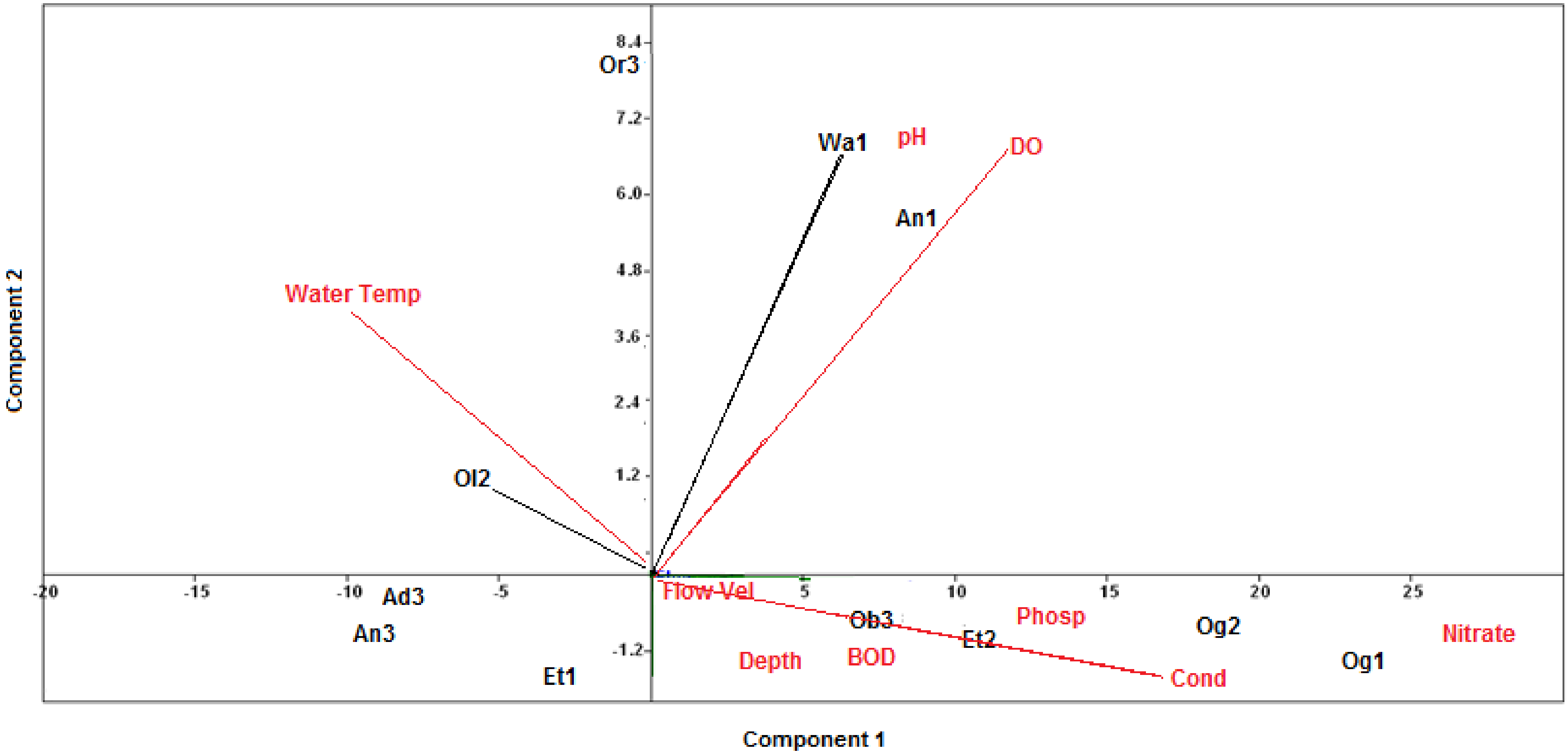



Figure 3. 1: PCA showing the correlations between sampling stations and physico-chemical variables analysed for rivers draining urban catchments in the Niger Delta. Water Temp=Water Temperature, Flow Vel=Flow velocity, DO=Dissolved oxygen, BOD=Five-day bochemical oxygen demand, Cond=Electrical conductivity, Phosp=Phosphate.

### 3.3.2 Delineating sampling stations within the urban-agriculture catchments along an impact gradient

Physico-chemical variables analysed for urban-agriculture dominated river catchments revealed that Axis 1 explained 76.2% with Eigen value of 472.5. Eriora River station 3 (Er3) and Ossiomo River station 3 (Os3) were positively correlated with EC and $BOD_5$ on Axis 1, while Anwai River station 2 (An2), Obosh River station 2 (Ob2), Ogba River station 3 (Og3) and Umu River station 2 (Uu2) were negatively correlated with DO, water temperature and flow velocity on Axis 1 (Figure 3.2). Axis 2 of the PCA explained 22.5% with an Eigen value of 139.6. Edor River station 4 (Ed4), Ethiope River station 4 (Et4), Ogba River station 4 (Og4) and Ossiomo River station 4 (OS4) were positively correlated with pH, nitrate and phosphate on Axis 2 of the PCA (Figure 3.2). Still on Axis 2, stations such as Edor River station 2 (Ed2), Ethiope River station 3 (Et3), Obosh River station 1 (Ob1), Orogodo River station 2 (Or2), Umaluku River stations 1 and 2 (Um1 and Um2) were negatively correlated with pH, nitrate and phosphate on Axis 2. (Figure 3.2).

Based on the method developed for delineating the stations into impact categories, the results indicate that, of the 17 stations within urban-agriculture rivers, two were designated LIS, seven MIS, and eight HIS (Table 3.2).

Table 3. 2: PCA coordinates for Axis 1, interstation distances, percent interstation distance and categorisation of sampling stations within urban-agriculture catchments during the study period (2008–2012). Note: LIS=least impacted stations, MIS=moderately impacted stations, HIS=heavily impacted stations.

| **Land use type** | **River/ Station codes** | **Station coordinates on PCA Axis 1** | **Inter-station distance** | **% Inter-station distance** | **Station impact category** |
|---|---|---|---|---|---|
| Urban-agriculture | Uu2 | -38.44 | 87.58 | 100 | LIS |
| | Ob2 | -18.48 | 67.62 | 77.21 | LIS |
| | Um1 | -15.97 | 65.11 | 74.34 | MIS |
| | An2 | -12.86 | 62.01 | 70.80 | MIS |
| | Ob1 | -12.68 | 61.83 | 70.59 | MIS |
| | Or2 | -11.72 | 60.86 | 69.49 | MIS |
| | Ed2 | -9.25 | 58.39 | 66.67 | MIS |
| | Os4 | -7.18 | 56.33 | 64.31 | MIS |
| | Um2 | -3.58 | 52.73 | 60.20 | MIS |
| | Ed4 | -2.95 | 52.09 | 59.48 | HIS |
| | Oa3 | 3.87 | 45.27 | 51.69 | HIS |
| | Et4 | 5.29 | 43.86 | 50.07 | HIS |
| | Et3 | 6.21 | 42.94 | 49.02 | HIS |
| | Og4 | 8.50 | 40.65 | 46.41 | HIS |
| | Og3 | 14.41 | 34.73 | 39.66 | HIS |
| | Er3 | 45.69 | 3.46 | 3.95 | HIS |
| | Os3 | 49.14 | 0.00 | 0.00 | HIS |

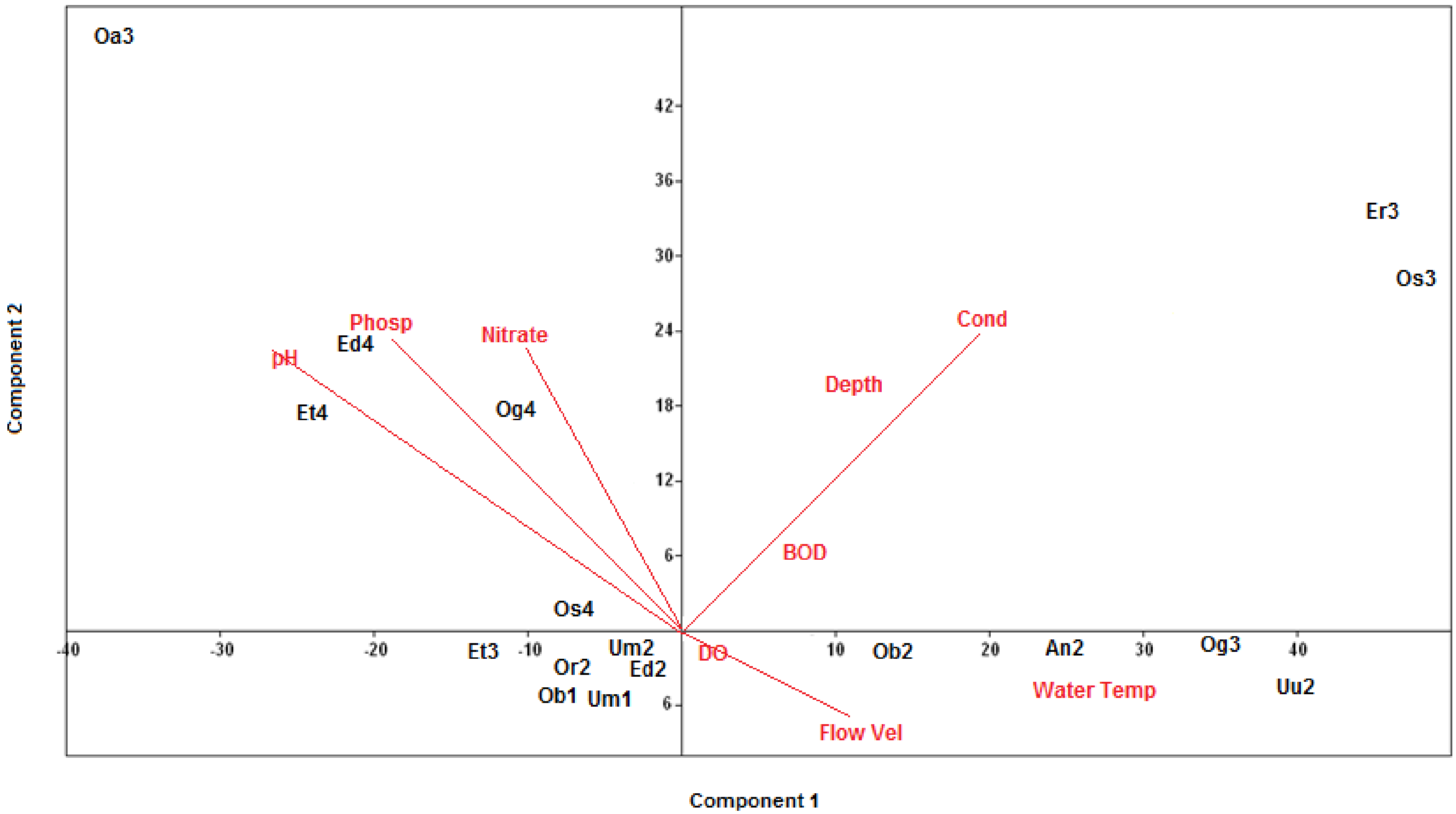



Figure 3. 2: PCA showing the correlations between sampling stations and physico-chemical variables analysed for rivers draining urban-agriculture catchments in the Niger Delta. Water Temp=Water Temperature, Flow Vel=Flow velocity, DO=Dissolved oxygen, BOD=Five-day bochemical oxygen demand, Cond=Electrical conductivity, Phosp=Phosphate.

### 3.3.3 Delineating sampling stations within the urban-forestry catchments along an impact gradient

Physico-chemical variables analysed for rivers draining urban-forestry catchments were correlated with the sampling stations using PCA. Axis 1 explained a total variance of 99.1% with an Eigen of value 1887.1. Benin River station 1 (Be1), Ossiomo River stations 1 and 2 (Os1 and Os2), Eriora River station 4 (Er4), Umaluku River stations 3 and 4 (Um3 and Um4) were positively correlated with $BOD_5$, water temperature, nitrate and phosphate on Axis 1, while Umomi River stations 1 and 2 (Ui1 and Ui2) were negatively correlated with with EC, pH, depth and flow velocity on Axis 1 (Figure 3.3). Axis 2 of the PCA explained a total variance of 0.36% with an Eigen value of 6.8. Adofi River station 1 (Ad1), Ase River station 2 (As2), Benin River station 3 (Be3), Owan River station 2 (Oa2), Warri River stations 2 and 3 (Wa2 and Wa3) were negatively correlated with DO on Axis 2 (Figure 3.3). Ase River station 1 (As1), Orogodo River station 1 (Or1), Iyiukwu River stations 1, 2 and 3 (Iy1, Iy2 and Iy3) were negatively correlated with DO on Axis 2 (Figure 3.3).

Based on the method developed for delineating the stations into impact categories, the results indicate that, of the 18 stations within urban-forested rivers, three were designated LIS, seven MIS, and ten HIS (Table 3.3).

Table 3. 3: PCA coordinates for Axis 1, interstation distances, percent interstation distance and categorisation of sampling stations within urban-forestry catchments during the study period (2008–2012). Note: LIS=least impacted stations, MIS=moderately impacted stations, HIS=heavily impacted stations.

| **Land use type** | **River/ Station codes** | **Station coordinates on PCA Axis 1** | **Inter-station distance** | **% Inter-station distance** | **Station impact category** |
|---|---|---|---|---|---|
| Urban-forestry | Wa3 | -28.31 | 188.97 | 100 | LIS |
| | Wa2 | -27.98 | 188.64 | 99.82 | LIS |
| | Ad1 | -26.06 | 186.72 | 98.81 | LIS |
| | Or1 | -24.18 | 184.84 | 97.81 | MIS |
| | As2 | -22.46 | 183.12 | 96.90 | MIS |
| | Iy3 | -22.36 | 183.02 | 96.85 | MIS |
| | Iy1 | -21.15 | 181.81 | 96.21 | MIS |
| | As1 | -20.69 | 181.35 | 95.97 | MIS |
| | Iy2 | -20.33 | 180.99 | 95.77 | MIS |
| | Be3 | -17.07 | 177.73 | 94.04 | MIS |
| | Os2 | -14.83 | 173.52 | 91.82 | HIS |
| | Be1 | -12.86 | 173.52 | 91.82 | HIS |
| | Os1 | -12.20 | 172.86 | 91.47 | HIS |
| | Oa2 | -8.61 | 169.27 | 89.57 | HIS |
| | Um4 | -2.29 | 162.95 | 86.23 | HIS |
| | Er4 | 19.03 | 141.63 | 74.95 | HIS |
| | Ui2 | 24.71 | 135.95 | 71.94 | HIS |
| | Um3 | 32.89 | 127.77 | 67.61 | HIS |
| | Ui1 | 44.08 | 116.58 | 61.69 | HIS |
| | Be2 | 160.66 | 0.00 | 0.00 | HIS |

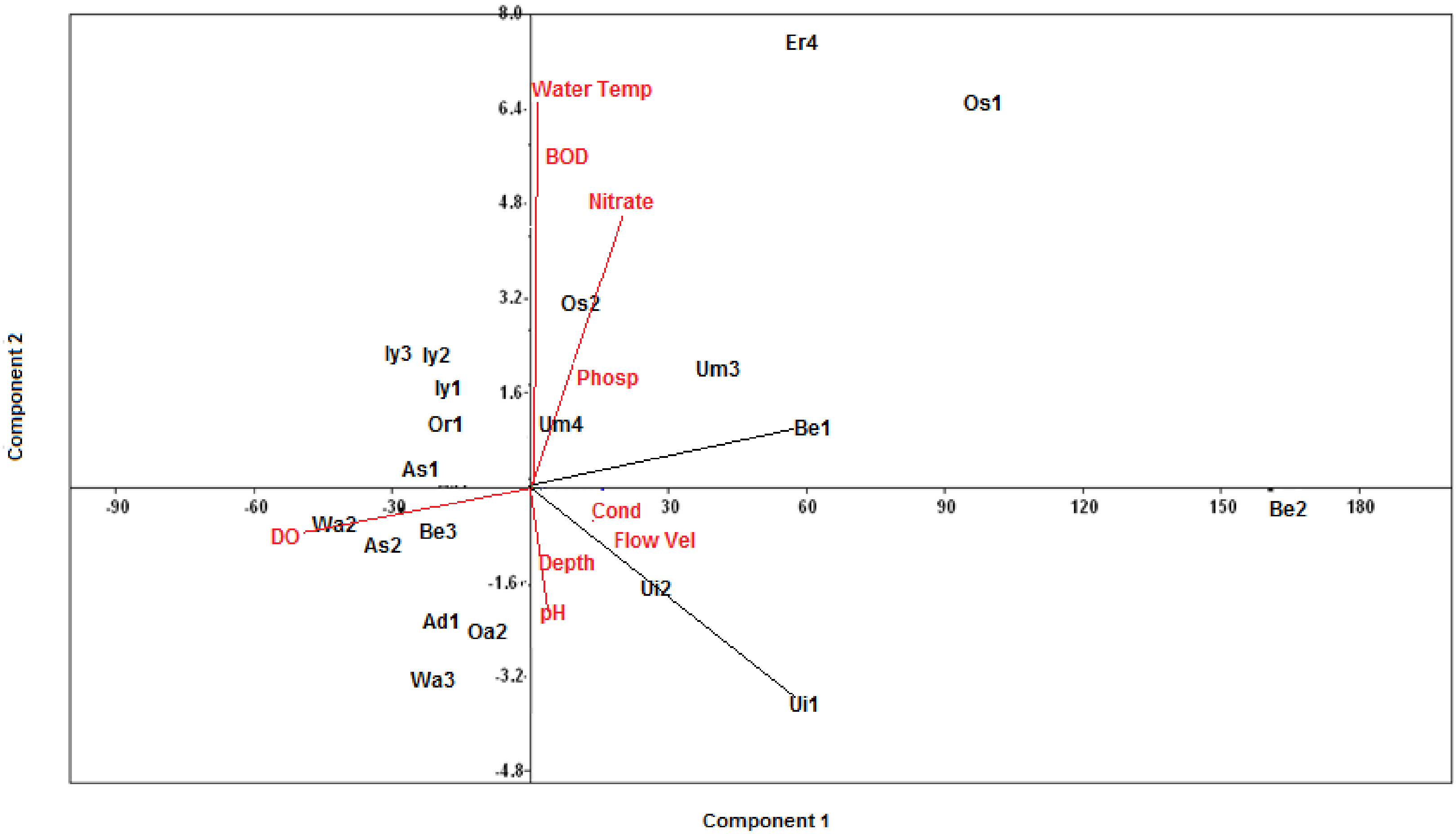



Figure 3. 3: PCA showing the correlations between sampling stations and physico-chemical variables analysed for rivers draining urban-forested catchments in the Niger Delta. Water Temp=Water Temperature, Flow Vel=Flow velocity, DO=Dissolved oxygen, BOD=Five-day bochemical oxygen demand, Cond=Electrical conductivity, Phosp=Phosphate

### 3.4 Discussion

#### 3.4.1 Physico-chemical classification of selected rivers in the Niger Delta into impact categories

Rivers in the Niger Delta region of Nigeria were characterised into impact categories using physico-chemical variables. For each dominant land use type, river stations were categorised into one of three impact gradients, LIS, MIS, or HIS.

Urbanisation, agricultural and forestry activities are key drivers of water quality changes in the Niger Delta. Within the urban and urban-agriculture catchments, stations designated as MIS and HIS had significantly elevated concentrations of nutrients. Most of these stations were situated a few metres away from the outlets of urban stormwater return flows. This may be the reason why most of the stations categorised as HIS in the urban, urban-agriculture and urban-forestry catchments were strongly positively correlated with nutrients, $BOD_5$ and EC. The observed results are consistent with the postulation of the urban river syndrome, in which urban rivers experience consistent, increased concentrations of nutrients, suspended solids, and alteration of channel morphology and stability (Paul & Meyer, 2001; Meyer *et al.,* 2005; Walsh *et al.,* 2005).

Alteration of channel morphology and stability was evident through visual observation at the MIS and HIS in the urban and urban-agriculture catchments. These stations were also associated with increased flow velocity. Altered channel morphology and stability can lead to reduced habitat complexity and heterogeneity, with direct effects on micro-habitat diversity and the provision of refugia for aquatic macroinvertebrates (Sedell *et al.,* 1990; Ladle & Ladle, 1992). For example, the adapted river habitat template concept (HTC) suggests that adequate refugia can buffer the effects of anthropogenic stressors as biota are able to take shelter in such refugia during periods of maximum disturbance (Townsend & Hildrew, 1994). The biological implication is that MIS and HIS stations in these catchments are unable to support a diverse array of biodiversity, as will be fully discussed in subsequent chapters. Further, diverse microhabitats and systems with high habitat complexity can moderate the wash-off effects of increased velocity occasioned by urban surface imperviousness, as experienced at MIS and HIS in the urban and urban-agriculture catchments. Overall, alteration of stream habitat morphology has significant implications for both the physical and biological properties of riverine systems (Paul & Meyer, 2001; Meyer *et al.,* 2005).

Apart from alteration of the morphology and habitat stability of urban and agricultural dominated river systems, nutrient enrichment and low DO concentrations are typical characteristics of urban systems, as was observed in the present study. Nutrient enrichment and low DO concentrations have been reported to affect the functionality of biota such as macroinvertebrate taxa and trait distribution in effluent- and agricultural-impacted stations (Arimoro & Ikomi, 2008; Pallottini *et al.,* 2017). Nutrient enrichment stimulates agal growth and limits macrophyte growth, which may not favour organisms with a preference for vegetation and grazing (O'Hare *et al.,* 2018). Nutrient enrichment reduces light penetration in freshwater systems, resulting in macrophyte and other plant loss (Moss, 1998). Once macrophytes and other plants are lost, established algal growth booms, limiting the structural diversity and composition of macroinvertebrates and other aquatic orgnaisms that depend on vegetation for their food, and increasing the diversity of macroinvertebrates and other organsims that depend on organic substances in the freshwater system (Scheffer & van Nes, 2007). Apart from stimulating algal growth and increased microbial activities, nutrient enrichment also limits light penetration in a freshwater system, which leads to dissolved oxygen (DO) depletion and an increase in biochemical oxygen demand (BOD) concentration. Dissolved oxygen depletion in a freshwater system negatively affects macroinvertebrates and other aquatic organisms that depend on dissolved oxygen for their breathing mechanism, a process that leads to adaptive air breathing mechanisms in some macroinverbrates such as the *Eristalis* (Arimoro & Ikomi, 2008). All these negative effects occasioned by urbanisation of the riverine catchments are present in the Niger Delta Rivers, as observed in the present research.

The effects of urbanisation on riverine systems have been reported elsewhere (e.g. Davies *et al.*, 2010; Wang *et al.*, 2012; Tchakonte *et al.*, 2015). Davies *et al.* (2010) documented the effect of urbanisation on a stream in the south-eastern part of Australia. The authors observe that urbanisation significantly impaired the ecological health condition of the rivers, thereby affecting the composition and richness of aquatic biota in aquatic ecosystems. Reduction in richness and relative abundance of macroinvertebrates has also been reported in urban polluted streams in China (Wang *et al.*, 2012), while Tchakonte *et al.* (2015) reported the structural and functional effects of urbanisation on streams in Douala, Cameroun which led to the decline in macroinvertebrate taxa abundance in the studied streams.

Overall, the physico-chemical approach used in categorising the selected stations in the present study proved useful as the approach classified most of the urban, urban-agriculture and urban-forestry stations into HIS. A similar approach has previously been used to calculate species sensitivity scores by Murphy *et al.* (2013) and Odume *et al.* (2016). A critical limitation of the approach, from an ecological perspective, is that it seems to classify the rivers into distinct impact zones rather than a continuum. However, the distinct impact zone can assist decision makers in rehabilitation efforts. In South Africa for example, rivers are classified into ecological categories A-F, according to their degree of perturbation (DWAF, 2008), but the South African system has transition categories such as A/B to reflect the idea of a continuum, which was not included in the approach developed here. However, given that the science of biomonitoring and its practical application in freshwater management is new in Nigeria, the approach developed here is an important step forward in stimulating thinking about how rivers might be managed and conserved – an issue that deserves urgent attention.

### 3.5 Conclusion

Physico-chemical characterisation of rivers in urban, urban-agriculture, and urban-forestry catchments in the Niger Delta, Nigeria was undertaken. The selected rivers were classified into three impact categories namely, LIS, MIS and HIS. Most of the stations designated as MIS and HIS in the urban and urban-agriculture catchments were found to be significantly correlated with increased nutrients, EC and $BOD_5$. From an ecological point of view, this approach is limited in that it classifies the rivers into distinct impact zones rather than a continuum. Nevertheless, the distinct impact zone can assist decision makers in rehabilitation efforts. The approach used in South Africa, where rivers are classified into ecological categories A-F according to their degree of perturbation (DWAF, 2008), but transition categories, such as A/B, that are used to reflect the idea of a continuum, was not included in this study. The science of biomonitoring and its practical application in freshwater management is new in Nigeria, and the approach developed here is an important start in stimulating ideas about the management and conservation of rivers – an issue that deserves urgent attention. An important aspect not taken into consideration in this research is the effect of seasonal change on rivers, and future development of this approach needs to take into account seasonal mediated pollution in riverine systems.

# CHAPTER 4: EXPLORING THE PATTERN AND DISTRIBUTION OF MACROINVERTEBRATE TRAITS AND ECOLOGICAL PREFERENCES IN RELATION TO URBAN, URBAN-AGRICULTURE AND URBAN-FORESTRY POLLUTION IN RIVERS WITHIN THE NIGER DELTA, NIGERIA.

**Publication based on this chapter**

Edegbene, A.O., Arimoro, F.O. and Odume, O.N. (2020). Exploring the distribution patterns of macroinvertebrate signature traits and ecological preferences and their responses to urban and agricultural pollution in selected rivers in the Niger Delta ecoregion, Nigeria. *Aquatic Ecology*. DOI: 10.1007/s10452-020-09759-9.

## 4.1 Introduction

Urbanisation, coupled with industrialisation, agricultural activities and a growing human population pose a significant threat to the health and functionality of riverine ecosystems (Pallottini *et al.,* 2017; Berger *et al.,* 2018; Desrosiers *et al.,* 2019). Rivers in urban and agricultural landscapes have been observed to consistently display impaired water quality conditions, depleted biota, modified channels, and altered micro-habitat complexity and heterogeneity (Olden *et al.,* 2004, Kuzmanovic *et al.,* 2017, Desrosiers *et al.,* 2019). In Africa, where urbanisation and associated rural-urban migration is on the rise, there is a significant risk that rivers in urban landscapes would be seriously impaired, particularly because of poor planning and lack of environmental safeguards (Arimoro *et al.,* 2015; Odume, 2020). The Niger Delta region is not an exception, as the region is urbanising rapidly due to the presence of oil exploration companies and other industrial activities.

The Niger Delta is situated within the tropical rainforest zone of Nigeria (Arimoro *et al.,* 2015) and wadeable riverine systems within the region rely heavily on allochthonous materials such as leaf litter as a primary organic carbon source (Vannote *et al.,* 1980; Sedell *et al.,* 1990). Further, from a functional ecological perspective, macroinvertebrates that are shredders are often common in forested streams, breaking down coarse particulate organic materials (CPOM), and accelerating their conversion into fine organic materials (Desrosiers *et al.*, 2019) for ecological process such as energy transfer and nutrient cycling. Thus, the established and expected functional assemblages and pattern in forested streams are critical to their functioning and for supporting the delivery of ecosystem services to society. In addition, most forested rivers in the Niger Delta are shaded, presenting a consistently cooler temperature pattern than non-forested streams. However, encroaching urbanisation and agricultural activities onto forested riverine catchment present a critical challenge, threatening the alteration of the natural physical, chemical, and biological processes of riverine systems in forested catchments. For example, increased temperature due to run-off from urban settlements has been reported in forested riverine systems subject to urbanisation (Ogbeibu & Oribhabor, 2002; Arimoro *et al.*, 2015), and a shift in the proportion of grazers to shredders due to increased algal growth as a result of nutrients from urban and agricultural pollution sources. Given the potential complex interaction between urban, agriculture and forestry pollution, how does urban and agricultural pollution influence traits and ecological preferences of the river systems in the Niger Delta regions of Nigeria?

The trait-based approach is useful because it provides an indirect assessment of ecosystem function, for example, through analysis of functional feeding groups, and has the potential to turn descriptive ecology into predictive tool through a discernible mechanistic relationship between traits and a specific stressor of interest. Therefore, in this chapter the influence of urban pollution on the pattern of distribution of traits and ecological preferences in agricultural and forested rivers is explored, with a view to identifying potential trait-based indicators suitable for monitoring urban pollution effects in rivers in agricultural and forested catchments.

Traditionally, effects of urbanisation, and of agricultural and forestry activities on riverine systems, particularly in Africa, have been monitored by analysing physico-chemical variables, combined with structural assessments of resident biota such as vegetation, macroinvertebrates and fish (Odume *et al.,* 2011; Edegbene & Arimoro, 2012; Arimoro *et al.,* 2014; Moges *et al.,* 2016).

Macroinvertebrates are among the most widely used biota for assessing the ecological health conditions of river systems (Mereta *et al.,* 2013; Fierro *et al.,* 2017). Studies focusing on taxonomic assessment of macroinvertebrate structure have shown that urban, agricultural and urban-forestry pollution impact on the diversity, richness, composition, and abundance of macroinvertebrates (Arimoro & Muller, 2010; Odume *et al.,* 2011; Lakew & Moog, 2015; Edegbene *et al.,* 2019). Through the effects of the so-called urban stream syndrome, including depleting dissolved oxygen, increases in metal, solids, and nutrient concentrations, macroinvertebrate structures have been shown to be negatively affected, with certain pollution tolerant taxa such as the Diptera culicids and syrphids larvae being favoured, to the detriment of sensitive taxa such as species of the Ephemeroptera, Plecoptera and Trichoptera (EPT). However, despite the growing recognition of the complementarity of traits with taxonomic analysis, studies on macroinvertebrate traits in relation to urban, agricultural and forestry pollution in Africa are sparse (Odume *et al.,* 2014; Akamagwuna *et al.,* 2019; Odume, 2020). An investigation into the differential responses of macroinvertebrate traits to urban, urban-agriculture and urban-forestry pollution is particularly useful because traits enable organisms to adapt to prevailing environmental alteration (Poff *et al.,* 2006; Kuzmanonic *et al.,* 2017). Traits mediate the responses of organisms to environmental perturbation, and their composition and distribution have been assessed in order to gain insights into how organisms respond and adapt to their environment (Poff *et al.,* 2006; Kuzmanovic *et al.,* 2017).

The increasing recognition accorded to the trait-based approach (TBA) could be attributed to empirical evidence suggesting that: i) it is less spatially constrained than the taxonomic approach, ii) has a more direct link to ecosystem function, and iii) has potential for impact diagnosis and predictive ecology (Ding *et al.,* 2017; Milosevic *et al.,* 2018; Desrosiers *et al.,* 2019). Given the growing recognition of the TBA as complementary to the taxonomic analysis, (Pallottini *et al.,* 2017; Akamagwuna *et al.,* 2019), it was asked whether urban, urban-agriculture and urban-forestry pollution in the Niger Delta, would differentially influence the distribution and pattern of macroinvertebrate traits, and their ecological preferences. If a consistent pattern is observed, trait-based indicators of urban, urban-agriculture and urban-forested pollution may be possible to identify, similar to taxonomic-based indicators such as the diversity and richness of EPT. Identifying trait-based indicators is useful, particularly because of the environment-organismal mediation role traits play, as well as their potential to inform prediction of organismal response to environmental perturbations (Akamagwuna *et al.,* 2019; Odume, 2020). Further, ecological preferences and trait-based indicators can also partly address the lack of taxonomic expertise in Afrotropical regions (Barber-James *et al.,* 2008; Odume, 2020) as not all traits are tightly tied to taxonomic identities of organisms. Therefore, it was hypothesised that urban, urban-agriculture and urban-forestry pollution would differentially influence macroinvertebrate traits and ecological preferences so that a pattern attributable to urban, urban-agriculture and urban-forestry pollution effect is discernible. This chapter addresses objective two outlined in Chapter 1; the objectives of this chapter were i) to explore the pattern and distribution of macroinvertebrate traits and ecological preferences in relation to a gradient of urban, urban-agriculture and urban-forestry pollution in selected rivers within the Niger Delta, and ii) on the basis of the observed pattern of trait and ecological preference distribution, identify potential trait-based indicators suitable for monitoring urban, urban-agriculture, and urban-forestry pollution in rivers within the Niger Delta.

## 4.2 Materials and methods

### 4.2.1 Selection of traits and ecological preferences

A stressor-based approach was followed in selecting appropriate traits and ecological preferences. First, the literature was reviewed to identify stressors linked to urbanisation, agriculture, and forestry. The main reported stressors include suspended sediment/storm water return flow (Roy *et al.,* 2005; Walsh *et al.,* 2005; Jones *et al.,* 2012; Odume *et al.,* 2018a); organic/nutrient pollution, (Lee & Bang; 2000; Hatt *et al.*, 2004, Walsh *et al.*, 2005; Heino, 2013; Krynak & Yates, 2018), and potential metals, pesticides pollution and allochthonous food materials (Doledec & Stazner, 2008; Ogren & King, 2008; Kuzmanovic *et al.,* 2017), all of which were evident in rivers of the Niger Delta region of Nigeria (Table 2.4 Chapter 2). Secondly, traits and ecological preferences potentially mechanistically linked to the stressors' modes of urban, agricultural and forestry stress were then selected. In this study, 12 categories of traits and ecological preferences, including respiration, body armouring, turbidity preference, voltinism, attachment mechanism, mobility, body shape, food preference, sensitivity to organic pollution, body size, aquatic stages and feeding habit, were selected for the rivers experiencing urban, urban-agriculture and urban-forestry pollution (Chapter 2, Table 2.3). The traits and ecological preference categories were resolved into 53 attributes. Traits and ecological preferences data were obtained primarily from the South African macroinvertebrate trait database (Odume *et al.,* 2018b), supplemented by data obtained from elsewhere (e.g. Bonada & Doledec, 2011; Kuzmanovic *et al.,* 2017; Desrosiers *et al.,* 2019) and consultation with invertebrate experts at the Albany Museum, Grahamstown, South Africa. Information on traits and ecological preferences was retrieved at the family level because species level information is sparse in the Afrotropical region. Based on the limitations in the use of family level information as species within a family may exhibits different traits and ecological preferences, a fuzzy coding method was employed. Fuzzy coding method accounts for the plasticity, variability within species between a family and between the different life history stages of macroinvertebrates (Chevenet *et al.,* 1994). A fuzzy coding system of 0, 1, 2, and 3 was used to award affinity scores to macroinvertebrate taxa corresponding to no affinity, low affinity, moderate affinity and high affinity, respectively, to traits and ecological preferences (Chevenet *et al.*, 1994). Each trait and ecological preference score was multiplied by the logarithm transformed relative abundance of the macroinvertebrate taxa.

## 4.3 Statistical and data analyses

### 4.3.1 Exploring the pattern and distribution of traits and ecological preferences in relation to urban, urban-agriculture and urban-forestry pollution

To explore the patterns of trait attributes and ecological preferences in relation to urban, urban-agricultural, and urban-forestry pollution, an RLQ analysis was performed. An RLQ is a multivariate ordination analysis developed by Doledec *et al.* (1996) which performs an ordination on three datasets: environmental variables (R), taxa (L) and traits (Q). In this chapter, it was used to relate physico-chemical parameters (R), macroinvertebrates taxa (L) and the traits and ecological preferences (Q) in relation to a gradient of urban, urban-agriculture and urban-forestry pollution derived through the PCA analysis in Chapter 3, which categorised stations into three impact categories: LIS (least impacted stations), MIS (moderately impacted stations) and HIS (heavily impacted stations). Correspondence ordination analysis (dudi.COA) was applied to the macroinvertebrate taxa table (Dray & Dufour, 2007) and a principal component analysis (dudi. PCA) was applied to the trait table, while the Hillsmith (dudi.Hillsmith) function was applied to the environmental (physico-chemical) matrix (Dray & Dufour, 2007). On the RLQ ordination planes, trait attributes and ecological preferences associated with stations categorised as highly impacted were deemed urban, urban-agriculture, and urban-forestry pollution tolerant traits and ecological preferences, and those associated with the LIS, were deemed urban and urban-agriculture pollution sensitive traits and ecological preferences. A similar approach has been used to identify signature and sensitive traits and ecological preferences of urban pollution by Odume (2020). Statistical significance of the two axes of the RLQ was tested using the Monte Carlo permutation test at 999 permutations at $P$=0.05.

To further explore the pattern of traits and ecological preferences in relation to urban, urban-agricultural and urban-forestry pollution, a fourth-corner analysis was computed. The fourth-corner test is a multivariate analysis that elucidates the respective correlations between multiple traits and environmental variables. It reveals the trait attributes that either negatively or positively relate to a given physico-chemical variable. In this chapter, the fourth-corner analysis was used to reveal the relationship between traits and ecological preferences, and physico-chemical variables. In addition to being associated with the LIS based on the RLQ results, traits and ecological preferences that were either significantly positively associated with DO, or negatively associated with any two of the physico-chemical indicators of urban and urban-agriculture pollution (e.g. five-day Biochemcial Oxygen Demand ($BOD_5$), Electrical Conductivity (EC) and nutrients) were deemed urban pollution sensitive traits and ecological preferences, and those either significantly positively associated with any two of increasing nutrients, EC and $BOD_5$ or significantly negatively associated with DO were deemed urban and urban-agriculture pollution tolerant traits and ecological preferences. For urban-forestry polluted rivers, in addition to being associated with LIS based on the RLQ results, traits and ecological preferences that were either significantly associated with DO or negatively associated with any two of increased flow velocity, water temperature, $BOD_5$ and nutrients were deemed urban-forestry pollution sensitive traits and ecological preferences, and those either significantly negatively associated with DO or positively associated with any two of increased flow velocity, water temperature, $BOD_5$ and nutrients were deemed urban-forestry pollution tolerant traits and ecological preferences. The fourth-corner, RLQ, and associated analyses (COA, Hillsmith and PCA) were computed using the ade4 package for R-statistics version 2.5.4 within the R programming environment (Dray & Dufour, 2007).

## 4.4 Results

### 4.4.1 Pattern of distribution of traits and ecological preferences in relation to urban pollution gradient

Of the 66 sampling stations considered in this study, 11 stations were impacted by urban pollution. Of the 11 stations impacted by urban activities, two stations were considered LIS, four MIS, and five HIS.

The least impacted stations (Anwai River station 1 and Warri River station 1) were associated with Axis 1, while most of the heavily impacted stations were associated with Axis 2 of the RLQ ordination plane (Figure 4.1). Stations categorised as LIS that were associated with Axis 1, positively correlated with dissolved oxygen (DO) and depth (Figure 4.1). The LIS, MIS and HIS are depicted as A, B and C, respectively, on the RLQ ordination (Figure 4.1).

Traits and ecological preferences that were associated with the LIS include the possession of hardshell, moderate and high sensitivities to oxygen depletion, very large body sized individuals (>20-40mm), swimmers, flattened body shape, a preference for temporary attachment, crawling, respiration with aerial/vegetation, possession of breathing tubes, possession of strap or other apparatus for respiration, streamlined body, and a high sensitivity to oxygen depletion. Most of the heavily impacted stations (Ethiope River Station 2, Ogba River Station 2 and Orogodo River Station 3) were positioned on the second axis of the RLQ biplot (Figure 4.1). Physico-chemical indicators of urban pollution such EC, nitrate, phosphate and $BOD_5$ were positively correlated with pupa aquatic stage, the possession of a large body size, larva aquatic stage, free-living, skating, soft and exposed body, cased/tubed body, sprawling and burrowing in the heavily impacted stations (HIS) on the RLQ biplot (Figure 4.1).

The Eigen values of the first two RLQ axes were 6.57 and 0.36, respectively (Table 4. 1) while the RLQ Axes 1 and 2 explained 91.17% and 5.02% with a projected total inertia of 7.21. For physico-chemical variables, the variances for Axes 1 and 2 were 3.83 and 6.17, respectively, while the traits and ecological preferences variance for Axis 1 was 11.18 and Axis 2 was 18.45. A Monte Carlo test at 999 permutations revealed Axes 1 and 2 to be statistically significant in terms of the relationship between the macroinvertebrate traits and ecological preferences, and the physico-chemical variables ($P < 0.05$).

Table 4. 1: Properties of the RLQ analysis of the correlation between macroinvertebrate traits and ecological preferences, stations and physico-chemical variables in relation to urban pollution gradient in rivers in the Niger Delta, Nigeria.

| **RLQ Properties** | **Axis 1** | **Axis 2** |
|---|---|---|
| Variance RLQ-COA, PCA (%) | 91.17 | 5.02 |
| Eigen value | 6.57 | 0.36 |
| Variance (Hillsmith transformed) of the macroinvertebrate traits/ecological preferences | 11.18 | 18.45 |
| Variance of the physico-chemical parameters (PCA) | 3.83 | 6.17 |
| Monte Carlo permutation | 0.026 | 0.003 |
| Total inertia | 7.21 | |

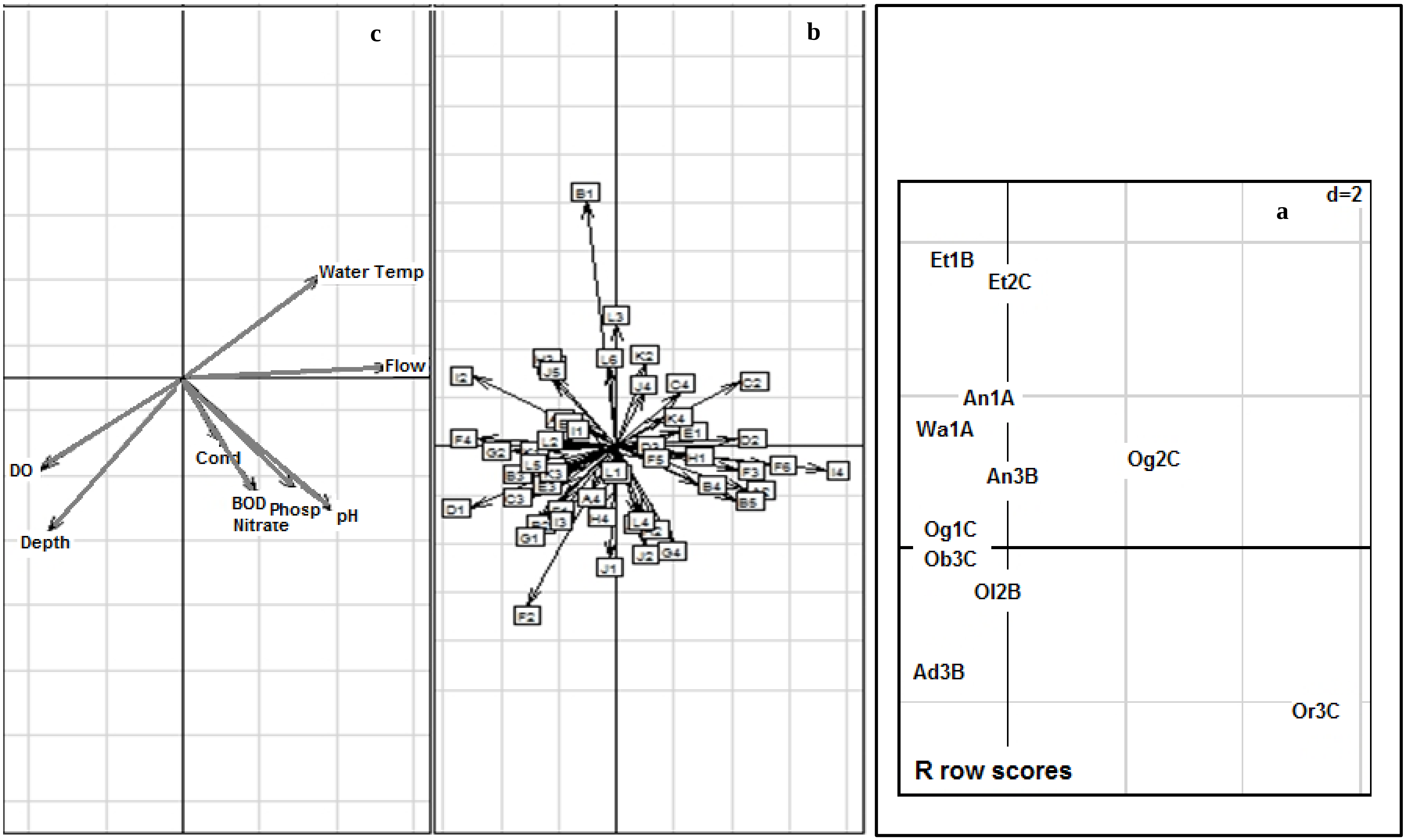



Figure 4. 1: RLQ analysis showing the co-variation of the 11 river stations (a), macroinvertebrate traits and ecological preferences (b), and physico-chemical variables (c) in relation to the first two axes of the RLQ. Abbreviations: Stations: An1A (Anwai River station

1), (Wa1A (Warri River station 1), Ad3B (Adofi River station 3), An3B (Anwai River station 3), Ol2B (Oleri River station 2), Et1 (Ethiope River station 1), Et2C (Ethiope River station 2), Ob3C (Obosh River station 3), Og1C (Ogba River station 1), Og2C (Ogba River station 2) and Or3C (Orogodo River station 3). Physico-chemical variables: Water Temp=Water Temperature, Flow=Flow velocity, DO= Dissolved oxygen, BOD=Five-day biochemical oxygen demand ($BOD_5$), Cond=Electrical conductivity (EC), Phosp=Phosphate. Stations impact categories: A=least impacted stations (LIS), B=moderately impacted stations (MIS), C=heavily impacted stations (HIS). Traits and ecological preferences: A1=Gills, A2=Tegument/cutaneous, A3=Aerial: spiracle, A4=Aerial/vegetation: breathing tube, strap/other apparatus, B1=Hardshell, B2=Completely sclerotized, B3=Partly sclerotized, B4=Soft and exposed, B5=Cased/tubed, C1=Clear and transparent waters, C2=Silty, C3=Turbid waters, C4=No preference, D1=1 year (Univoltine), D2=2 years (Bivoltine), D3= >2years (Multivoltine), D4=longer than one year (Semivoltine), E1=Free-living, E2=Temporary attachment, E3=Permanent attachment, F1=Climber, F2=Crawler, F3=Sprawler, F4=Swimmer, F5=Skater, F6=Burrower, G1=Streamlined, G2=Flattened, G3=Spherical, G4=Cylindrical/tubular, H1=Detritus (FPOM), H2=Detritus (CPOM), H3=Macrophytes/algae, H4=Animal materials, I1=Highly sensitive to oxygen depletion, I2=Moderately sensitive to oxygen depletion, I3=Moderately tolerant of oxygen depletion, I4= Highly tolerant of oxygen depletion, J1=Very small (<5 mm), J2=Small (>5-10 mm), J3=Medium (>10-20 mm), J4=Large (>20-40 mm), J5=Very large (>40-80 mm), K1=Egg, K2=Larva, K3=Nymph, K4=Pupa, L1=Predator, L2=Scraper, L3=Grazer, L4=Filter feeder, L5=Deposit feeder, L6=Shredder.

Following the RLQ analysis, a fourth-corner analysis was performed to explore the correlation between traits and ecological preferences, and physico-chemical variables. Traits and ecological preferences, which were positively associated with the LIS and were also significantly positively correlated with either DO, or significantly negatively correlated with at least two physico-chemical indicators of increasing urban pollution (i.e. EC, nutrients, and $BOD_5$) were identified as urban pollution-sensitive traits and ecological preferences. These traits and preferences include univoltinism, permanent attachment, crawling, swimming, and a moderate sensitivity to oxygen depletion (Figure 4.2). Traits and ecological preferences positively associated with HIS on the RLQ ordination plane that were either significantly positively correlated with at least two of the physico-chemical indicators of urban pollution (i.e. EC, nutrients, and $BOD_5$), or significantly negatively correlated with DO, were deemed urban pollution-tolerant traits and ecological preferences. These traits and ecological preferences include tegument/cutaneous respiration, cased/tubed protection, a preference for silty waters, bivoltinism, burrowing, and a high tolerance for oxygen depletion (Figure 4.2). A summary of the traits and ecological preferences deemed sensitive to and tolerant of urban pollution is provided in Table 4.2.

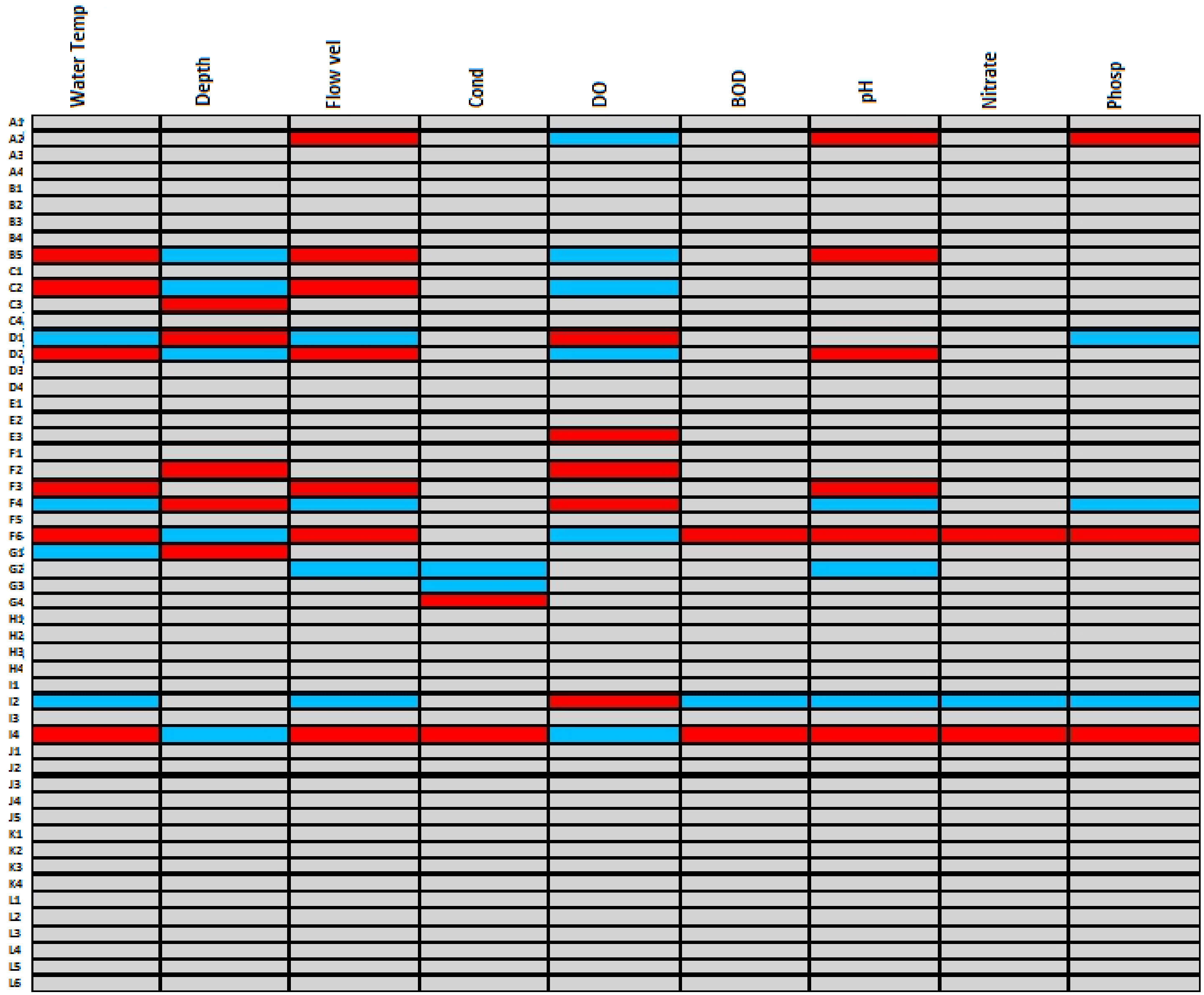



Figure 4. 2: Summary of the fourth-corner test performed for macroinvertebrate traits and ecological preferences, and physico-chemical variables in the selected urban rivers. Significant positive correlations are shown in red cells while the significant negative correlations are shown in blue cells. The grey cells represent non-significant correlations.

Table 4. 2: Traits and ecological preferences deemed sensitive to and tolerant of urban pollution in rivers in the Niger Delta, Nigeria.

| **Traits and ecological preferences** | | | |
|---|---|---|---|
| **Pollution sensitive** | | **Pollution tolerant** | |
| **Traits and ecological preferences attributes** | **Codes** | **Traits and ecological preferences attributes** | **Codes** |
| Univoltinism | D1 | Tegument/cutaneous respiration | A2 |
| Permanent attachment | E3 | Cased/tubed protection | B5 |
| Crawling | F2 | A preference for silty waters | C2 |
| Swimming | F4 | Bivoltinism | D2 |
| Moderately sensitive to oxygen depletion | I2 | Burrowing | F6 |
| | | Highly tolerant of oxygen depletion | I4 |

### 4.4.2 Pattern of distribution of traits and ecological preferences in relation to urban-agriculture pollution

Of the 66 sampling stations considered in this study, 17 stations were impacted by urban-agriculture pollution. Of the 17 stations impacted by urban-agricultural activities, two stations were considered LIS, seven MIS, and eight HIS.

The two least impacted river stations (Obosh River station 2 and Umu River station 2) were associated with Axis 1 of the RLQ ordination plane (Figure 4.3). Also associated with Axis 1 were all the moderately impacted river stations except station 2 of Umaluku River, which was positioned at the centre of the RLQ biplot, and station 2 of Edor River, which was associated with Axis 2 (Figure 4.3). All the heavily impacted river stations were associated with Axis 2, except station 3 of Owan River which was associated with Axis 1 (Figure 4.3). Dissolved oxygen (DO), pH, depth and flow velocity were positively correlated with stations categorised as LIS and MIS, whereas EC, $BOD_5$, water temperature, phosphate and nitrate, were positively correlated with the HIS (Figure 4.3). The LIS, MIS and HIS are depicted as A, B and C, respectively, on the RLQ ordination plot (Figure 4.3).

Traits such as gills, tegument/cutaneous respiration, possession of hardshell, soft and exposed bodies, a preference for clear and transparent waters, univoltinism, free-living, a preference for temporary attachment, a preference for permanent attachment, crawling, cylindrical/tubular body shape, and large (>20-40 mm) body size were associated with the LIS. Physico-chemical indicators of pollution such as $BOD_{5,}$ phosphate, nitrate and EC explained the distribution of traits and ecological preferences such as aerial respiration: spiracle, cased/tubed body armouring, permanent attachment, swimming, a preference for detritus (FPOM) as food, high tolerance of oxygen depletion and deposit feedings on Axis 2 (Figures 4.3). These traits were associated with the HIS (Figure 4.3).

Axes 1 and 2's Eigen values were 4.98 and 3.5, respectively (Table 4.3). A projected total inertia of 9.46 was explained by the RLQ ordination. Axis 1 explained 52.62% of the variance while Axis 2 explained 37.063%. Hillsmith transformed variance for the macroinvertebrate traits and ecological preferences were 12.24 and 19.99 for Axes 1 and 2, respectively (Table 4.3). There were no significant differences for Axes 1 and 2 of the RLQ ordination plot between macroinvertebrate traits and ecological preferences, and physico-chemical variables ($P$>0.05) as revealed by the Monte Carlo permutation test at 999 permutations.

Table 4. 3: Properties of RLQ analysis of the correlation between macroinvertebrate traits and ecological preferences, stations and physico-chemical variables in relation to urban-agriculture pollution.

| **RLQ Properties** | **Axis 1** | **Axis 2** |
|---|---|---|
| Variance RLQ-COA, PCA (%) | 52.62 | 37.063 |
| Eigen value | 4.98 | 3.5 |
| Variance (Hillsmith transformed) of the macroinvertebrate traits/ecological preferences | 12.24 | 19.99 |
| Variance of the physico-chemical parameters (PCA) | 3.84 | 5.83 |
| Monte Carlo permutation | 0.235 | 0.115 |
| Total inertia | 9.46 | |

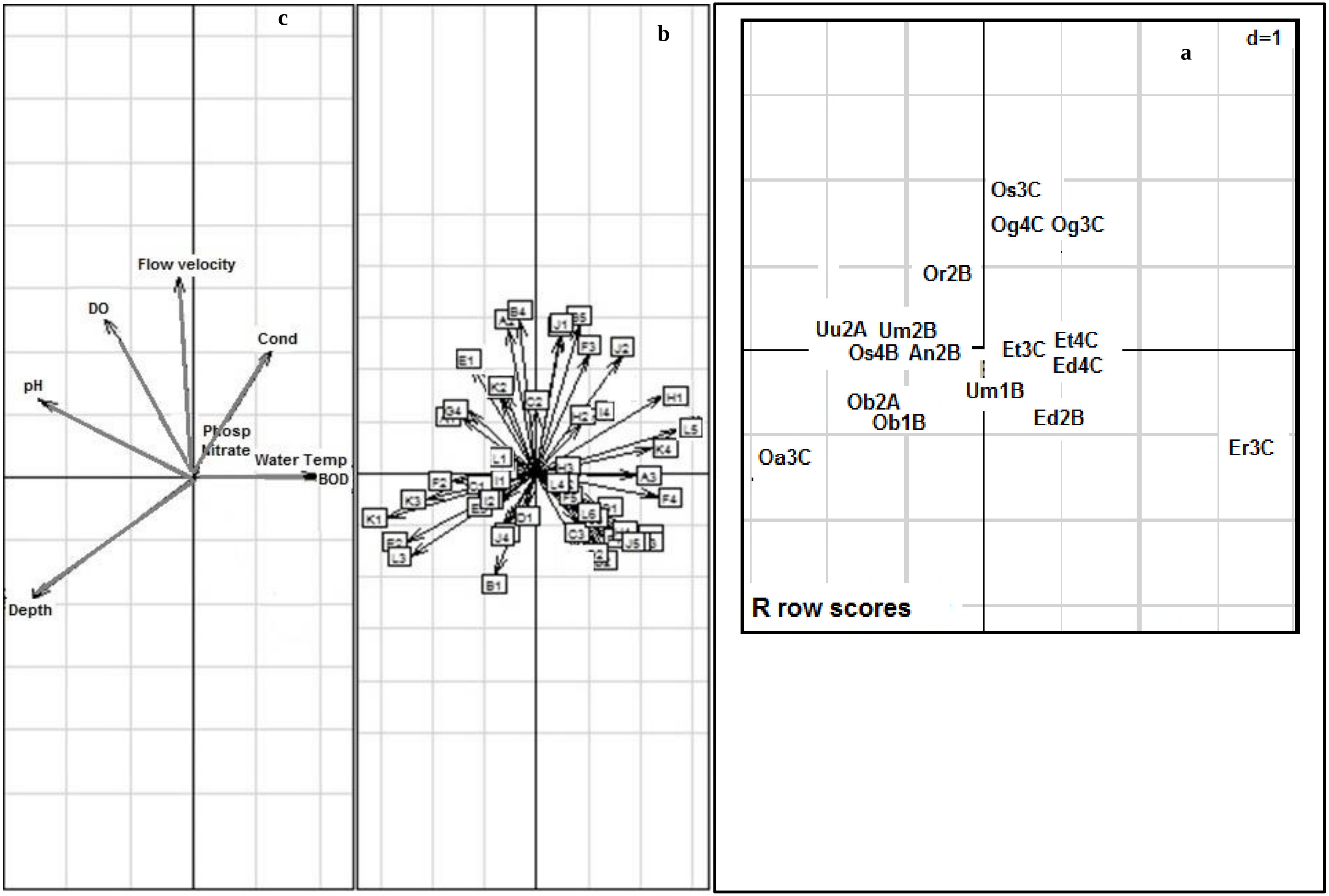

c
Flow velocity
DO
Cond
pH
Phosp
Nitrate
Water Temp
BOD
Depth
b
a
d=1
Os3C
Og4C Og3C
Or2B
Uu2A Um2B
Os4B An2B
Et3C Et4C
Ed4C
Um1B
Ob2A
Ob1B
Ed2B
Oa3C
Er3C
R row scores

Figure 4. 3: RLQ analysis showing the co-variation of the 17 river stations (a), macroinvertebrate traits and ecological preferences (b) and physico-chemical variables (c) in relation to the first two axes of the RLQ. Abbreviations: Stations: Ob2A (Obosh River station 2),Uu2A (Umu River station 2), An2B (Anwai River station 2), Ed2B (Edor River station 2), Ob1B (Obosh River station 1), Or2B (Orogodo River station 2), Os4B (Ossiomo River station 4), Um1B (Umaluku River station 1), Um2B (Umaluku River station 2), Ed4C (Edor River station 4), Er3C (Eriora River station 3), Et3C (Ethiope River, station 3), Et4C (Ethiope River station 4), Og3C (Ogba River station 3), Og4C (Ogba River station 4), Os3C (Ossiomo River station 3) and Oa3C (Owan River station 3). Physico-chemical variables: Water Temp=Water Temperature, Flow velocity=Flow velocity, DO=Dissolved oxygen, BOD=Five-day biochemical oxygen demand ($BOD_5$), Cond=Electrical conductivity (EC), Phosp=Phosphate. Stations impact categories: A=least impacted stations (LIS), B=moderately impacted stations (MIS), C=heavily impacted stations (HIS). Traits and ecological preferences: A1=Gills, A2= Tegument/cutaneous, A3=Aerial: spiracle, A4=Aerial/vegetation: breathing tube, strap/other apparatus, B1=Hardshell, B2=Completely sclerotized, B3=Partly sclerotized, B4=Soft and exposed, B5=Cased/tubed, C1=Clear and transparent waters, C2=Silty, C3=Turbid waters, C4=No preference, D1=1 year (Univoltine), D2=2 years (Bivoltine), D3= >2years (Multivoltine), D4=longer than one year (Semivoltine), E1= Free-living, E2=Temporary attachment, E3=Permanent attachment, F1=Climber, F2=Crawler, F3=Sprawler, F4= Swimmer, F5=Skater, F6=Burrower, G1=Streamlined, G2=Flattened, G3=Spherical, G4=Cylindrical/tubular, H1=Detritus (FPOM), H2=Detritus (CPOM), H3=Macrophytes/algae, H4=Animal materials, I1=Highly sensitive to oxygen depletion, I2=Moderately sensitive to oxygen depletion, I3=Moderately tolerant of oxygen depletion, I4=Highly tolerant of oxygen depletion, J1=Very small (<5 mm), J2=Small (>5-10 mm), J3=Medium (>10-20 mm), J4=Large (>20-40 mm), J5=Very large (>40-80 mm), K1=Egg, K2=Larva, K3=Nymph, K4=Pupa, L1=Predator, L2=Scraper, L3=Grazer, L4=Filter feeder, L5=Deposit feeder, L6=Shredder.

The fourth-corner analysis performed after the RLQ ordination showed that permanent attachment, high and moderate sensitivity to oxygen depletion, and the possession of large body size (>20-40 mm), which were associated with the LIS, were also significantly positively correlated with DO concentration (Figure 4.4). These four traits and ecological preferences were deemed urban-agricultural pollution sensitive traits. On the other hand, traits and ecological preferences which were associated with the HIS on the RLQ plane, and were also either significantly negatively correlated with DO concentration or significantly positively correlated with at least two physico-chemical indicators of pollution were deemed urban-agricultural pollution tolerant traits. These traits include a preference for detritus (CPOM) as food, and possession of very small body size (<5mm) (Table 4.4).

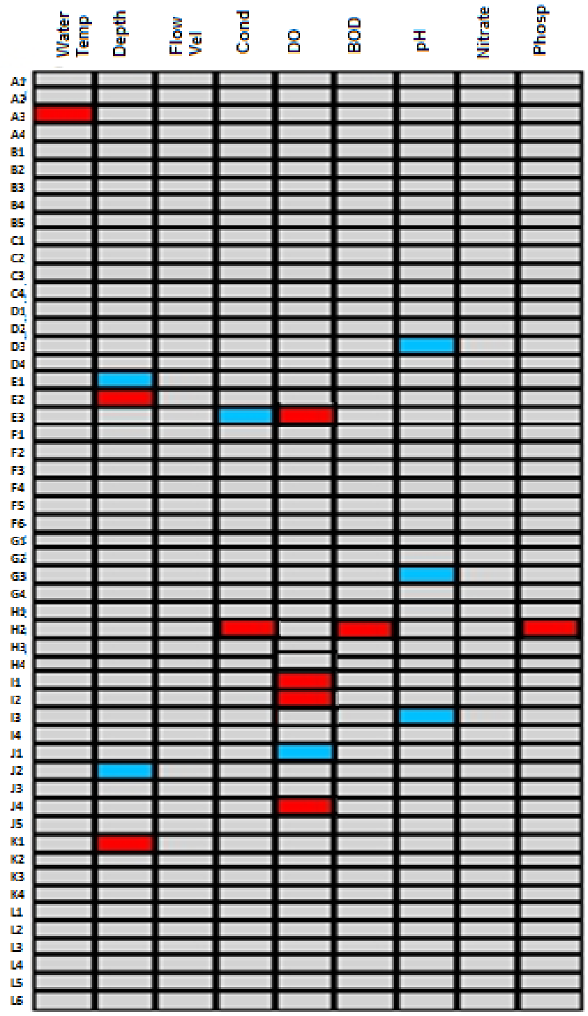



Figure 4. 4: Summary of the fourth-corner test performed for macroinvertebrate traits and ecological preferences, and physico-chemical variables in the selected urban-agricultural rivers. Significant positive correlations are shown in red cells, while the significant negative correlations are shown in blue cells. The grey cells represent non-significant relationships.

Table 4. 4: Traits and ecological preferences deemed sensitive to and tolerant of urban-agriculture pollution in rivers in the Niger Delta, Nigeria.

| **Traits and ecological preferences** | | | |
|---|---|---|---|
| **Pollution sensitive** | | **Pollution tolerant** | |
| **Traits and ecological preferences attributes** | **Codes** | **Traits and ecological preferences attributes** | **Codes** |
| Permanent attachment | E3 | Preference for Detritus (CPOM) | H2 |
| Highly sensitive to oxygen depletion | I1 | Very small body size (<5 mm) | J1 |
| Moderately sensitive to oxygen depletion | I2 | | |
| Large body size (>20-40 mm) | J4 | | |

**4.4.3 Pattern of distribution of traits and ecological preferences in relation to urban-forestry pollution**

Of the 66 sampling stations selected in this study, 20 stations were within forested catchments impacted by urban pollution. Of the 20 stations impacted by urban-forestry pollution, three stations were considered LIS, seven were MIS, and 10 were HIS.

Two of the three LIS (stations 2 and 3 of Warri River) were positioned on the first axis of the RLQ ordination along with MIS (stations 3 of Ase and Benin Rivers, and station 1 of Orogodo River) (Figure 4.5). At the centre of the RLQ ordination plot were two MIS which include stations 1 of both Ase and Orogodo Rivers. All the HIS were positioned on Axis 2 of the RLQ ordination, except station 2 of Owan River and stations 1 and 2 of Umomi River, which were at the centre of the RLQ plane (Figure 4.5). Also on the second axis of the RLQ ordination were one LIS (station 1 of Adofi River) and three MIS (stations 1, 2 and 3 of Iyiukwu River).

Axis 1 of the RLQ ordination explained 92.60% of variance, while Axis 2 explained 5.90% of the ordination (Table 4.5). Axes 1 and 2 had Eigen values of 10.67 and 0.68, respectively (Table 4.5), with a total inertia of 11.53. For the physico-chemical variables, the variance for Axes 1 and 2 was 4.60 and 6.18, respectively, while the traits and ecological preferences variance for Axis 1 was 17.70 and Axis 2 was 10.49. A Monte Carlo test at 999 permutations revealed that Axes 1 and 2 had no significant differences between the macroinvertebrate traits and ecological preferences and physico-chemical variables ($P$ >0.05). Stations classified as HIS were associated with increasing temperature, flow velocity, $BOD_5$, nutrient and EC, whereas those classified as LIS were associated with increasing DO (Figure 4.5).

Table 4. 5: Properties of RLQ analysis of the correlation between macroinvertebrate traits and ecological preferences, stations and physico-chemical variables in relation to stations in forested catchments experiencing urban pollution.

| **RLQ Properties** | **Axis 1** | **Axis 2** |
|---|---|---|
| Variance RLQ-COA, PCA (%) | 92.60 | 5.9 |
| Eigen value | 10.67 | 0.68 |
| Variance (Hillsmith transformed) of the macroinvertebrate traits/ecological preferences | 10.49 | 17.70 |
| Variance of the physico-chemical parameters (PCA) | 4.60 | 6.18 |
| Monte Carlo permutation | 0.23 | 0.11 |
| Total inertia | 11.53 | |

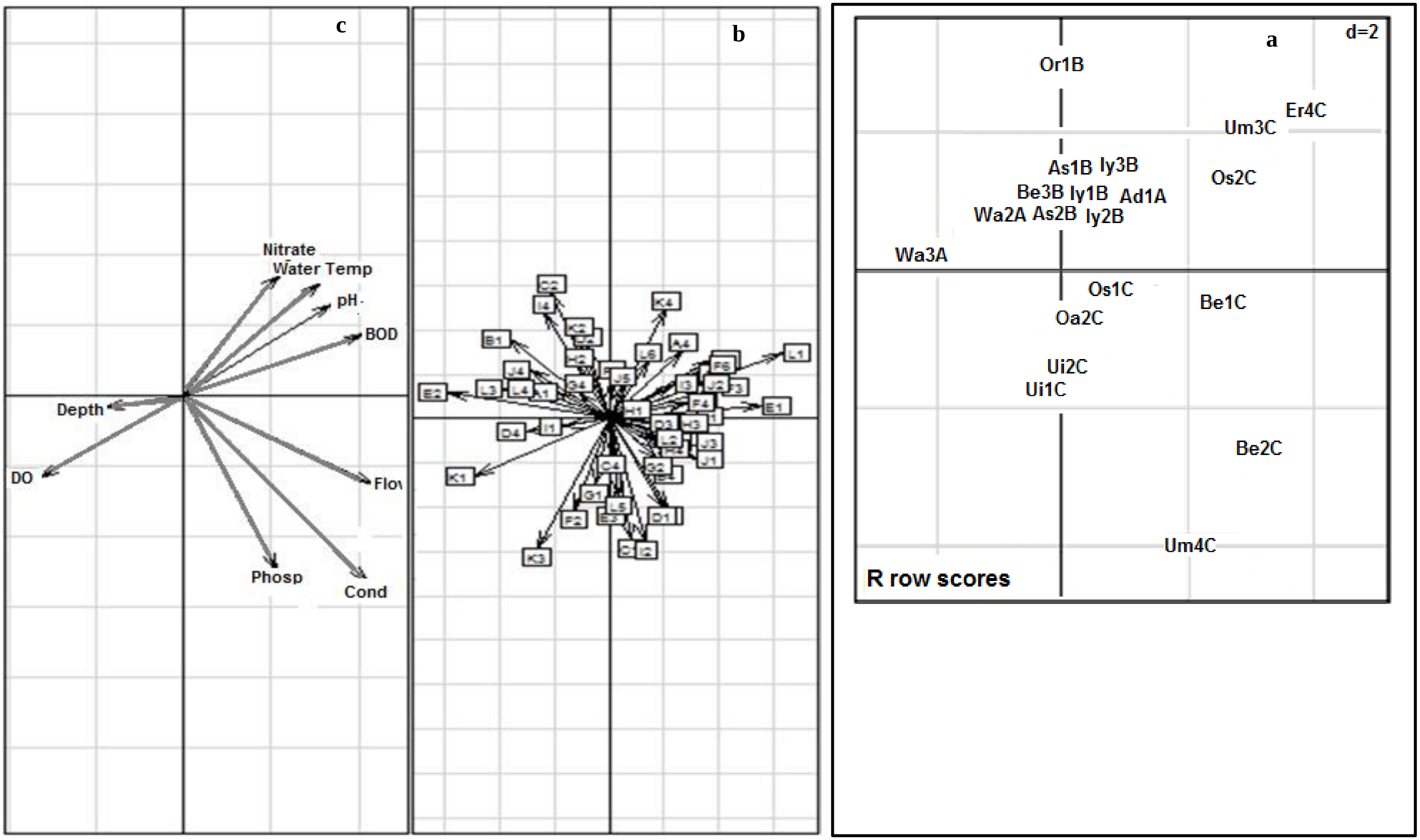



Figure 4. 5: RLQ analysis showing the co-variation of the 20 river stations (a), macroinvertebrate traits and ecological preferences (b), and physico-chemical variables (c), in relation to the first two axes of the RLQ. Abbreviations: Stations: Ad1A (Adfofi River station

1), Wa2A (Warri River station 2),Wa3A (Warri River station 3), As1B (Ase River station 1), As2B (Ase River station 2), Iy1B (Iyiukwu River station 1), Iy2B (Iyiukwu River station 2), Iy3B (Iyiukwu River station 3), Or1B (Orogodo River station 1), Be1C (Benin River station 1), Be2C (Benin River station 2), Be3C (Benin River station 3), Er4C (Eriora River station 4), Os1C (Ossiomo River station 1), Os2C (Ossiomo River station 2), Oa2C (Owan River station 2), Um3C (Umaluku River station 3), Um4C (Umaluku River station 4), Ui1C (Umoni River station 1) and Ui2C (Umomi River station 2). Physico-chemical variables: Water Temp=Water Temperature, Flow=Flow velocity, DO=Dissolved oxygen, BOD=Five-day biochemical oxygen demand ($BOD_5$), Cond=Electrical conductivity, Phosp=Phosphate. Station impact categories: A=least impacted stations (LIS), B=moderately impacted stations (MIS), C=heavily impacted stations (HIS). Traits and ecological preferences: A1=Gills, A2=Tegument/cutaneous, A3=Aerial: spiracle, A4= Aerial/vegetation: breathing tube, strap/other apparatus, B1=Hardshell, B2=Completely sclerotized, B3=Partly sclerotized, B4=Soft and exposed, B5=Cased/tubed, C1=Clear and transparent waters, C2=Silty, C3=Turbid waters, C4=No preference, D1=1 year (Univoltine), D2=2 years (Bivoltine), D3= >2years (Multivoltine), D4=longer than one year (Semivoltine), E1=Free-living, E2=Temporary attachment, E3=Permanent attachment, F1=Climber, F2=Crawler, F3=Sprawler, F4=Swimmer, F5=Skater, F6=Burrower, G1=Streamlined, G2=Flattened, G3=Spherical, G4=Cylindrical/tubular, H1=Detritus (FPOM), H2=Detritus (CPOM), H3= Macrophytes/algae, H4=Animal materials, I1=Highly sensitive to oxygen depletion, I2=Moderately sensitive to oxygen depletion, I3= Moderately tolerant of oxygen depletion, I4=Highly tolerant of oxygen depletion, J1=Very small (<5 mm), J2=Small (>5-10 mm), J3= Medium (>10-20 mm), J4=Large (>20-40 mm), J5=Very large (>40-80 mm), K1=Egg, K2=Larva, K3=Nymph, K4=Pupa, L1= Predator, L2=Scraper, L3=Grazer, L4=Filter feeder, L5=Deposit feeder, L6=Shredder.

The fourth-corner analysis performed to further explore the correlation between traits and ecological preferences, and physico-chemical variables revealed that hardshell, large (>20-40 mm) body size and grazing, which were significantly positively associated with the LIS, were also either significantly positively correlated with DO, or significantly negatively correlated with increasing any two of flow velocity, water temperature, $BOD_5$ and nutrient (Figure 4.6). Hence, these traits and ecological preferences were deemed sensitive in forested rivers receiving urban pollution (Table 4.6). Further, burrowing, the pupa aquatic stage, and predation were significantly positively associated with HIS on the RLQ ordination, or were significantly negatively associated with DO. These traits were deemed tolerant of forested systems receiving urban pollution (Table 4.6).

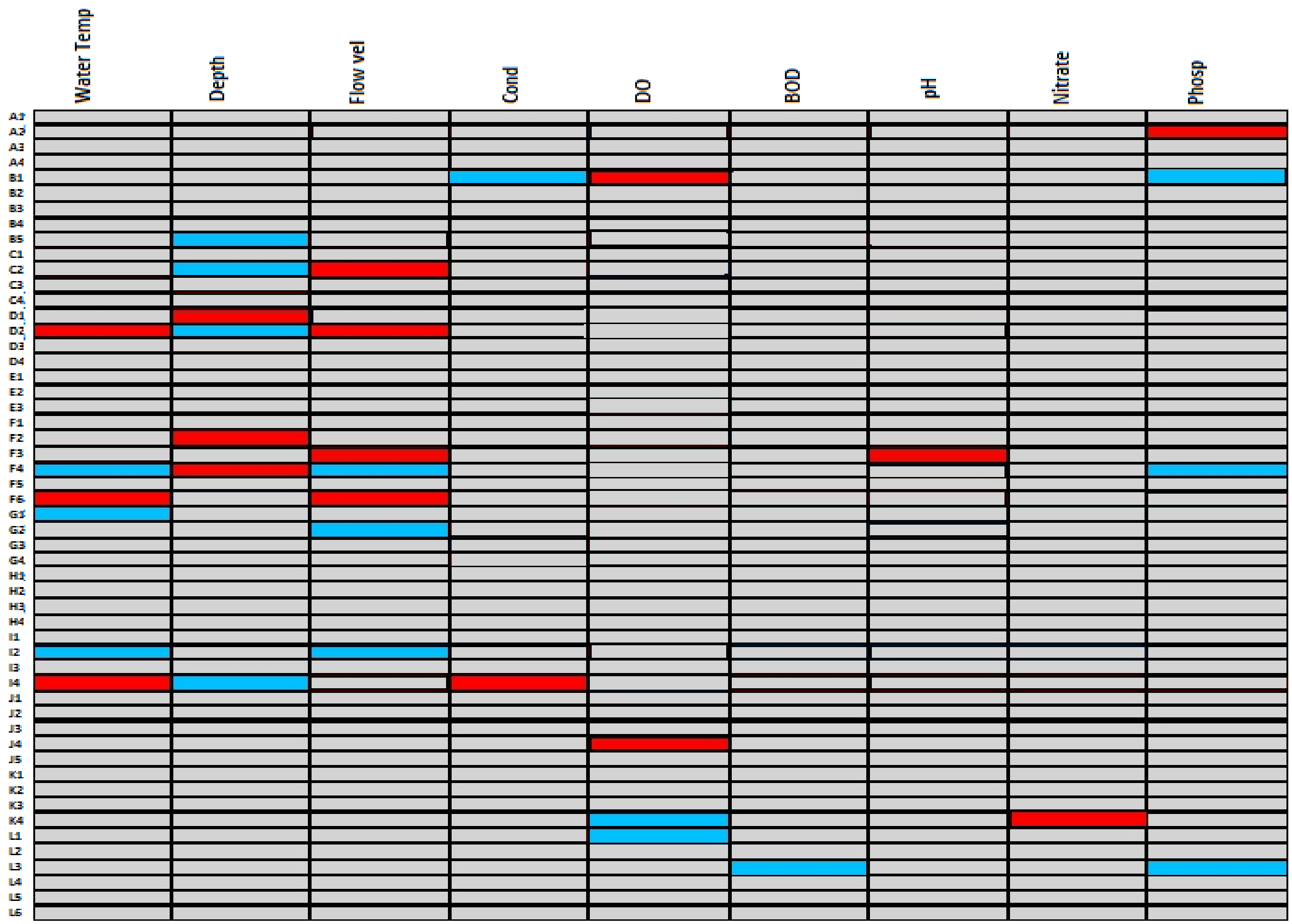



Figure 4. 6: Summary of the fourth-corner test performed for macroinvertebrate traits and ecological preferences, and physico-chemical variables in the selected forested rivers receiving urban pollution in the Niger Delta. Significant positive correlations are shown in red cells, while the significant negative correlations are shown in the blue cells. The grey cells represent non-significant relationships.

Table 4. 6: Traits and ecological preferences deemed sensitive and tolerant in forested rivers receiving urban pollution.

| **Traits and ecological preferences** | | | |
|---|---|---|---|
| **Pollution sensitive** | | **Pollution tolerant** | |
| **Traits and ecological preferences attributes** | **Codes** | **Traits and ecological preferences attributes** | **Codes** |
| Possession of hardshell | B1 | Burrowing | F6 |
| Large body size (>20-40 mm) | J4 | Pupal aquatic stage | K4 |
| Grazing | L3 | Predation | L1 |

## 4.5 Discussion

### 4.5.1 Pattern of distribution of traits and ecological preferences in relation to urban pollution

An exploration of the distribution of macroinvertebrate traits and ecological preferences to urban pollution was undertaken in the selected rivers in the Niger Delta, Nigeria. The results indicate that urban pollution differentially influences traits and ecological preferences. Traits and ecological preferences such as univoltinism, permanent attachment, crawling, and a moderate sensitivity to oxygen depletion were associated with the LIS, and were also positively correlated with DO. These traits were deemed urban pollution-sensitive traits and ecological preferences. For instance, crawlers are slow walkers and it may be difficult for them to move away from sedimentation, which is one of the potential stressors of urban rivers, unlike swimmers which can quickly relocate in a perturbed environment (Buendia *et al.,* 2013).The pattern of distribution of crawlers and swimmers in this study is in line with earlier studies by Mather *et al.* (2017) and Murphy *et al.* (2017) who reported crawling and swimming organisms responded negatively to fine sediment stress. It has also been argued by Wilkes *et al.* (2017) that crawlers are relatively sedentary, hence they may not be able to escape an area of increased urban pollution input.

Univoltinism, a sensitive trait, is an important reproductive trait of organisms that undergo one life cycle in a year. It has been hypothesised that organisms exhibiting more than one life cycle per year, for example bivoltinism or mutivoltinism, are likely to be more tolerant of pollution than those exhibiting one life cycle per year (univoltinism) (Townsend & Hildrew, 1994; Doledec *et al.,* 2006; Tomanova *et al.,* 2008; Kuzmanovic *et al.,* 2017). Kuzmanovic *et al.* (2017) earlier reported the presence of organisms that exhibit bivoltinism in pesticide-laden sites in some polluted Iberian rivers.

Organisms that have more generations in their lifetime have been reported to adapt faster to deteriorating water conditions (Mondy *et al.,* 2012; Kuzmanovic *et al.,* 2017; Berger *et al.,* 2018). This assertion was attributed to ecological theory which states that plurivoltinism exhibits high recolonisation potential after perturbation due to the faster capacity to multigeneration potential within a year, serving as an important adaptive and resilience mechanism (Mondy *et al.,* 2012; Berger *et al.,* 2018). In the present study, the univoltine taxa were found to be associated with the LIS, and taxa exhibiting more than one life cycle per year were found to be associated with the HIS on the RLQ plot. Univoltinism was found to be positively correlated with DO in the fourth-corner test performed in the present study. Similarly, it has been reported that organisms with one life cycle per year (univoltine) associate positively with high DO while plurivoltinistic organisms thrive in disturbed sites with increased nutrient concentration (Kuzmanovic *et al.,* 2017; Desrosiers *et al.,* 2019). The implication is that univoltine taxa seemed to be less resilient to pollution because of the long-time taken during the reproductive cycle. Similar results have been reported by Serra *et al.* (2017), who indicate that univoltine organisms were found to be less associated with polluted sites than were the multivoltine taxa.

In the present study, traits such cased/tubed protection, burrowing, and a high tolerance of oxygen depletion were deemed pollution tolerant traits. The differential responses of the identified pollution sensitive and tolerant traits, and ecological preferences suggest that traits can be used as indicators of urban pollution. This study thus contributes to the growing body of knowledge indicating the usefulness of the trait-based approach in freshwater biomonitoring (Liess *et al.,* 2008; Ding *et al.,* 2017; Pallottini *et al.,* 2017; Desrosiers *et al.,* 2019).

Cased/tubed protection, burrowing, and a high tolerance for oxygen depletion were associated with HIS on the RLQ and among these three traits, cased/tubed protection and burrowing were negatively correlated with increased DO. Cased/tubed protection, burrowing traits and ecological preferences confer resilience and resistance on macroinvertebrates in the face of environmental disturbances because, for example, organisms can easily retract into their tubes in the face of harsh environmental conditions (Pallottini *et al.,* 2017; Desrosiers *et al.,* 2019). This observation is in line with results reported by Pallottini *et al.* (2017) and Desrosiers *et al.* (2019), who indicated that taxa that possessed cased/tubed protection were associated with increased nutrient enrichment and EC. Further, burrowing, which was found to be associated with the HIS in the present study, has also been reported to be associated with increasing sedimentation (Lamouroux *et al.,* 2004; Tomanova & Usseglio-Polatera, 2007) and relatively disturbed sites (Tomanova *et al.,* 2008), suggesting that differential distribution of burrowing could be a trait-based indicator of urban pollution.

The pollution tolerant trait attribute, tegument/cutaneous respiration, was associated with HIS on the RLQ. Tegumental respiration has been reported to associate positively with heavily polluted stations in other studies (Tomanova *et al.,* 2008; Ding *et al.,* 2017; Desrosiers *et al.,* 2019).

### 4.5.2 Pattern of distribution of trait and ecological preferences in relation to urban-agriculture pollution

An exploration of the distribution of macroinvertebrate traits and ecological preferences to urban-agriculture pollution was carried out in selected rivers in the Niger Delta, Nigeria. The results revealed urban-agriculture pollution sensitive traits and ecological preferences such as permanent attachment, a high sensitivity to oxygen depletion, a moderate sensitivity to oxygen depletion and large body size (>20-40 mm) to be associated with LIS and the distribution of these traits and ecological preferences were defined by high DO concentration. Permanently attached organisms have been reported to be positively correlated to increased DO concentration (Berger *et al.,* 2018), corroborating the finding of the present study as permanent attachment, which was associated with the LIS on the RLQ ordination was also found to be positively correlated with high DO on the fourth-corner test.

Large body size (>20–40 mm) in the present study was identified as a sensitive trait as it was associated with LIS on the RLQ ordination and positively correlated with high DO, which contradicts the findings of Statzner & Beche, (2010), Odume *et al.* (2014) and Odume, (2020) who reported large body size to be associated with heavily impacted urban river stations. Nevertheless, the finding in the present study is consistent with the hypothesis of the habitat template concept, which predicts large body size to be less associated with impacted sites because it is often associated with the production of fewer offspring per reproductive event (Townsend & Hildrew, 1994). Further, a large body size presents a reduced surface area to volume ratio, which is likely to accelerate diffusion of toxicants, making organisms with such body size more likely to be vulnerable to urban-agriculture pollution (Odume, 2020), particularly if concentrations of dissolved solids are high, as is evident in the present study. Overall, the finding in this study, which suggests that a large body size is sensitive to pollution, is ecologically meaningful as per the postulation of the habitat template concept and filtering concept (Townsend & Hildrew 1994; Poff *et al.*, 2006).

The tolerant traits and ecological preferences were a preference for detritus (CPOM) and very small body size (<5 mm). Apart from being associated with HIS, CPOM was positively correlated with EC, $BOD_5$ and phosphate, while very small body size (<5 mm) was negatively correlated with DO on the fourth-corner test. The present findings are in agreement with the habitat template concept (HTC), which predicted small body sized organisms to be associated with disturbed habitats (Townsend & Hildrew, 1994). Resilience features often associated with the possession of a small body size include many offspring per reproductive event, large body surface area to volume ratio and multivoltinism (Townsend & Hildrew, 1994), all of which are likely to contribute to the persistence of species in the face of harsh environmental conditions (Buendia *et al.,* 2013; Verberk *et al.,* 2013; Akamagwuna *et al.,* 2019).

### 4.5.3 Pattern of distribution of traits and ecological preferences in relation to urban pollution in forested riverine systems

Natural processes, including biological assemblage distribution of traits, can be altered in forested streams exposed to urban pollution. In the present study, the results revealed that the possession of large body size was particularly vulnerable to urban pollution in forested streams as it was dominant in the LIS compared to MIS and HIS. As already argued, urban pollution can introduce elevated dissolved solids into forested rivers, increasing the risk of absorbing potentially toxic dissolved materials such as metals. A large body size presents a large surface area to volume ratio, increasing the likelihood of increased chemical exposure and adsorption due to the increased surface area to volume ratio compared to smaller body sized individuals (Statzner & Beche, 2010; Odume *et al.,* 2014). A large body has been further predicted to be particularly vulnerable because it is often associated with the production of fewer offspring per reproductive cycle (Townsend & Hildrew, 1994) – supporting the results of this study, which revealed that a reduced ratio of large body sized organisms to smaller body sized ones could potentially serve as a trait-based indicator of urban pollution in forested systems.

With regard to small body size, studies have confirmed the resilience of small body sized organisms in environments subjected to high disturbances (Mondy & Usseglio-Polatera 2014; Serra *et al.,* 2017; Castro *et al.,* 2018). These studies attribute the dominance of the small body sized organisms in polluted sites to their relatively short life cycles, which makes them to recover quickly after a post-disturbance activites (Serra *et al.,* 2017; Castro *et al.,* 2018).

Grazing as a feeding mode was significantly higher in the LIS compared to the MIS and HIS, and was also significantly negatively correlated with physico-chemical indicators of urban pollution in forested rivers. The association of grazing with the LIS instead of the HIS and MIS was unexpected as grazers are often expected to prefer stations with potential for algal growth as a result of increased nutrient concentration which characterised HIS and MIS in the studied river systems. Nevertheless, results similar to those observed in the present study have been reported elsewhere by Odume (2020) who reported a preference for grazing as a feeding mode to be associated with control site in an industrially impacted river in South Africa.

Predation as a feeding mode was expected to be vulnerable to urban pollution in forested streams because most predators are specialist feeders (Pallottini *et al.*, 2017; Desrosiers *et al.*, 2019). However, in the present study, predators were associated with the impacted stations, and proved to be tolerant. The majority of the predators collected in the HIS were coleopterans and hemipterans, which also have mechanisms for the intake of atmospheric oxygen (Odume, 2020). It is thus possible that the possession of traits enabling intake of atmospheric oxygen may confer resilience on these groups of organisms, enabling them to thrive in the HIS and thus serve as indicators of urban pollution in forested stream systems.

## 4.6 Conclusion

The present study suggests that urban, urban-agricultural and urban-forestry pollution differentially influence macroinvertebrate traits and ecological preferences. The implication is that an analysis of the distribution of traits and ecological preferences can provide a basis for identifying potential trait-based indicators. In the present study, univoltinism, permanent attachment, crawling, swimming, and a moderate sensitivity to oxygen depletion were identified as potential urban pollution sensitive traits, whereas tegument/cutaneous respiration, cased/tubed protection, a preference for silty waters, bivoltinism, burrowing, and a high tolerance of oxygen depletion were deemed urban pollution-tolerant traits. Traits such as permanent attachment, a high sensitivity to oxygen depletion, a moderate sensitivity to oxygen depletion and a large body size (>20-40 mm) were also deemed sensitive to urban-agriculture pollution, whereas a preference for detritus (CPOM) and a very small body size (<5 mm) were identified as potentially tolerant traits. For forested rivers impacted by urban pollution, traits such as possession of hardshell, large body size (>20-40 mm) and grazing were deemed sensitive to urban-forestry pollution, while burrowing, pupal aquatic stage and predation were deemed tolerant of urban-forestry pollution.

Overall, this study contributes to the growing body of knowledge suggesting the usefulness of the TBA in freshwater biomonitoring. A critical limitation of the study is that traits and ecological preferences were coded at the family level because of sparse life-history information on Afrotropical species, as well as taxonomic expertise. While other studies have demonstrated the utility of the TBA, it is argued here that as more life-history information becomes available, the approach developed here would need to be refined to take account of new data. Recommendation is made to accelerate life-history studies of Afrotropical macroinvertebrates and investment in taxonomic expertise, a field that is rapidly dying in the region. Nevertheless, the finding in this study proves that the TBA has utility in biomonitoring Afrotropical freshwater systems impacted by urban pollution and a combination of urban, agriculture and forestry pollution.

# CHAPTER 5: DEVELOPING AND APPLYING MACROINVERTEBRATE-BASED MULTIMETRIC INDICES FOR ASSESSING IMPACT OF URBAN, URBAN-AGRICULTURE AND URBAN-FORESTRY POLLUTION IN SELECTED RIVERS IN THE NIGER DELTA


**Publication based on this chapter**

Edegbene, A.O., Arimoro, F.O. and Odume, O.N. (2019). Developing and applying a macroinvertebrate-based multimetric index for urban rivers in the Niger Delta, Nigeria. *Ecology and Evolution* 9: 12869 - 12885. DOI:10.1002/ece3.5769.


## 5.1 Introduction

Human population increase, food scarcity and industrialisation are driving urbanisation and agricultural activities in sub-Saharan Africa at an alarming rate (Parienté, 2017). While rapid urbanisation and farming are necessary to provide employment and an adequate food supply for sub-Saharan Africa's growing population, the unintended consequences of such developments are the pollution and degradation of freshwater ecosystems, particularly because most urban developments and farms are situated close to major rivers (Parienté, 2017). The consequences of such pollution include deteriorating water quality, impaired ecological conditions and overall functionality of impacted urban rivers and streams (Mereta *et al.,* 2013; Edegbene *et al.,* 2015; Edegbene *et al.,* 2019; Gieswein *et al.,* 2019). The Niger Delta, which is home to a range of creeks, rivers and streams, is no exception, as most of the urban rivers in the region are seriously impacted (Arimoro & Ikomi, 2008).

Natural forestry is also an important component of river catchments in the Niger Delta as the region is situated within the tropical rain forest zone. Many forested rivers within the region are naturally influenced by forest processes such as leaf litter, shading, and the forest influence on hydrology (Vannote *et al.,* 1980; Sedell *et al.,* 1990). For naturally forested river systems, allochthonous food materials and shading are critical processes determining the structuring of biological assemblages in such riverine systems (Vannote *et al.,* 1980; Sedell *et al.,* 1990). In forested streams, a predictable pattern of soluble organic compounds, substrate size, nutrient availability, proportion of CPOM/FPOM and dominance of shredders and collectors is expected, based on the knowledge of relevant ecological concepts and theories such as the concepts of river geomorphology and river continuum (Vannote *et al.*, 1980; Rowntree *et al*., 2000). However, urbanisation presents a critical threat to the natural processes, structures and function of forested riverine systems in the Niger Delta. For example, as already stated in Chapter 4, increased temperature due to run-off from urban settlements has been reported in forested riverine systems subject to the effects of urbanisation (Odume, 2020). A shift in the dominance of shredders and alteration of substrate sizes and soluble organic compounds have also been reported in forested riverine systems impacted by urbanisation (Akamagwuna *et al.,* 2019; Desrosieres *et al.,* 2019). Given that urbanisation is growing exponentially in the Niger Delta, there is a need to develop appropriate biomonitoring tools for monitoring the effects of urbanisation, agricultural activities and urban pollution in forested riverine systems. The development of an appropriate biomonitoring tool can contribute to managing pollution through monitoring and assessing urban and agricultural pollution, and urban-forestry effects in riverine systems within the region.

A range of biomonitoring tools and approaches exist for monitoring pollution effects on riverine ecosystems, tools and approaches that include biotic indices, functional feeding groups, multivariate approaches, and multimetric indices (Bonada *et al.,* 2006; Monaghan & Soares, 2012; Mereta *et al.,* 2013; Edegbene *et al.,* 2019). Of these approaches, multimetric indices have been shown to perform extremely well, particularly because they integrate information and data from multiple dimensions of aquatic biota and from the ecosystem as a whole (Bonada *et al.*, 2006). Multimetric indices have been developed based on aquatic macrophytes (Aguiar *et al.*, 2011; Zervas *et al.,* 2018), diatoms (Stevenson *et al.*, 2013), phytoplankton (Lugoli *et al.*, 2012; Wu *et al.,* 2012; Katsiapi *et al.,* 2016; Tsiaoussi *et al.*, 2017), macroinvertebrates (Ntislidou *et al.*, 2018; Edegbene *et al.,* 2019; Gieswein *et al.*, 2019; Lu *et al.*, 2019), and fish (Petriki *et al.*, 2017).

While most macroinvertebrate-based multimetric indices developed are used to assess general water quality deterioration, in this study, the intention is to develop pollution-type-specific multimetric indices for assessing water quality impairment. The significance of developing indices specifically for urban, urban-agricultural, and urban-forested pollution is based on the recognition that Nigeria is urbanising rapidly, and rivers in the Niger Delta region, particularly those which are mainly forested, suffer the effects of serious urban and agricultural pollution. The aim of this chapter was to develop and apply macroinvertebrate-based multimetric indices suitable for assessing and monitoring ecological impairment of rivers impacted by urban, urban-agricultural and urban-forestry pollution. This study is the first to posit regional, macroinvertebrate-based, multimetric indices in Nigeria, where studies on biomonitoring methods are still scanty. The present study adds to the few existing studies on macroinvertebrate multimetric indices for biomonitoring freshwater ecosystems in sub-Saharan Africa (e.g. Odume *et al.,* 2012; Mereta *et al.,* 2013; Lakew & Moog 2015; Aura *et al.,* 2017; Chirwa & Chilima, 2017; Edegbene *et al.*, 2019). This chapter thus fulfils objective three in Chapter 1 of this thesis.

## 5.2 Materials and Methods

### 5.2.1 Selection of candidate metrics

Metrics selected for index development represent measures of abundance, composition, richness, diversity, traits, and ecological preferences. For the urban multimetric index development, a total of 77 metrics was selected for evaluation; for the urban-agricultural index, a total of 67 metrics was selected for evaluation and for the urban-forestry index, a total of 59 metrics was selected for evaluation (Table 5.1). The rationale for the selection of metrics in each category is provided in Chapter 2, section 2.7.

Table 5. 1: Selected urban, urban-agriculture and urban-forestry candidate taxonomic, traits and ecological preferences metrics. X depicts selected metrics for urban, urban-agriculture and urban-forestry impacted rivers.

| **S/N** | **Candidate metrics** | **Metric codes** | **Metrics selected for urban** | **Metrics selected for urban-agriculture** | **Metrics selected for urban-forestry** | **Predicted response to disturbance** |
|---|---|---|---|---|---|---|
| **Abundance measures (Absolute abundance of individuals in macroinvertebrate groups)** | | | | | | |
| 1 | Ephemeroptera Plecoptera and Trichoptera abundance | EPT Abun | X | X | X | Decrease |
| 2 | Ephemeroptera family abundance | Eph Abun | X | X | X | Decrease |
| 3 | Trichoptera family abundance | Tri Abun | X | X | X | Decrease |
| 4 | Ephemeroptera Trichoptera Odonata and Coleoptera abundance | ETOC Abun | X | X | X | Decrease |
| 5 | Chironomidae abundance | Chi Abun | X | X | X | Increase |
| 6 | Chironomidae + Oligochaeta abundance | Chi+Oli Abun | X | X | X | Increase |
| 7 | Oligochaeta family abundance | Oli Abun | X | X | X | Increase |
| 8 | Diptera family abundance | Dip Abun | X | X | X | Increase |
| 9 | Mollusca +Diptera family | Mol+Dip | X | X | X | Increase |

| | | | | | | |
|---|---|---|---|---|---|---|
| | abundance | Abun | | | | |
| 10 | Decapoda family abundance | Dec Abun | X | X | X | Decrease |
| 11 | Mollusca family abundance | Mol Abun | X | X | X | Increase |
| 12 | Mollusca +Decapoda family abundance | Mol+Dec Abun | X | X | X | Variable |
| 13 | Coleoptera family abundance | Col Abun | X | X | X | Decrease |
| 14 | Odonata family abundance | Odo Abun | X | X | X | Decrease |
| 15 | Hemiptera family abundance | Hem Abun | X | X | X | Decrease |
| 16 | Coleoptera + Hemiptera abundance | Col+Hem Abun | X | X | X | Decrease |
| 17 | Ephemeroptera Plecoptera and Trichoptera family/Chironomidae abundance | EPT/Chi Abun | X | X | X | Decrease |
| 18 | Ephemeroptera Trichoptera Odonata and Coleoptera family /Chironomidae abundance | ETOC/Chi Abun | X | X | X | Decrease |
| 19 | Ephemeroptera Trichoptera Odonata and Coleoptera family /Diptera abundance | ETOC/Dip Abun | X | X | X | Decrease |
| 20 | Chironomidae/Diptera family | Chi/Dip | X | X | X | Increase |

| | | | | | | |
|---|---|---|---|---|---|---|
| | abundance | Abun | | | | |
| **Composition measures (relative abundance of individual macroinvertebrates in the entire sample)** | | | | | | |
| 21 | % Ephemeroptera, Plecoptera and Trichoptera | %EPT | X | X | X | Decrease |
| 22 | % Ephemeroptera | %Eph | X | X | X | Decrease |
| 23 | % Ephemeroptera, Trichoptera, Odonata and Coleoptera | %ETOC | X | X | X | Decrease |
| 24 | % Trichoptera | %Tri | X | X | X | Decrease |
| 25 | % Chironomidae | %Chi | X | X | X | Increase |
| 26 | % Chironomidae+Oligochaeta | %Chi+Oli | X | X | X | Increase |
| 27 | % Oligochaeta | %Oli | X | X | X | Increase |
| 28 | % Diptera | %Dip | X | X | X | Increase |
| 29 | % Decapoda | %Dec | X | X | X | Decrease |
| 30 | % Mollusca | %Mol | X | X | X | Increase |
| 31 | % Mollusca +Decapoda | %Mol+Dec | X | X | X | Variable |
| 32 | % Odonata | %Odo | X | X | X | Decrease |
| 33 | % Hemiptera | %Hem | X | X | X | Decrease |

| | | | | | | |
|---|---|---|---|---|---|---|
| 34 | % Coleoptera | %Col | X | X | X | Decrease |
| 35 | % Coleoptera+Hemiptera | %Col+Hem | X | X | X | Decrease |
| 36 | % Mollusca +Diptera | %Mol+Dip | X | X | X | Increase |
| **Richness measures (absolute number of taxa in macroinvertebrate groups)** | | | | | | |
| 37 | Ephemeroptera, Plecoptera and Trichoptera richness | EPT Rich | X | X | X | Decrease |
| 38 | Ephemeroptera richness | Eph Rich | X | X | X | Decrease |
| 39 | Trichoptera richness | Tri Rich | X | X | X | Decrease |
| 40 | Diptera richness | Dip Rich | X | X | X | Increase |
| 41 | Ephemeroptera, Trichoptera, Odonata and Coleoptera richness | ETOC Rich | X | X | X | Decrease |
| 42 | Chironomidae richness | Chi Rich | X | X | X | Increase |
| 43 | Chironomidae + Oligochaeta richness | Chi+Oli Rich | X | X | X | Increase |
| 44 | Mollusca richness | Mol Rich | X | X | X | Increase |
| 45 | Coleoptera + Hemiptera richness | Col+Hem Rich | X | X | X | Decrease |
| 46 | Coleoptera richness | Col Rich | X | X | X | Decrease |

| | | | | | | |
|---|---|---|---|---|---|---|
| 47 | Hemiptera richness | Hem Rich | X | X | X | Decrease |
| 48 | Odonata richness | Odo Rich | X | X | X | Decrease |
| 49 | Oligochaeta richness | Oli Rich | X | X | X | Increase |
| 50 | Decapoda richness | Dec Rich | X | X | X | Decrease |
| | | | **Diversity measures** | | | |
| 51 | Simpson diversity (1-D) **(weighted towards the abundance of commonest taxa (Ogbeibu, 2005))** | Sim Div | X | X | X | Decrease |
| 52 | Evenness index (e^H/S) (**evenness of taxa within sample (Clarke and Warwick 1994; Edegbene *et al.*, 2019))** | Eve Ind | X | X | X | Decrease |
| 53 | Margalef index (Taxa diversity index) **(account for both number of taxa and individuals and is independent of sample size (Ogbeibu, 2005))** | Mar Ind | X | X | X | Decrease |
| 54 | Shannon-Weiner diversity index (H) **(information statistics index taking account of contribution of individual taxa to the diversity while** | Sha Ind | X | X | X | Decrease |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **assigning greater weight to dominant taxa (Ogbeibu, 2005))** | | | | | |
| **Traits and ecological preferences attributes** | | | | | | |
| 55 | Logarithm of relative abundance of hardshell | Log HaS | X | | X | Decrease |
| 56 | Logarithm of relative abundance of 1 year, (Univoltine) | Log Uni | X | | | Decrease |
| 57 | Logarithm of relative abundance of permanent attachment | Log PeA | X | | | Decrease |
| 58 | Logarithm of relative abundance of crawler | Log Cra | X | | | Decrease |
| 59 | Logarithm of relative abundance of moderately sensitive oxygen depletion | Log MoS | X | | | Decrease |
| 60 | Logarithm of relative abundance of very large (>40-80) | Log VeL | X | | | Decrease |
| 61 | Logarithm of relative abundance of large (>20-40mm) | Log Lar | | | X | Decrease |

| | | | | | | |
|---|---|---|---|---|---|---|
| 62 | Logarithm of relative abundance of tegument/cutaneous | Log Teg | X | | | Increase |
| 63 | Logarithm of relative abundance of soft and exposed | Log SoE | X | X | | Increase |
| 64 | Logarithm of relative abundance of cased/tubed | Log CaT | X | X | | Increase |
| 65 | Logarithm of relative abundance of silty turbidity preference | Log SiT | X | | | Increase |
| 66 | Logarithm of relative abundance of no preference to turbidity | Log NoT | X | | | Increase |
| 67 | Logarithm of relative abundance of 2 years (Bivoltine) | Log Biv | X | | | Increase |
| 68 | Logarithm of relative abundance of free-living | Log FrL | X | X | | Increase |
| 69 | Logarithm of relative abundance of sprawler | Log Spr | X | X | | Increase |
| 70 | Logarithm of relative abundance of skater | Log Ska | X | | | Increase |

| | | | | | | |
|---|---|---|---|---|---|---|
| 71 | Logarithm of relative abundance of burrower | Log Bur | X | | | Increase |
| 72 | Logarithm of relative abundance of cylindrical/tubular | Log CyT | X | | | Increase |
| 73 | Logarithm of relative abundance of detritus (FPOM) | Log DeF | X | X | | Increase |
| 74 | Logarithm of relative abundance of highly tolerant of oxygen depletion | Log HiT | X | | | Increase |
| 75 | Logarithm of relative abundance of small, >5-10mm | Log Sma | X | X | | Increase |
| 76 | Logarithm of relative abundance of nymph | Log Nym | | | X | Decrease |
| 77 | Logarithm of relative abundance of larva | Log Lav | X | X | | Increase |
| 78 | Logarithm of relative abundance of pupa aquatic stage | Log Pup | X | | X | Increase |
| 79 | Logarithm of relative abundance of filter feeder | Log FiF | X | | | Increase |
| 80 | Logarithm of relative | Log Aer | | X | | Increase |

| | | | | | | |
|---|---|---|---|---|---|---|
| | abundance of aerial: spiracle | | | | | |
| 81 | Logarithm of relative abundance of preference for clear waters | Log Opa | | X | | Increase |
| 82 | Logarithm of relative abundance of swimmer | Log Swi | | X | | Increase |
| 83 | Logarithm of relative abundance of predator | Log Pre | | | X | Increase |
| 84 | Logarithm of relative abundance of detritus (CPOM) | Log DeC | | X | | Increase |
| 85 | Logarithm of relative abundance of very small (<5mm) | Log VeS | | X | | Increase |
| 86 | Logarithm of relative abundance of shredder | Log Shr | | X | | Increase |

### 5.2.2 Development of the multimetric indices for assessing urban, urban-agriculture and urban-forestry pollution

Five steps were followed in developing the multimetric indices: (i) metric sensitivity test, (ii) metric seasonality test, (iii) metric redundancy test, (iv) integration of selected metrics into the multimetric index, and (v) index validation and application. The macroinvertebrate data were split into two datasets: 2008–2010 and 2011–2012. Datasets collected from 2008–2010 were used for the development of the indices, and those collected between 2011–2012 were used for the validation and application.

***Metric sensitivity test***

Candidate metrics were tested for their potential to discriminate between the least impacted stations (LIS) from the moderately impacted stations (MIS) and the heavily impacted stations (HIS). Box plots were used to visualise metrics, and two levels of discrimination were considered satisfactory. First, a metric was deemed sensitive if there was an overlap between the interquartile ranges (IQRs) of the MIS and HIS, and those of the LIS, but the medians were outside the interquartile ranges (Odume *et al.,* 2012; Edegbene *et al.,* 2019). Second, metrics were considered sensitive if the IQR of the LIS did not overlap with those of the MIS and HIS (Odume *et al.,* 2012; Edegbene *et al.,* 2019). Metrics that met two, or any of the criteria, were selected for further testing.

Selected metrics based on the box plot visualisation were further tested for significant differences using the Mann-Whitney (U) test. The Mann-Whitney (U) test was used because the Kolmogorov-Smirnov test indicated that metrics were non-normally distributed. Metrics exhibiting a significant difference between the LIS, and the MIS and HIS at $P$ <0.05 were retained for further analysis (Barbour *et al.,* 1996). Box plots were generated using Statistica version 13.4.14 (TIBCO Software Inc., 2018) and the Kolmogorov-Smirnov test of normality and Mann-Whitney tests were computed using Paleontological statistical package, PAST (Hammer *et al.,* 2001).

***Metric seasonality test***

Metrics that were deemed sensitive after confirmation with the Mann-Whitney test were further subjected to a seasonality test for seasonal stability. The seasonality test was undertaken to filter out metrics that are highly sensitive to natural seasonal variation as such variations may confound variations occasioned by human activities. Box plots were used to visualise the metrics' seasonal stability and the Kruskal-Wallis test was further used to confirm seasonally stable metrics (Baptista *et al.,* 2007). Metrics with a Kruskal-Wallis P-value >0.05 were deemed seasonally stable (Melo *et al.,* 2015; Aura *et al.,* 2017). Only metrics from the LIS were used for the seasonality test to avoid the confounding effect of pollution on the seasonal variation of metrics.

***Metric redundancy test***

Redundant metrics convey the same or similar information (Odume *et al.,* 2012). Spearman's rank correlation coefficient (r) was performed on the seasonally stable metrics to explore co-linearity between the metrics. Metrics with Spearman correlation values of $r \geq 0.78$, $P < 0.05$ were considered redundant (Edegbene *et al.,* 2019). Non-redundant metrics were selected for integration into the multimetric indices. Where two or more metrics were redundant, only one of such metrics was selected for inclusion in the multimetric indices, based on the ecological importance of the metrics to be selected (Mereta *et al.,* 2013).

***Integration of the metrics into a multimetric indices***

Prior to integration, selected metrics were standardised by using the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%) and maximum value of each metric dataset according to the method described by Baptista *et al.* (2007). Lower, mid- and upper quartiles were computed with Microsoft Excel, 2010 version. Metrics that were predicted to increase with increasing urban, urban-agriculture and urban-forestry pollution were assigned a score of 5 if the metric value was below the upper quartile (75%) of the LIS; a score of 3 was awarded if the metric value was above 75%, and a score of 1 was awarded if the metric value was above the maximum value of the LIS. In the case of metrics that were predicted to decrease with increasing urban, urban-agriculture, and urban-forestry pollution, a score of 5 was awarded if the metric value of LIS was greater than or equal to the lower quartile (25%); a score of 3 was assigned if the metric value was between the minimum value and less than 25% of the LIS, while score of 1 was assigned if the metric value was lower than the minimum value of LIS.

***Validation and application of the multimetric indices***

To test the efficacy of the developed multimetric indices, the scores were calculated for the station per sampling occasion from the period 2011–2012. The indices' performance was assessed by calculating the percentage correspondence between the index result and the initial station categorisation, based on the physico-chemical variables. The respective index performance for the LIS was determined by assessing the percent correspondence of LIS falling in the very good to good water quality categories; that for MIS was gauged by assessing the correspondence of MIS falling in the good to fair water quality categories, and that for HIS was measured by assessing the correspondence of HIS falling in the fair to very poor water quality categories. Two-way analysis of variance (ANOVA) was used to test for significant difference between the indices scores for LIS, MIS, HIS, taking station and season as explanatory factors. ANOVA was computed using the Paleontological statistical package, PAST (Hammer *et al.*, 2001).

### 5.3. Relating the selected metrics to physico-chemical variables

Metrics selected for integration into the multimetric indices were correlated with physico-chemical variables to visualise their distribution on an RDA ordination plane. A test of unimodality and linearity using a detrended correspondence analysis (DCA) returned a gradient length <3 indicating that the dataset was linear (ter Braak, 1995), and thus an RDA was used for the final ordination. A Monte Carlo test at 999 permutations was used to test for the level of significance between the RDA axes (Legendre & Legendre, 2012). Redundancy analysis (RDA), and the Monte Carlo test was computed using vegan package within the R programming environment (Oksanen *et al.*, 2019 - R Core-Team, 2019). All co-linear physico-chemical variables ($r \geq 0.80$) were removed from the RDA ordination analysis.

## 5.4 Results

### 5.4.1 Developing a multimetric index (MMI-urban) for assessing urban pollution effects

### 5.4.2 Metric sensitivity and seasonality tests

Of the 77 candidate metrics, only 26 satisfactorily discriminated between the LIS, the MIS, and the HIS (Appendix D; Table D1). After subsequent analysis, only five metrics were integrated into the final index; their discrimination potential is depicted in Figure 5.1. Sensitive and non-sensitive metrics not integrated into the MMI-urban are presented in Appendix D (Figures D1–D5).

The seasonality test indicated that 15 metrics were seasonally stable. The 15 metrics were Chironomidae abundance, Chironomidae + Oligochaeta abundance, Oligochaeta abundance, Hemiptera abundance, Diptera abundance, Mollusca + Diptera abundance, % Chironomidae + Oligochaeta, % Oligochaeta, % Diptera, % Hemiptera, % Coleoptera, % Coleoptera + Hemiptera, % Mollusca + Diptera, Evenness index and logarithm relative abundance of very large body size (Appendix D; Figures D6-D8). Seasonal stability of the five metrics integrated into the multimetric index is shown in Figure 5.2.

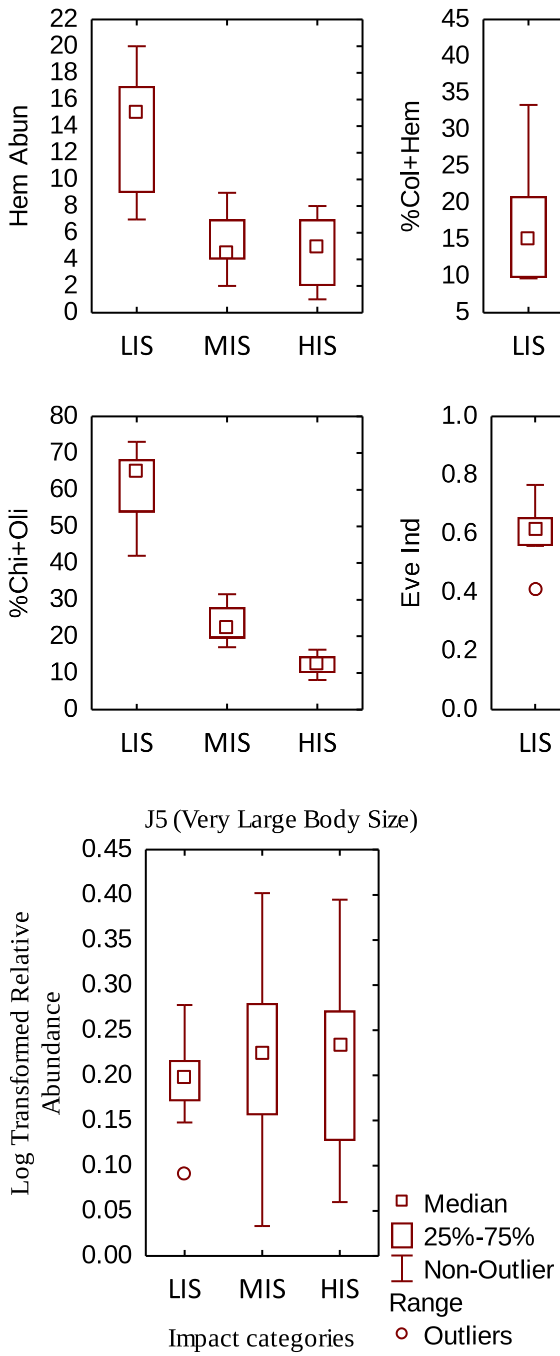



Figure 5. 1: Box plots showing metric discrimination potential of the five metrics integrated into the final multimetric index (MMI-urban) for urban river assessment in the Niger Delta, Nigeria.

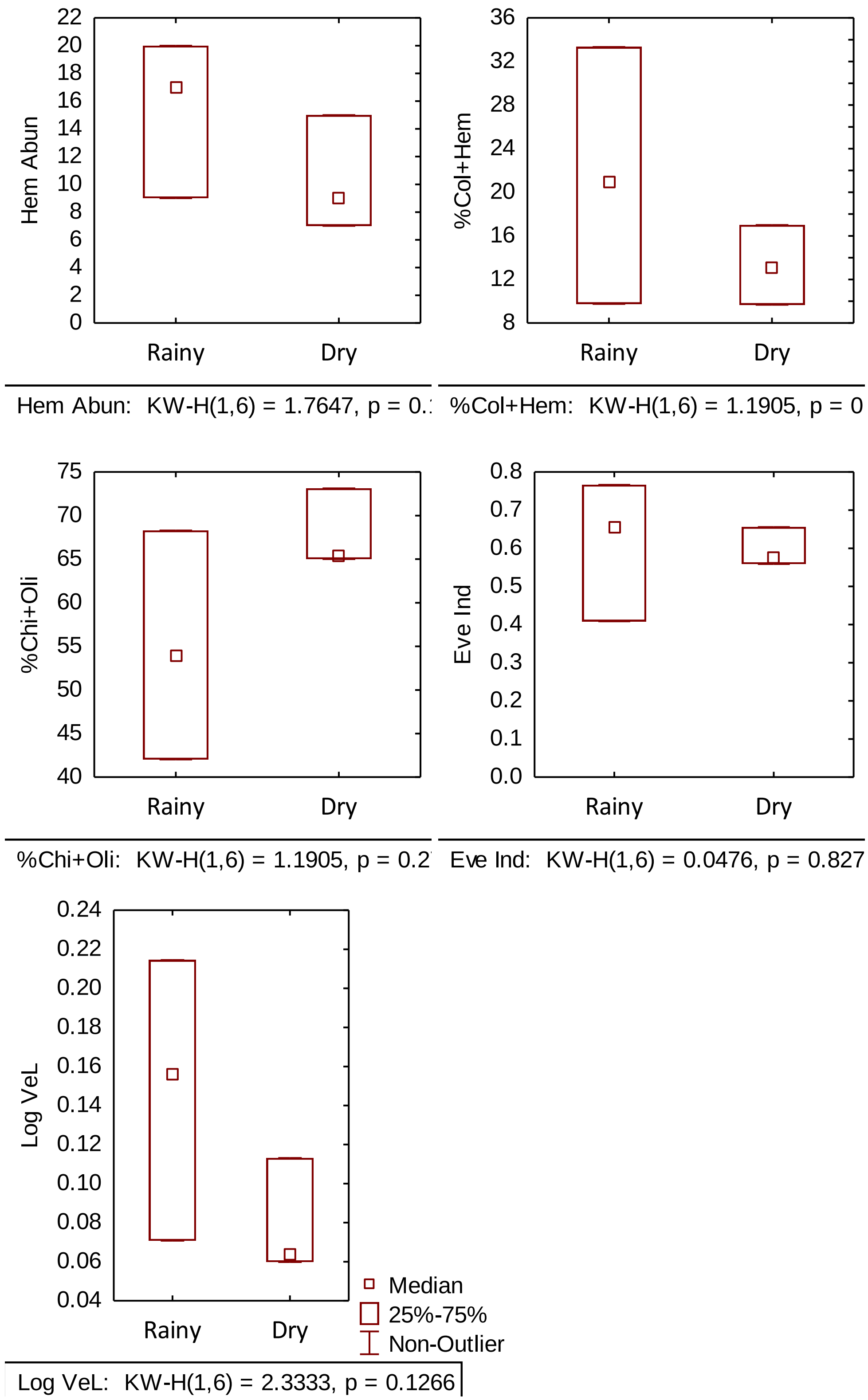



Figure 5. 2: Box plots showing seasonal stability of the five metrics integrated into the final multimetric index (MMI-urban) for assessing urban rivers in the Niger Delta, Nigeria.

### 5.4.3 Metric redundancy test

Apart from the trait measure of very large body size (log VeL), all other sensitive and seasonally stable metrics were found to be redundant (Table 5.2). Of the 26 metrics that satisfactorily discriminated between the LIS, the MIS, and the HIS, 15 metrics were retained and 14 proved to be redundant. However, because those 14 metrics represent different measures, four of them have been retained in addition to log VeL. The four metrics selected in addition to log VeL were Hemiptera abundance, % Coleoptera + Hemiptera, % Chironomidae + Oligochaeta and Evenness index (Table 5.2).

### 5.4.4 Integrating the selected metrics into the urban multimetric index (MMI-urban)

To conclude the development of the multimetric index, the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%), and maximum value of each metric for the LIS metric assemblage values were used as thresholds for calculating the metric scores (Table 5.3). The multimetric index was computed by summing the scores of the five metrics component, and the index value range (5–25), since five metrics were used (5 X 5=25). The index value range then reflects five water quality categories, as shown in Table 5.4.

Table 5. 2: Spearman's rank correlation of macroinvertebrate metrics showing co-linearity between metrics in the urban impacted rivers ($r \geq 0.78$, $P < 0.05$). Metric abbreviations: Chi Abun (Chironomidae abundance), Chi+Oli Abun (Chironomidae+Oligochaeta abundance), Oli Abun (Oligochaeta abundance), Hem Abun (Hemiptera abundance), Dip Abun (Diptera abundance), Mol+Dip (Mollusca+Diptera abundance), % Chi+Oli (percentage Chironomidae+Oligochaeta), % Oli (percentage Oligochaeta), % Dip (percentage Diptera), % Hem (percentage Hemiptera), % Col (percentage Coleoptera), % Col+Hem (percentage Coleoptera+Hemiptera), % Mol+Dip (percentage Mollusca +Diptera), Eve Ind (Evenness index), LogVeL (Logarithm of relative abundance of very large body size (>40-80 mm)).

| | **Chi Abun** | **Chi+Oli Abun** | **Oli Abun** | **Hem Abun** | **Dip Abun** | **Mol+ Dip Abun** | **%Chi +Oli** | **%Oli** | **%Dip** | **%Hem** | **%Col** | **%Col+ Hem** | **%Mol+ Dip** | **Eve Ind** | **LogVeL** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chi Abun** | 0 | 2.9E-15 | 6.0E-06 | 0.0077 | 1.9E-16 | 1.1E-15 | 8.7E-05 | 2.2E-05 | 8.1E-05 | 0.00014 | 0.00054 | 0.013 | 3.2E-05 | 0.00023 | 0.23 |
| **Chi+Oli Abun** | **0.97** | 0 | 3.1E-08 | 0.0017 | 1.8E-15 | 9.0E-19 | 3.5E-05 | 5.3E-06 | 6.5E-05 | 2.0E-05 | 0.00011 | 0.0144 | 2.3E-05 | 0.00015 | 0.19 |
| **Oli Abun** | **0.78** | **0.87** | 0 | 0.00017 | 1.2E-06 | 3.0E-07 | 0.0010 | 5.2E-06 | 0.0022 | 1.3E-06 | 13E-05 | 0.014 | 0.0014 | 8.6E-06 | 0.081 |
| **Hem Abun** | 0.53 | 0.61 | 0.69 | 0 | 0.0028 | 0.030 | 0.055 | 0.00078 | 0.023 | 5.02E-06 | 0.0011 | 0.041 | 0.020 | 0.00016 | 0.66 |
| **Dip Abun** | **0.98** | **0.97** | **0.82** | 0.58 | 0 | 6.4E-19 | 0.00043 | 7.9E-06 | 0.00040 | 1.4E-05 | 0.0015 | 0.034 | 0.00021 | 9.8E-05 | 0.35 |
| **Mol+Dip Abun** | **0.97** | **0.98** | **0.84** | 0.58 | **0.99** | 0 | 7.6E-05 | 1.3E-06 | 0.00011 | 2.7E-05 | 0.00052 | 0.016 | 4.4E-05 | 6.2E-05 | 0.32 |
| **%Chi+Oli** | 0.71 | 0.74 | 0.63 | 0.40 | 0.66 | 0.72 | 0 | 0.0018 | 7.4E-14 | 0.039 | 0.0010 | 0.031 | 2.4E-15 | 0.0057 | 0.013 |
| **%Oli** | 0.75 | **0.79** | **0.79** | 0.64 | **0.78** | **0.81** | 0.60 | 0 | 0.0039 | 8.0E-05 | 6.0E-06 | 6.7E-05 | 0.0022 | 7.1E-08 | 0.36 |
| **%Dip** | 0.72 | 0.72 | 0.59 | 0.46 | 0.66 | 0.71 | **0.96** | 0.57 | 0 | 0.025 | 0.0041 | 0.034 | 1.4E-22 | 0.0045 | 0.022 |
| **%Hem** | 0.70 | 0.76 | **0.81** | **0.79** | 0.76 | 0.75 | 0.42 | 0.72 | 0.46 | 0 | 0.001 | 0.14 | 0.019 | 5.7E-05 | 0.54 |
| **%Col** | -0.65 | -0.71 | -0.77 | -0.62 | -0.61 | -0.65 | -0.63 | **-0.78** | -0.56 | -0.63 | 0 | 3.4E-06 | 0.0021 | 3.9E-05 | 0.076 |
| **%Col+Hem** | -0.50 | -0.49 | -0.49 | -0.42 | -0.43 | -0.48 | -0.44 | -0.72 | -0.43 | -0.31 | **0.80** | 0 | 0.033 | 7.1E-05 | 0.15 |
| **%Mol+Dip** | 0.74 | 0.75 | 0.61 | 0.47 | 0.69 | 0.73 | **0.97** | 0.59 | **0.99** | 0.48 | -0.60 | -0.43 | 0 | 0.0047 | 0.024 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Eve Ind** | -0.68 | -0.69 | -0.78 | -0.69 | -0.71 | -0.72 | -0.55 | **-0.86** | -0.56 | -0.73 | 0.74 | 0.72 | -0.56 | 0 | 0.47 |
| **LogVeL** | -0.26 | -0.28 | -0.36 | -0.093 | -0.20 | -0.21 | -0.50 | -0.19 | -0.47 | -0.13 | 0.37 | 0.30 | -0.46 | 0.15 | 0 |

**Note:** Bold values were significant at $P < 0.05$.

Table 5. 3: Score of metric thresholds of the selected metrics for their integration into the MMI-urban for assessing urban pollution in the Niger Delta, Nigeria.

| **Urban metrics** | **Statistics** | | | | | **Score** | | |
|---|---|---|---|---|---|---|---|---|
| | **Min. value** | **25%** | **50%** | **75%** | **Max. value** | **5** | **3** | **1** |
| Hem Abun | 7 | 9 | 12 | 16.5 | 20 | ≥9 | 7- <9 | <7 |
| %Col+Hem | 9.68 | 10.60 | 15.05 | 19.91 | 33.33 | ≥10.60 | 9.68- <10.60 | <9.68 |
| %Chi+Oli | 42.03 | 56.63 | 65.24 | 67.60 | 73.12 | <67.60 | >67.60 - 73.12 | >73.12 |
| Even Ind | 0.41 | 0.56 | 0.61 | 0.66 | 0.77 | ≥0.56 | 0.41- < 0.56 | <0.41 |
| Log VeL | 0.060 | 0.065 | 0.092 | 0.145 | 0.21 | ≥0.065 | 0.060-<0.065 | <0.060 |

Table 5. 4: Thresholds of MMI-urban index score corresponding to water quality categories for urban pollution effects in the Niger Delta, Nigeria

| **Ecological category** | **Very poor** | **Poor** | **Fair** | **Good** | **Very good** |
|---|---|---|---|---|---|
| MMI-urban score | 5-9 | 10-13 | 14-17 | 18-21 | 22-25 |
| Water quality class | F | E | D | C | B |

### 5.4.5 Validation and application of the multimetric index (MMI-urban)

The index validation results showed that 25% of the time, stations designated as LIS had very good water quality, and 58.3% of the time, stations designated as LIS had good water quality (Figure 5.3), thus the agreement between the index results and the physico-chemically based classification was 83.3%, indicating a good index performance for the LIS. For the MIS, the index validation results showed that 50% of the time, stations designated as MIS had good water quality and 25% of the time, these MIS stations had fair water quality, indicating a 75% correspondence between the MMI-urban results and the physico-chemically-based classification (Figure 5.3). The validation results for HIS indicated that the index performed poorly with only 22.2% (Figure 5.3) correspondence between the index results and the physico-chemically-based classification, that is, 18.5% of the time, stations designated as HIS fell within the fair water quality category, and only 3.7% of the time, stations designated as HIS fell within the poor water quality category. Surprisingly, the index indicated that most of the time, stations classified as

HIS had very good and good water quality compared to the number of times the index indicated that the HIS stations had fair and poor water quality. Nevertheless, the index performed well for the LIS and MIS stations, as it did not indicate that these stations had poor water quality throughout the sampling period.

Seasonally, the MMI-urban results showed that during the wet season, stations designated as LIS had very good water quality 8.3% of the time, and good water quality 33.3% of the time (Figure 5.4). The performance of the MMI-urban was thus 41.6% for the wet season. The dry season performance was 41.7%, with readings for LIS stations for very good and good water quality of 16.7% and 25% of the time, respectively (Figure 5.4). The performance for the MMI-urban was thus was consistent for both the wet and dry seasons.

The seasonal validation results show that 20% of the time stations designated as MIS registered good water quality and 15% of the time, these same stations were in the fair water quality category in the wet season. In the dry season, 35% of the time water quality condition was good, and 5% of the time, water quality registered as fair (Figure 5.4). It can be said that the MMI-urban performed better in the dry season (40%) than the wet season (35%) for stations designated MIS.

The results for the wet season showed that 3.7% of the time, stations designated as HIS recorded fair water quality based on the MMI-urban, whereas during the dry season 18.5% of the time, the stations had fair water quality. Nevertheless, in the dry season, the results indicated that 14.8% of the time, the HIS station recorded poor water quality (Figure 5.4). It can be said that the MMI-urban performed better in the dry season than in the wet season for stations designated as HIS. The two-way ANOVA indicated no significant differences for the MMI-urban index values between the LIS, MIS and HIS (*P*>0.05), while a significant difference existed between wet and dry seasons in terms of the MMI-urban index values (*P*<0.05).

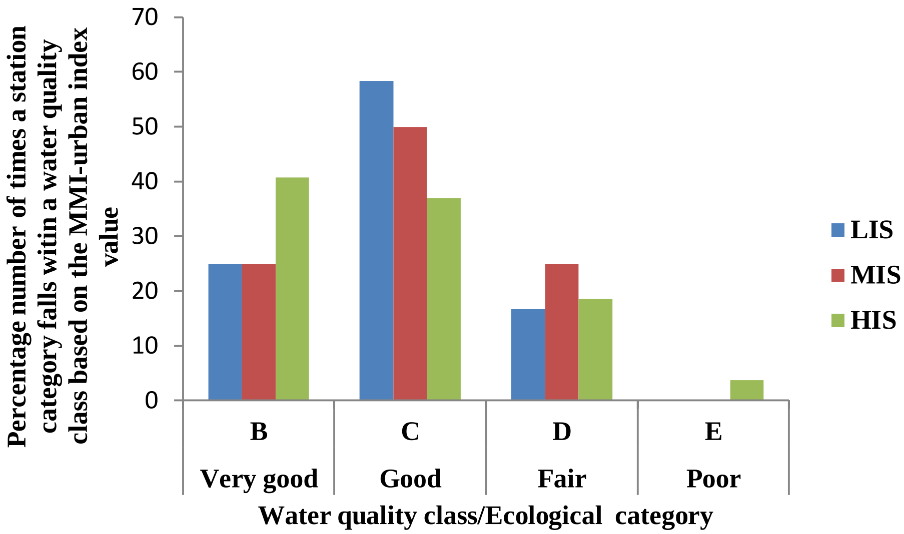



Figure 5. 3: Percentage number of times station categories fell within a water quality class based on the MMI-urban index value. Abbreviations: LIS=least impacted stations, MIS=moderately impacted stations and HIS=heavily impacted stations. MMI-urban-based water quality class: B (Very good), C (Good), D (Fair), E (Poor).

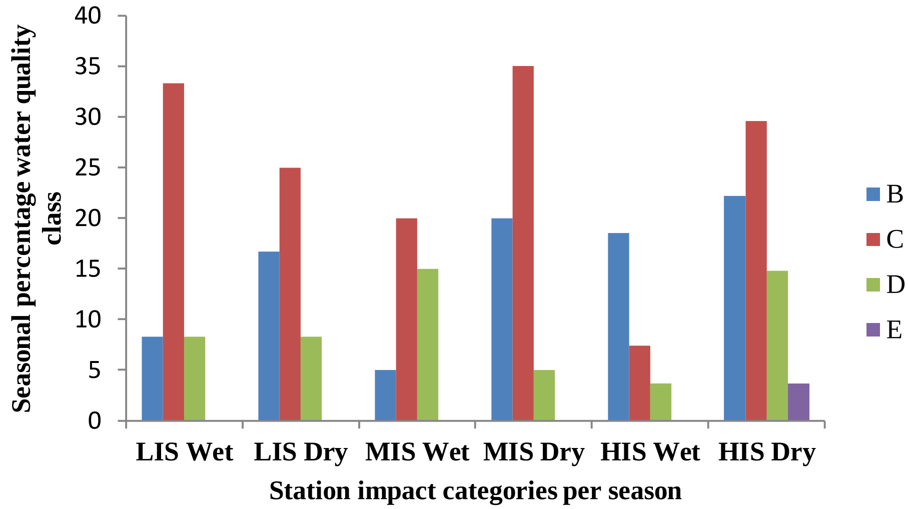



Figure 5. 4: Percentage number of times station categories fell within a water quality class based on the MMI-urban-based water quality class per season (wet and dry). Abbreviation: LIS=least impacted stations, MIS=moderately impacted stations and HIS=heavily impacted stations. MMI-urban-based water quality class: B (Very good), C (Good), D (Fair), E (Poor)

### 5.4.6 Relating the selected metrics in the MMI-urban to physico-chemical variables

The first RDA axis explains 86.98% of the variance, while the second axis explains 13.02%. The Eigen value of the first axis was higher (6.409) than that of the second axis (0.409). There was no significant difference between the two RDA axes' correlation with metrics and the physico-chemical variables ($P > 0.05$) as revealed by the Monte Carlo test at 999 permutations. A strong positive correlation existed between dissolved oxygen and the Evenness index and %Coleoptera + Hemiptera on Axis 1 (Figure 5.5). The logarithm of relative abundance of very large body size was positioned at the centre of the RDA triplot and was positively correlated with depth on Axis 1. Five-day biochemical oxygen demand and EC were strongly negatively correlated to % Chironomidae + Oligochaeta at the HIS on Axis 2. Hemiptera abundance was correlated to water temperature and flow velocity at LIS (Figure 5.5).

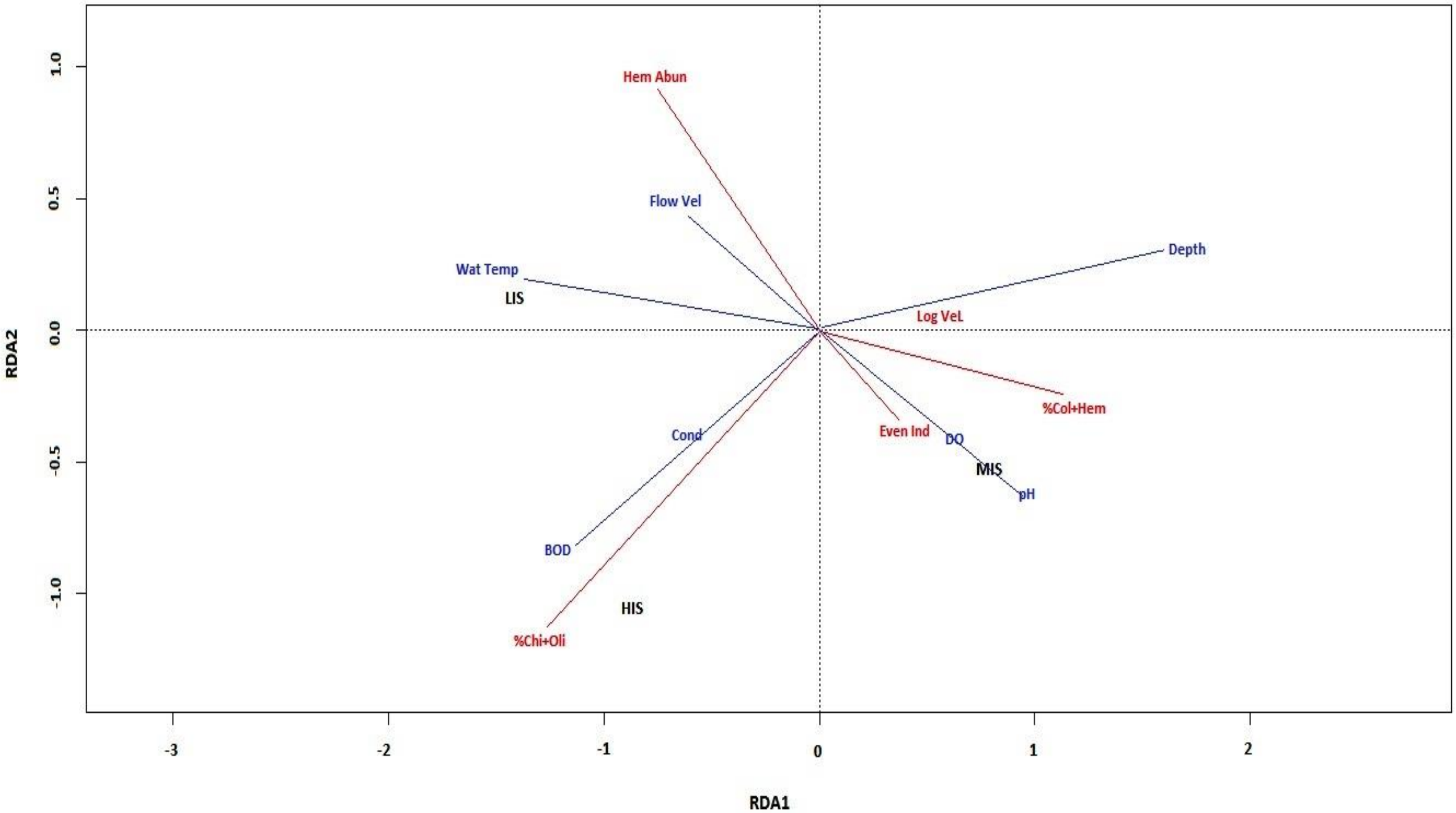



Figure 5. 5: Redundancy ordination plot showing the relationship between macroinvertebrate metrics and physio-chemical variables for the MMI-urban. Metrics: Hem Abun (Hemiptera Abundance), %Col+Hem (%Coleoptera+Hemiptera), %Chi+Oli (%Chironomidae+Oligochaeta), Eve Ind (Evenness Index), Log VeL (Logarithm of relative abundance of very large body size). Physico-chemical variables: Wat Temp (water temperature), Flow vel (flow velocity), Cond (electrical conductivity), DO (dissolved oxygen), BOD (five-day biochemical oxygen demand, $BOD_5$), Depth and pH. Station impact categories: LIS (least impacted stations), MIS (moderately impacted stations) and HIS (heavily impacted stations).

### 5.4.7 Developing a multimetric index (MMI-urban-agric) for assessing urban-agricultural pollution effect

### 5.4.8 Metric sensitivity and seasonality tests

Of the 67 candidate metrics, only 18 satisfactorily discriminated between the LIS, and the MIS and HIS (Appendix E, Table E1). From the results of subsequent analysis, only five metrics were integrated into the final index; their discrimination potential is presented in Figure 5.6. Sensitive and non-sensitive metrics not integrated into the MMI-urban-agric are presented in Appendix E (Figures E1–E5).

The seasonality test indicated that 12 metrics were seasonally stable. The 12 metrics were Chironomidae abundance, Chironomidae, Diptera abundance, %Odonata, Diptera richness, Chironomidae richness, Chironomidae + Oligochaeta richness, Oligochaeta richness, Simpson diversity, Evenness index, Margalef index, logarithm relative abundance of sprawler (Appendix E, Figures E6–E10). Seasonal stability of the five metrics integrated into the multimetric index is shown in Figure 5.7.

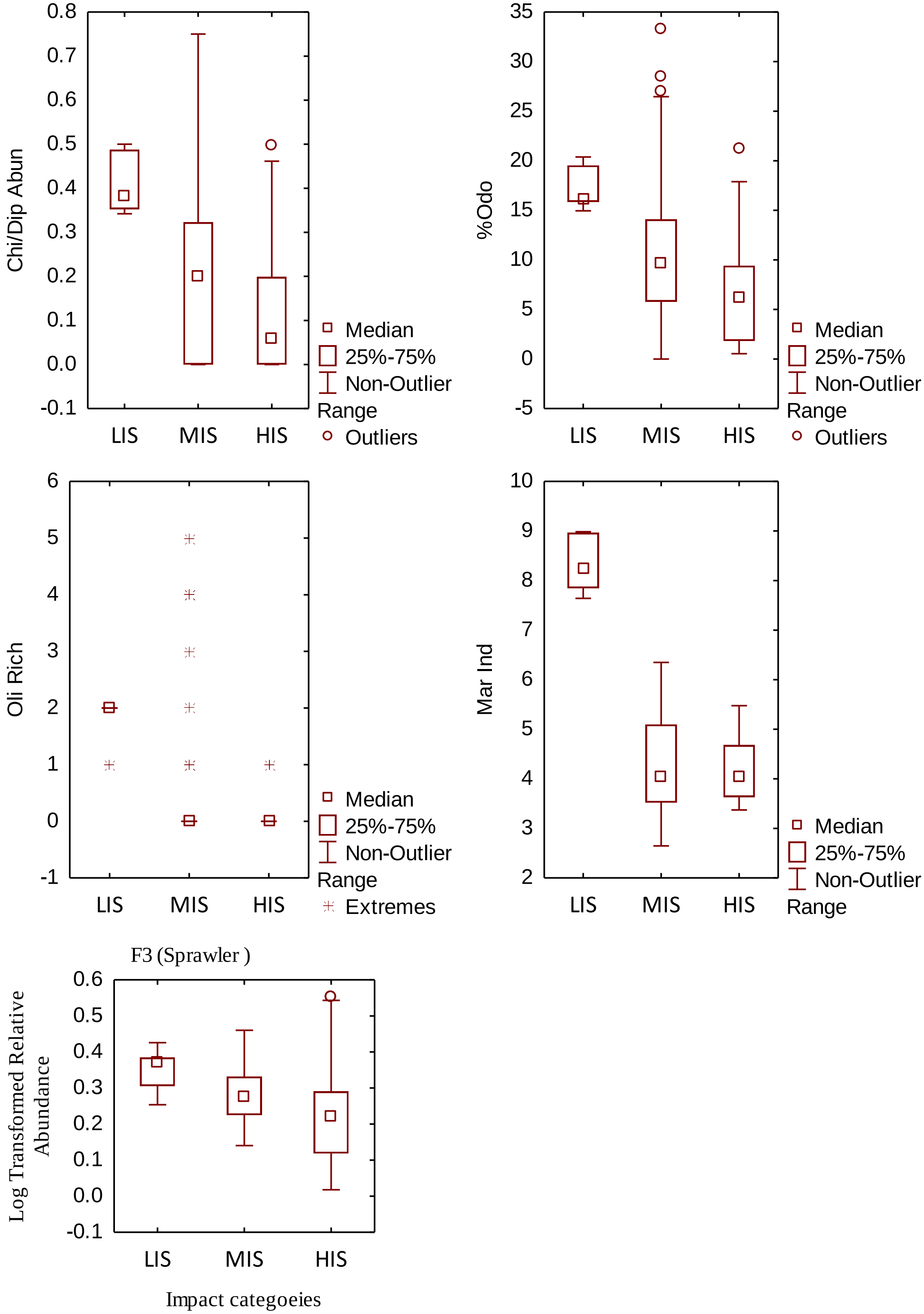



Figure 5. 6: Box plots showing metric discrimination potential of the five metrics integrated into the final multimetric index for urban-agricultural river assessment in the Niger Delta (MMI-urban-agric), Nigeria.

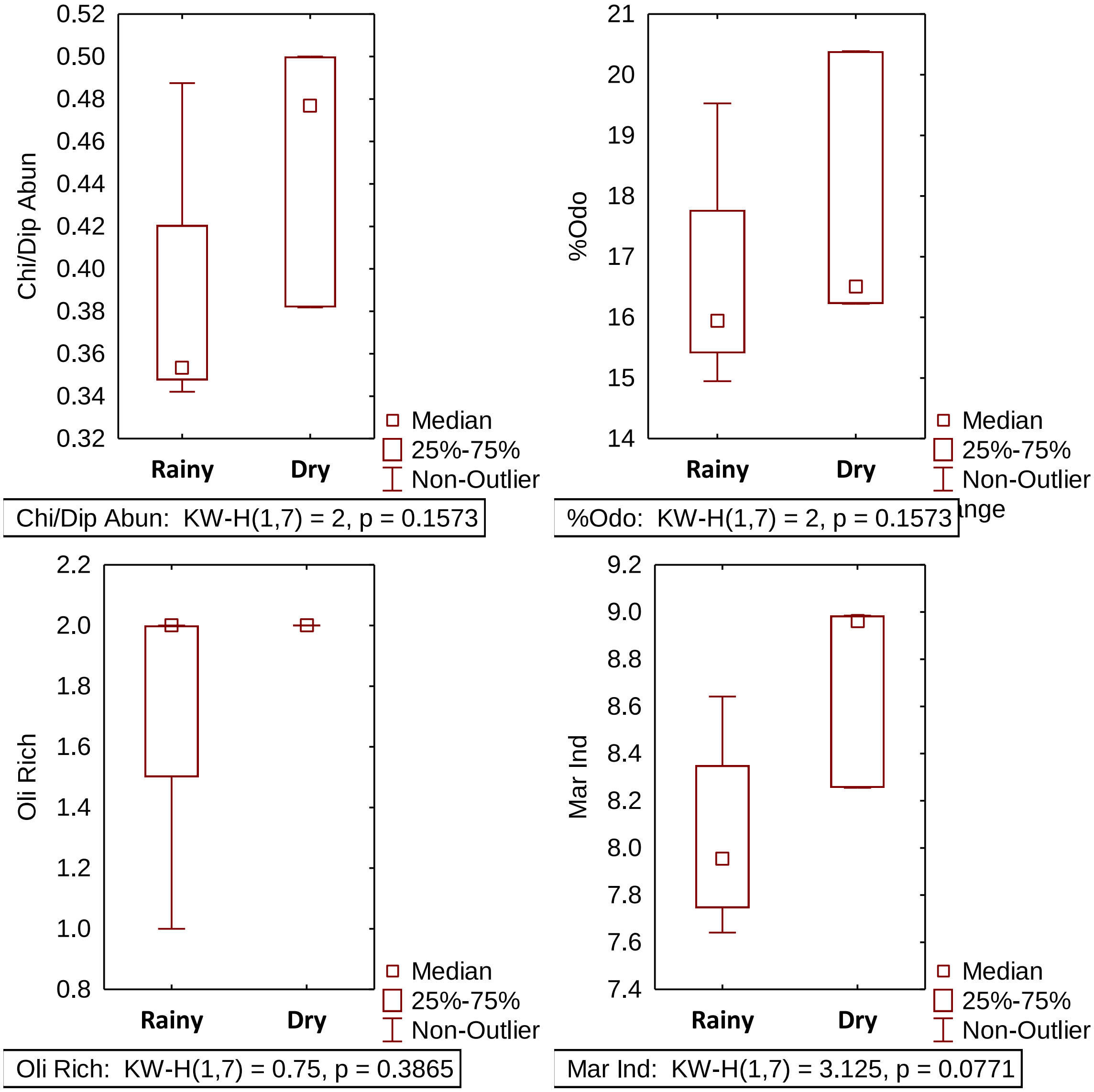



Figure 5. 7: Box plots showing seasonal stability of the five metrics integrated into the final multimetric index development for assessing urban-agricultural rivers in the Niger Delta (MMI-urban-agric), Nigeria.

### 5.4.9 Metric redundancy test

Of all the seasonally stable metrics in all categories of metric measures selected, Chironomidae/Diptera abundance, %Odonata and Oligochaeta richness were not redundant (Table 5.5). Of the 18 metrics that satisfactorily discriminated between the LIS, the MIS, and the HIS, only 12 metrics were retained and nine proved to be redundant. However, because those nine metrics represent different measures, two of them have been retained in addition to

Chironomidae/Diptera abundance, % Odonata and Oligochaeta richness. The two metrics selected in addition to the Chironomidae/Diptera abundance, % Odonata and Oligochaeta richness were the Margalef index and the logarithm of relative abundance of sprawler (Table 5.5).

### 5.4.10 Integrating the selected metrics into the urban-agriculture multimetric index (MMI-urban-agric)

To complete the development of the multimetric index, the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%), and maximum value of each metric for the LIS metric assemblage values were used as thresholds for calculating the metric scores (Table 5.6). The multimetric index was computed by summing the scores of the five metric components, and the index value range (5–25), since five metrics were used (5 X 5=25). The index value range then reflected five water quality categories, as shown in Table 5.7.

Table 5. 5: Spearman's rank correlation of macroinvertebrate metrics showing co-linearity between metrics in the urban-agricultural impacted rivers (r≥0.78, $P$<0.05). Metric abbreviations: Chi Abun (Chironomidae abundance), Chi/Dip Abun (Chironomidae/Diptera abundance), %Odo (percentage Odonata), Dip Rich (Diptera richness), Chi Rich (Chironomidae richness), Chi+Oli Rich (Chironomidae+Oligochaeta richness), Oli Rich (Oligochaeta richness), Sim Div (Simpson diversity), Sha Div (Shannon diversity), Eve Ind (Evenness index), Mar Ind (Margalef index), Log Spr (Logarithm of relative abundance of sprawler)

| | **Chi Abun** | **Chi/Dip Abun** | **%Odo** | **Dip Rich** | **Chi Rich** | **Chi+Oli Rich** | **Oli Rich** | **Sim Div** | **Sha Div** | **Eve Ind** | **Mar Ind** | **Log Spr** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chi Abun** | 0 | 1.2E-14 | 0.0049 | 5.2E-10 | 4.5E-10 | 1.1E-09 | 0.0179 | 1.1E-05 | 8.5E-08 | 0.20 | 0.00056 | **0.81** |
| **Chi/Dip Abun** | 0.76 | 0 | 0.063 | 0.0024 | 0.00049 | 3.5E-05 | 0.0055 | 2.2E-05 | 0.00028 | 0.051 | 0.030 | 0.052 |
| **%Odo** | 0.33 | 0.22 | 0 | 0.07 | 0.19 | 0.26 | 0.26 | 3.2E-06 | 1.1E-06 | 0.097 | 0.00050 | 0.030 |
| **Dip Rich** | 0.66 | 0.36 | 0.22 | 0 | 1.2E-30 | 2.1E-19 | 0.092 | 1.0E-06 | 3.1E-09 | 0.019 | 1.6E-10 | 0.41 |
| **Chi Rich** | 0.66 | 0.40 | 0.16 | **0.92** | 0 | 2.9E-18 | 0.53 | 3.8E-06 | 3.3E-08 | 0.061 | 3.4E-08 | 0.24 |
| **Chi+Oli Rich** | 0.65 | 0.47 | 0.13 | **0.83** | **0.82** | 0 | 3.4E-07 | 6.9E-08 | 1.9E-10 | 0.21 | 1.5E-10 | 0.32 |
| **Oli Rich** | 0.28 | 0.33 | 0.13 | 0.20 | 0.076 | 0.56 | 0 | 0.00057 | 0.00053 | 0.64 | 0.00035 | 0.099 |
| **Sim Div** | 0.49 | 0.48 | 0.52 | 0.54 | 0.52 | 0.59 | 0.39 | 0 | 6.2E-32 | 0.011 | 1.7E-13 | 0.021 |
| **Sha Div** | 0.58 | 0.42 | 0.54 | 0.63 | 0.59 | 0.67 | 0.41 | **0.93** | 0 | **0.84** | 5.6E-20 | 0.30 |
| **Eve Ind** | -0.15 | 0.23 | 0.20 | -0.28 | -0.22 | -0.15 | 0.057 | 0.30 | 0.024 | 0 | 0.048 | 0.00084 |
| **Mar Ind** | 0.39 | 0.26 | 0.40 | 0.67 | 0.59 | 0.67 | 0.41 | 0.74 | **0.84** | -0.24 | 0 | 0.10 |
| **Log_Spr** | 0.03 | 0.23 | 0.26 | 0.10 | 0.14 | 0.12 | 0.19 | 0.27 | 0.12 | 0.39 | 0.20 | 0 |

**Note:** Bold values were significant at $P$ < 0.05.

Table 5. 6: Score of metric thresholds of the selected metrics for the development of the multimetric index to assess urban-agricultural pollution (MMI-urban-agric) in the Niger Delta, Nigeria.

| **Urban-agricultural metrics** | **Statistics** | | | | | **Score** | | |
|---|---|---|---|---|---|---|---|---|
| | **Min. value** | **25%** | **50%** | **75%** | **Max. value** | **5** | **3** | **1** |
| Chi/Dip Abun | 0.342105 | 0.3533 | 0.381818 | 0.482212 | 0.5 | <0.482212 | ≥0.482212-<0.5 | >0.5 |
| %Odo | 14.94662 | 15.9407 | 16.22419 | 18.02119 | 20.38835 | ≥15.9407 | 14.94662-<15.9407 | <14.94662 |
| Oli Rich | 1 | 2 | 2 | 2 | 2 | <2 | 2 | >2 |
| Mar Ind | 7.641 | 7.954 | 8.255 | 8.802 | 8.985 | ≥7.954 | 7.641-<7.954 | <7.641 |
| Log Spr | 1.363636 | 1.365579 | 1.423611 | 1.56568 | 1.666667 | <1.56568 | ≥1.56568 - <1.666667 | >1.666667 |

Table 5. 7: Thresholds of MMI-urban-agric index score corresponding to water quality categories for urban pollution effects in the Niger Delta, Nigeria

| **Ecological category** | **Very poor** | **Poor** | **Fair** | **Good** | **Very good** |
|---|---|---|---|---|---|
| MMI-urban-agric score | 5-9 | 10-13 | 14-17 | 18-21 | 22-25 |
| Water quality class | F | E | D | C | B |

### 5.4.11 Validation and application of the multimetric index (MMI-urban-agric)

The index validation results showed that 16.7% of the time, stations designated as LIS had good water quality, and 83.3% of the time, they recorded fair water quality condition (Figure 5.8); thus the agreement between the index results and the physico-chemically based classification was 100%, indicating a good index performance for the LIS. For the MIS, the index validation results showed that 42.9% of the time, stations designated as MIS registered a fair water quality condition (Figure 5.8). This indicated a 42.9% correspondence between the MMI-urban-agric results and the physico-chemically-based classification (Figure 5.8). HIS validation results indicated that 70% of the time, stations designated as HIS had poor water quality condition, and

7.5% of the time, had very poor water quality condition (Figure 5.8). These results indicate a 77.5% performance agreement between the MMI-urban-agric the physico-chemically based classification.

Seasonally, MMI-urban-agric results showed that, during the wet season, 16.7% of the time, the LIS registered fair water quality (Figure 5.9). Hence, the performance of the index in the wet season for the LIS was 16.7%. For the dry season, 16.7% and 66.6% of the time, the LIS fell within the good and fair water quality categories, respectively (Figure 5.9). The performance of the MMI-urban-agric for the LIS during the dry season was thus 83.3%.

The seasonal validation results for MIS stations showed that 14.3% of the time these stations had fair water quality in the wet season while the results for the dry season showed the stations had fair water quality 28.6% of the time (Figure 5.9). The MMI-urban-agric performed better in the dry season than in the wet season.

Results for the wet season for stations designated as HIS showed that 27.5% of the time, water quality was poor, while in the dry season, water quality was poor (42.5%) and very poor (7.5%) (Figure 5.9). These results indicate that MMI-urban-agric performed better in the dry season than in the wet season for the HIS. The two-way ANOVA indicated that there was a significant difference for the MMI-urban-agric index values between LIS, MIS and HIS ($P$>0.05) whereas there were no statistically significant differences between the wet and dry seasons in terms of the MMI-urban-agric index values ($P$>0.05).

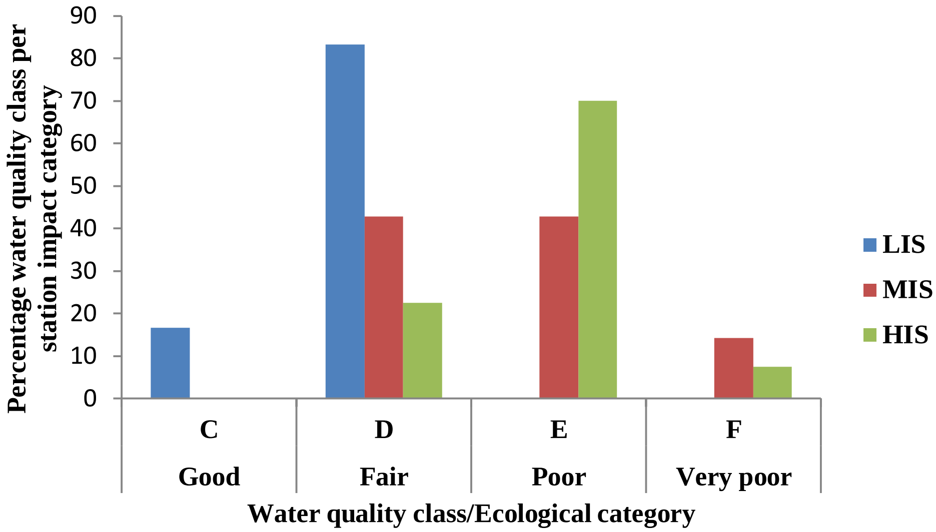



Figure 5. 8: Percentage number of times station categories fell within a water quality class based on the MMI-urban-agric index value. Abbreviations: LIS=least impacted stations, MIS= moderately impacted stations and HIS=heavily impacted stations. MMI-urban-agric-based water quality class: C (Good), D (Fair), E (Poor), F (Very poor).

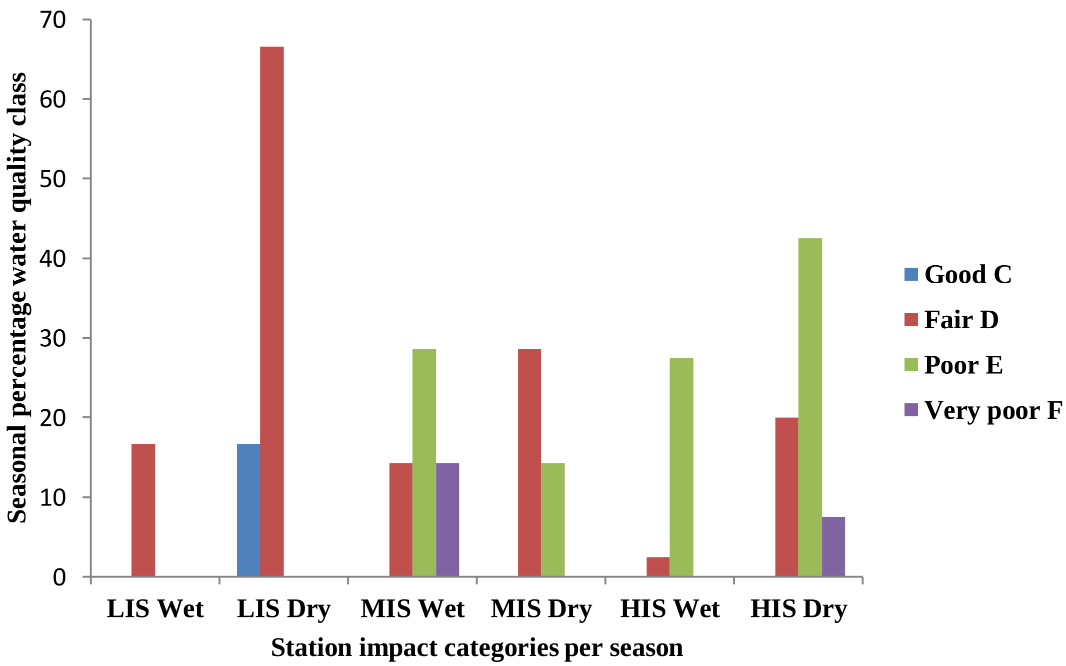



Figure 5. 9. Percentage number of times station categories fell within a water quality class based on the MMI-urban-agric-based water quality class per season (wet and dry). Abbreviations: LIS= least impacted stations, MIS=moderately impacted stations and HIS=heavily impacted stations. MMI-urban-agric-based water quality class: C (Good), D (Fair), E (Poor), F (Very poor).

### 5.4.12 Relating the selected metrics in the MMI-urban-agric to physico-chemical variables

Axes 1 and 2 of the RDA explained 80.55% and 19.45% of the variance, respectively. The Eigen value of Axis 1 was higher (31.087) than that of Axis 2 (0.064). A Monte Carlo test at 999 permutations revealed that Axes 1 and 2 of the RDA were not statistically significant ($P > 0.05$). Logarithmic relative abundance of the sprawlers and %Odonata were positively correlated with pH and negatively correlated with LIS on Axis 1 and MIS on Axis 2 (Figure 5.10). Dissolved oxygen and depth were strongly positively correlated with the Margalef index at LIS on Axis 1 (Figure 5.10). Five-day biochemical oxygen demand ($BOD_5$), nitrate and water temperature were strongly positively correlated with Oligochaete richness and Chironomidae/Diptera abundance at HIS on Axis 1 (Figure 5.10).

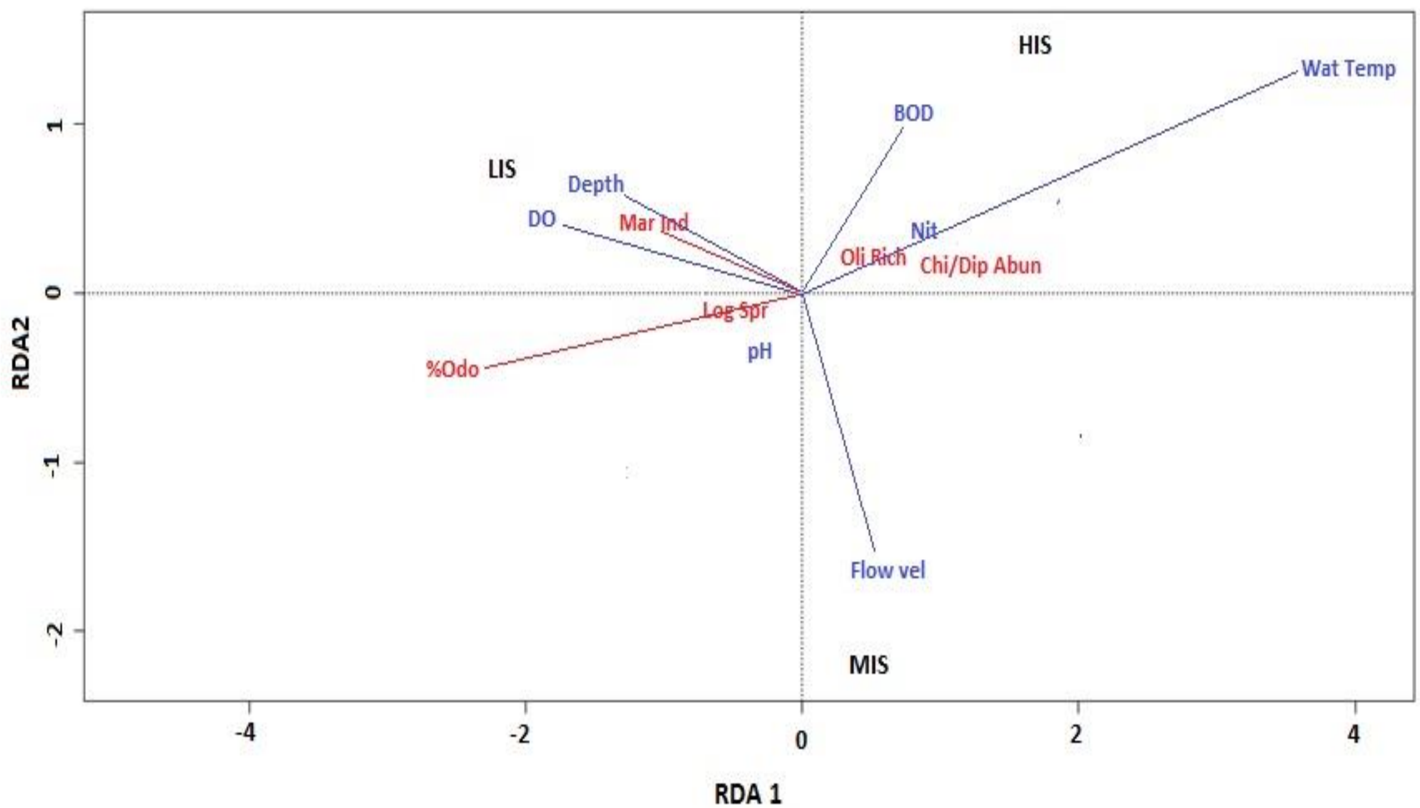



Figure 5. 10: Redundancy ordination plot showing the relationship between macroinvertebrate metrics and physio-chemical variables for the MMI-urban-agricultural. Metrics: Chi/DipAbun (Chironomidae/Diptera Abundance), %Odo (%Odonata), Oli Rich (Oligochaeta Richness), Mar Ind (Margalef Index), Log Spr (Logarithm of relative abundance of Sprawler). Physico-chemical variables: Wat Temp (Water temperature), Flow vel (Flow velocity), DO (Dissolved oxygen), BOD (five-day biochemical oxygen demand, $BOD_5$), Nit (Nitrate), Depth and pH. Station impact categories: LIS (least impacted stations), MIS (moderately impacted stations) and HIS (heavily impacted stations).

### 5.4.13 Developing a multimetric index (MMI-urban-forest) for assessing urban pollution in forested riverine systems

### 5.4.14 Metric sensitivity and seasonality tests

Of the 59 candidate metrics tested, only 14 satisfactorily discriminated between the LIS, and the MIS and HIS (Appendix F; Table F1). Following the results of subsequent analyses, only four metrics were integrated into the final index, and the discrimination between the LIS and the MIS and HIS is depicted in Figure 5.11. Sensitive and non-sensitive metrics not integrated into the MMI-urban-forest are presented in the Appendix F (Figures F1–F5).

The seasonality test indicated that 12 metrics were seasonally stable. The 12 metrics were Trichoptera abundance, Ephemeroptera Plecoptera Trichoptera/Chironomidae abundance, %Chironomidae, %Chironomidae+Oligochaeta, %Diptera, %Odonata, %Mollusca+Diptera, Coleoptera+Hemiptera richness, Coleoptera richness, Simpson dominance, Shannon diversity, Margalef index (Appendix F; Figures F6-F10). Seasonal stability of the four metrics integrated into the multimetric index is shown in Figure 5.12.

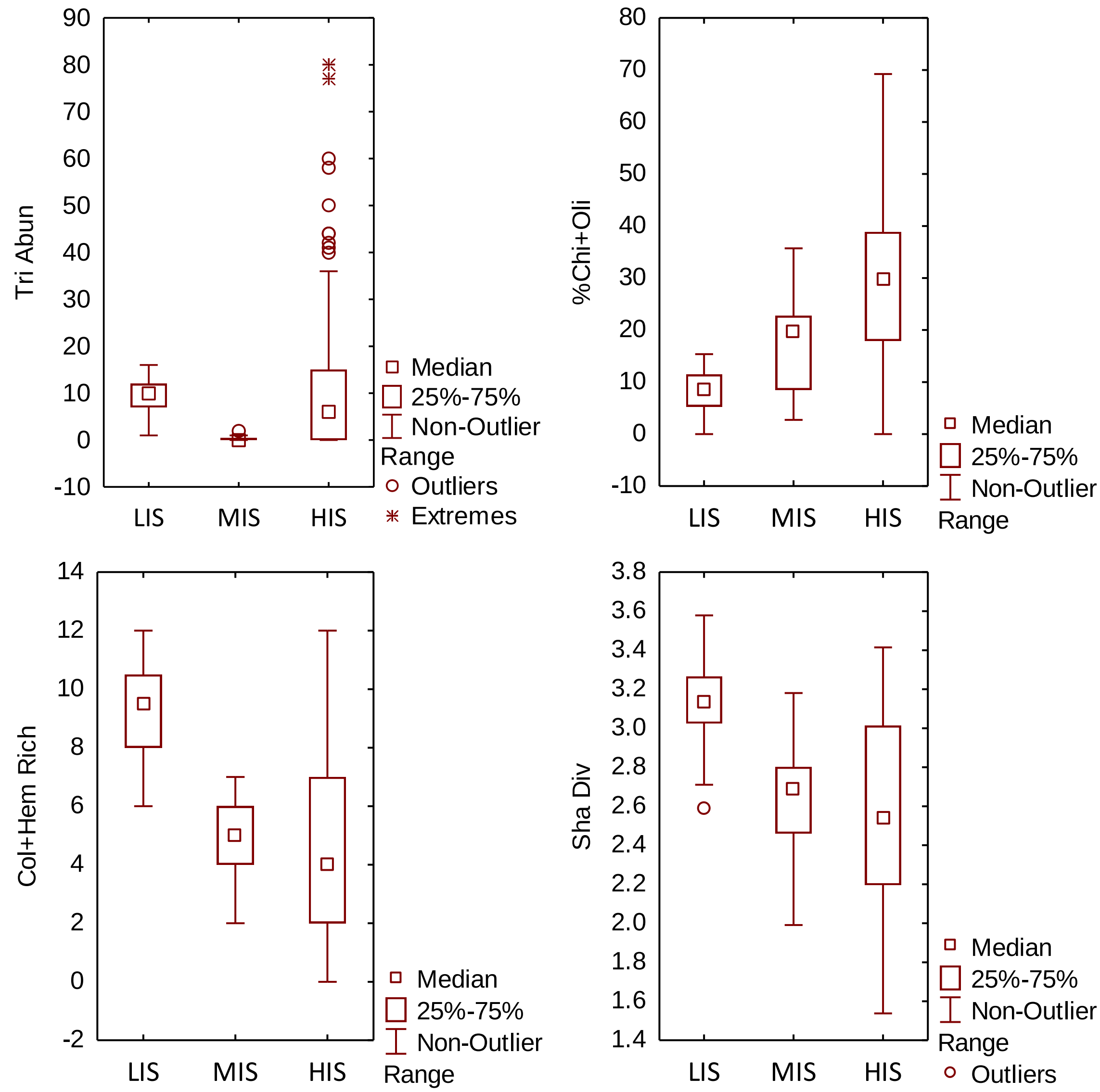



Figure 5. 11: Box plots showing metric discrimination between the LIS, MIS and HIS of the four metrics integrated into the final multimetric index (MMI-urban-forested) for the urban-forested river systems in the Niger Delta, Nigeria.

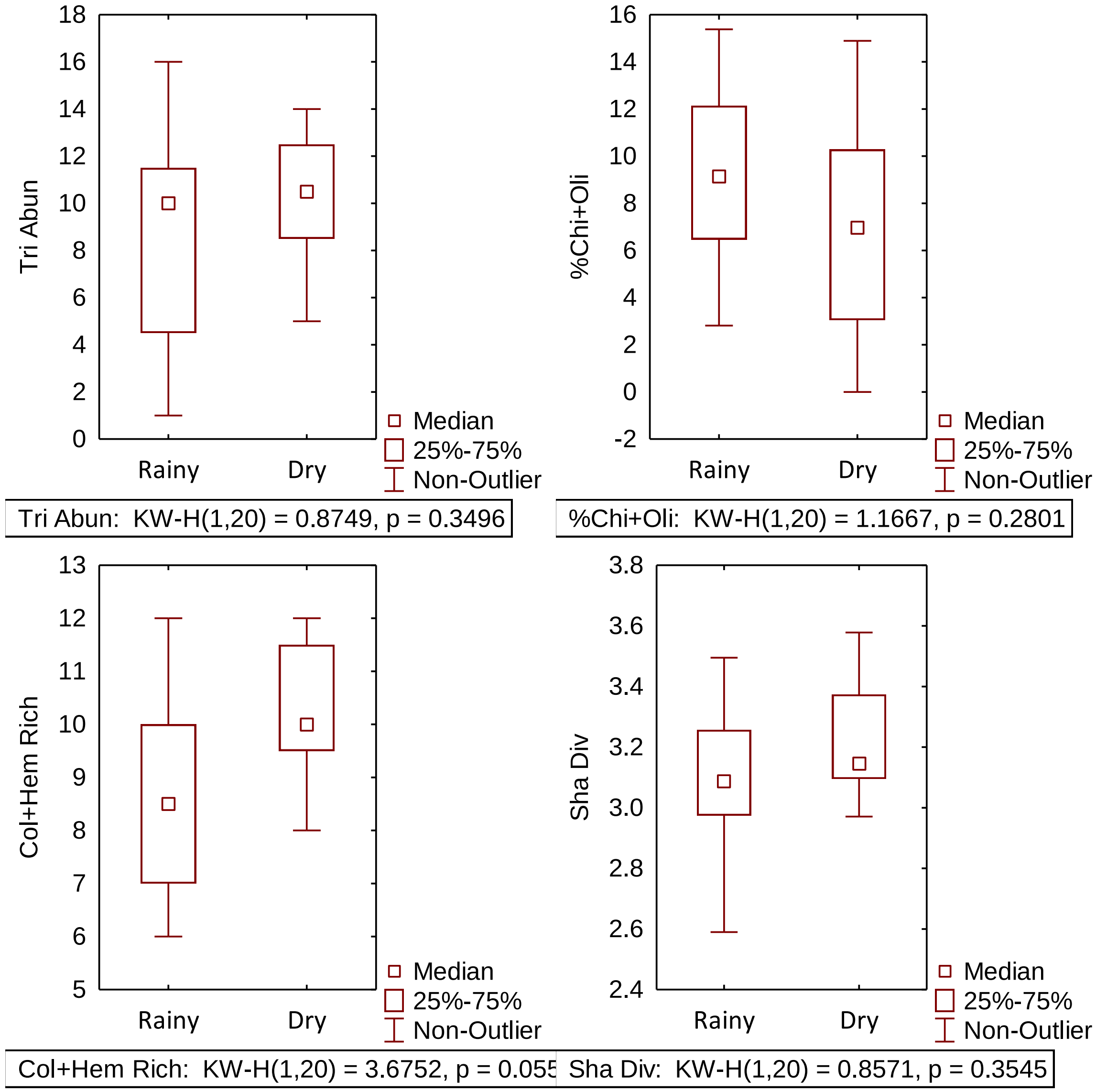



Figure 5. 12: Box plots showing seasonal stability of the four metrics integrated into the final multimetric index (MMI-urban-forested) for assessing urban pollution in forested riverine systems in the Niger Delta, Nigeria.

### 5.4.15 Metric redundancy test

Of all the seasonally stable metrics in all categories of metric measures selected, only Trichoptera abundance was not redundant (Table 5.8). Of the 14 metrics that satisfactorily discriminated between the LIS, the MIS, and the HIS, only 12 metrics were retained and 11 proved to be redundant. However, because those 11 metrics represent different measures, three of them have been retained in addition to Trichoptera abundance. The three metrics selected in addition to the Trichoptera abundance were the % Chironomidae + Oligochaeta, Coleoptera + Hemiptera richness and Shannon diversity (Table 5.8).

### 5.4.16 Integrating the selected metrics in the urban-forestry multimetric index (MMI-urban-forest)

To develop the multimetric index, the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%), and maximum value of each metric for the LIS metric assemblage values were used as thresholds for calculating the metric scores (Table 5.9). The multimetric index was computed by summing the scores of the four metric components and the index value range (4–20) since four metrics were used (4 X 5=20). The index value range reflected five water quality categories as shown in Table 5.10.

Table 5. 8: Spearman's rank correlation of macroinvertebrate metrics showing co-linearity between metrics in the urban-forestry impacted rivers (r≥0.78, *P*<0.05). Metric abbreviations: Tri Abun (Trichoptera abundance), EPT/Chi Abun (Ephemeroptera Plecoptera Trichoptera/Chironomidae abundance), %Chi (percentage Chironomidae ), %Chi+Oli Rich (percentage Chironomidae+Oligochaeta), %Dip (percentage Diptera), %Odo (percentage Odonata), %Mol+Dip (percentage Mollusca+Diptera), Col+Hem Rich (Coleoptera+Hemiptera richness), Col Rich (Coleoptera richness), Sim Div (Simpson diversity), Sha Div (Shannon diversity), Mar Ind (Margalef index)

| | **Tri Abun** | **EPT/Chi Abun** | **%Chi** | **%Chi+ Oli** | **%Dip** | **%Odo** | **%Mol+ Dip** | **Col+Hem Rich** | **Col Rich** | **Sim Div** | **Sha Div** | **Mar Ind** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Tri Abun** | 0 | 0.41 | 0.33 | 0.51 | 0.37 | 0.55 | 0.37 | 0.51 | 0.66 | 0.077 | 0.077 | 0.137 |
| **EPT/Chi Abun** | 0.19 | 0 | 3.7E-08 | 4.9E-06 | 5.6E-07 | 0.57 | 5.6E-07 | 0.77 | **0.99** | 0.28 | 0.32 | 0.31 |
| **%Chi** | -0.23 | **-0.91** | 0 | 6.0E-07 | 1.5E-07 | **0.92** | 1.5E-07 | **0.88** | **0.91** | 0.052 | 0.058 | 0.047 |
| **%Chi+Oli** | -0.15 | **-0.83** | **0.87** | 0 | 2.4E-07 | 0.62 | 2.1E-07 | 0.65 | **0.89** | 0.046 | 0.079 | 0.095 |
| **%Dip** | -0.21 | **-0.87** | **0.89** | **0.89** | 0 | **0.87** | 0 | **1** | 0.71 | 0.039 | 0.034 | 0.026 |
| **%Odo** | -0.14 | -0.14 | 0.024 | 0.12 | 0.038 | 0 | **0.87** | 0.186 | 0.26 | 0.188 | 0.24 | 0.29 |
| **%Mol+Dip** | -0.21 | **-0.87** | **0.89** | 0.89 | 1 | 0.038 | 0 | 1 | 0.71 | 0.039 | 0.034 | 0.026 |
| **Col+Hem Rich** | -0.155 | 0.070 | -0.038 | -0.11 | 0 | 0.31 | 0 | 0 | 3.5E-06 | 0.018 | 0.0026 | 0.0029 |
| **Col Rich** | -0.10 | 0.0027 | 0.026 | -0.033 | 0.088 | 0.27 | 0.088 | **0.84** | 0 | 0.018 | 0.0091 | 0.0095 |
| **Sim Div** | -0.40 | -0.25 | 0.44 | 0.45 | 0.47 | 0.31 | 0.47 | 0.52 | 0.52 | 0 | 1.8E-11 | 3.4E-09 |
| **Sha Div** | -0.41 | -0.23 | 0.43 | 0.4 | 0.47 | 0.28 | 0.47 | 0.64 | 0.57 | **0.96** | 0 | 1.0E-14 |
| **Mar_Ind** | -0.34 | -0.24 | 0.45 | 0.38 | 0.49 | 0.25 | 0.49 | 0.63 | 0.56 | **0.93** | **0.98** | 0 |

**Note:** Bold values were significant at *P* < 0.05.

Table 5. 9: Score of metric thresholds of the selected metrics for the development of the multimetric index (MMI-urban-forested) for assessing urban pollution in forested riverine systems in the Niger Delta, Nigeria.

| **Metrics** | **Statistics** | | | | | **Score** | | |
|---|---|---|---|---|---|---|---|---|
| | **Min. value** | **25%** | **50%** | **75%** | **Max. value** | **5** | **3** | **1** |
| Tri Abun | 1 | 7 | 10 | 12 | 16 | ≥7 | <7 | 1 |
| %Chi+Oli | 0 | 5.538302 | 8.581769 | 11.16942 | 15.38462 | <11.16942 | ≥11.16942 -<15.38462 | >15.38462 |
| Col+Hem Rich | 6 | 8 | 9.5 | 10.25 | 12 | ≥8 | 6 - <8 | <6 |
| Sha Div | 2.59 | 3.0275 | 3.1375 | 3.2615 | 3.578 | ≥3.0275 | 2.59- <3.0275 | <2.59 |

Table 5. 10: Threshold MMI-urban-forested index score corresponding to water quality categories for urban pollution effects in forested riverine systems in the Niger Delta, Nigeria.

| **Ecological category** | **Very poor** | **Poor** | **Fair** | **Good** | **Very good** |
|---|---|---|---|---|---|
| MMI-urban-forested score | 4-7 | 8-11 | 12-14 | 15-17 | 18-20 |
| Water quality class | F | E | D | C | B |

#### 5.4.17 Validation and application of the multimetric index (MMI-urban-forested)

Data sets used for MMI-urban-forest validation and application were derived from MIS and HIS only. The validation results for the MMI-urban-forested index showed that 42.8% of the time, stations designated as MIS had very good water quality, and 7.2 % of the time the MIS water quality was good. The MIS had fair water quality 50% of the time (Figure 5.13). The agreement between the physico-chemically-based station classification and the MMI-urban-forested index was 100%, indicating good index performance for the MIS. For HIS, the validation result for the index showed that 83.3% of the time, HIS stations had fair water quality, 8.3% of the time water quality was poor, and 8.3% of the time water quality was very poor (Figure 5.13), indicating a 100% agreement between the physico-chemically-based station designation and that of the newly developed MMI-urban-forested.

Seasonally, results showed that, during the wet season, 35.7% of the time, stations designated as MIS had very good water quality, and 7.1% of the time the water quality was good. The MIS had fair water quality 28.6% of the time during the wet season (Figure 5.14). Thus, the performance of the MMI-urban-forested in the wet season for MIS stations was 71.4%. For the dry season, 7.2% of the time, stations designated as MIS had very good water quality and 21.4% of the time, had fair water quality (Figure 5.14), indicating 28.6% agreement between the physico-chemically based station classification and those of the MMI-urban-forested.

The results showed that stations designated as HIS had fair water quality 50.7% of the time, and 8.3% of the time, they had poor water quality (Figure 5.14). The agreement between the physico-chemically based classification and the MMI for the wet season for the HIS stations was thus 59%. For the dry season, stations designated as HIS fell within the fair water quality class 32.6% of the time, and 8.3% of the time, they fell within the very poor water quality category (Figure 5.14), indicating an agreement of 40.9% between the physico-chemically based designation and the MMI during the dry season.

Statistically, there was a significant difference between the dry and wet seasons in terms of the MMI-urban-forested index values ($P<0.05$) but the index values for the MIS and HIS were not significantly different ($P>0.05$).

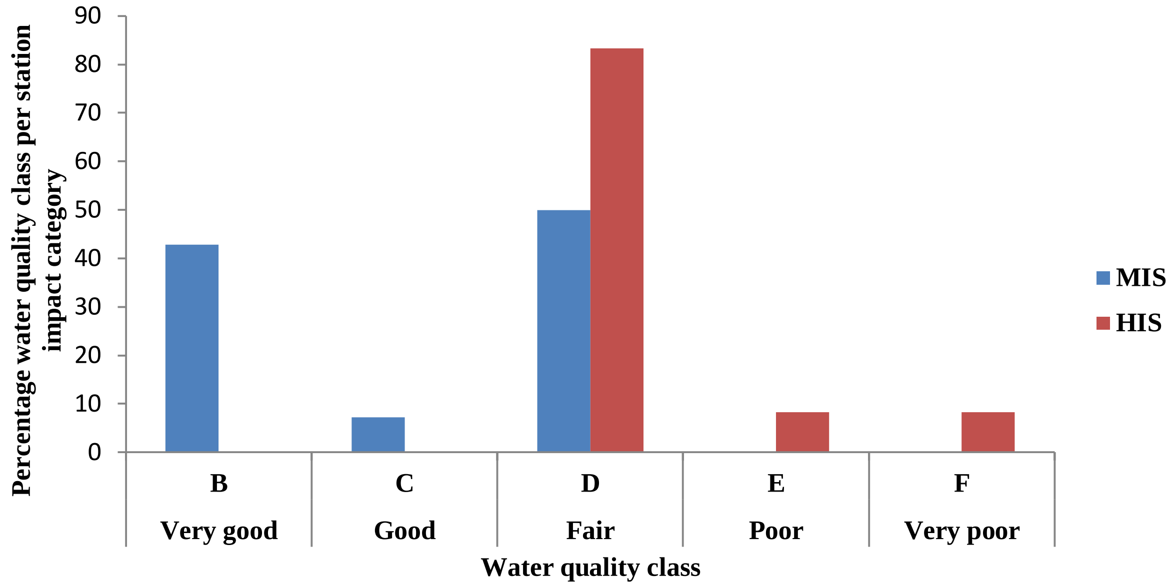



Figure 5. 13: Percentage number of times the LIS, MIS and HIS fell within a water quality class based on the MMI-urban-forested value. Abbreviations: MIS=moderately impacted stations and HIS=heavily impacted stations. MMI-urban-forested water quality class: B (Very good), C (Good), D (Fair), E (Poor), F (Very poor)

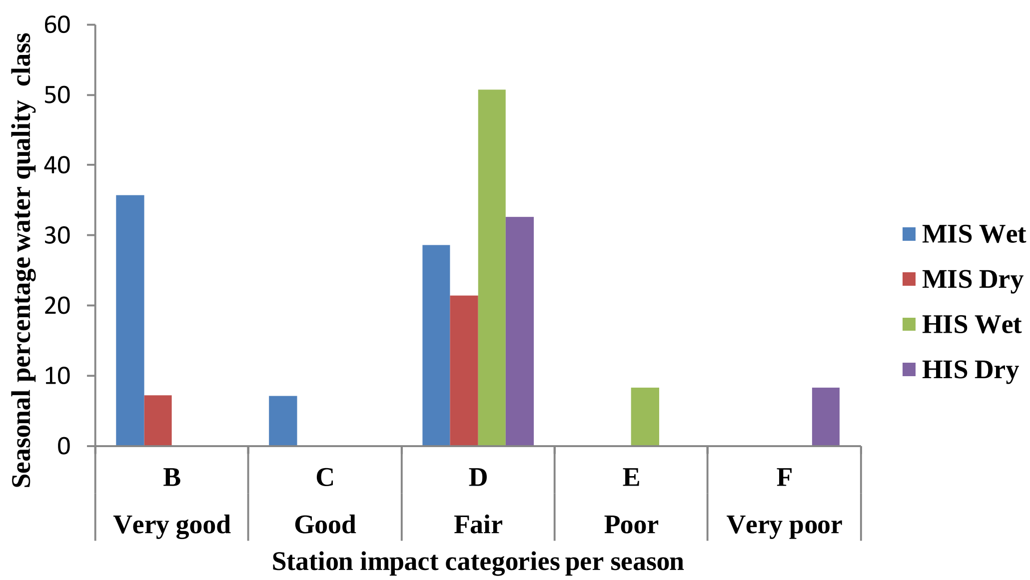



Figure 5. 14: Percentage number of times the LIS, MIS and HIS fell within a water quality class based on the MMI-urban-forested water quality class per season (wet and dry). Abbreviations: MIS=moderately impacted stations and HIS=heavily impacted stations. MMI-urban-forest-based water quality class: B (Very good), C (Good), D (Fair), E (Poor), F (Very poor).

**5.4.18 Relating the selected metrics in the MMI-urban-forest to physico-chemical variables**

Axes 1 and 2 of the RDA triplot explained 90.1% and 9.6% of the variance, respectively. The Eigen value of Axis 1 was higher (102.49) than that of Axis 2 (34.16). A Monte Carlo test at 999 permutations revealed that Axes 1 and 2 of the RDA were not statistically significant ($P > 0.05$). Dissolved oxygen, depth, and pH were strongly positively correlated with the Shannon diversity index, Coleoptera+Hemiptera richness, and Trichoptera abundance at the LIS on Axis 2 (Figure 5.15). Water temperature, flow velocity, EC, $BOD_5$, nitrate and phosphate were strongly negatively correlated with %Chironomidae+Oligochaeta at the HIS on Axis 2 (Figure 5.15).

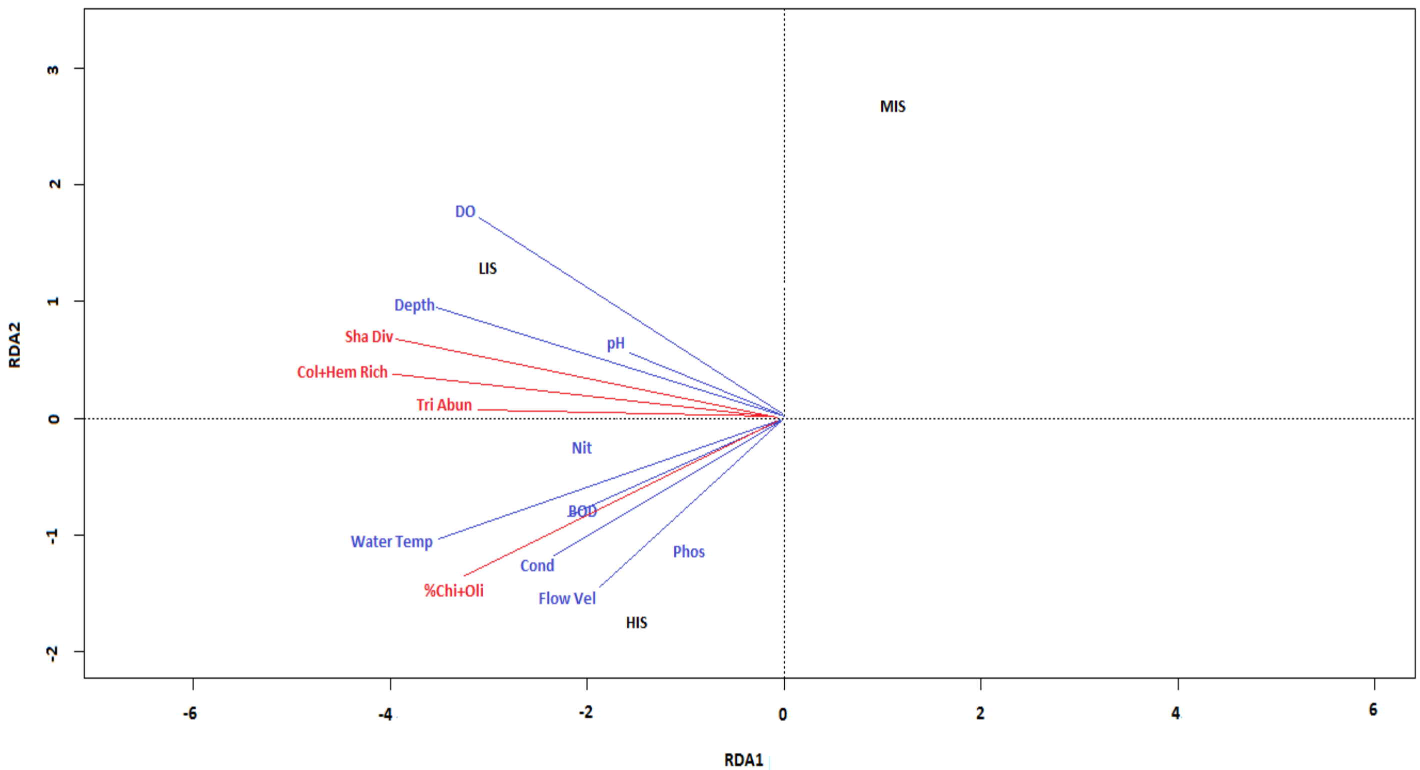



Figure 5. 15: Redundancy ordination plot showing the relationship between macroinvertebrate metrics and physio-chemical variables for the MMI-urban-forested. Metrics: Tri Abun (Trichoptera Abundance), %Chi+Oli (% Chironomidae+Oligochaeta), Col + Hem Rich (Coleoptera + Hemiptera Richness), Sha Div (Shannon Diversity). Physico-chemical variables: Wat Temp (Water temperature), Flow vel (Flow velocity), DO (Dissolved oxygen), BOD (five-day biochemical oxygen demand, $BOD_5$), Nit (Nitrate), Phos (Phosphate), Cond (electrical conductivity), Depth and pH. Station impact categories: LIS (least impacted stations), MIS (moderately impacted stations) and HIS (heavily impacted stations).

## 5.5 Discussion

### 5.5.1 The multimetric index (MMI-urban) for monitoring urban pollution effects in rivers in the Niger Delta

A total of 77 macroinvertebrate candidate metrics were tested, of which only five representing trait, composition, diversity and abundance measures were retained and integrated into the final MMI-urban. Of all the candidate metrics in the various measures considered for integration into the MMI-urban, 26 metrics discriminated the LIS from the MIS and HIS and were confirmed sensitive. Most of the sensitive metrics were in the abundance and composition measures. The abundance and composition metrics are widely recognised as being sensitive to pollution and therefore often integrated into multimetric indices (Baptista *et al.,* 2013; Huang *et al.,* 2015; Melo *et al.,* 2015; Lu *et al.,* 2019; Gieswein *et al.,* 2019).

Of the diversity measures used, Margalef's index, the Shannon-Wiener, Simpson diversity and Evenness index were all discriminatory; only the Evenness index was confirmed sensitive. Similar studies elsewhere have reported most diversity metrics have high discriminatory potential (Ntislidou *et al.,* 2018; Edegbene *et al.,* 2019). Edegbene *et al.* (2019) integrated two diversity measures, namely the Margalef and Shannon diversity indices, into a multimetric index developed for a river in Nigeria. This attests to the fact that diversity indices are useful biomonitoring tools. Other studies have also integrated the Margalef index (Mereta *et al.,* 2013) and Shannon diversity index (Aura *et al.,* 2017) into multimetric indices. In the present study, the Evenness index was the only diversity measure integrated into the final index because the remaining measures were found to be either seasonally unstable or redundant. Zamora-Muniz *et al.* (1995) caution against using metrics that are seasonally unstable because of the difficulty of disentangling variation occasioned by natural seasonal dynamics from that occasioned by anthropogenic activities.

One of the five metrics integrated into the final MMI-urban was a trait measure, that is, very large body size (>40–80 mm). Organisms with a body size ranging between >40–80 mm proved highly sensitively to urban pollution and the trait was non-redundant with the rest of the taxonomic metrics. Abundances of very large-bodied macroinvertebrates have been hypothesised to decrease in response to environmental stress because they are often associated with a long reproductive cycle and fewer offspring per reproductive event than small-bodied individuals (Townsend & Hildrew, 1994; Serra *et al.*, 2017; Castro *et al.*, 2018). Studies testing metrics for integration into multimetric indices have often included one or two trait-based metrics in final indices, indicating that the present study, which found only a single trait to be highly sensitive and non-redundant, was consistent with most other studies (e.g. Baptista *et al.,* 2007; Fierro *et al.,* 2018; Ntislidou *et al.,* 2018; Gieswein *et al.,* 2019). The inclusion of the trait-based metric into the final MMI-urban is particularly useful because, while taxonomic metrics relate to structural measures, traits relate to the functional aspects of the biota (Monaghan & Soares, 2012; Ding *et al.,* 2017; Desrosiers *et al.,* 2019).

The five candidate metrics integrated into the MMI-urban are sparsely reported as metrics for development of a multimetric index except the %Hemiptera+Coleoptera and Hemiptera abundance (Aura *et al.,* 2017; Edegbene *et al.,* 2019). The frequent integration of Hemiptera+Coleoptera and Hemiptera abundance in multimetric index elsewhere informed the selection of %Coleoptera+Hemiptera and Hemiptera abundance for integration into the MMI-urban, even when they were redundant. Furthermore, two metrics from the composition measures were retained, though redundant. The %Coleoptera+Hemiptera reflect moderately tolerant macroinvertebrate taxa while %Chironomidae + Oligochaeta reflect taxa that are tolerant of pollution. Mereta *et al.* (2013) also selected final metrics based on their degree of sensitivity to water quality impairment.

The validation of the performance of the developed MMI-urban with separate datasets revealed that the index performed better for LIS and MIS than for the HIS. The relatively good performance of the index for the LIS and MIS stations indicate that using the index may not lead to under- or over-protection, whereas the poor performance of the index for the HIS could be because pollution at these stations is seasonally mediated, such that macroinvertebrate recovery and recolonization are rapid, reducing the cumulative effects of pollution. Even though seasonal stability was tested for during the selection of the metrics, it appears that a “flushing effect” aggravated the effects of urban pollution during the wet season. During the wet season, water quality at the HIS was generally poor compared with the dry season. Increased urban storm water run-off, and run-off from settlements carrying pollutants may have led to the poor water quality at the HIS during the wet season. Similar findings have been reported by Speak *et al.* (2013) who indicated that increased urban run-off caused by increased precipitation led to increased pollution of riverine ecosystems. Water quality at the HIS seems to recover during the dry season, mediating the overall performance of the developed index. The implication, therefore, is that monitoring needs to be structured to take account of seasonality, and data should be interpreted in the light of the season-mediating effects of urban pollution.

The developed MMI-urban performed better in the dry season than in the wet season except for the LIS. The reason for the better performance of the index during the dry season could be attributed to reduced urban run-off during the dry season. Urban run-off is one of the major factors influencing the water quality of rivers in the Niger Delta. In addition, heavy rains have an impact on water quality because debris and other pollutants are carried into urban river systems during the downpour. In contrast to the findings in the present study, Edegbene *et al.* (2019) found that a similar index developed for a river in north central Nigeria did not exhibit much seasonal variation in terms of performance. This may be connected to longer wet seasons in the Niger Delta area compared to the north central part of Nigeria where Edegbene *et al.* (2019) reported contrary findings.

### 5.5.2 The multimetric index (MMI-urban-agric) for monitoring urban-agricultural impact in rivers in the Niger Delta

Of the 67 macroinvertebrates candidate metrics that were tested, only five representing abundance, composition, richness, diversity and trait measures were retained and integrated into the final MMI-urban-agric. Of the 67 candidate metrics, 18 satisfactorily discriminated LIS from MIS and HIS, and were confirmed sensitive. Four metrics each of abundance, composition, richness and diversity measures were sensitive.

Most of the metrics that discriminated LIS from MIS and HIS were the metrics that respond positively to deteriorating water quality. For example, pollution-tolerant metrics such as Chironomidae, Chironomidae+Oligochaete and Oligochaete were able to discriminate and confirm their sensitivity for the abundance, composition and richness measures. Pollution-tolerant candidate metrics have been reported to increase with anthropogenic impacts in agricultural and urban dominated rivers (Melo *et al.*, 2015). The relationships of Chironomidae and Oligochaete with nutrient enrichment have received attention in biomonitoring of freshwater bodies (Arimoro *et al.,* 2015; Edegbene *et al.,* 2015; Melo *et al.,* 2015). Genera of Chironomidae, for example, *Chironomus,* have been reported to thrive in organically enriched water bodies and in polluted systems because they are able to trap atmospheric oxygen via blood-trapping tissues (haemoglobin) (Kleine & Trivinho-Strixino, 2005). More recently, Macedo *et al.* (2016) reported dominance of Chironomidae in Neotropical savanna headwater streams within catchments that had a hydroelectric power plant. The researchers assert that catchment disturbances impact negatively on the functionality of their studied streams (Macedo *et al.,* 2016) as more pollution-tolerant taxa, e.g. Chironomidae, which can thrive in polluted water, were dominant in the streams.

The effect of agricultural activities and urbanisation on water quality of rivers and their biological communities (Allan, 2004) calls for serious attention as these stressors have been implicated in negatively affecting the ecosystem balance of rivers located in such areas (Laini *et al.,* 2018). This is the case in the present study, because of the growing rural-urban migration and increased agricultural activities in the studied river catchments in the Niger Delta. Further confirming the deterioration of the rivers studied for the MMI-urban-agric development is the inclusion of Chironomidae/Diptera abundance and Oligochaete richness candidate metrics which were non-redundant and thereby qualified for integration into the MMI-urban-agric.

Simpson diversity, Shannon-Weiner, Margalef's index and the Evenness index were all discriminatory and confirmed sensitive. Studies elsewhere in the temperate region have reported diversity measures to have high discriminatory potential (Ntislidou *et al.*, 2018). Ntislidou *et al.* (2018) integrated Simpson diversity into a macroinvertebrate-based multimetric index developed in Greece, thus confirming that diversity metrics are useful for biomonitoring freshwater bodies. Other studies have also integrated diversity metrics into multimetric indices (Mereta *et al.,* 2013; Aura *et al.,* 2017).

Of the macroinvertebrate trait metrics, only the logarithm of relative abundance of sprawler was integrated into the MMI-urban-agric, as other trait metrics were either non-sensitive or seasonally unstable. Macroinvertebrate traits as candidate metrics for integration into multimetric indices are sparse in the literature (Baptista *et al.,* 2007; Mereta *et al.,* 2013). Some studies which incorporate the trait category of metrics do not finally integrate them into the developed multimetric indices (Fierro *et al.,* 2018, Ntislidou *et al.,* 2018).

Three of the five metrics integrated into the final MMI-urban-agric were in the categories of abundance, composition and richness measures, that is, Chironomidae/Diptera abundance, %Odonata and Oligochaete richness.

Most of the five metrics integrated into the MMI-urban-agric have been integrated into multimetric indices elsewhere (Baptista *et al.,* 2013, Ntislidou *et al*., 2018). A recently developed multimetric index for an urban and agriculture-laden catchment in the north central part of Nigeria integrated component metrics of Chironomidae, Diptera, Odonata and diversity (Margalef index) into a multimetric index (Edegbene *et al.,* 2019).

Validation of the performance of the developed MMI-urban-agric with separate datasets revealed MMI-urban-agric performed better for LIS (100%), followed by HIS (77.5%), while the MIS performance was 42.9%.

Seasonally, there was no significant difference between the water quality conditions in the wet and dry seasons. A similar study in Nigeria also revealed that seasonal variation had no effect on the applicability of the developed multimetric index (Edegbene *et al.,* 2019). In contrast, elsewhere in the neotropics, seasonal variation was noted in the applicability of the final neotropical lowland stream multimetric index (Helson & Williams, 2013).

Agricultural activities and urbanisation negatively affect rivers in the Niger Delta area, as revealed by the present study. Other studies have reported alteration of water quality of rivers/streams by industrial, municipal and agricultural discharges (Ndaruga *et al.,* 2004, Kasangali *et al.,* 2008), which means that agricultural and urban pollution are altering macroinvertebrate assemblage and functionality, as has been noted in this study in the dominance of pollution-tolerant taxa, for example, Chironomidae and Oligochaete.

Correlation of the integrated metrics with physico-chemical variables revealed Chironomidae/Diptera abundance and Oligochaete richness were positively associated with HIS and strongly positively correlated with pollution indicator physico-chemical variables: $BOD_5$ and nitrate. These findings confirm that the applicability of the developed MMI-urban-agric as pollution-tolerant metrics relate positively to pollution indicator physico-chemical variables. Studies elsewhere report the variation of pollution-tolerant metrics with prevailing environmental variables portray a pollution gradient of human disturbance (Baptista *et al.,* 2011; Chowdhury *et al.,* 2016). Diptera and Oligochaete have been reported to relate negatively to a developed multimetric index (Chowdhury *et al.,* 2016), a phenomenon the authors ascribe to increasing pollution-related environmental factors, such as conductivity, total phosphorus, and chlorophyll-a (Chowdhury *et al.,* 2016). Again, Baptista *et al.* (2011) assert that the Mollusca+Diptera metric increased in assemblage following the impact of organic pollution and other related human pressures. This assemblage can result in a deteriorating state of rivers, as also revealed in this study where Chironomidae/Diptera abundance and Oligochaete richness were found to be strongly positively correlated with pollution indicator environmental factors (nitrate and $BOD_5$) on the ordination plot.

### 5.5.3 The multimetric index (MMI-urban-forest) for monitoring urban-forestry impact in rivers in the Niger Delta

Of the 59 candidate metrics selected for the development of MMI-urban-forested, only four metrics (abundance, composition, richness and diversity measures) were retained and integrated into the MMI-urban-forested. Of all the selected candidate metrics, 14 satisfactorily discriminated LIS from MIS and HIS. Most of the metrics that were sensitive were in the composition and richness measures. Rivers that are least impacted are known to support a diverse array of macroinvertebrates because they tend to provide heterogenous habitats, favouring diverse niche partitioning and thus high composition and richness (Pallottini *et al.*, 2017). When such systems are impacted, richness and a diverse composition tend to be sensitive, thus indicating why they proved sensitive in this study. Given that composition and richness measures are highly sensitive, they have also been included in most multimetric indices (Baptista *et al.*, 2007; Helson & Williams, 2013; Huang *et al.*, 2015; Lakew & Moog, 2015). The performance of metrics in the richness, abundance and composition measures have also been reported to be widely used for biomonitoring freshwater ecosystems (Barbour *et al.*, 1999; Suriano *et al.*, 2011; Odume e*t al.*, 2012; Pond *et al.*, 2013). Macroinvertebrate taxa that constitute metrics in the categories of richness, abundance and composition have been claimed to structure the aquatic ecosystem community balance (Rizo-Patron *et al.*, 2013).

Four metrics were finally integrated into the MMI-urban-forested: Trichoptera abundance, Coleoptera+Hemiptera richness, Shannon diversity and %Chironomidae+Oligochaete. In pristine forested systems in the tropics, Trichoptera are often reported as critical biological features because of their high sensitivity to changes in oxygen as well as their use of forest leaves and litter for building cases (e.g. the case-building Trichopterans). Tropical forested systems present ideal habitat for the case-building Trichopterans because of the availability of suitable materials and because Trichopterans are shredders and collectors (Arimoro *et al.,* 2015). Because of their sensitivity to oxygen, food quality, and case-building materials, the Trichopterans are often among the first set of macroinvertebrates to reduce in abundance in response to urban pollution in forested river systems. Thus, their ecology and biology present them as a group of macroinvertebrates potentially vulnerable to urban pollution; this characteristic explains why the metric, Trichopteran abundance, proved sensitive in the present study and was thus integrated into the final MMI developed. Trichoptera abundance, Coleoptera+Hemiptera richness and Shannon diversity are well documented pollution-sensitive metrics (Mereta *et al.,* 2013, Edegbene *et al.,* 2019). Although %Chironomidae + Oligochaete was redundant, it was selected for integration into the urban-forestry multimetric index because of its importance in defining deteriorating water conditions, as their assemblage increased with anthropogenic influence (Melo *et al.,* 2015). The pollution indication of Chironomidae and Oligochaetes in flowing water ecosystems has received attention for some time; their dominance and composition in nutrient-rich systems with depleting dissolved oxygen is well reported (Odume *et al.,* 2014, Melo *et al.,* 2015); for instance, the genus *Chironomus* is an organically polluted waterbody dweller as their preponderance in deteriorating water conditions shows (Kleine & Trivinho-Strixino; 2005; Melo *et al.,* 2015).

The validation of the performance of the developed MMI-urban-forest with separate datasets revealed that the index performed better for MIS than for the HIS. The relatively good performance of the index for the MIS and HIS stations indicates the less impaired status of the urban-forestry impacted rivers used in this study. The developed MMI-urban-forest performed better in the wet season than in the dry season for both MIS and HIS.

As already mentioned, the probable reason for the better performance of the MMI-urban-forest in the wet season over the dry season could be attributed to increased flow velocity during the period of rainfall. Rains and floods increase flow velocity and water volume and could change microhabitats of macroinvertebrates in aquatic systems (Aura *et al.*, 2017). Therefore, the effects of urban pollution on forested riverine systems in the Niger Delta could be said to define the temporal variation of the MMI-urban-forest applicability, as has earlier been reported by Aura *et al.* (2017). Thus it can be argued that the difference in the multimetric index with regard to season is a pointer to the urbanisation of the selected rivers catchments used for the development of the MMI-urban-forest in the present study.

### 5.6 Conclusion

In this chapter, multimetric indices for urban (MMI-urban), urban-agricultural (MMI-urban-agric) and urban-forestry (MMI-urban-forest) pollution were developed for monitoring urban, urban-agricultural, and urban-forestry pollution effects in the Niger Delta. Five metrics representing abundance, composition, diversity, traits, and ecological preferences measures were integrated into the final urban multimetric index; five metrics (abundance, composition, richness, diversity and traits) were integrated into the urban-agricultural multimetric index, and four metrics (abundance, composition, richness and diversity) were integrated into the urban-forestry multimetric index.

For the MMI-urban, LIS stations showed that the metric performed very well, recording 83.3% correspondence with physico-chemically based classification. For the MIS, 75% correspondence between the index results and physico-chemically-based classification was recorded, while for the HIS, only 22.2% correspondence was recorded. In terms of the developed MMI-urban-agric, the index recorded 100% correspondence between the index results and the physico-chemically based classification for LIS, 42.9% for the MIS, and 77.5% for the HIS. For the MMI-urban-forest, the performance was 100% for both MIS and HIS stations.

The newly developed multimetric indices proved useful as biomonitoring tools for monitoring river health in the Niger Delta region of Nigeria and can thus be used by environmental managers and government officials for routine monitoring of rivers and streams. It is important to mention that no such regional indices exist in Nigeria, and the effort here can thus stimulate routine monitoring of rivers, which is currently lacking.

# CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 6.1 Introduction

Rivers and streams are common features of landscapes in the Niger Delta region, thus sustaining and managing them is critical for maintaining their integrity and ensuring the delivery of ecosystem services to society. However, as already argued, industrialisation and urbanisation around river catchments in Nigeria are a major threat to the integrity of rivers and streams within the Niger Delta region, causing deteriorating water quality and ecological impairment (Adekola & Mitchell, 2011). Urbanisation in the Niger Delta, for example, has resulted in elevated nutrient enrichment, accumulation of dissolved solids, depletion of dissolved oxygen, algal bloom, macroinvertebrate and fish kills, and excessive macrophyte growth in riverine systems within the region (Adekola & Mitchell, 2011; Jonathan *et al.*, 2016). Because of the negative effects of water quality deterioration occasioned by human-induced pressures such as agriculture, removal of natural forestry, urbanisation and industrialisation, developing appropriate biomonitoring tools is pertinent, as is exploring the effects of such pressures on the functional aspects of these systems via traits and ecological preferences. The objective of this chapter, then, is to present a critical, synthesised and integrative discussion of the results of this study and to reflect whether or not the set research objectives have been met.

## 6.2 Physico-chemical characterisation of the selected rivers in the Niger Delta, Nigeria

Chapter 3 of this thesis described the classification of the selected rivers in the Niger Delta in impact categories. Three impact categories, namely, least impacted stations (LIS), moderately impacted stations (MIS) and heavily impacted stations (HIS) were designated in the present study. The categorisation of the rivers was based on the different three land uses delineated in the present study. The three land use types were: urban, urban-agriculture and urban-forestry.

Urbanisation and agricultural activities around river catchments are a source of concern as they alter the ecological balance of freshwater bodies, most especially in sub-Saharan Africa (Pariente, 2017). For the urban and urban-agricultural rivers, most of the HIS were strongly correlated with increased nutrient, $BOD_5$ and EC (Figures 3.1 and 3.2). Of the 11 stations classified for the urban-dominated riverine systems, five of the stations were categorised as HIS (Table 3.1), and for the urban-agricultural rivers, eight of the 17 stations were categorised as HIS (Table 3.2). Of the 20 stations delineated in the urban-forested rivers, six stations – Benin River station 1 (Be1), Eriora River station 4 (Er4), Ossiomo River stations 1 and 2 (OS1 and Os2) and Umaluku River stations 3 and 4 (Um3 and Um4) – were strongly positively correlated with biochemical oxygen demand, nitrate, phosphate and water temperature (Figure 3.3). The result signifies that pollution indicators, that is, biochemical oxygen demand and nutrients, are determinants of the impact of urbanisation in forested river systems in the Niger Delta.

Physico-chemical monitoring of riverine systems remains a critical pillar of monitoring and managing the health of river systems; its criticality is based on the recognition that it provides insights into chemical processes such as speciation, transport, sources, and the kinds of stressors in riverine systems. In many jurisdictions, regulatory guidelines and standards for monitoring and managing riverine systems are developed based on the physico-chemical approach. In the present study, the physico-chemical approach was used to organise the stations along a gradient of potential impact, with the stations grouped into least impacted stations (LIS), moderately impacted stations (MIS), and highly impacted stations (HIS) following an approach developed by Murphy *et al.* (2013) and Odume *et al.* (2016).

The approach followed in this study made possible the evaluation of the extent to which rivers and streams in the Niger Delta have been degraded. For example, of the 11 stations within urban catchments, four were moderately impacted and five were heavily impacted (Table 3.1). Only two could be considered least impacted, suggesting that urgent measures need to be taken to revise the current trajectories of urban rivers within the region. Most of the MIS and HIS within rivers in urban catchments were characterised by relatively high nutrients, EC, and BOD, as well as flow velocity. These observed features are indicative of the so-called urban stream syndrome, which describes a state of persistent degradation of urban streams (Paul & Meyer, 2001; Meyer *et al.*, 2005). Overall, the approach developed by this research would allow action to be taken for management purposes, contributing to sustaining the health and integrity of rivers within the Niger Delta.

### 6.3 Trait-based biomonitoring – the use of traits and ecological preferences in freshwater biomonitoring

In Nigeria, biomonitoring of freshwater bodies is based on physico-chemical variables and their relationship with macroinvertebrate taxa distribution, abundance, and diversity (Adakole & Anunne, 2003; Ojutiku & Kolo, 2011). Because of the gap in developing appropriate tools to biomonitor freshwater bodies in Nigeria, Chapter 4 was devoted to developing a trait-based biomonitoring approach (TBA) for assessing urban, urban-agriculture and urban-forestry pollution in the Niger Delta. It was hypothesised that macroinvertebrate traits and ecological preferences would be differentially influenced by urban, agriculture and urban-forestry pollution. To discern the distribution pattern of traits and ecological preferences, an RLQ and fourth-corner analyses were conducted to select sensitive and tolerant traits in the urban, urban-agriculture and urban-forested rivers landscapes (Figures 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6). From the RLQ analyses, more traits and ecological preferences were strongly associated with the heavily impacted stations (HIS) compared to the least impacted stations (LIS) (Figures 4.1, 4.3 and 4.5). For example, five traits and ecological preferences were deemed sensitive to urban pollution. The traits and ecological preferences were: univoltinism, permanent attachment, crawling, swimming, and moderate sensitivity to oxygen depletion, while six traits and ecological preferences were deemed tolerant traits and ecological preferences to urban pollution: tegument/cutaneous respiration, cased/tubed protection, a preference for silty waters, bivoltinism, burrowing and a high tolerance of oxygen depletion (Table 4.2).

Traits and ecological preferences play a critical mediating role in determining the persistence of organisms in their external environments (Lange *et al.,* 2014; Berger *et al.,* 2018; Desrosiers *et al.,* 2019). The habitat template concept (HTC) and the habitat filtering concept (HFC) are the two primary ecological concepts informing the use of traits in freshwater biomonitoring, based on the understanding that the environment/habitat exert pressure on organisms that live in them, and in response, the organisms evolve adaptive features, usually in the form of traits; thus organisms with the right combination of traits survive, while those without the appropriate trait combination strive for survival (Townsend & Hildrew, 1994). Apart from the mechanistic and predictive insights the trait-based approach provides, it provides an indirect means for assessing the functionality of biological assemblages and ecosystems (Townsend & Hildrew, 1994). For example, food type, body size, and feeding modes are all traits and ecological preferences linked to ecosystem function such as top-down control, nutrient cycling and energy flow (Statzner & Beche, 2010; Pallottini *et al.,* 2017). Given that traits play a critical role in determining organisms' persistence in their habitat, exploring how specific pressures influence their distribution is an important ecological and management enterprise for sustaining riverine ecosystems. Chapter 4 of this study examined the influence of urban, urban-agriculture and urban-forestry pollution in riverine systems. The results showed that these stressors differentially influence the pattern of distribution of traits and ecological preferences. For example, urban pollution selected small body size. Body size is a critical adaptive feature of organisms, determining internal metabolism and the size of food consumed, thus serving to constrain other important traits (Serra *et al.,* 2017; Castro *et al.,* 2018). The small body size selected by urban pollution, for example, is predicted by the HTC, as small body size is often associated with rapid reproduction, many offspring per reproductive event, and agility, enabling the organisms to move into patches of refuge in polluted streams. The finding in the present study of the link between body sizes as a response to pollution impacts is an important step forward that can be used in routine monitoring of riverine systems subjected to urban, agricultural and urbanisation impacts on forested rivers in the Niger Delta. Thus, body sizes can serve as a yardstick to assess the level of perturbation of riverine systems. Sections 4.5.1, 4.5.2 and 4.5.3 of Chapter 4 provide a detailed discussion of the implications of traits and ecological preferences deemed sensitive to and tolerant of pollution in urban, urban-agriculture and urban-forested river landscapes in the Niger Delta, Nigeria.

### 6.4 Developing multimetric indices as an approach to freshwater biomonitoring

Multimetric indices are robust biomonitoring techniques as they take into account a combination of different metrics and measures (Bonada *et al.,* 2006). Macroinvertebrate multimetric indices play an important role in determining the extent of pollution to which riverine systems are subjected as the approach accounts for different taxonomic groups with regard to their level of sensitivity and tolerance in an ecosystem (Baptista *et al.*, 2007; Shull *et al.*, 2019).

Chapter 5 of this thesis was devoted to developing macroinvertebrate-based multimetric indices for urban, urban-agriculture, and urban-forested rivers. The multimetric indices were developed using family-level taxonomy, and traits and ecological preferences. The complementary integration of taxonomy, and trait and ecological preferences at family-level into the multimetric indices employed in Chapter 5 strengthens the applicability potential of the developed multimetric indices over multimetric indices based on the macroinvertebrate-based taxonomic approach only. The approach employed in this study proved effective as the traits and ecological preferences were integrated into the urban and urban-agriculture multimetric indices. For example, five metrics in four measures in the categories of abundance, composition, diversity, and traits were integrated into the MMI-urban (Table 5.3), and five metrics in five measures in the categories of abundance, composition, richness, diversity, and traits were integrated into the MMI-urban-agric (Table 5.6). The integrated metrics measures in the present study are well documented for the development of multimetric indices globally (Baptista *et al.,* 2013; Lu *et al.,* 2019; Gieswein *et al.,* 2019, Shull *et al.,* 2019), with the exception of the trait measures that were included in the present study. This inclusion makes the trait-based approach devised in the present study novel in the field of biomonitoring in sub-Saharan Africa. For instance, very large body size was integrated into the MMI-urban, and large body size has proved sensitive to pollution (Townsend & Hildrew, 1994; Castro *et al.,* 2018). The very large body size trait measure was the only non-redundant metric among the metrics integrated into the MMI-urban. In the findings, very large body size satisfactorily discriminated LIS from MIS and HIS, and was also seasonally stable and non-redundant, confirming the importance of the trait and ecological preference metrics component in developing multimetric indices for biomonitoring river health of freshwater systems in the Niger Delta. This accord with the suggestion in Chapter 4 of this thesis of large body-sized organisms for biomonitoring of freshwater systems in the Niger Delta.

As well as the usefulness of the TBA in developing multimetric indices in this study, the abundance, composition, richness and diversity measures were also well represented in the integrated metrics; these metrics are widely applicable in biomonitoring polluted urban and industrial riverine systems (Barbour *et al.,* 1999; Odume *et al.,* 2012; Edegbene *et al.,* 2019).

The significance of the discriminatory potential of abundance, composition, richness and diversity measures in the present study is that most of the metrics in these measures are sensitive to pollution. Similar studies have reported the integration of abundance, composition, richness and diversity measures into multimetric indices due to their sensitivity, seasonal stability and non-redundancy (Baptista *et al.,* 2007; Mereta *et al.,* 2013; Aura *et al.,* 2017). For example, the Ephemeroptera, Plecoptera and Tichoptera are known to respond negatively to deteriorating water conditions (Bonada *et al.,* 2006; Golfieri *et al.,* 2018). The discriminatory potential, seasonal stability and non-redundancy of the EPT in this study confirmed their usefulness in biomonitoring freshwater systems. Details of the significance of abundance, composition, richness and diversity metrics measures in biomonitoring river health is fully discussed in Chapter 5 of this thesis.

Seasonally, the developed indices performed better in the wet season than the dry season. For example, the better performance of the MMI-urban-forest in the wet rather than in the dry season was discovered during the validation and application of the MMI-urban-forest, and was attributed to increased run-off as a result of heavy rainfall in the studied area. Similarly, Speak *et* *al.* (2013) earlier reported that increased run-off due to precipitation could lead to pollution of rivers.

### 6.5 Application and management implications of the study outcomes in relation to riverine systems in Nigeria

A critical motivation for this study was to ensure that the study outcomes not only deepen understanding of the ecological processes of rivers subject to human-induced stressors in the Niger Delta, but also stimulate and contribute to effective monitoring of riverine systems in order to ensure their long-term sustainability. Earlier, it was argued that most biological indices applied in freshwater biomonitoring are general in nature such as the Shannon, Margalef and Simpson indices, and that specific regional multimetric indices and an approach based on macroinvertebate traits and ecological preference have not be developed for rivers and streams in the Niger Delta region. Further,  current monitoring tools are based mainly on physico-chemical variables , and even so, only occasional monitoring is undertaken in response to specific events, such as the need for an EIA (Environmental Impact Assessment), or oil spills. But physico-chemical monitoring alone is limited for several reasons already argued in Chapter 1. It is hoped that the developed biomonitoring tools in this study will be taken up by the relevant agencies and incorporated into EIA requirements, and that these tools will stimulate the need for routine monitoring of riverine systems as occurs elsewhere, such as in South Africa (Dickens & Graham, 2002), the United States of America (Shull *et al*., 2019) and Europe (Hering *et al.*, 2006). To be successful in this regard, a number of measures are required, which are briefly listed below.

- Training and education about the relevance of freshwater biomonitoring to sustainable management of water resources. Personnel in the Federal Ministries of Environment and

Water Resources should be trained and educated in the importance of using biomonitoring tools such as those developed in this study. This training will broaden their regulatory capacity for assessing the pollution impact on the Nigeria environment.

- Inter-agency collaboration and co-operation. Co-operation between agencies of government in charge of regulating the activities of industries and other multi-national organisations in Nigeria's environment is necessary. Duties of agencies of government that overlap should be streamlined so that every agency recognises their regulating boundaries as far as Nigeria's environment is concerned.
- Further development of biomonitoring tools to include other regions of Nigeria. Nigeria is divided into five ecological regions, of which Niger Delta ecoregion is one, and on which the present study was focused. Biomonitoring tools developed in this study should be replicated for the remaining four ecoregions and a comprehensive national biomonitoring protocol should also be developed for Nigeria, as is already implemented in developed countries in Europe and North America.
- Setting up routine programmes for physical, chemical and biological monitoring of riverine systems. Routine monitoring programmes that take into consideration the physical, chemical and biological communities for effective management and sustainability of the freshwater resources are essential to the river health of freshwater systems in Nigeria.

## 6.6 Study contribution to the science and practice of freshwater biomonitoring

The present study was able to contribute the following to the science and practice of freshwater biomonitoring:

- Phsyico-chemistry was used to categorise stations into impact categories namely; least impacted stations (LIS), moderately impacted stations (MIS) and heavily impacted stations (HIS).
- Stressor based-specific traits and ecological preferences were developed for three land use types namely urban, urban-agriculture abd urban-forestry.
- Signature traits and ecological preferences sensitive to and tolerant of specific and combined key stresors were identified in the course of this study. This has advanced the science of biomonitoring globally and in the Afrotropics particularly where study of this kind is sparse.
- An attempt was made to develop multimetric indices in specific and combined key stressors as against the popular development of multimetric indices using combined stressors.

## 6.7 Conclusion and Limitations

This study makes an important contribution to understanding the ecology of riverine systems in the Niger Delta, particularly those systems subject to urban stresses, agricultural activities and urban pollution in forested systems. For example, the study shed light on the differential effects of multiple stressors on macroinvertebrate traits and ecological preferences, as well as their integration into multimetric indices. The present study thus makes an important contribution to the science and practice of biomonitoring in Nigeria, where such studies are sparse. The combination of the three approaches deployed in the study, that is, physico-chemistry, the trait-based approach and multimetric indices, was useful in advancing the science and practice of biomonitoring. For example, the physico-chemical approach provides an organising framework for the classification of the stations in terms of their exposure to impacts, while the trait-based approach proved useful in discerning the potential effects of specific pressures on the functional ecology of macroinvertebrates. The developed multimetric indices serve as practical tools that can stimulate sustainable management of water resources within the region. However, a critical limitation of the entire study is that all biological tools were developed at the family-level taxonomic resolution because of the lack of taxonomic keys for macroinvertebrate fauna of Nigerian riverine systems. Even though family-level taxonomy is more suitable for rapid application among resource managers who may not always be ecologists, species-level tools have been noted as being more useful from an ecological point of view. Nevertheless, this study makes an important contribution in advancing riverine ecological studies in the Niger Delta, and Nigeria as a whole.

### 6.8 Recommendations for future study and management

Based on the results of the present study, the following are recommended for future study and management:

- The lack of Afrotropical macroinvertebrate identification guides is a serious concern; much effort should be made by the appropriate body to train ecologists in macroinvertebrate taxonomy and trait identification.
- The trait-based approach cannot be fully realised in the Afrotropical region if the life history of organisms is not well known, so more research needs to be conducted with regard to life history as life history gives critical information on an organism's reproduction.

- There is need to compile a macroinvertebrate database at family, genus and species levels to facilitate the identification and probable development of more accurate biomonitoring tools in the Afrotropical region.
- More research into developing biomonitoring tools using other biota, for example, fish, plankton and birds, is recommended.
- More research on the complementary use of taxonomic and trait-based approaches in assessing health conditions of riverine systems in Nigeria is recommended.

## REFERENCES


Aazami, J., Sari, A.E., Abdoli, A., Sohrabi, H. and Van den Brink, P. J. (2015). Assessment of ecological quality of the Tajan River in Iran using a multimetric macroinvertebrate index and species traits. *Environmental Management* 56, 260 – 269.

Abam, T.K.S. (2016). Engineering geology of the Niger Delta. *Journal of Earth Sciences and Geotechnical Engineering* 6(3), 65-89.

Adakole, J.A. and Anunne, P.A. (2003). Benthic macroinvertebrates as indicators of environmental quality of an urban stream in Zaria, Northern Nigeria. *Journal of Aquatic Sciences* 18(2), 85 -92.

Adekola, O. and Mitchell, G. (2011). The Niger Delta wetlands: threats to ecosystem services, their importance to dependent communities and possible management measures. *International Journal of Biodiversity Science, Ecosystem Service and Management* 7, 50–68.

Aguiar, F.C., Feio, M.J. and Ferreira, M.T. (2011). Choosing the best method for stream bioassessment using macrophyte communities: indices and predictive models. *Ecological indicators* 11, 379 - 388.

Akamagwuna, F.C. (2018). Taxonomic and trait-based responses of the Orders Ephemeroptera, Plecoptera, Odonata, and Trichoptera (EPOT) to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. M.Sc. thesis, Rhodes University, South Africa, 218Pp.

Akamagwuna, F.C., Mensah, P.K., Nnadozie, C. F. and Odume, O.N. (2019). Traits-based responses of Ephemeroptera, Plecoptera and Trichoptera to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. *River Research and Applications* 35(7), 999 – 1012.

Akpokodje, EG (1989). Preliminary Studies on the geotechnical characteristics of the Niger Delta subsoil. *Engineering Geology* 26, 247–257.

Allan, J.D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology and Systematics* 35, 257–284.

Allen, J. R. L. (1965). Late Quatenary Niger Delta and adjacent areas: sedimentary environment and lithofacies. *American Association of Petroleum Geologists* 49, 549-600.

APHA, (1995). Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Arimoro, F.O. (2017). Macroinvertebrates and physico-chemical variables of selected rivers in the Niger Delta area of Nigeria. An unpublished database, Department of Animal Biology, Federal University of Technology, Minna, Nigeria.

Arimoro, F.O. and Ikomi, R.B. (2008). Response of macroinvertebrate communities to abattoir wastes and other anthropogenic activities in a municipal stream in the Niger Delta, Nigeria. *Environmentalist* 28 85 - 98.

Arimoro, F.O. and Muller, W.J. (2010): Mayfly (Insect: Ephemeroptera) community structure as an indicator of the ecological status of a stream in Niger Delta, Nigeria. *Environmental Monitoring Assessment*, 165, 581-594.

Arimoro, F.O. Odume, O.N., Uhunoma, S.I. and Edegbene, A.O. (2015). Anthropogenic impact on water chemistry and benthic macroinvertebrate associated changes in a southern Nigeria stream. *Environtal Monioring and Assessment* 187(2), 1 – 14.

Arimoro, F.O., Ikomi, R.B., Nwadukwe, F.O., Eruotor, O.D. and Edegbene, A.O. (2014). Fluctuating salinity levels and increasing pollution gradient on fish community structure and trophic levels in a small creek in the Niger Delta, Nigeria. *International Aquatic Research* 6(4), 187 – 202.

Aura, C.M., Kimani, E., Musa, S., Kundu, R. and Njiru, J.M. (2017). Spatio-temporal macroinvertebrate multi-index of biotic integrity (MMiBI) for a coastal river basin: A case study of River Tana, Kenya. *Ecohydrology & Hydrobiology* 17, 113–124.

Baptista, D.F., Buss, D.F., Egler, M., Giovanelli, A., Silveira, M.P. and Nessimian, J.L. (2007). A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. *Hydrobiologia* 575, 83–94.

Baptista, D.F., deSouza, R. S.G., Viera, C. A., Mugnai, R., Souza, A.S. and de Oliveira, R.B.S. (2011). Multimetric index for assessing ecological condition of running waters in the upper reaches of the Piabanha-Paquequer-Preto Basin, Rio de Janeiro, Brazil. *Zoologia* 5, 619-628.

Baptista, D.F., Henriques-Oliveira, A.L., Oliveira, R.B.S., Mugnai, R., Nessimian, J.L. and Buss, D.F. (2013). Development of a benthic multimetric index for Serra da Bocaina bioregion in Southeast Brazil. *Brazillian Journal of Biology* 73(3), 573-583.

Barber-James, H.M., Gattolliat, J., Sartori, M. and Hubbard, M.D. (2008). Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. *Hydrobiologia* 595, 339 – 350.

Barbour, M.T., Gerritsen, J., Griffith, G.E., Frydenbourg, R., McCarron, E., White, J.S. and Bastian, M.L. (1996). A framework for biological criteria for Florida streams using benthic macroinvertebrates. *Journal of the North American Benthological Society 15*, 185-211.

Barbour, M.T., Gerritsen, J., Synder, B.D. and Stribling, J.B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates and Fish. Washington, D.C., U.S. Environmental Protection Agency, Office of Water, EPA 841-B-99-002, 2nd ed.

Berger, E., Haase, P., Schafer, R.B. and Sundermann, A. (2018). Towards stressor-specific macroinvertebrate indices: which traits and taxonomic groups are associated with vulnerable and tolerant taxa? *Science of the Total Environment* 619, 144 – 154.

Bird, M. (2010). Aquatic invertebrates as indicators of human impacts in South African Wetlands. Wetlands Health and Importance Research Programme (WRC Project No.K5/1584).

Bonada, N. and Doledec, S. (2011). Do Mediterranean genera not included in Tachet *et al.*, 2002 have Mediterranean trait characteristics? *Limnetica* 30, 129-142.

Bonada, N., Prat, N., Resh, V.H. and Statzner, B. (2006). Development in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology* 51, 495 – 523.

Bringezur, S., Schutz, H., Pengue, W., Obrien, M., Garcia, F., Sims, R., Howarth, R., Kauppi, I., Swilling, M. and Herrick, J. (2014). Assessing global land use: balancing consumption with sustainability supply. A report of the working group on land and soils of the international resource panel.

Buendia, C., Gibbins, C.N., Vericat, D., Batalla, R.J. and Douglas, A. (2013). Detecting the structural and functional impacts of fine sediment on stream invertebrates. *Ecological Indicators* 38, 351 – 367.

Carmago, J.A. (2017). Multimetric assessment of macroinvertebrate responses to mitigation measures in a dammed and polluted river of Central Spain. *Ecological Indicators* 83, 356 – 367.

Castro, D.M.P., Dolédec, S. and Callisto, M. (2018). Land cover disturbance homogenizes aquatic insect functional structure in neotropical savanna streams. *Ecological Indicators* 84, 573–582.

Charvet, S., Kosmala, A. and Statzner, B. (1998). Biomonitoring through biological traits of benthic macroinvertebrates: perspectives for a general tool in stream management. *Archaecological Hydrobiology* 142, 415- 432.

Chevenet, F., Dolédec, S. and Chessel, D. (1994). A fuzzy coding approach for analysis of longterm ecological data. *Freshwater Biology* 31, 295 – 309.

Chirwa, E.R. and Chilima, L. (2017). Use of benthic macroinvertebrate indices as bioindication of ecosystem health for the detection of degraded landscape in Malawi. *International Journal of Agriculture, Forestry and Fisheries* 5(6), 128-134.

Chowdhury, G.W., Gallardo, B. and Aldridge, D.C. (2016). Development and testing of a biotic index to assess the ecological quality of lakes in Bangladesh. *Hydrobiologia* 765, 55-69.

Culp, J.M., Armanini, D.G., Dunbar, M.J., Orlofske, J.M., Poff, N.L., Pollard, A.I., Yates, A.G. and Hose, G.C. (2011). Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. *Integrated Environmental Assessment and Management* 7 (2), 187 – 197.

Dallas, H.F., Lowe, S., Kennedy, M.P., Saili, K. and Murphy, K.J. (2018). Zambian invertebrate scoring system (ZISS): A macroinvertebrate-based biotic index for rapid bioassessment of southern tropical African river systems. *African Journal of Aquatic Science* 43(4), 325 – 344.

Davies, P.J., Wright, I.A., Findlay, S.J., Jonasson, O.J. and Burgin, S. (2010). Impact of urban development on aquatic macroinvertebrates in South Eastern Australia: degradation of in-stream habitats and comparison with non-urban streams. *Aquatic Ecology* 44, 685 – 700.

Day, J.A., Harrison, A.D. and De Moor, I.J. (2003). Guides to the freshwater invertebrates of Southern Africa: Diptera. Water Research Commission, Pretoria.

de Moor, I.J., Day, J.A. and de Moor, F.C. (2003). Guides to the Freshwater Invertebrates of Southern Africa. Volume 7: Insecta I. Ephemeroptera, Odonata and Plecoptera. Water Research Commission, Report No. TT207/03.

Department of Water Affairs and Forestry (DWAF) (2008). National Aquatic Ecosystem Health Monitoring Programme (NAEHMP): River Health Programme (RHP) Implementation Manual. Version 2. ISBN No. 978-0-621- 383343-0, Department of Water Affairs and Forestry, Pretoria, South Africa.

Desrosiers, M., Usseglio-Polatera, P., Archaimbault, V., Larras, F. Methot, G. and Pinel-Alloul, B. (2019). Assessing anthropogenic pressure in the St. Lawrence River using traits of benthic macroinvertebrates. *Science of the Total Environment* 649, 233 – 246.

Dickens, C.W.S. and Graham, P.M. (2002). The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science* 27, 1 - 10.

Ding, N., Yang, W., Zhou, Y., Gonzalez-Bergonzoni, I., Zhang, J., Chen, K., Vidal, N., Jeppesen, E., Liu, Z. and Wang, B. (2017). Different responses of functional traits and diversity of stream macroinvertebrates to environmental and spatial factors in the Xishuangbanna watershed of the upper Mekong River Basin, China. *Science of the Total Environment* 574, 288 – 299.

Dolédec, S. and Statzner, B. (2008). Invertebrate traits for the biomonitoring of large European rivers: an assessment of specific types of human impact. *Freshwater Biology* 53, 617 – 634.

Dolédec, S., Chessel, D., ter Braak, C. J. F. and Champely, S. (1996). Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics* 3(2), 143–166.

Dolédec, S., Phillips, N., Scarsbrook, M., Riley, R. H., and Townsend, C. R. (2006). Comparison of structural and functional approaches to determining land use effects on grassland stream invertebrate communities. *Journal of the North American Benthological Society 25*(1), 44–60.

Dray, S. (2013). A tutorial to perform fourth-corner and RLQ analyses in R. Accessed 22nd February, 2019. Pp 1-11.

Dray, S. and Dufour, A. B. (2007). The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22, 1–20.

Dray, S., Choler, P., Doledec, S., Peres-Neto, P.R., Thuiller, W., Pavoine, S. and ter Braak, C.J.F. (2014). Combining the fourth corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology* 95, 14 -21.

Edegbene, A.O. and Arimoro, F.O. (2012). Ecological Status of Owan River, Southern Nigeria Using Aquatic Insects as Bioindicators. *Journal of Aquatic Sciences* 27, 99 -111.

Edegbene, A.O., Arimoro, F.O., Odoh, O. and Ogidiaka, E. (2015). Effect of anthropogenicity on the composition and diversity of aquatic insects of a municipal river in North Central Nigeria. *Biosciences Research in Today's World* 1, 69 – 80.

Edegbene, A.O., Elakhame, L.A., Arimoro, F.O., Osimen, E.C. and Odume, O.N. (2019). Development of Macroinvertebrates Multimetric Index for Ecological Evaluation of a River in North Central Nigeria. *Environmental Monitoring and Assessment* 191(5), 274. https://doi.org/10.1007/s10661-019-7438-8.

Edokpayi, C.A., Okenyi, J.C., Ogbeibu, A.E. and Osimen, E.C. (2000). The effects of human activities on the macrobenthos invertebrates of Ibiekuma stream, Ekpoma, Nigeria. *Bioscience Research Communications* 12(1), 79 – 87.

Egborge, A.B.M., Ezemonye, L.I. and Awoze, G.E. (2003). Macroinvertebrate fauna of Udu-Ughoevwen Wetlands, Southern Nigeria. *Journal of Aquatic Sciences* 18(1), 1 – 8.

Elbrecht, V., Beermann, A.J., Goessler, G., Neumann, J., Tollrian, R., Wlecklik, A., Piggott, J.J., Matthaei, C.D. and Leese, F. (2016). Multiple-stressor effects on stream invertebrates: a mesocosm experiment manipulating nutrients, fine sediment and flow velocity. *Freshwater Biology* 61, 362-375.

Emere, M.C. and Nasiru, C.E. (2007). Macroinvertebrates as indicators of the water quality of an urbanized stream, Kaduna, Nigeria. *Journal of Fisheries International* 2(2), 152 – 157.

Environmental Impact Assessment Act (1992). An Act set out to the general principles, procedure and methods to enable the prior consideration of environmental impact assessment on certain public and private projects- enacted by the National Assembly of the Federal Republic of Nigeria, 10th Day of December, 1992.

European Council (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. 72Pp.

FEPA (Federal Environmental Protection Agency) (1991). Guidelines and standards for environmental pollution control in Nigeria. Abuja: FEPA.

Fierro, P., Arismendi, I., Hughes, R.M., Valdovinos, C. and Jara-Flores, A. (2018). A benthic macroinvertebrate multimetric index for Chilean Mediterranean streams. *Ecological Indicators* 91, 13-23.

Fierro, P., Bertran, C., Hauenstein, E., Pena-Cortes, F., Vergara, C. Cerna, C. and Vargas-Chaoff, I. (2017). Effects of local land-use on riparian vegetation, water quality and the functional organisation of macroinvertebrate assemblages. *Science of the Total Environment* 609, 724-734.

Forio, M.A.E., Goethals, P.L.M., Lock, K., Asio, V., Bande, M. and Thas, O. (2018). Model-based analysis of the relationship between macroinvertebrate traits and environmental river conditions. *Environmental Modelling & Software* 106, 57-67.

Friberg, N., Bonada, N., Bradley, D. C., Dunbar, M. J., Edwards, F. K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M. and Woodward, G. (2011). Biomonitoring of Human Impacts in Freshwater Ecosystems. The Good, the Bad and the Ugly. *Advances in Ecological Research* 44, 2 -68.

Gieswein, A., Hering, D., Lorens and A.W. (2019). Development and validation of a macroinvertebrate-based biomonitoring tool to assess fine sediment impact in small mountain streams. *Science of the Total Environment* 652, 1290 -1301.

Golfieri, B., Surian, N. and Hardersen, S. (2018). Towards a more comprehensive assessment of river corridor conditions: A comparison between the morphological quality index and three biotic indices. *Ecological Indicators*, 84, 525 – 534.

Gordon, N.D., McMahon, T.A. and Finlayson, B.L. (1994). *Stream Hydrology, an introduction for Ecologists*. New York: John Wiley & Sons Ltd, 526Pp.

Guilpart, A., Roussel, J.M., Aubin, M., Caquet, T., Marle, M. and Le Bris, H. (2012). The use of bethic invertebrate community and water quality analyses to assess ecological consequences of fish farm effluents in rivers. *Ecological Indicators* 23, 356 – 365.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1), 9Pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm Accessed 1 July 2015

Hatt, B.E., Flecther, T.D., Walsh, C.J. and Taylor, S.L. (2004). The influence of urban density and drainage infrastructure on the concentrations and loads of pollutants in small streams. *Environmental Management* 34, 112 – 124.

Heino, J. (2013). The importance of metacommunity ecology for environmental assessment research in the freshwater realm. *Biological Review* 88, 166 – 178.

Helson, J.E. and Williams, D.D. (2013). Development of a macroinvertebrate multimetric index for the assessment of low-land streams in the neotropics. *Ecological Indicators* 29, 167 - 178.

Herbst, D.B., Cooper, S.D., Medhurst, R.B., Wiseman, S.W. and Hunsaker, C.T. (2018). A comparison of the taxonomic and trait structure of macroinvertebrate communities between the riffles and pools of montane headwater streams. *Hydrobiologia* 820, 115 – 133.

Hering, D., Feld, C.K., Moog, O. and Ofenbo, C.K. (2006). Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. *Hydrobiologia* 566, 311-324.

Huang, Q., Gao, J., Yin, H., Gao, Y., Zhao, J., Liu, L. and Huang, J. (2015). Development and application of benthic macroinvertebrate-based multimetric indices for the assessment of streams and rivers in the Taihu Basin, China. *Ecological Indicators* 48, 649-659.

Jonathan, D.T., Arimoro, F.O. and Haase, P. (2016). Exploring stream communities in a tropical biodiversity hotspot: biodiversity, regional occupancy, niche characteristics and environmental correlates. *Biodiversity Conservation* 25, 975-993.

Jones, J. I., Murphy, J. F., Collins, A. L., Sear, D. A., Naden, P. S., and Armitage, P. D. (2012). The Impact of Fine Sediment on macroinvertebrates. *River Research and Applications* 28(8), 1055–1071.

Jonnalagadda, S.B and Mhere, G. (2001). Water quality of the Odzi River in the Eastern Highlands of Zimbabwe. *Water Resource* 35, 2371 – 2376.

Kaaya, L.T., Day, J.A. and Dallas, H.F. (2015). Tanzania river scoring system (TARISS): a macroinvertebrate-based biotic index for rapid bioassessment or rivers. *African Journal of Aquatic Science* 40(2), 109-117.

Kamdem-Toham, A., D’Amico, J., Olson, D., Blom, A., Trowbridge, L., Burgess, N., Thieme, M., Abell, R., Carroll, R.W., Gartlan, S., Langrand, O., Mikala Mussavu, R., O’Hara, D. and Strand, H. (2006) A vision for biodiversity conservation in Central Africa: Biological priorities for conservation in the Guinean-Congolian Forest and Freshwater region. WWF-US/Central African Regional Program Office, Washington, DC, 113 Pp.

Karr, J. R. (1981). Assessment of biotic integrity using fish communities. *Fisheries* 6, 21 – 27.

Karr, J.R. (1991). Biological integrity: a long-neglected aspect of water resource management. *Ecological Application* 1, 66 – 84.

Kasangali, A., Chapman, L.J. and Balirwa, J. (2008). Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshwater Biology* 53, 681-697.

Katsiapi, M., Moustaka-Gouni, M. and Sommer, U. (2016). Assessing ecological water quality of freshwaters: Phycol-a new phytoplankton community index. *Ecological Information* 31, 22-29.

Kleine, P. and Trivinho-Strixino, S. (2005). Chironomidae and other aquatic macroinvertebrates of a first order stream: community response after habitat fragmentation. *Acta Limnologica Brasiliensia* 17, 81-90.

Krynak, E.M. and Yates, A.G. (2018). Benthic invertebrates taxonomic and trait associations with land use intensively managed watershed: implications for indicator identification. *Ecological Indicators* 93, 1050-1059.

Kuzmanovic, M., Doledec, S., deCatro-Catala, N., Ginebreda, A., Sabater, S., Munoz, I. and Barcelo, D. (2017). Environmental stressors as driver of the trait composition of benthic macroinvertebrates assemblages in polluted Iberian rivers. *Environmental Research* 156, 485-493.

Ladle, M. and Ladle, R.J. (1992). Life history patterns of river invertebrates. *Hydrobiologia* 248, 31 - 37.

Laini, A., Bolpagni, R., Cancellario, T., Guareschi, S. Racchetti, E. and Viaroli, P. (2018). Testing the response of macroinvertebrate communities and biomonitoring indices under multiple stressors in a lowland regulated river. *Ecological Indicators* 90, 47-53.

Lakew, A. and Moog, O. (2015). A multimetric index based on benthic macroinvretebrates for assessing the ecological status of streams and rivers in central and southeast highlands of Ethiopia. *Hydrobiologia* 751, 229-242.

Lamouroux, N., Dolédec, S. and Gayraud, S. (2004). Biological traits of stream macroinvertebrat e communities: effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society* 23(3), 449–466.

Lange, K., Townsend, C.R. and Matthaei, C.D. (2014). Can biological traits of stream invertebrates help disentangle the effects of multiple stressors in an agricultural catchment? *Freshwater Biology* 59, 2431–2446.

Lagrue, C., Kominoski, J., Danger, M., Baudoin, J., Lamothe, S., Lambrigot, D. and Lecerf, A. (2011). Experimental shading alters leaf litter breakdown in streams of contrasting riparian canopy cover. *Freshwater Biology* 56, 2059 – 2069.

Larsen, S., Pace, G. and Ormerod, S.J. (2011). Experimental effects of sediment deposition on the structure and function of macroinvertebrate assemblages in temperate streams. *River Research and Applications* 27(2), 257-267.

Lazorchak, J.M., Klemm, D.J. and Peck, D.V. (1998). Environmental monitoring and assessment program surface waters: field operations and methods manual for measuring the ecological condition of wadeable streams. EPA 620/R-94/004F. Washington , D.C. U.S. Environmental Protection Agency.

Lee, J.H. and Bang, K.W. (2000). Characterization of urban stormwater runoff. *Water Research* 34, 1773 – 1780.

Legendre, P. and Legendre, L. (2012). Numerical Ecology. Development in environmental modelling, 3rd edition. Vol.24, Elsevier science BV, Amsterdam 1006Pp.

Lenat, D.R. and Resh, V.H. (2001). Taxonomy and stream ecology: The benefits of genus-and species-level identifications. *Journal of North American Benthological Society* 20 (2), 287 - 298.

Lenat, D.R. and Resh, V.H. (2001). Taxonomy and stream ecology: The benefits of genus-and species-level identifications. *Journal of North American Benthological Society* 20 (2), 287 - 298.

Liess, M. and Von der Ohe, P.C. (2005). Analysing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry* 24 (4), 954 – 965.

Liess, M., Schafer, R.B. and Schriever, C.A. (2008). The footprint of pesticide stress in a communities-species traits reveal community effects of toxicants. *Science of the Total Environment* 406, 484-490.

Lu, K., Wu, H., Xue, Z., Lu, X. and Batzer, D.P. (2019). Development of a multi-metric index based on aquatic invertebrates to assess floodplain wetland condition. *Hydrobioloia* 827, 141-153.

Lugoli, F., Garmendia, M., Lehtinen, S., Kauppila, P., Moncheva, S., Revilla, M., Roselli, L., Slabakova, N., Valencia, V., Dromph, K.M. and Basset, A. (2012). Application of a new multi-metric phytoplankton index to the assessment of ecological status in marine and transitional waters. *Ecological Indicators* 23, 338-355.

Macedo, D.R., Hughes, R.M., Ferreira, W.R., Firmiano, K.R., Silva, D.R.O., Ligeiro, R., Kaufmann, P.R. and Callisto, M. (2016). Development of a benthic macroinvertebrate multimetric index (MMI) for Neotropical Savanna headwater streams. *Ecological Indicators* 64, 132-141.

Malherbe, W., van Vuren, J.H.J. and Wepener, V. (2018). The application of a macroinvertebrate indicator in Afrotropical regions for pesticide pollution. *Journal of Toxicology* ID 2581930, 6 pages. https://doi.org/10.1155/2018/2581930.

Mcgill, B. J., Enquist, B. J., Weiher, E. and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21(4), 178 – 185.

Melo, S., Stenert, C., Dalzochio, M.S and Maltchik, L. (2015). Development of a multimetric based on aquatic macroinvertebrate communities to assess water quality of rice fields in southern Brazil. *Hydrobiologia* 742, 1-14.

Mereta, S.T., Boets, P. De Meester, L. and Goethals, P.L.M.C. (2013). Development of multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in southwest Ethiopia. *Ecological Indicators* 29, 510 – 521.

Merritt, R.W. and Cummins, K.W. (1996). An introduction to the aquatic insects of North America. Kendall Hunt, St. Louis.

Meyer, j.l., Paul, M.J. and taulbee, W.K. (2005). Stream ecosystem function in urbanising landscapes. *Journal of North America Benthological Society* 24, 602 – 612.

Milosevic, D., Stojanovic, K., Djurdjevic, A., Markovic, Z., ,Piperac, M.S., Zivic, M. and Zivic, I. (2018). The response of chironomid taxonomy- and functional trait-based metric to fish effluent pollution in lotic systems. *Environmental Pollution* 242, 1058 – 1066.

Moges, A., Beyene, A., Kelbessa, E., Mereta, S.T. and Ambelu, A. (2016). Development of a multimetric plant-based index of biotic integrity for assessing the ecological state of forested, urban and agricultural natural wetlands of Jimma Highlands, Ethiopia. *Ecological Indicators* 71, 208-217.

Monaghan, K. and Soares, A.M.V.M. (2012). Bringing new knowledge to an old problem: Building a biotic index from lotic macroinvertebrates traits. *Ecological Indicators* 20, 213 - 220.

Mondy, C.P. and Usseglio-Polatera, P. (2014). Using fuzzy-coded traits to elucidate the non-random role of anthropogenic stress in the functional homogenisation of invertebrate assemblages. *Freshwater Biology* 59, 584–600.

Mondy, C.P., Villeneuve, B., Archaimbault, V. and Usseglio-Polatera, P. (2012). A new macroinvertebrate-based multimetric index ($I_2M_2$) to evaluate ecological quality of French wadeable streams fulfilling the WFD demands: a taxonomical and trait approach. *Ecological Indicators* 18, 452-467.

Moss, B. (1998). Ecology of Freshwaters, Man and Medium, Past to Future, 3rd Edition. Oxford: Blackwell Science.

Murphy, J.F., Davy-Bowker, J., McFarland, B. and Ormerod, S.J. (2013). A diagnostic biotic index for assessing acidity in sensitive streams in Britain. *Ecological Indicators* 24, 562–572.

Murphy, J.F., Jones, J.I., Arnold, A., Duerdoth, C.P., Pretty, J.L., Naden, P.S., Sear, D.A. and Collins, A.L. (2017). Can macroinvertebrate biological traits indicate fine-grained sediment conditions in streams? *River Research and Applications* 33, 1606 – 1617.

National Environmental Standards and Regulations Enforcement Agency (NESREA)-Establishment Act (2007). An Act to provide for the establishment of National Environmental Standards and Regulations Enforcement Agency charged with responsibility for the protection and development of the environment in Nigeria and for related matters- enacted by the National Assembly of the Federal Republic of Nigeria, 30th Day of July, 2007.

National Oil Spill Detection and Response Agency (NOSDRA)-Establishment Act (2006). An Act to provide for the establishment of the National Oil Spill Detection and Response Agency and related matters-enacted by the National Assembly of the Federal Republic of Nigeria, 18th Day of October, 2006.

Ndaruga, A.M., George, G.N., Nathan, N.G., Wamicha, W.N. (2004). Impact of water quality on macroinvertebrate assemblages along a tropical stream in Kenya. *Africa Journal of Ecology* 42, 208-216.

Ntislidou, C., Lazaridou, M., Tsiaoussi, V. and Bobori, D.C. (2018). A new multimetric index for the ecological assessment of Mediterranean lakes. *Ecological Indicators* 93, 1020-1033.

Odountan, O.H., de Bisthoven, L.J., Abou, Y. and Eggertmont, H. (2019). Biomonitoring of lakes using macroinvertebrates: recommended indices and metrics for use in West Africa and developing countries. *Hydrobiologia* 826, 1-23.

Odume, O. N. (2014). An evaluation of macroinvertebrate-based biomonitoring and ecotoxicological assessments of deteriorating environmental water quality in the Swartkops River, South Africa. Ph.D thesis, Rhodes University, South Africa, 396Pp.

Odume, O.N and Muller, W.J. (2011). Diversity and structure of Chironomidae communities in relation to water quality differences in the Swartkops River. *Journal of Physics and Chemistry of the Earth* 36, 929 - 938.

Odume, O.N. (2020). Searching for urban pollution signature and sensitive macroinvertebrate traits and ecological preferences in a river in the Eastern Cape of South Africa. *Ecological Indicators* 108(2020) 105759, https://doi.org/10.1016/j.ecolind.2019.105759.

Odume, O.N., Muller, W.J., Arimoro, F.O. and Palmer, C.G. (2012). The impact of water quality deterioration on macroinvertebrate communities in the Swartkops River, South Africa: a multimetric approach. *African Journal of Aquatic Science* 37, 191 – 200.

Odume, O.N., Ntloko P., Akamagwuana, F.C., Dallas, H.M. and Barber-James, H. (2018b). A trait database for South African macroinvertebrates. Unpublished WRC Report, Pretoria.

Odume, O.N., Ntokolo, P., Akamagwuna, F.C., Dallas, H.M. and Barber-James, H. (2018a). Development of macroinvertebrate trait-based approach for assessing and managing ecosystem health in South African Rivers- incorporating a case study of the Tsitsa River and its triturates, Eastern Cape. WRC, No.: K1/7157.

Odume, O.N., Palmer, C.G., Arimoro, F.O. and Mensah, P.K. (2014). Patterns of chironomid body-size distribution in an effluent-impacted river in the Eastern Cape, South Africa. *African Journal of Aquatic Science* 39(4), 377 – 386.

Odume, O.N., Palmer, C.G., Arimoro, F.O. and Mensah, P.K. (2016). Chironomid assemblage structure and morphological response topollution in an effluent-impacted river, Eastern Cape, South Africa. *Ecological Indicators*, 67, 391 – 402.

Ofenböck, T., Moog, O., Gerritsen, J. and Barbour, M. (2004). A stressor specific multimetric approach for monitoring running waters in Austria using benthic macro-invertebrates *Hydrobiologia* 516, 251 - 268.

Ogbeibu, A.E. (2005). Biostatistics: a practical approach to research and data handling. Benin City: Mindex publishing, 264Pp

Ogbeibu, A.E. and Oribhabor, B.J. (2002). Ecological impact of river impoundment using benthic macroinvertebrates as indicators. *Water Research* 36, 2427-2436.

Ogbogu, S.S. and Oladije, S.A. (2002). Effect of sewage oxidation pond effluent on macroinvertebrate communities of a tropical forest stream, Nigeria. *Journal of Aquatic Sciences* 17(1), 22 -27.

Ogren, S.A. and King, D.K. (2008). The effect of karge woody debris on macroinvertebrate communities and epilithon detritus composition in a channelized headwater stream. *Journal of Freshwater Ecology* 23, 65-77.

O'Hare, M.T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I.D.M., Lázár, A.N., Sinclair, R., Wade, A.J. and Bowes, M.J. (2018). Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. *Frontiers in Plant Science* 9, 451. Doi: 10.3389/fpls.2018.00451.

Ojutiku, R.O. and Kolo, R.J. (2011). Temporal and Spatial variations in some physico-chemical parameters of River Chanchaga, Niger State, Nigeria. *Journal of Applied Bioscience* 47, 3242 – 3255.

Oksanen, J., Blanchet, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Steven, M.H.M., Szoecs, E. and Wagner, H. (2019). Vegan: Community Ecology Package. Retrieved 11/02/2019 from https://cran.r-project.org,https://github.com/veganders/vegan. Published 04-02-2019, 11:50:04 UTC.

Olden, J.D., Poff, N.L., Douglas, M.E. and Fausch, K.D. (2004). Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution* 19, 18-24.

Olomukoro, J.O. and Ezemonye, L.I.N. (2007). Assessment of the macroinvertebrate fauna of rivers in southern Nigeria. *African Zoology* 42(1), 1 -11.

Pallottini, M., Cappelletti, D., Fabrizi, A., Gaino, E., Goretti, E., Selvaggi, R. and Cereghino, R. (2017). Macroinvertebrate functional trait responses to chemical pollution in agricultural landscapes. *River Research and Applications* 33, 505 – 513.

Paul, M.J. and Meyer, J.L. (2001). Streams in the urban landscape. *Annual Review of Ecology and Systematics* 32, 333-365.

Parienté, W. (2017). Urbanisation in Sub-Saharan Africa and challenges of access to basic services. *Journal of Demographic Economics*, 83(1), 31–39.

Pena-Cortes, F., Pincheira-Ulbrich, J., Bertran, C., Tapia, J., Hauenstein, E., Fernandez, E. and Rozas, D. (2011). A study of the geographic distribution of swamp forest in the coastal zone of the Araucania Region, Chile. *Applied Geography* 31, 545 – 555.

Petriki, O., Lazaridou, M. and Bobori, D.C. (2017). A fish-based index for the assessment of the ecological quality of temperate lakes. *Ecological Indicators* 78, 556-565.

Poff, N.L., Olden, J.D., Vieira, N.K.M., Finn, D.S., Simmons, M.P. and Konddratieff, B.C. (2006). Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society* 25, 730-755.

Poff, N.L., Pyne, M.I., Bledsoe, B.P., Cuhaciyan, C.C. and Carisle, D.M. (2010). Developing linkages between species traits and multiscaled environmental variation to explore vulnerability of stream benthic communities to climate change. *Journal of North American Benthological Society* 29 (4), 1441 – 1458.

Pond, G.J., Bailey, J.E., Lowman, B.M. and Whitman, M.J. (2013). Calibration and validation of a regionally and seasonally stratified macroinvertebrate index for West Virginia wadeable streams. *Environmental Monitoring and Assessment* 185, 1515-1540.

Rizo-Patron, F., Kumar, A., Colton, M.B., Springer, M. and Trama, F.A. (2013). Macroinvertebrate communities as bioindicators of water quality in conventional and organic irrigated rice fields in Guanacaste, Costa Rica. *Ecological Indicators* 29, 68-78.

Rosenberg, D.M. and Resh, V.H. (Eds.) (1993). Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall one penn plaza, New York, NY 10119.

Rowntree, K.M.; Wadeson, R.A. and O'Keeffe, J. (2000). The development of a geomorphological classification system for the longitudinal zonation of South African rivers. *South African Goegraphical Journal* 82 (3), 163 – 172.

Roy, A.H., Freeman, M.C., Freeman, B.J., Wenger, S.J., Ensign, W.E. and Meyer, J.L. (2005). Investigating hydrological alteration as a mechanism of fish assemblage shifts in urbanising streams. *Journal of the North American Benthological Society* 24, 656 – 678.

Scheffer, M. and van Nes, E. H. (2007). Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia* 584, 455–466.

Schmera, D., Heino, J., Podani, J., Erős, T. and Dolédec, S. (2017). Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. *Hydrobiologia* 787(1), 27-44.

Schoolmaster, D.R., James, B.G., Schweiger, E.W. Glenn, R.G., Mitchell, R.M., Kathryn, M. and Little, A.M. (2013). An algorithmic and information-theoritical approach to multimetric index construction. Ecological Indicators 26, 14 – 23.

Sedell, J.R., Reeves, G.H., Hauer, F.R., Stanford, J.A., Hawkins, C.P. (1990). Role of refugia in recovery from distrubances: modern fragmented and disconnected river systems. *Environmental Management* 14 (5), 711 – 724.

Serra, R.Q.S., Graca, M.A.S., Doledec, S. and Feio, M.J. (2017). Chironomidae traits and life history strategies as indicators of anthropogenic disturbance. *Environmental Monitoring and Assessment* 189. Doi: 10.1007/s10661-017-6027-y.

Shi, X., Liu, J., You, X., Bao, K., Meng, B. and Chen, B. (2017). Evlaution of river habitat integrity based on benthic macroinvertebrate-based multi-metric model. *Ecological Modelling* 353, 63 – 76.

Short, K.C. and Stauble, A.J. (1967). Outline of the Geology of Niger Delta. *America Association of Petroleum Geologists Bulletin*, 51, 761 – 779.

Shull, D.R., Smith, Z.M., and Selckmann, G.M. (2019). Development of a benthic macroinvertebrate multimeric index for large semiwadeable rivers in the Mid-Atlantic region of the USA. *Environmental Monitoring and Assessment*, 191(1), 22. https://doi.org/10.1007/s10661-018-7153-x.

Speak, A.F., Rothwell, J.J., Lindley, S.J. and Smith, C.L. (2013). Rainwater runoff retention on an aged intensive green roof. *Science of the Total Environment* 461-462, 28-38.

Statzner, B. and Beche, L. (2010). Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology* 55, 80–199.

Statzner, B., Resh, V.H. and Doledec, S. (1994). Ecology of the Upper Rhone River: a test of habitat templet theories. *Special Issue of Freshwater Biology* 31, 253-554.

Stevenson, R.J., Zalack, J.T. and Wolin, J. (2013). A multimetric index of lake diatom condition based on surface-sediment assemblages. *Freshwater Science* 32, 1005-1025.

Suriano, M.T., Fonseca-Gessner, A.A., Roque, F.O. and Froehlich, C.G. (2011). Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic Forest, Brazil. *Environmental Monitoring and Assessment* 175, 87-101.

Tchakonte, S., Ajeagah, G.A., Camara, A.I., Diomande, D., Tchatcho, N.L.N. and Ngassam, P. (2015). Impact of urbanisation on aquatic insects assembalges in the coastal zone of Cameroon: the use of biotraits and indicator taxa to assess environmental pollution. *Hydrobiologia* 755, 123-144.

ter Braak, C.J.F. (1995). Ordination. In: Jongman, R.H.J, terBraak, C.J.F. and van Tongeren, O.F.R. (Eds). Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, UK: 91 - 173.

ter Braak, C.J.F. and Verdonschot, P.F.M. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences* 57 (3), 256-289.

Tomanova, S. and Usseglio-Polatera, P. (2007). Patterns of benthic community traits in neotropical streams: relationship to mesoscale spatial variability. *Fundamental and Applied Limnology* 170, 243–255.

Tomanova, S., Moya, N. and Oberdorff, T. (2008). Using macroinvertebrate biological traits for assessing biotic integrity of neotropical streams. *River Research and Applications* 24(9), 1230–1239.

Townsend C.R. and Hildrew A.G., (1994). Species traits in relation to a habitat templet for river systems. *Freshwater Biology* 31, 265-275.

Tsiaoussi, V., Mavromatic, E. and Kemitzoglou, D. (2017). Report on the development of the national method for the assessment of the ecological status of natural lakes in Greece, using he biological quality element “phytoplankton”. 1st revision. Greek Biotope/Wetland Centre and special secretariat for waters, Ministry of Environment. Thermi, Greece

Uluocha, N. and Okeke, I. (2004). Implications of wetlands degradation for water resources management: lessons from Nigeria. *Geojournal* 61, 151-154.

Umoh, S.G. (2008). The promise of wetland farming; evidence from Nigeria. *Agricultural Journal*, 3 107-112.

USEPA (2016). National rivers and streams assessment, 2008-2009: A collaborative study. EPA-841-R-16-007. U.S. Environmental Protection Agency, Washington, DC.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. and Cushing, C.E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37, 130 - 137.

Verberk, W. C. E. P., van Noordwijk, C. G. E. and Hildrew, A. G. (2013). Delivering on a promise: integrating species traits to transform descriptive community ecology into a predictive science. *Freshwater Science 32*(2), 531–547.

Verberk, W.C.E.P., Siepel, H. and Esselink, H. (2008). Life-history strategies in freshwater macroinvertebrates. *Freshwater Biology* 53, 1722-1738.

Violle, C., Navas, M., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. and Garnier, E. (2007). Let the concept of trait be functional! *Oikos* 116(5), 882 - 892.

Walley, W.J. and Hawkes, H.A. (1996). A computer-based reappraisal of the biological monitoring working party scores using data from the 1990 river quality survey of England and Wales. *Water Research* 30, 2086 – 2094.

Walsh, C.J., Jack, W.F., Cottingham, P.D., Groffman, P.M. and Morgan, R.P. (2005). The urban syndrome: current knowledge and the search for a cure. *Journal of North American Benthological Society* 24(3), 706-723.

Wang, B.X., Liu, D.X., Liu, S.R., Zhang, Y., Lu, D.Q., and Wang, L.Z. (2012). Impacts of urbanization on stream habitats andmacroinvertebrate communities in the tributaries of Qiantang River, China. *Hydrobiologia* 680, 39 - 51.

Wilby, R., Orr, H., Watts, G., Battarbee, R., Berry, P., Chadd, R., Dugdale, S., Dunbar, M., Elliott, J., Extence, C. Hannah, D., Holmes, N., Johnson, A., Knights, B., Milner, N.J., Ormerod, S., Solomon, D., Timlett, R.P. and Wood, P. (2010). Evidence needed to manage freshwater ecosystems in a changing climate: Turning adaptation principles into practice. *The Science of the Total Environment*. 408(19), 4150 - 4164.

Wilkes, M. A., Mckenzie, M., Murphy, J. F. and Chadd, R. P. (2017). Assessing the Mechanistic Basis for Fine Sediment Biomonitoring: Inconsistencies among the Literature, Traits and Indices. *River Research and Applications* 33, 1618 – 1629.

Wu, N., Schmaz, B. and Fohrer, N. (2012). Development and testing of a phytoplankton index of biotic integrity (P-IBI) for a German lowland river. *Ecological Indicators* 13, 158-167.

Xu, L., Li, H., Liang, X., Yao, Y., Zhou, L. and Cui, X. (2012). Water quality parameters response to temperature change in small shallow lakes. *Physics and Chemistry of the Earth*, Parts A/B/C 47–48, 128–134.

Youdeowei, P.O. and Nwankwoala, H.O. (2012). Preliminary evaluation of some engineering geological properties of soils in the New Yenagoa Town, Bayelsa State, Central Niger Delta. *Journal of Applied Science and Environmental Management* 16(3), 227-231.

Zamora-Muniz, C., Sainz-Cantero, C. E., Sanchez-Ortega, A. and Alba- Tercedor, J. (1995). Are biological indices BMPW and APT and their significance regarding water quality seasonally dependent? Factor explaining their variations. *Water Research* 29, 285–290.

Zervas, D., Tsiaoussi, V. and Tsiripidis, I. (2018). HeLM: a macrophyte-based method for monitoring and assessment of Greek lakes. *Environmental Monitoring and Assessment* 190, 326. http://dx.doi.org/10.1007/s10661-018-6708-1.

## APPENDICES

**Appendix A: Average physico-chemical variables collected in selected rivers in urban catchment during the study period (2008 – 2012). Abbreviations:** Rivers/Stations: (Wa1 (Warri River station 1), An1 (Anwai River station 1), An3 (Anwai River station 3), Ad3 (Adofi River station 3), Ol2 (Oleri River station 2), Et1 (Ethiope River station 1), Or3 (Orogodo River station 3), Ob3 (Obosh River station 3), Et2 (Ethiope River station 2), Og1 (Ogba River station 1), Og2 (Ogba River station 2).

| **Physico-chemical variables** | **Rivers/Stations** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wa1 | An1 | An3 | Ad3 | Ol2 | Et1 | Or3 | Ob3 | Et2 | Og1 | Og2 |
| Water Temperature ($^{o}$C) | 23.4 | 23.9 | 24.4 | 20.8 | 25.6 | 25.2 | 26.8 | 23.4 | 26.1 | 24.7 | 25.8 |
| Depth (m) | 0.96 | 0.48 | 0.68 | 0.78 | 0.71 | 0.21 | 0.48 | 0.66 | 0.21 | 0.86 | 0.41 |
| Flow velocity (ms$^{-1}$) | 0.18 | 0.28 | 0.28 | 0.19 | 0.19 | 0.2 | 0.39 | 0.2 | 0.2 | 0.17 | 0.49 |
| Conductivity (µScm$^{-1}$) | 8.3 | 16.5 | 18.6 | 19.9 | 22.3 | 25.9 | 26.9 | 35.2 | 38.3 | 51 | 46 |
| Dissolved oxygen (mgl$^{-1}$) | 4.8 | 5.7 | 5.2 | 6.6 | 7 | 6 | 3 | 7 | 5 | 5.6 | 5.5 |
| Biochemical oxygen demand (mgl$^{-1}$) | 1.4 | 2.1 | 2.5 | 3.8 | 5.8 | 1.4 | 6.8 | 3 | 2.7 | 2.2 | 2.1 |
| pH | 7 | 6.2 | 6.6 | 6.6 | 6.2 | 5.3 | 7.1 | 6.2 | 5.4 | 6.2 | 6.8 |
| Nitrate (mgl$^{-1}$) | 0.04 | 0.1 | 0.66 | 1.6 | 2.2 | 0.03 | 4.7 | 1.1 | 0.07 | 0.02 | 0.13 |
| Phosphate (mgl$^{-1}$) | 0.04 | 0.03 | 0.06 | 1.5 | 0.1 | 0.13 | 6.3 | 1.3 | 0.18 | 1.1 | 1.2 |

**Appendix B: Overall abundance of macroinvertebrates collected in selected rivers in urban catchment during the study period (2008 – 2012). Abbreviations:** Rivers/Stations: (Wa1 (Warri River station 1), An1 (Anwai River station 1), An3 (Anwai River station 3), Ad3 (Adofi River station 3), Ol2 (Oleri River station 2), Et1 (Ethiope River station 1), Or3 (Orogodo River station 3), Ob3 (Obosh River station 3), Et2 (Ethiope River station 2), Og1 (Ogba River station 1), Og2 (Ogba River station 2).

| | Rivers/Stations | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Taxon** | Wa1 | An1 | An3 | Ad3 | Ol2 | Et1 | Or3 | Ob3 | Et2 | Og1 | Og2 |
| Naididae | 10 | 21 | 18 | 46 | 8 | 0 | 49 | 44 | 24 | 8 | 149 |
| Tubificidae | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 76 |
| Lumbricidae | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 0 | 0 |
| Lymnaidae | 0 | 8 | 15 | 0 | 0 | 0 | 7 | 0 | 1 | 0 | 0 |
| Planorbidae | 0 | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Thiaridae | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 2 | 3 |
| Amphullariidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Atyidae | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 8 | 0 | 0 |
| Desmocarididae | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 12 | 0 | 0 |
| Euryrhynchidae | 56 | 28 | 21 | 28 | 0 | 52 | 0 | 120 | 38 | 23 | 0 |
| Palaemonidae | 0 | 23 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 6 | 0 |
| Baetidae | 37 | 63 | 36 | 178 | 45 | 24 | 15 | 282 | 10 | 114 | 16 |
| Leptophlebiidae | 0 | 23 | 7 | 28 | 0 | 0 | 0 | 17 | 0 | 28 | 0 |
| Caenidae | 7 | 0 | 0 | 59 | 0 | 10 | 0 | 40 | 0 | 52 | 0 |
| Heptageniidae | 15 | 5 | 6 | 0 | 0 | 4 | 0 | 82 | 0 | 0 | 0 |
| Tricorythidae | 0 | 0 | 6 | 3 | 0 | 0 | 5 | 52 | 0 | 9 | 0 |
| Oligoneuridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 0 | 0 | 0 |
| Perlidae | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae | 8 | 8 | 5 | 19 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Hydropsychidae | 12 | 10 | 18 | 9 | 4 | 0 | 0 | 314 | 0 | 0 | 0 |
| Ecnomidae | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 148 | 0 | 0 | 0 |
| Leptoceridae | 0 | 25 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 6 |
| Pyraustidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Notonectidae | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| Pleidae | 7 | 4 | 4 | 12 | 0 | 0 | 0 | 43 | 0 | 12 | 0 |
| Mesoviliidae | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nepidae | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 6 |
| Naucoridae | 15 | 16 | 17 | 23 | 10 | 12 | 2 | 180 | 0 | 7 | 0 |
| Belostomatidae | 0 | 18 | 11 | 0 | 6 | 0 | 71 | 102 | 0 | 16 | 24 |
| Gerridae | 0 | 0 | 17 | 2 | 0 | 9 | 0 | 0 | 0 | 9 | 4 |
| Dytiscidae | 36 | 16 | 25 | 132 | 42 | 16 | 8 | 277 | 8 | 33 | 8 |
| Hydrophilidae | 18 | 9 | 8 | 10 | 2 | 0 | 6 | 162 | 0 | 20 | 2 |
| Elmidae | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrinidae | 7 | 0 | 0 | 0 | 0 | 12 | 0 | 114 | 0 | 16 | 5 |
| Noteridae | 0 | 0 | 0 | 26 | 0 | 0 | 0 | 0 | 0 | 12 | 0 |
| Hydraenidae | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aeschnidae | 9 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Gomphidae | 0 | 11 | 0 | 6 | 0 | 0 | 4 | 24 | 0 | 16 | 0 |
| Coenagrionidae | 17 | 19 | 45 | 117 | 0 | 3 | 3 | 116 | 6 | 142 | 96 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Libellulidae | 18 | 0 | 0 | 34 | 15 | 4 | 0 | 234 | 8 | 24 | 0 |
| Calopterygidae | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Macromidae | 3 | 0 | 0 | 0 | 12 | 7 | 0 | 0 | 2 | 0 | 0 |
| Chlorocyphidae | 0 | 0 | 8 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Culicidae | 0 | 0 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 35 |
| Simulidae | 0 | 0 | 3 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Tabanidae | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 2 | 0 | 8 |
| Ceratopogonidae | 9 | 15 | 92 | 13 | 8 | 0 | 0 | 178 | 6 | 4 | 6 |
| Athericidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 94 | 0 | 6 | 0 |
| Chaoboridae | 0 | 16 | 13 | 3 | 0 | 0 | 11 | 0 | 0 | 0 | 0 |
| Tipulidae | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Sryphidae | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 94 |
| Chironomidae | 40 | 32 | 89 | 177 | 52 | 11 | 252 | 370 | 31 | 57 | 383 |

**Appendix C: Fuzzy coding of selected macroinvertebrate traits/ecological preferences, taxon-station matrix and traits/ecological preferences-station matrix for the sampling stations for selected rivers in urban catchment in the Niger Delta, Nigeria during the study period (2008 –2012).**

**Table C1:** Fuzzy coding of macroinvertebrate traits and ecological preferences for selected rivers in urban catchment in the Niger Delta. **Codes:** A1=Gills, A2=Tegument/cutaneous, A3=Aerial: spiracle, A4= Aerial/vegetation: breathing tube, strap/other apparatus, B1=Hardshell, B2=Completely sclerotized, B3=Partly sclerotized, B4=Soft and exposed, B5=Cased/tubed, C1=Clear and transparent waters, C2=Silty, C3=Turbid waters, C4=No preference, D1=1 year (Univoltine), D2=2 years (Bivoltine), D3= >2years (Multivoltine), D4=longer than one year (Semivoltine), E1=Free-living, E2=Temporary attachment, E3=Permanent attachment, F1=Climber, F2=Crawler, F3=Sprawler, F4=Swimmer, F5=Skater, F6=Burrower, G1=Streamlined, G2=Flattened, G3=Spherical, G4=Cylindrical/tubular, H1=Detritus (FPOM), H2=Detritus (CPOM), H3= Macrophytes/algae, H4=Animal materials, I1=Highly sensitive to oxygen depletion, I2=Moderately sensitive to oxygen depletion, I3= Moderately tolerant of oxygen depletion, I4=Highly tolerant of oxygen depletion, J1=Very small (<5 mm), J2=Small (>5-10 mm), J3= Medium (>10-20 mm), J4=Large (>20-40 mm), J5=Very large (>40-80 mm), K1=Egg, K2=Larva, K3=Nymph, K4=Pupa, L1= Predator, L2=Scraper, L3=Grazer, L4=Filter feeder, L5=Deposit feeder, L6=Shredder.

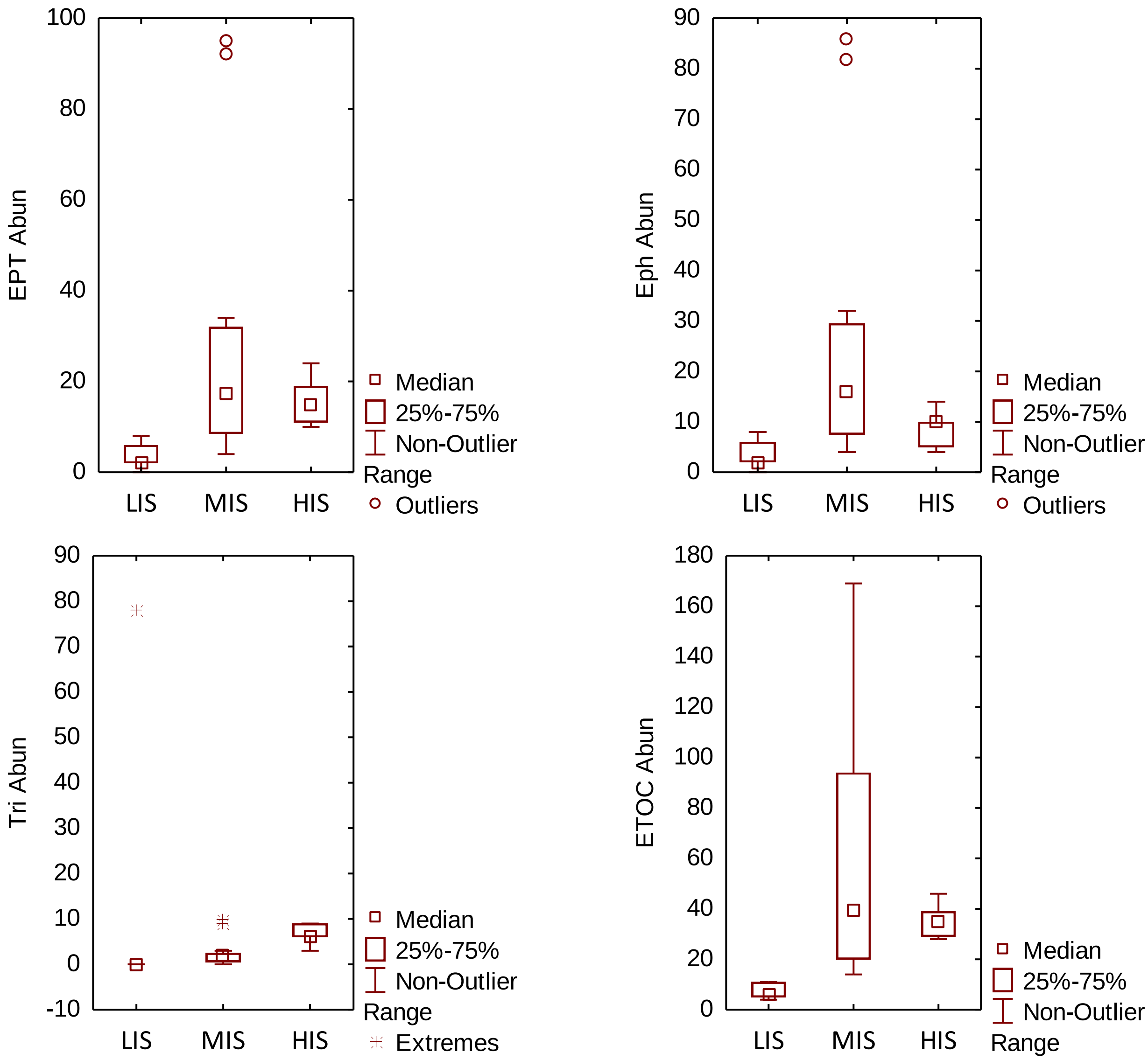
| Traits/ecological preferences | Respiration | | | | Body armouring | | | | | Turbidity preference | | | | Voltinism | | | | Attachment | | | Mobility | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Codes** | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 | B5 | C1 | C2 | C3 | C4 | D1 | D2 | D3 | D4 | E1 | E2 | E3 | F1 | F2 | F3 | F4 | F5 | F6 |
| **Taxon** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Naididae | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Tubificidae | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Lumbricidae | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Lymnaidae | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| Planorbidae | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 2 | 3 | 1 | 0 | 0 | 0 |
| Thiaridae | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Amphullariidae | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Atyidae | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Desmocarididae | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Euryrhynchidae | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Palaemonidae | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Baetidae | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 |
| Leptophlebiidae | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 |
| Caenidae | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 1 | 2 | 0 | 0 |

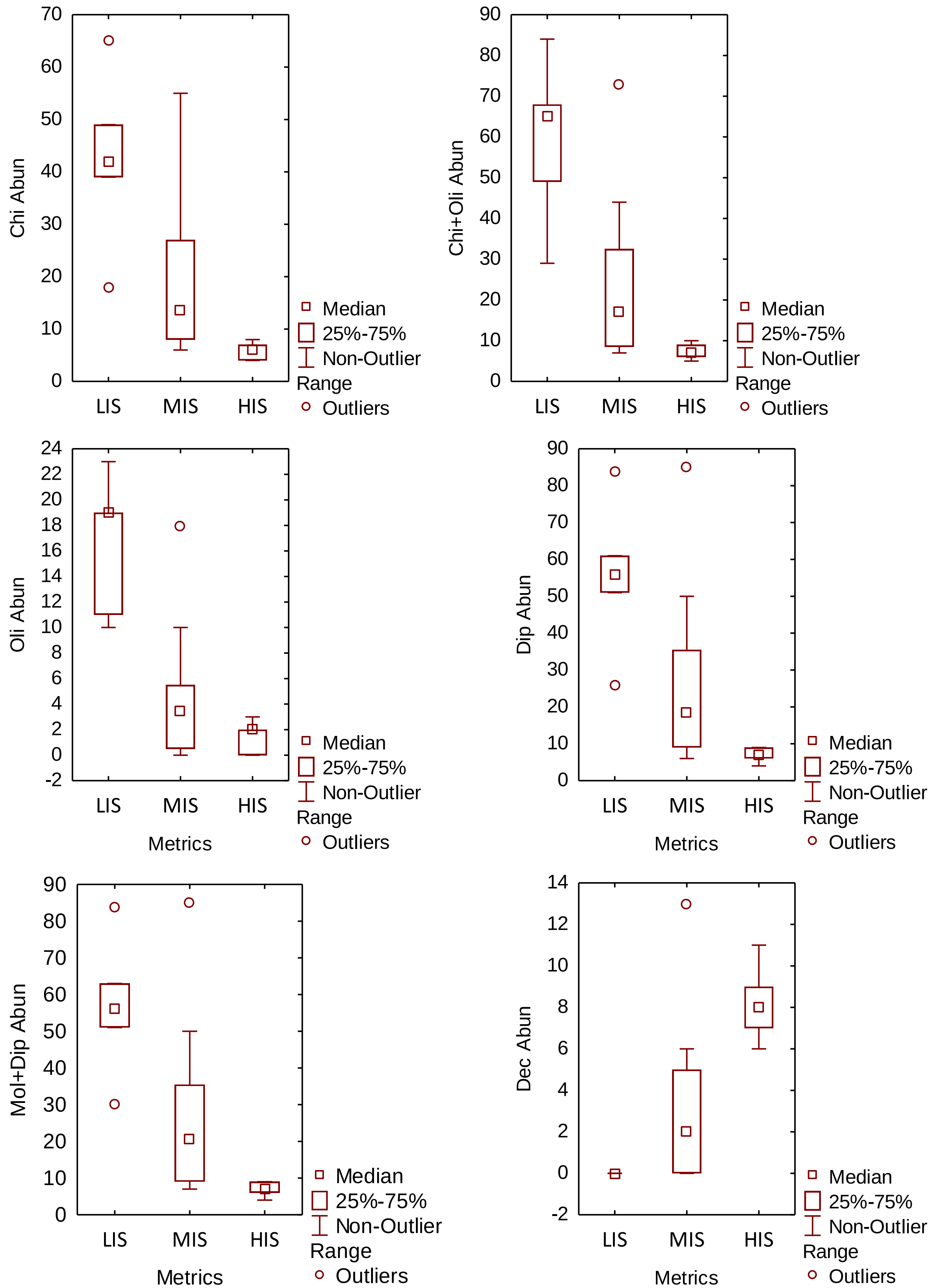
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Heptageniidae | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 2 | 1 | 0 | 0 |
| Tricorythidae | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Oligoneuridae | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Perlidae | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Hydroptilidae | 1 | 3 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 |
| Hydropsychidae | 3 | 1 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 1 | 0 | 0 |
| Ecnomidae | 1 | 3 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 1 | 0 | 0 |
| Leptoceridae | 3 | 1 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 2 | 3 | 0 | 0 |
| Pyraustidae | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 3 | 0 | 1 | 0 | 1 |
| Notonectidae | 0 | 0 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 0 |
| Pleidae | 0 | 0 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 0 |
| Mesoviliidae | 0 | 0 | 3 | 1 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Nepidae | 0 | 0 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 1 | 0 | 2 | 3 | 0 | 3 | 0 | 0 |
| Naucoridae | 0 | 0 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 2 | 0 | 3 | 0 | 0 |
| Belostomatidae | 0 | 0 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 2 | 2 | 0 | 3 | 0 | 0 |
| Gerridae | 0 | 0 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Dytiscidae | 0 | 0 | 3 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 3 | 3 | 0 | 3 | 0 | 0 |
| Hydrophilidae | 2 | 1 | 3 | 3 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 3 | 0 | 3 | 0 | 0 |
| Elmidae | 3 | 1 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 0 |
| Gyrinidae | 3 | 2 | 0 | 3 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 3 | 0 | 3 | 0 | 1 |
| Noteridae | 0 | 1 | 1 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Hydraenidae | 2 | 2 | 3 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 2 | 0 | 3 | 0 | 0 |
| Aeschnidae | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 1 | 1 | 1 | 0 | 3 | 0 | 3 | 3 | 0 | 2 | 0 | 0 |
| Gomphidae | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 1 | 3 | 0 | 2 | 0 | 0 |
| Coenagrionidae | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 1 | 1 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 2 | 0 | 0 |
| Libellulidae | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 1 | 2 | 0 | 0 | 2 | 0 | 1 | 3 | 1 | 2 | 0 | 1 |
| Calopterygidae | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Macromidae | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Chlorocyphidae | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Culicidae | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Simulidae | 1 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| Tabanidae | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 2 | 1 | 1 | 0 | 2 | 0 |
| Ceratopogonidae | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 1 | 0 | 0 | 2 | 1 | 1 | 0 | 2 |
| Athericidae | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 2 | 0 | 0 | 3 | 0 |
| Chaoboridae | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 3 | 0 |

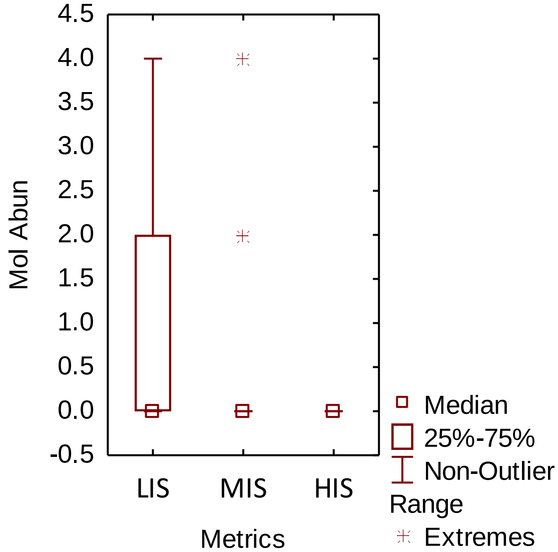
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tipulidae | 2 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 3 | 0 | |
| Sryphidae | 0 | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 1 | 2 | 3 | 0 | 0 | 0 | |
| Chironomidae | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 3 | |
| | **Body shape** | | | | **Food preference** | | | | **Response to oxygen depletion** | | | | **Body size** | | | | | **Aquatic stages** | | | | **Feeding habit** | | | | | |
| **Codes** | G1 | G2 | G3 | G4 | H 1 | H 2 | H 3 | H 4 | I1 | I2 | I3 | I4 | J1 | J2 | J3 | J4 | J5 | K 1 | K 2 | K 3 | K 4 | L 1 | L 2 | L 3 | L 4 | L 5 | L6 |
| **Taxon** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Naididae | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 |
| Tubificidae | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lumbricidae | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 |
| Lymnaidae | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 2 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 |
| Planorbidae | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 2 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 |
| Thiaridae | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 2 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 |
| Amphullariidae | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 1 | 2 | 0 | 3 | 2 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 |
| Atyidae | 0 | 1 | 3 | 0 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| Desmocarididae | 0 | 1 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 0 | 3 | 3 | 0 | 1 | 0 | 0 | 0 |
| Euryrhynchidae | 0 | 1 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 0 | 3 | 3 | 0 | 1 | 0 | 0 | 0 |
| Palaemonidae | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| Baetidae | 1 | 1 | 0 | 3 | 3 | 1 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 3 | 0 |
| Leptophlebiidae | 1 | 1 | 0 | 3 | 3 | 1 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 3 | 2 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Caenidae | 3 | 1 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 3 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Heptageniidae | 0 | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Tricorythidae | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Oligoneuridae | 3 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Perlidae | 1 | 2 | 0 | 0 | 1 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 3 | 0 | 1 | 0 | 2 | 0 | 1 | 0 |
| Hydroptilidae | 1 | 2 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| Hydropsychidae | 0 | 0 | 0 | 3 | 3 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 1 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Ecnomidae | 0 | 0 | 0 | 3 | 1 | 1 | 1 | 3 | 0 | 3 | 0 | 0 | 1 | 3 | 2 | 0 | 0 | 1 | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| Leptoceridae | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 2 | 0 | 3 | 3 | 2 | 1 | 1 | 3 | 2 |
| Pyraustidae | 0 | 0 | 0 | 3 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 1 | 2 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 |
| Notonectidae | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Pleidae | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 0 |
| Mesoviliidae | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Nepidae | 0 | 3 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |

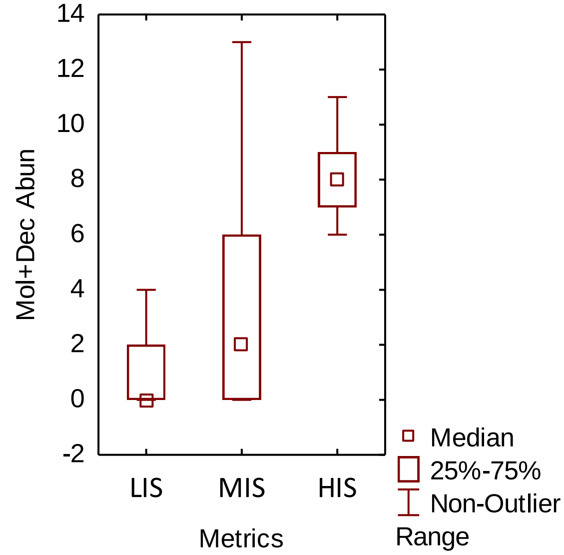
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Naucoridae | 0 | 2 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Belostomatidae | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Gerridae | 0 | 3 | 0 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 1 | 3 | 2 | 1 | 1 | 1 | 2 | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Hydrophilidae_ | 0 | 0 | 3 | 3 | 1 | 1 | 2 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Elmidae | 0 | 2 | 2 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 3 | 0 | 1 | 0 | 0 | 3 | 1 |
| Gyrinidae | 0 | 1 | 2 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 3 | 2 | 1 | 1 | 1 | 2 | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Noteridae | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Hydraenidae | 0 | 1 | 0 | 2 | 3 | 1 | 1 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 1 |
| Aeschnidae | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 3 | 2 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Gomphidae | 0 | 2 | 0 | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 5 | 3 | 1 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Coenagrionidae | 0 | 0 | 0 | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | 0 | 2 | 0 | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 3 | 1 | 1 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Calopterygidae | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Macromidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Chlorocyphidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Culicidae | 0 | 0 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| Simulidae | 0 | 0 | 0 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| Tabanidae | 0 | 0 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| Ceratopogonidae | 0 | 1 | 0 | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 |
| Athericidae | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Chaoboridae | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | 0 | 0 | 0 | 3 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Sryphidae | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 2 | 2 | 0 | 0 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 1 | 0 | 0 |
| Chironomidae | 0 | 0 | 0 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |

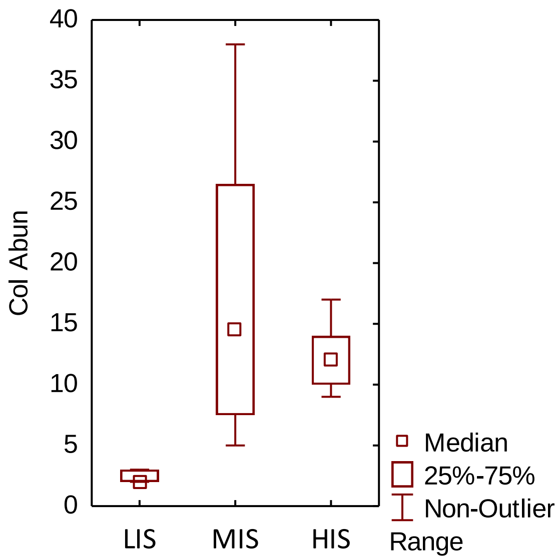
**Appendix D: Sensitive/non- sensitive and seasonally stable/non-seasonally stable metrics not integrated into the urban multimetrc index (MMI-urban)**

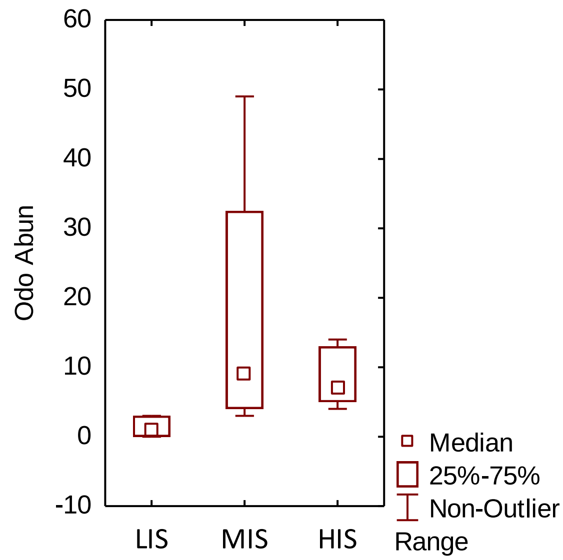


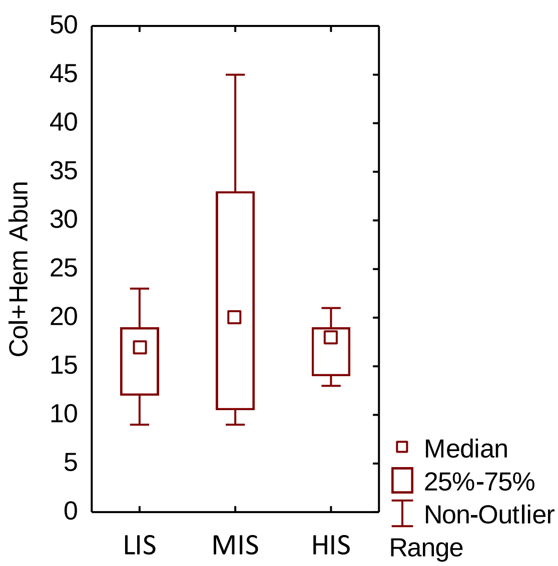

Chi Abun
70
60
50
40
30
20
10
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Chi+Oli Abun
90
80
70
60
50
40
30
20
10
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Oli Abun
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Metrics
Median
25%-75%
Non-Outlier Range
Outliers
Dip Abun
90
80
70
60
50
40
30
20
10
0
LIS
MIS
HIS
Metrics
Median
25%-75%
Non-Outlier Range
Outliers
Mol+Dip Abun
90
80
70
60
50
40
30
20
10
0
LIS
MIS
HIS
Metrics
Median
25%-75%
Non-Outlier Range
Outliers
Dec Abun
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Metrics
Median
25%-75%
Non-Outlier Range
Outliers

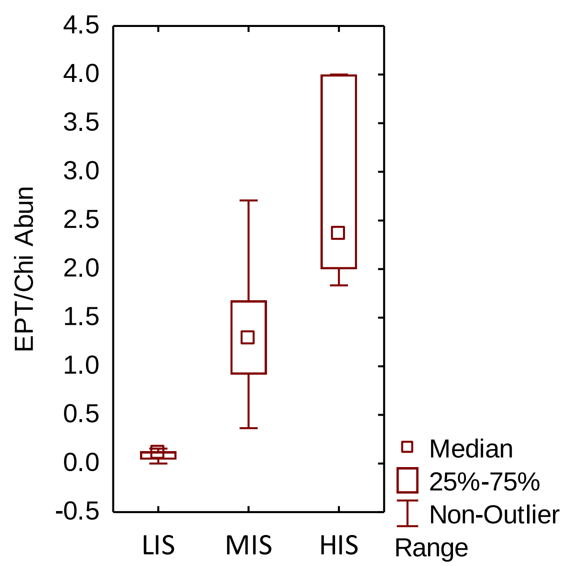

Mol Abun
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
LIS
MIS
HIS
Metrics
Median
25%-75%
Non-Outlier Range
Extremes


Mol+Dec Abun
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Metrics
Median
25%-75%
Non-Outlier Range


Col Abun
40
35
30
25
20
15
10
5
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


Odo Abun
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


Col+Hem Abun
50
45
40
35
30
25
20
15
10
5
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


EPT/Chi Abun
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range

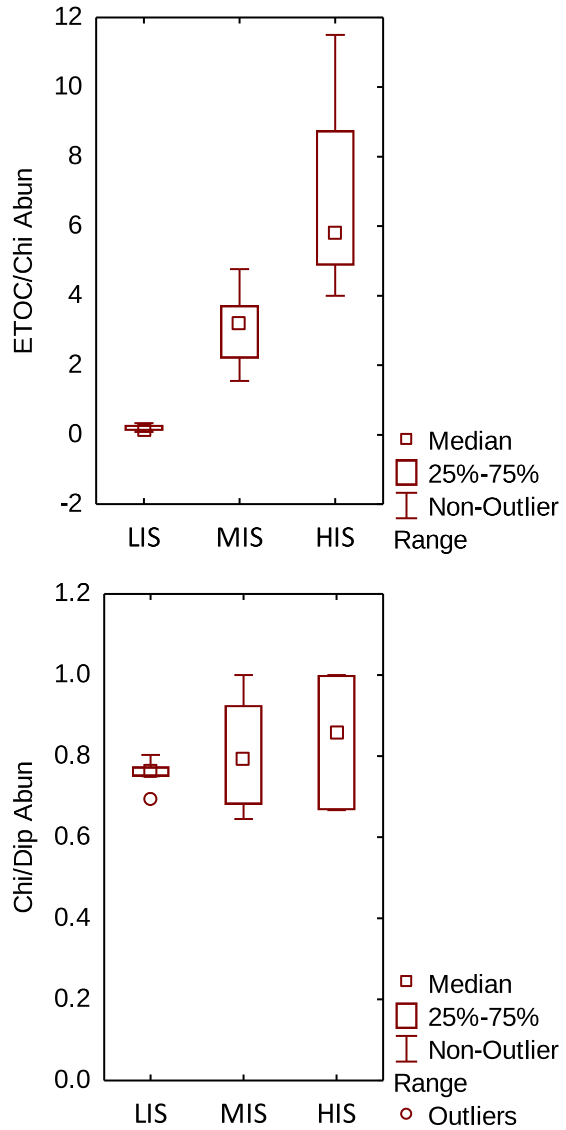
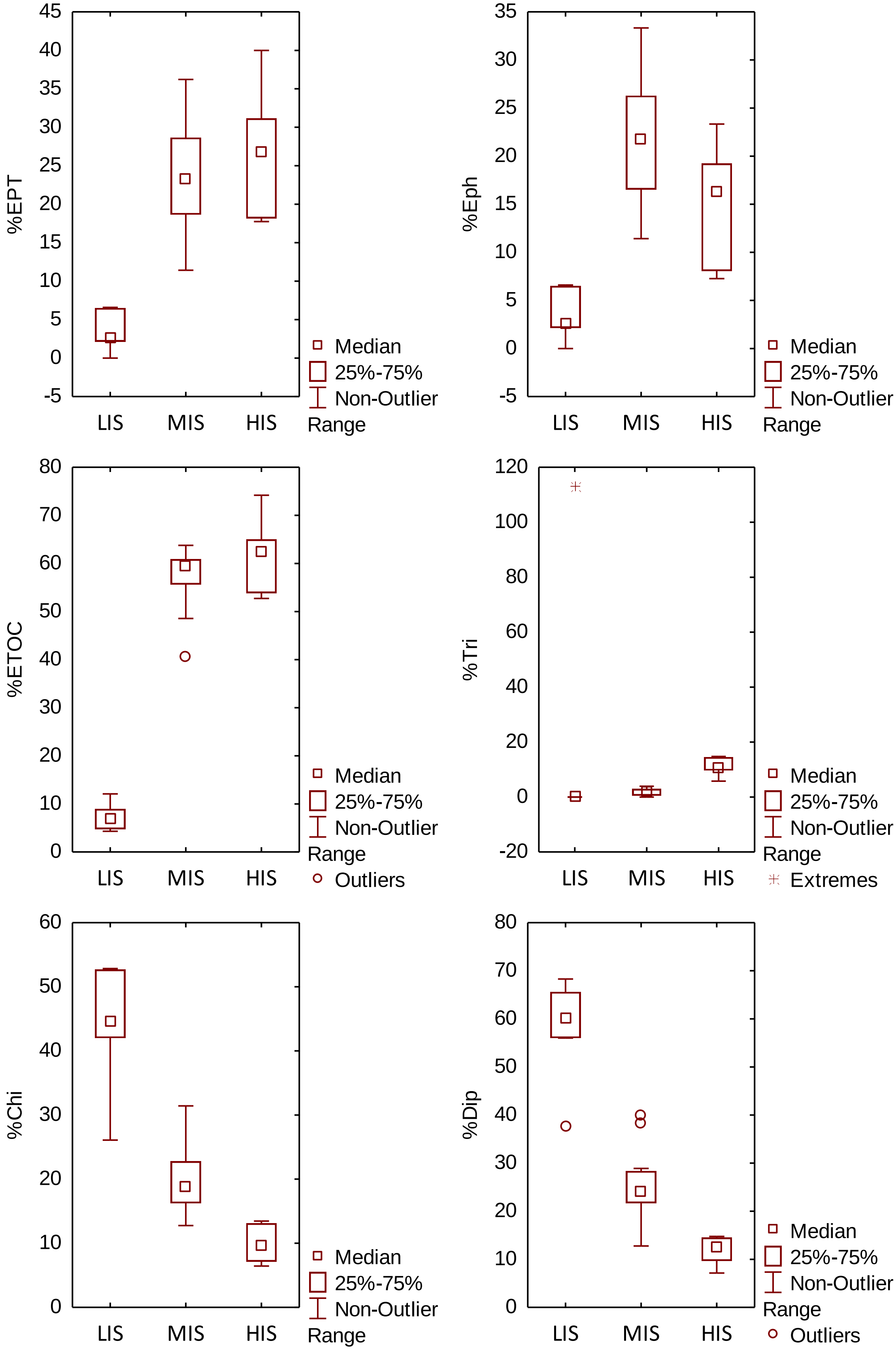
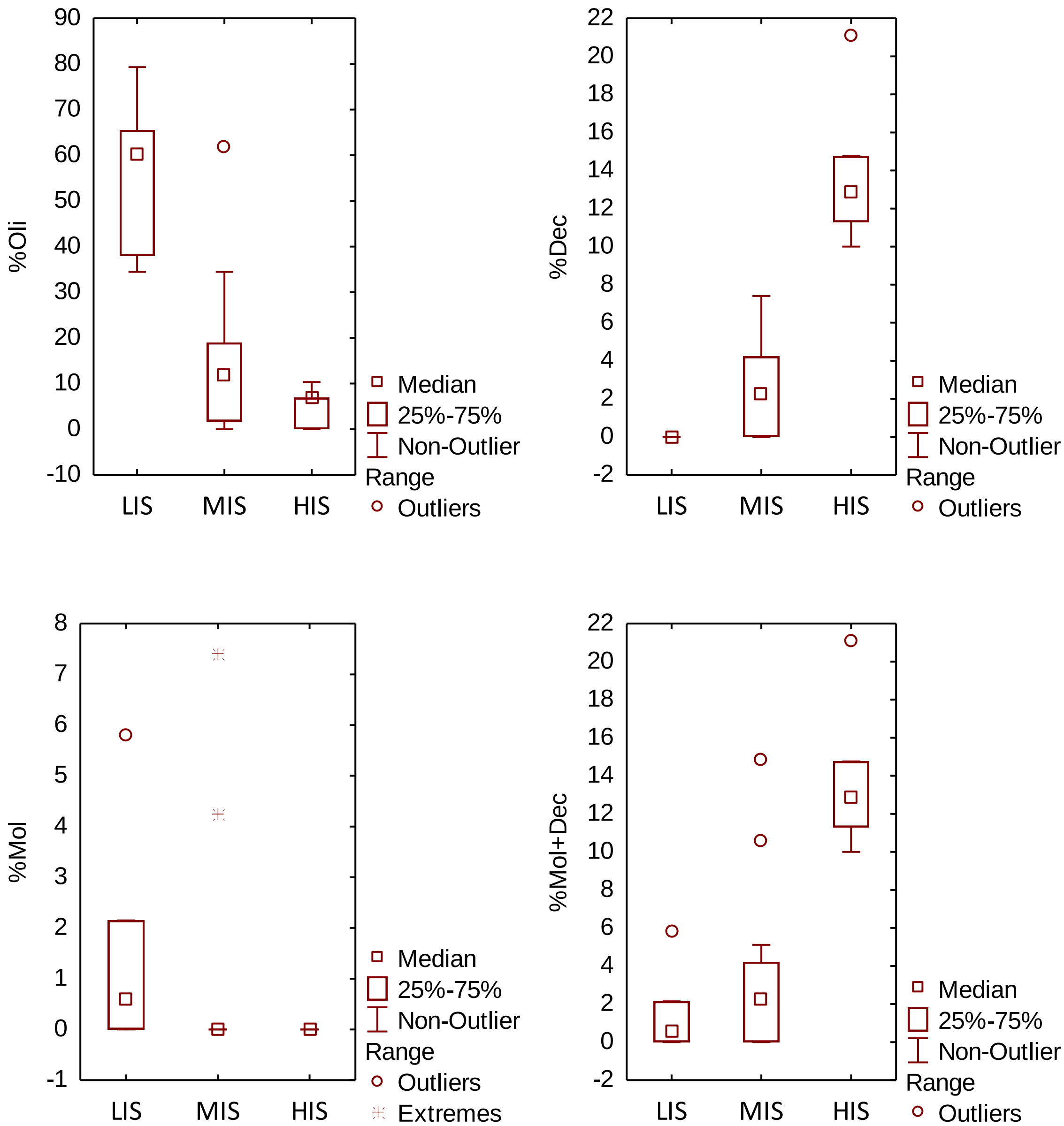



**Figure D1:** Sensitive and non sensitive metrics in the abundance measures not integrated into the urban multimetric index (MMI-urban)


%EPT
%Eph
%ETOC
%Tri
%Chi
%Dip
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


%Oli
90
80
70
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
%Dec
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
%Mol
8
7
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
%Mol+Dec
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers

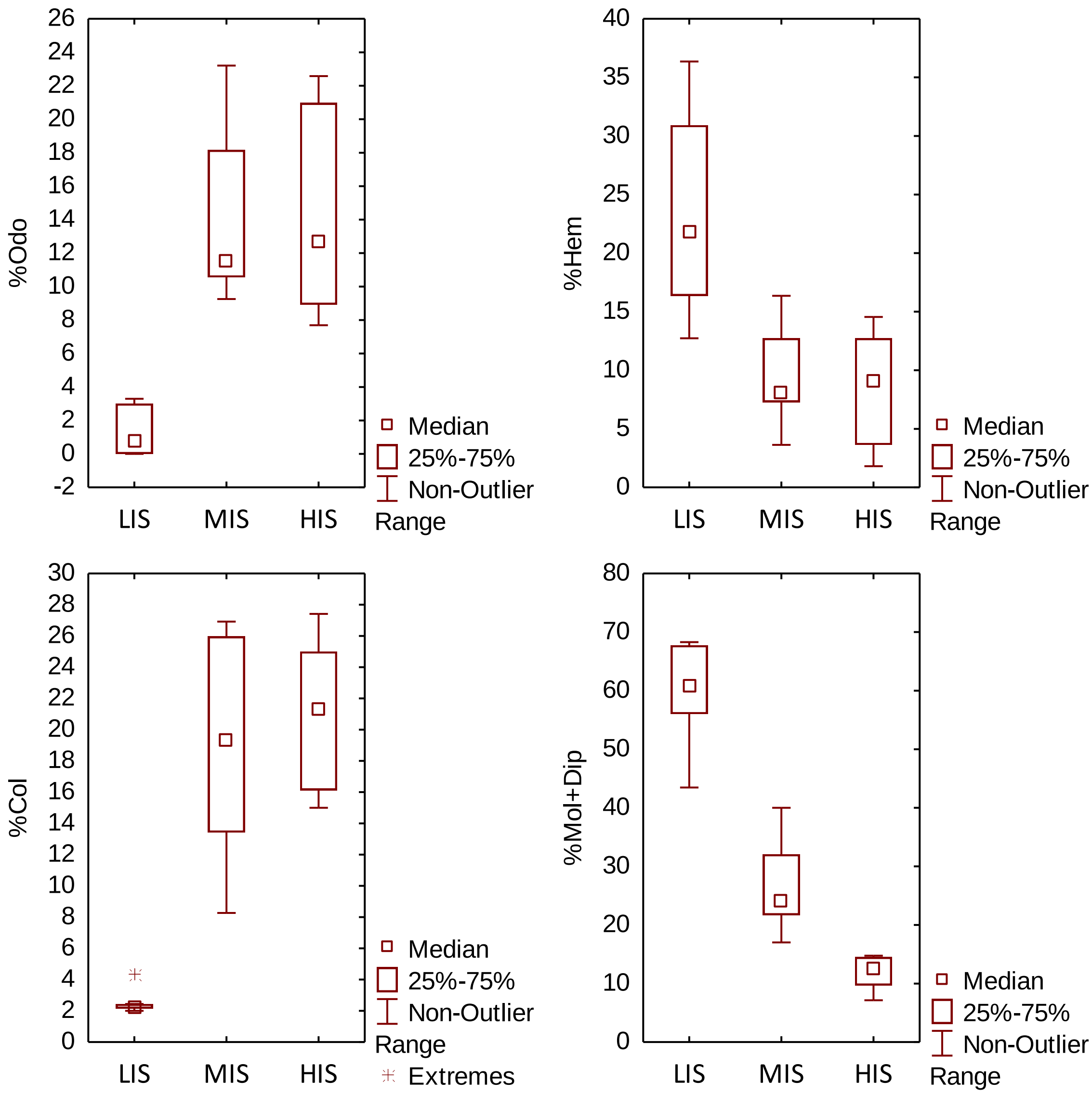
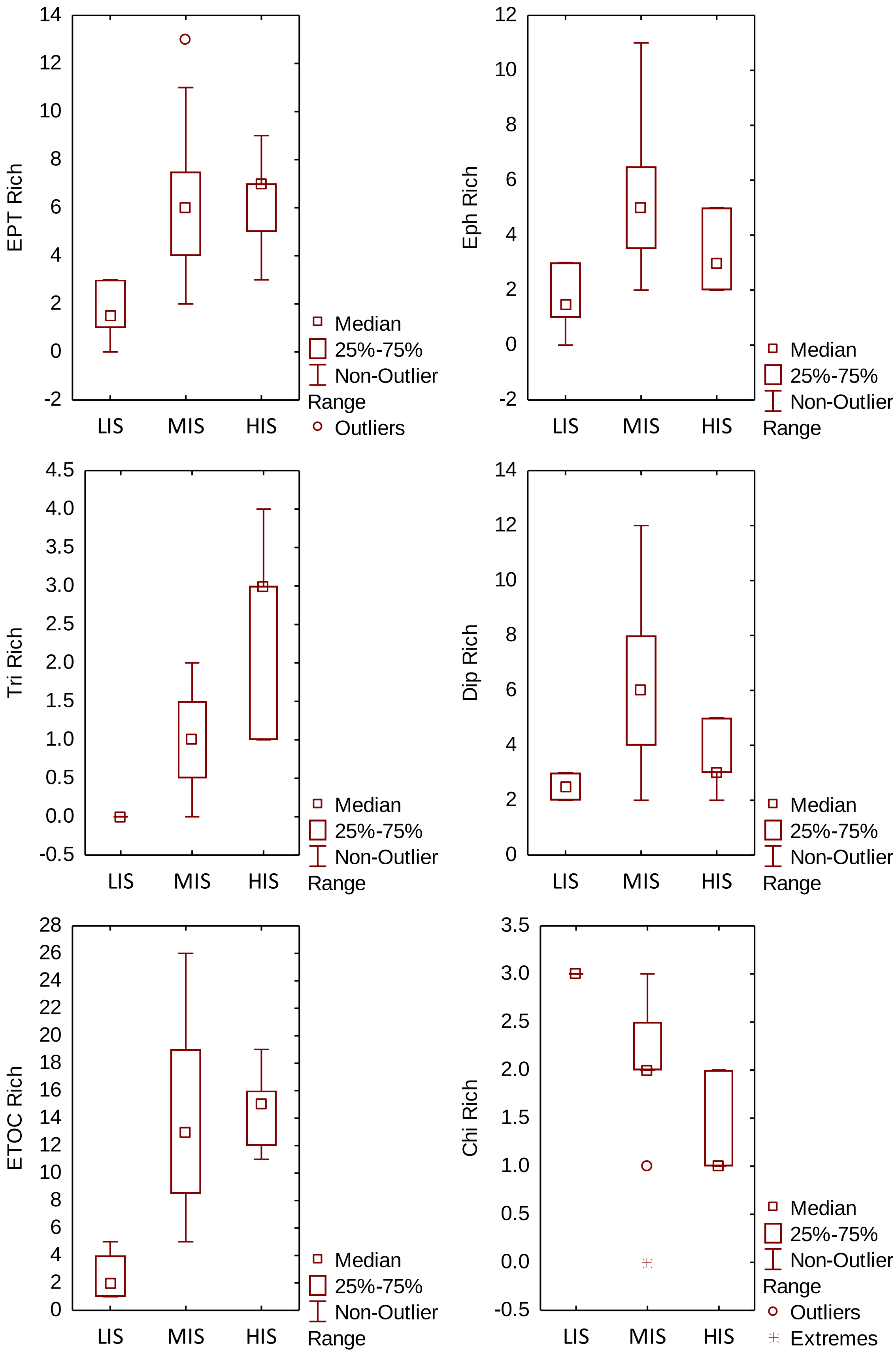
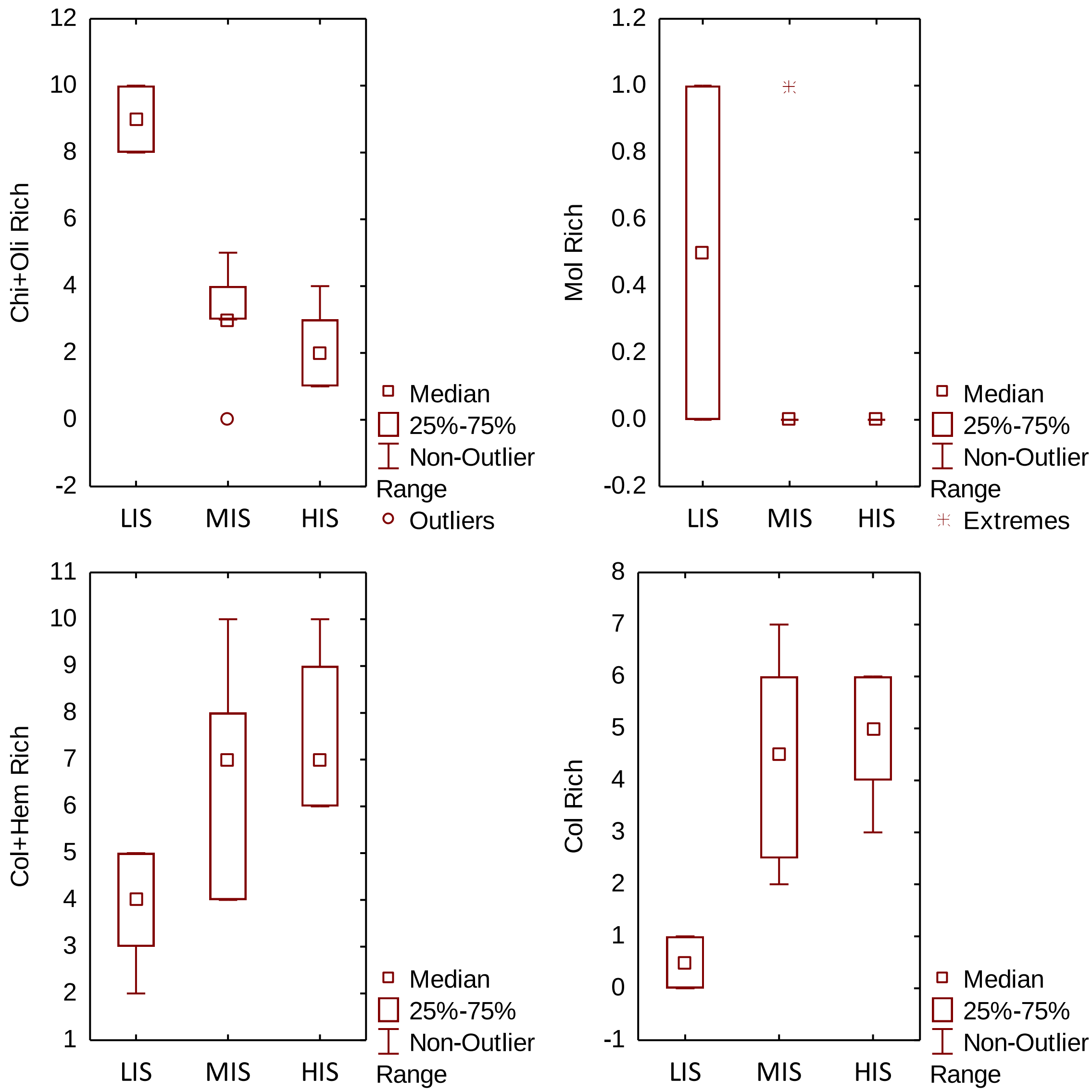



**Figure D2:** Sensitive and non sensitive metrics in the composition measures not integrated into the urban multimetric index (MMI-urban)


EPT Rich
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Eph Rich
Tri Rich
Dip Rich
ETOC Rich
Chi Rich
Extremes


Chi+Oli Rich
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Mol Rich
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes
Col+Hem Rich
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Col Rich
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range

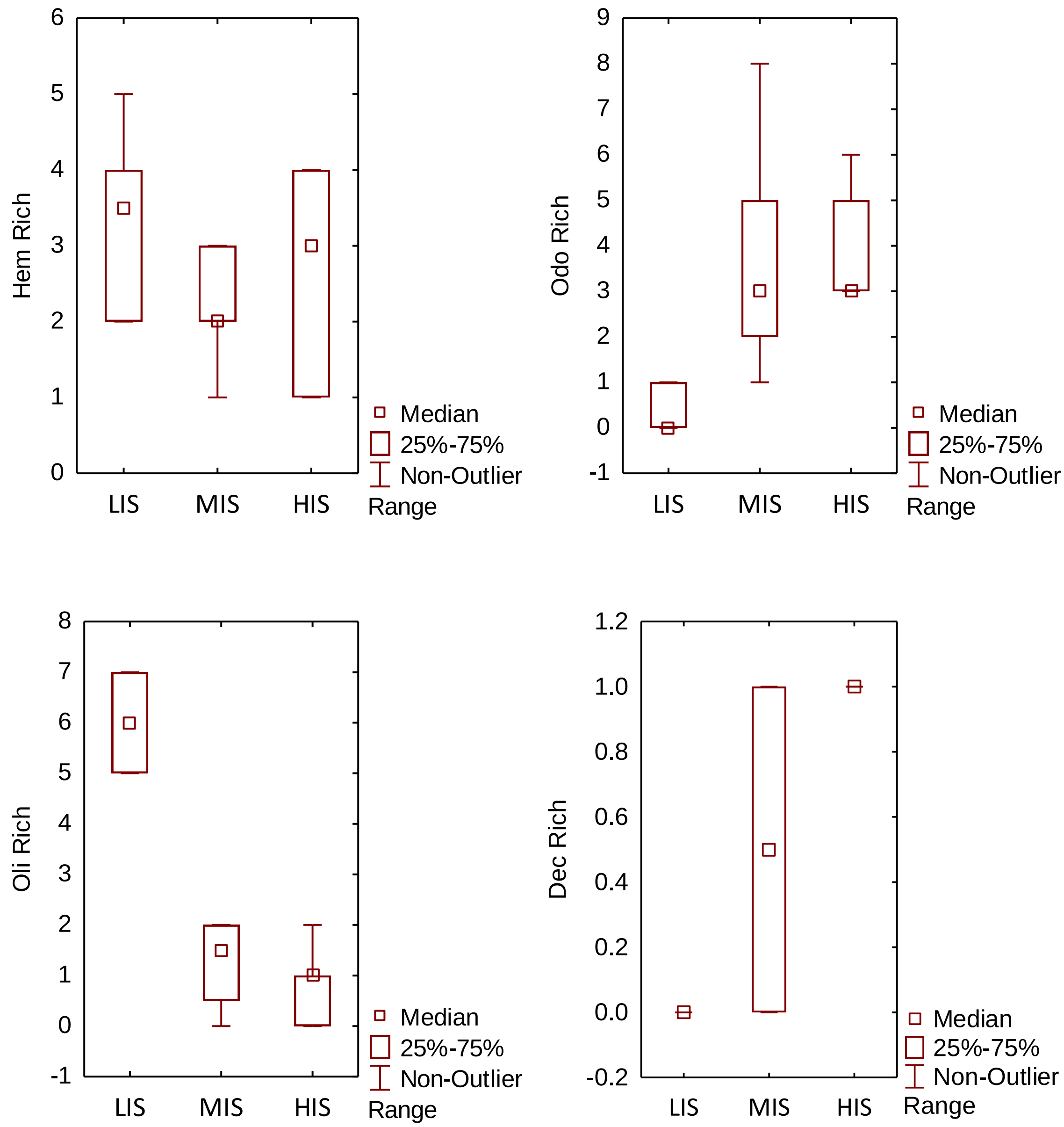



**Figure D3:** Sensitive and non sensitive metrics in the richness measures not integrated into the urban multimetric index (MMI-urban)

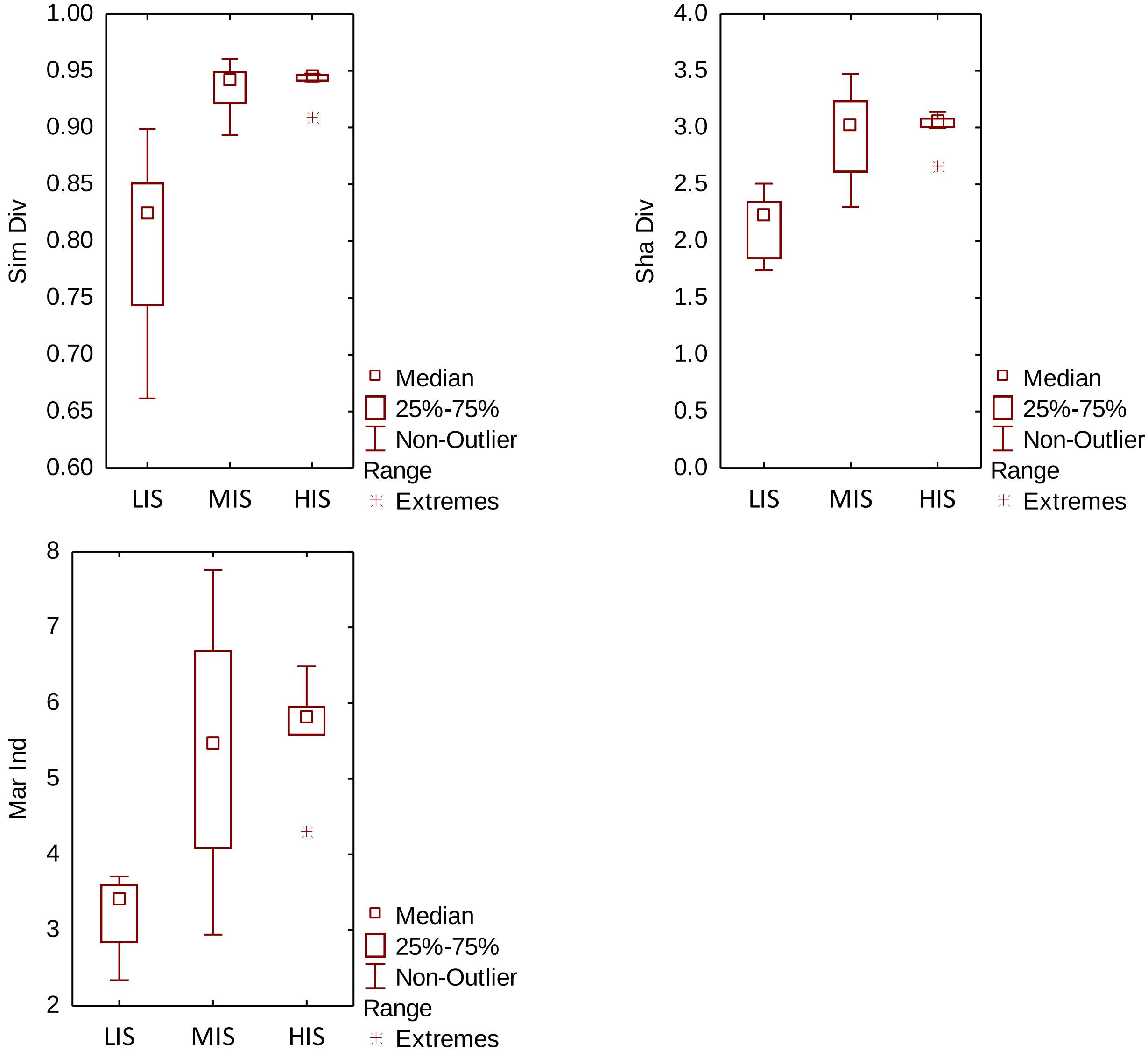
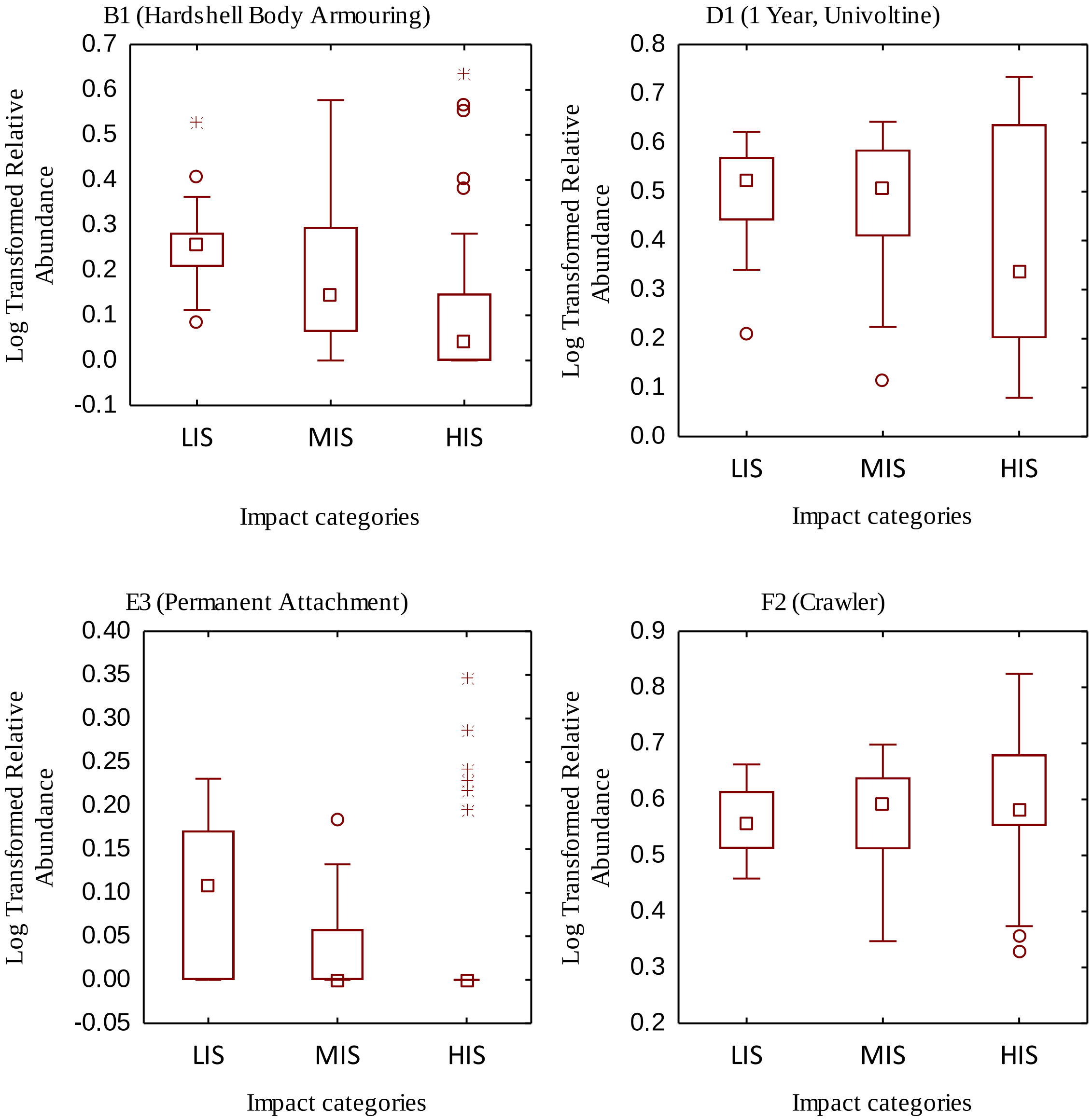
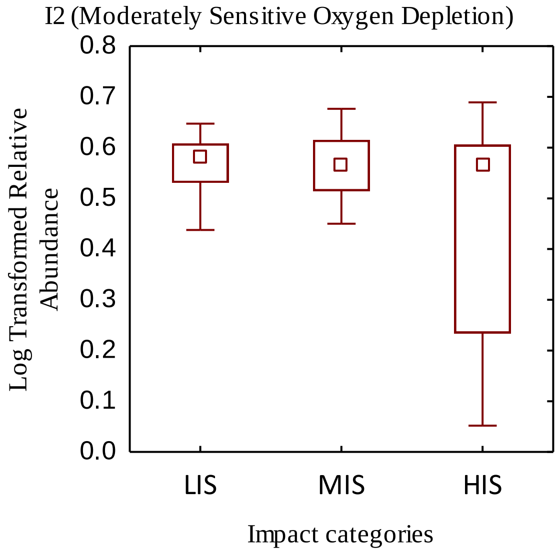
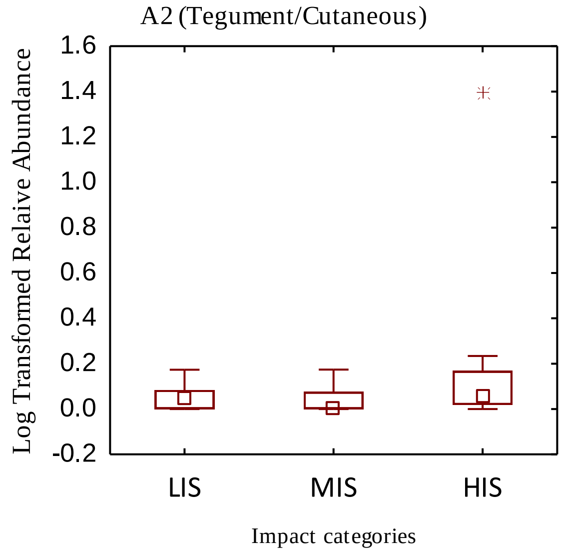
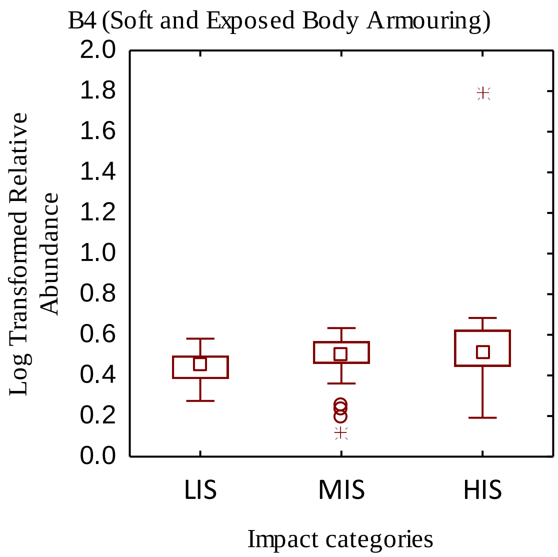


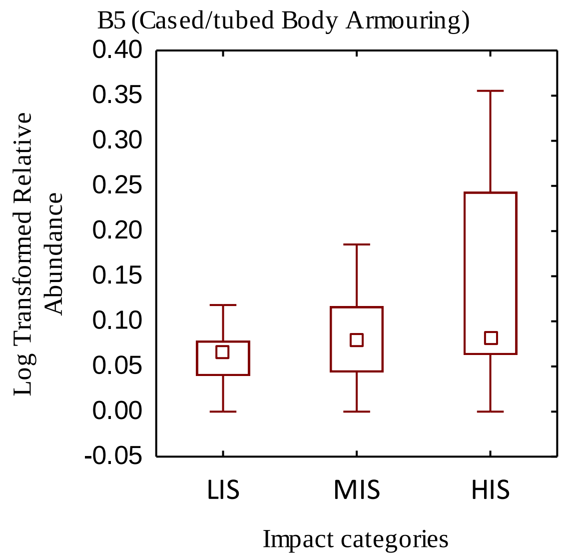

**Figure D4:** Sensitive and non sensitive metrics in the diversity measures not integrated into the urban multimetric index (MMI-urban)

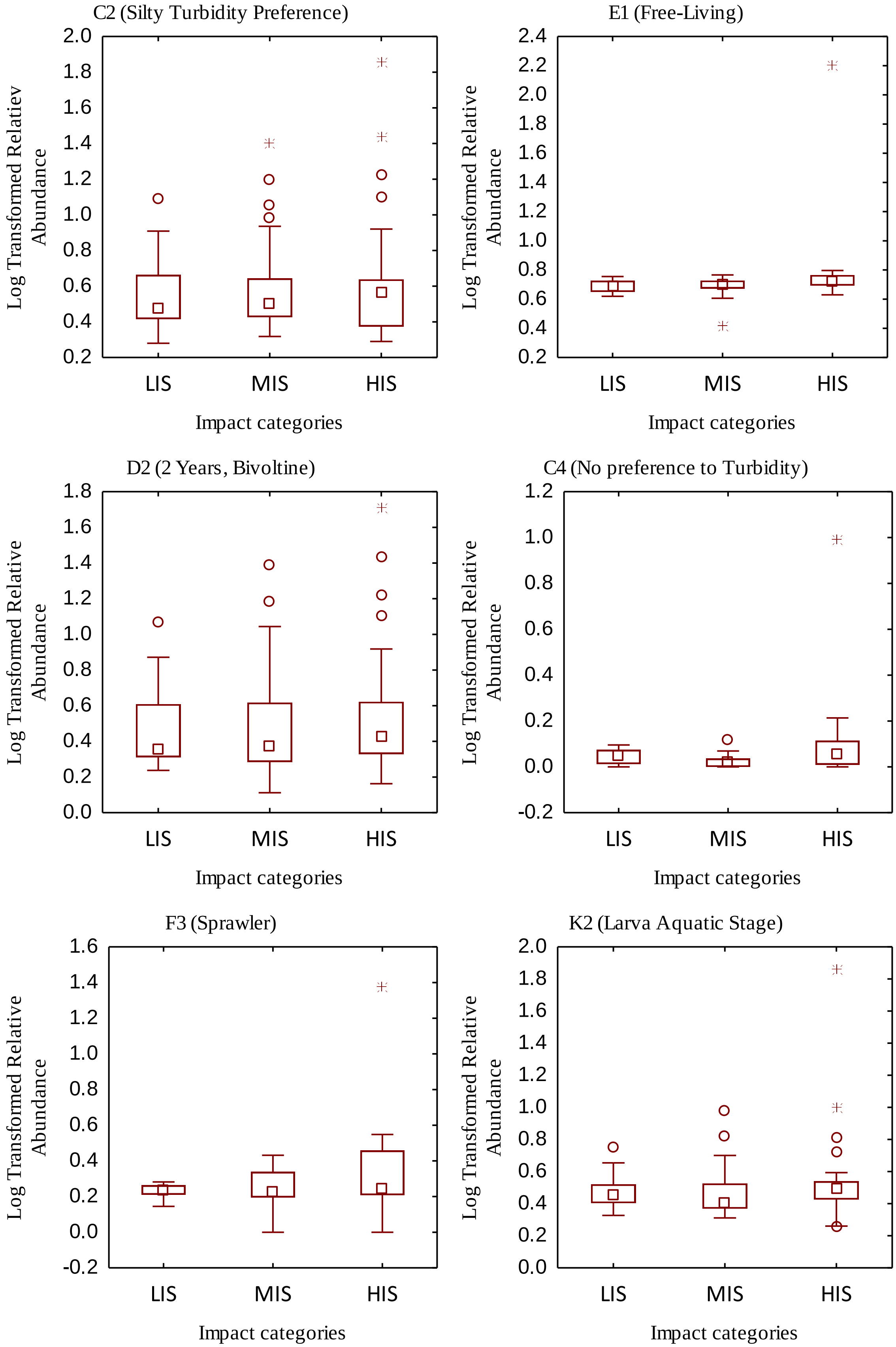

B1 (Hardshell Body Armouring)
D1 (1 Year, Univoltine)
E3 (Permanent Attachment)
F2 (Crawler)
Log Transformed Relative Abundance
Impact categories
LIS
MIS
HIS
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0.8
0.9
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05


I2 (Moderately Sensitive Oxygen Depletion)
Log Transformed Relative Abundance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
LIS
MIS
HIS
Impact categories


A2 (Tegument/Cutaneous)
Log Transformed Relaive Abundance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LIS
MIS
HIS
Impact categories


B4 (Soft and Exposed Body Armouring)
Log Transformed Relative Abundance
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LIS
MIS
HIS
Impact categories


B5 (Cased/tubed Body Armouring)
Log Transformed Relative Abundance
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
LIS
MIS
HIS
Impact categories


C2 (Silty Turbidity Preference)
Log Transformed Relatiev Abundance
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
LIS
MIS
HIS
Impact categories
E1 (Free-Living)
Log Transformed Relative Abundance
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
LIS
MIS
HIS
Impact categories
D2 (2 Years, Bivoltine)
Log Transformed Relative Abundance
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LIS
MIS
HIS
Impact categories
C4 (No preference to Turbidity)
Log Transformed Relative Abundance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LIS
MIS
HIS
Impact categories
F3 (Sprawler)
Log Transformed Relative Abundance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LIS
MIS
HIS
Impact categories
K2 (Larva Aquatic Stage)
Log Transformed Relative Abundance
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LIS
MIS
HIS
Impact categories

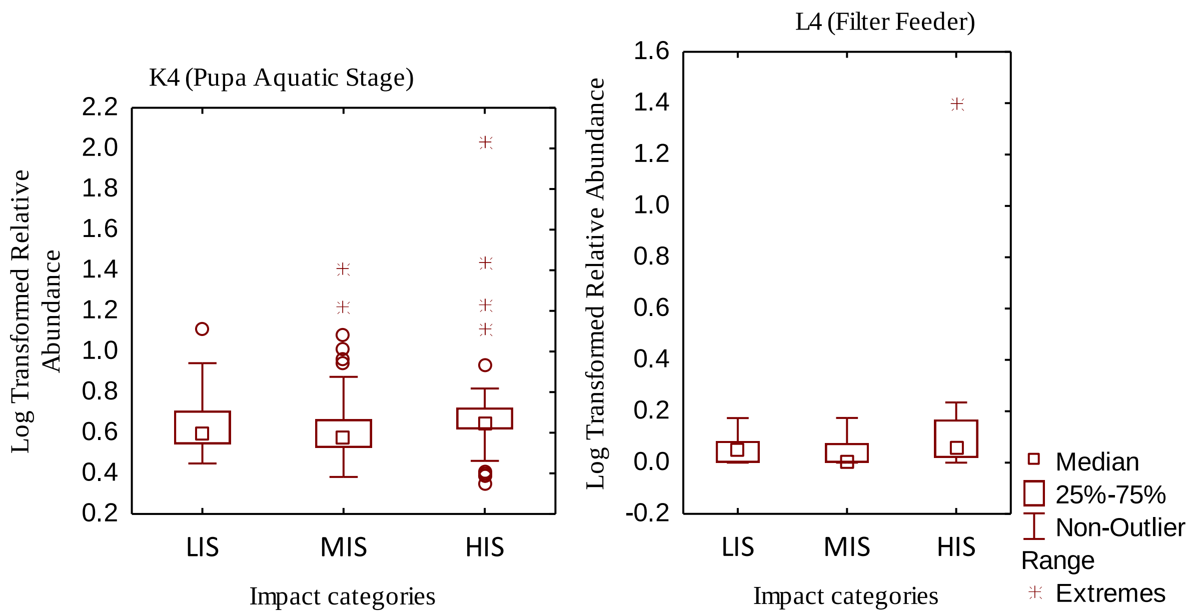
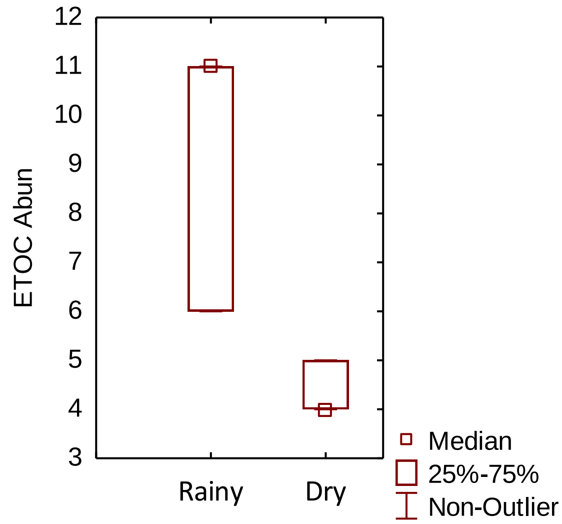



**Figure D5:** Sensitive and non sensitive metrics in the traits and ecological preferences measures not integrated into the urban multimetric index (MMI-urban)

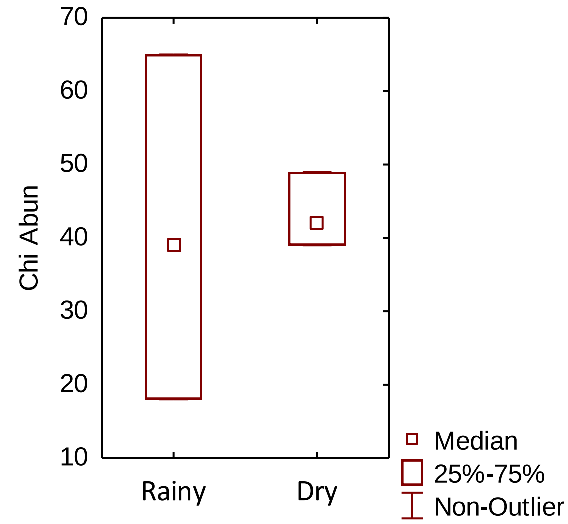
**Table D1:** Sensitive metrics selection for urban multimetric index (MMI-urban). **Note: a metric sensitivity is confirmed if significant at *P*<0.05**

| **Discriminatory metrics** | **Mann-Whitney test (U-test)** | ***P*-value** | **Sensitivity confirmed** |
|---|---|---|---|
| | **Abundance measures** | | |
| EPT Abun | 70.5 | 0.9539 | No |
| Eph | 62.0 | 0.582 | No |
| ETOC Abun | 72.0 | 0.02889 | Yes |
| Chi Abun | 3.0 | 7.36E-05 | Yes |
| Chi+Oli Abun | 6.0 | 0.0001517 | Yes |
| Oli Abun | 15.0 | 0.0009811 | Yes |
| Dip Abun | 2.0 | 5.722E-05 | Yes |
| Mol+Dip Abun | 2.0 | 5.722E-05 | Yes |
| Dec Abun | 34.5 | 0.02636 | Yes |
| Col Abun | 70.5 | 0.9539 | No |
| Odo Abun | 69.5 | 0.9077 | No |
| Hem Abun | 22.5 | 0.004344 | Yes |
| EPT/Chi Abun | 25.5 | 0.007858 | Yes |
| ETOC/Chi Abun | 27.0 | 0.01019 | Yes |
| ETOC/Dip Abun | 19.0 | 0.002437 | Yes |
| Chi/Dip Abun | 32.5 | 0.02367 | Yes |

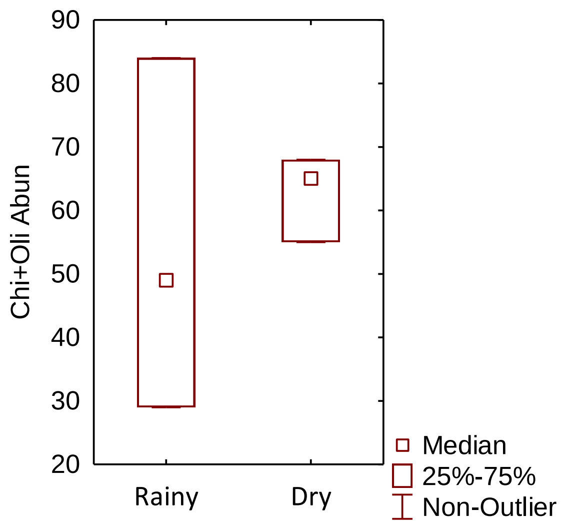
| | | | |
|---|---|---|---|
| **Composition measures** | | | |
| %EPT | 43.0 | 0.09988 | No |
| %Eph | 52.0 | 0.2601 | No |
| %ETOC | 44.0 | 0.1123 | No |
| %Chi | 33.0 | 0.0262 | Yes |
| %Chi+Oli | 29.0 | 0.01414 | Yes |
| %Oli | 4.0 | 8.232E-05 | Yes |
| %Dip | 25.0 | 0.00726 | Yes |
| %Dec | 25.0 | 0.004756 | Yes |
| %Odo | 52.0 | 0.2598 | No |
| %Hem | 8.0 | 0.0002155 | Yes |
| %Col | 24.0 | 0.006099 | Yes |
| %Col+Hem | 26.0 | 0.008616 | Yes |
| %Mol+Dip | 24.0 | 0.006099 | Yes |
| **Richness measures** | | | |
| EPT Rich | 64.5 | 0.684 | No |
| Tri Rich | 41.0 | 0.06599 | No |
| Dip Rich | 55.5 | 0.3417 | No |
| ETOC Rich | 64.0 | 0.6843 | No |
| Chi Rich | 43.0 | 0.08061 | No |
| Chi+Oli Rich | 12.0 | 0.0004453 | Yes |
| Col+Hem Rich | 67.0 | 0.7917 | No |
| Col Rich | 60.5 | 0.5202 | No |
| Odo Rich | 63.0 | 0.6173 | No |
| Oli Rich | 6.5 | 0.0001184 | Yes |
| Dec Rich | 36 | 0.01805 | Yes |
| **Diversity measures** | | | |
| Sim Div | 62.5 | 0.6033 | No |
| Sha Div | 71.0 | 0.977 | No |
| Eve Div | 1.0 | 4.695E-05 | Yes |
| Mar Ind | 68 | 0.8399 | No |
| **Trait attributes measures** | | | |
| Log HaS | 10.0 | 0.931 | No |
| Log VeL | 70.0 | 0.0002652 | Yes |



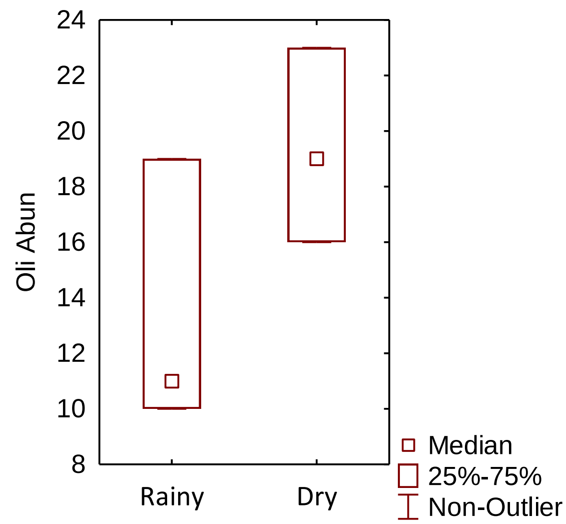

ETOC Abun: KW-H(1,6) = 4.0909, p = 0.0431



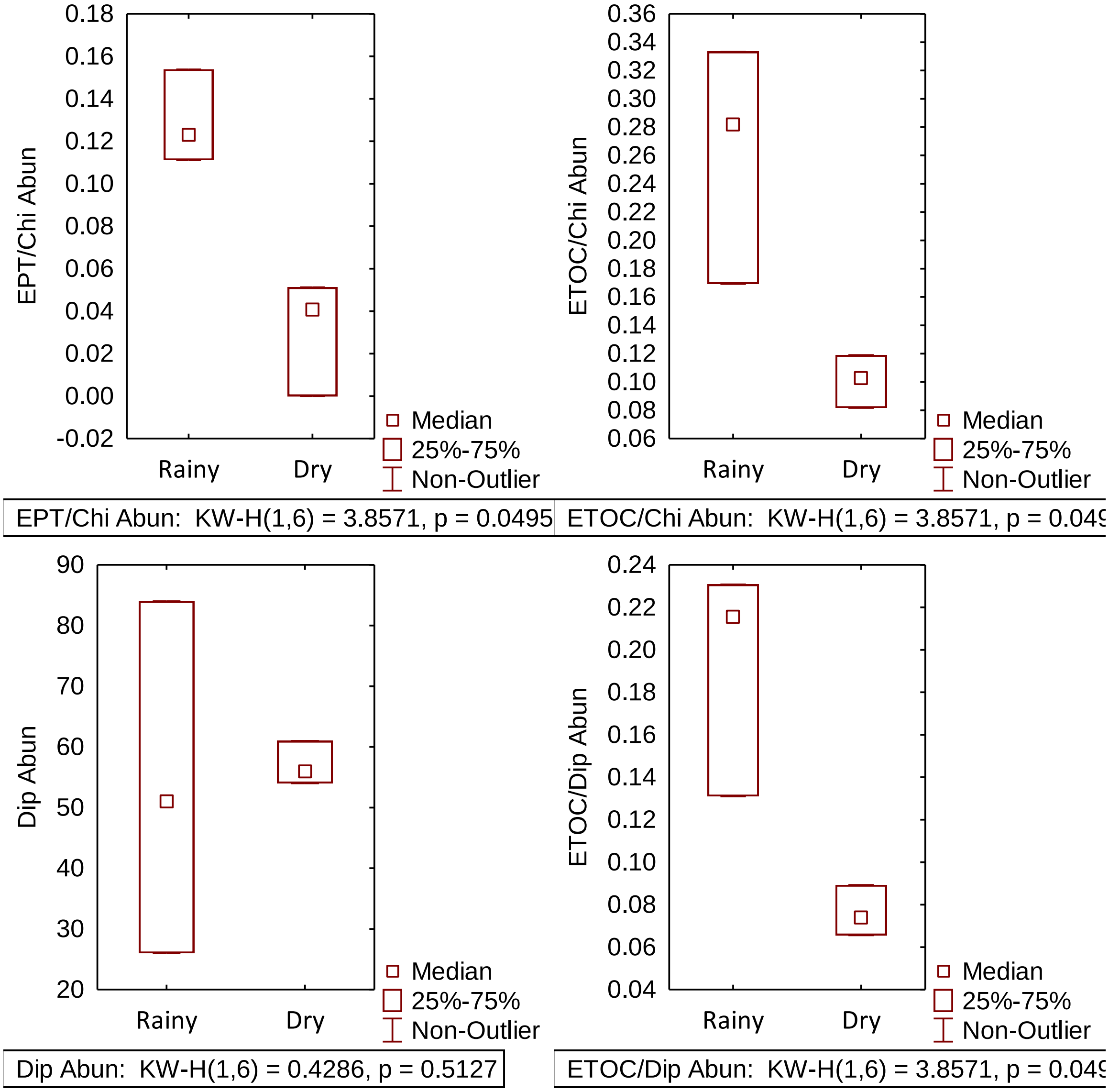

Chi Abun: KW-H(1,6) = 0.1961, p = 0.6579




Chi+Oli Abun: KW-H(1,6) = 0.4286, p = 0.5127




Oli Abun: KW-H(1,6) = 1.7647, p = 0.1840


EPT/Chi Abun
Rainy
Dry
Median
25%-75%
Non-Outlier
EPT/Chi Abun: KW-H(1,6) = 3.8571, p = 0.0495
ETOC/Chi Abun
ETOC/Chi Abun: KW-H(1,6) = 3.8571, p = 0.049
Dip Abun
Dip Abun: KW-H(1,6) = 0.4286, p = 0.5127
ETOC/Dip Abun
ETOC/Dip Abun: KW-H(1,6) = 3.8571, p = 0.049

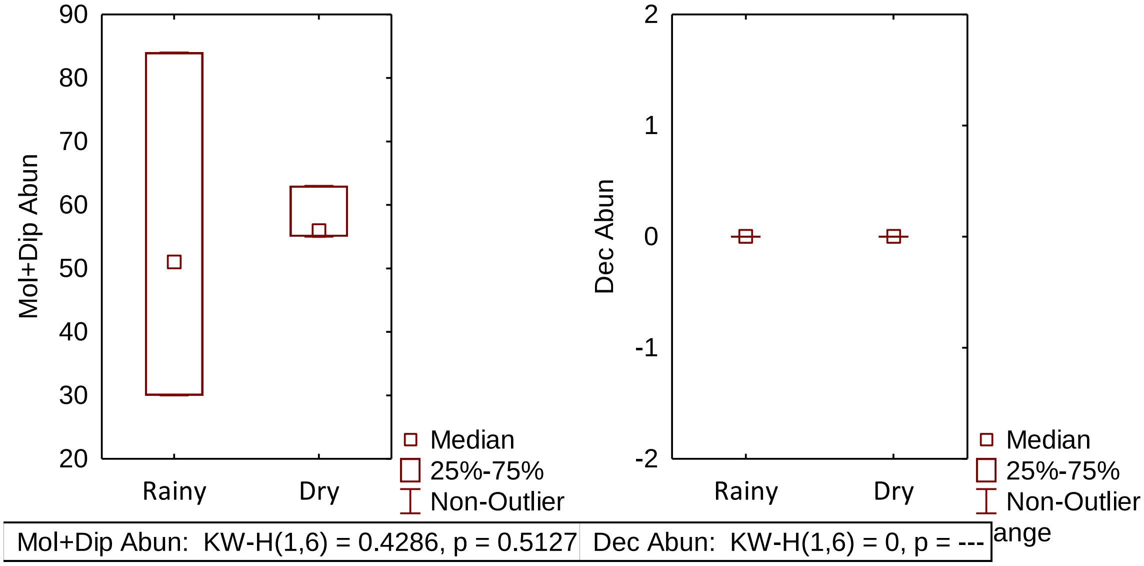
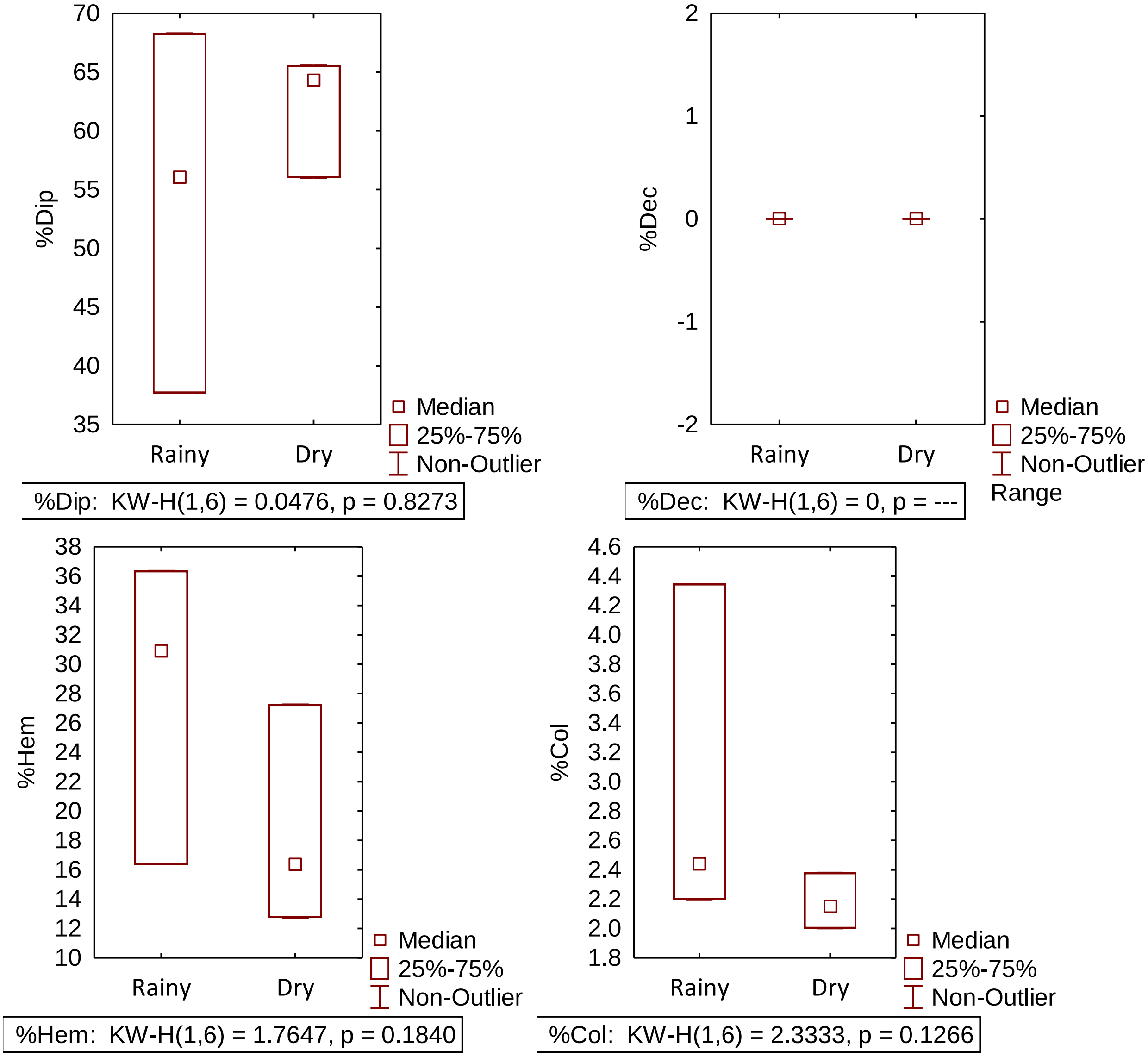



**Figure D6:** Seasonally and non-seasonally stable metrics in the abundance measures not integrated into the urban multimetric index (MMI-urban)


%Dip
70
65
60
55
50
45
40
35
Rainy
Dry
Median
25%-75%
Non-Outlier
%Dip: KW-H(1,6) = 0.0476, p = 0.8273
%Dec
2
1
0
-1
-2
Rainy
Dry
Median
25%-75%
Non-Outlier
Range
%Dec: KW-H(1,6) = 0, p = ---
%Hem
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
Rainy
Dry
Median
25%-75%
Non-Outlier
%Hem: KW-H(1,6) = 1.7647, p = 0.1840
%Col
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
Rainy
Dry
Median
25%-75%
Non-Outlier
%Col: KW-H(1,6) = 2.3333, p = 0.1266

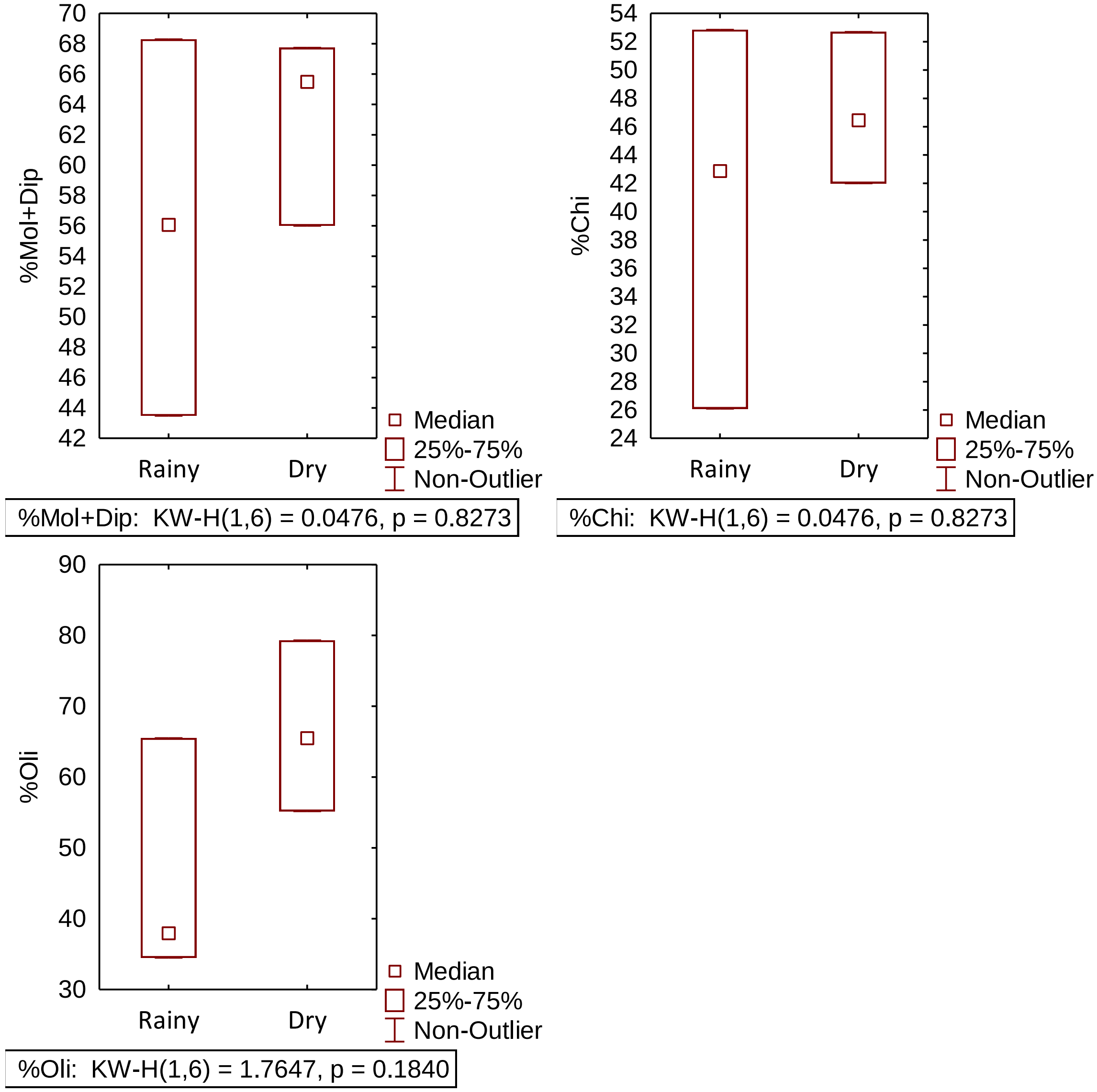



**Figure D7:** Seasonally and non-seasonally stable metrics in the composition measures not integrated into the urban multimetric index (MMI-urban)

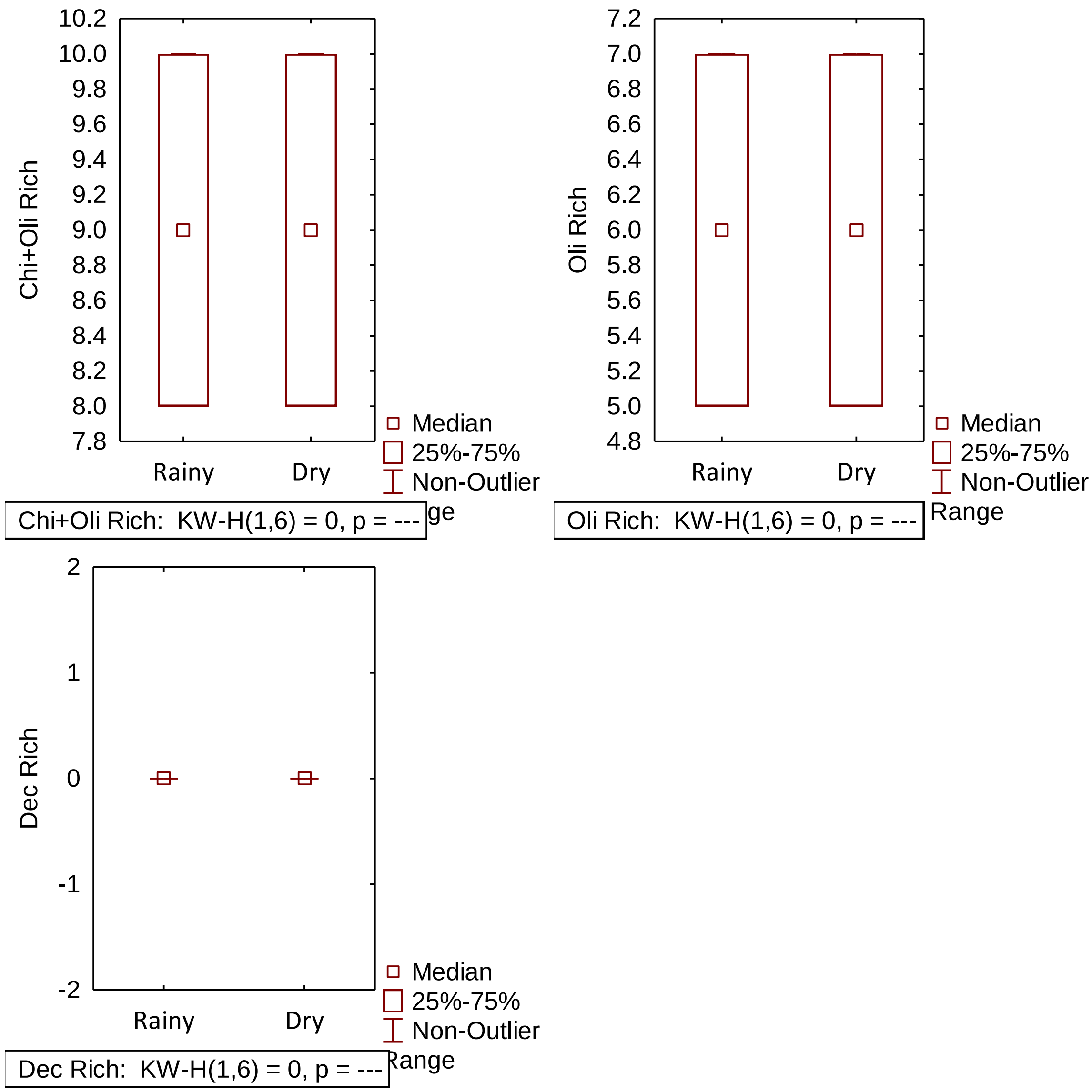
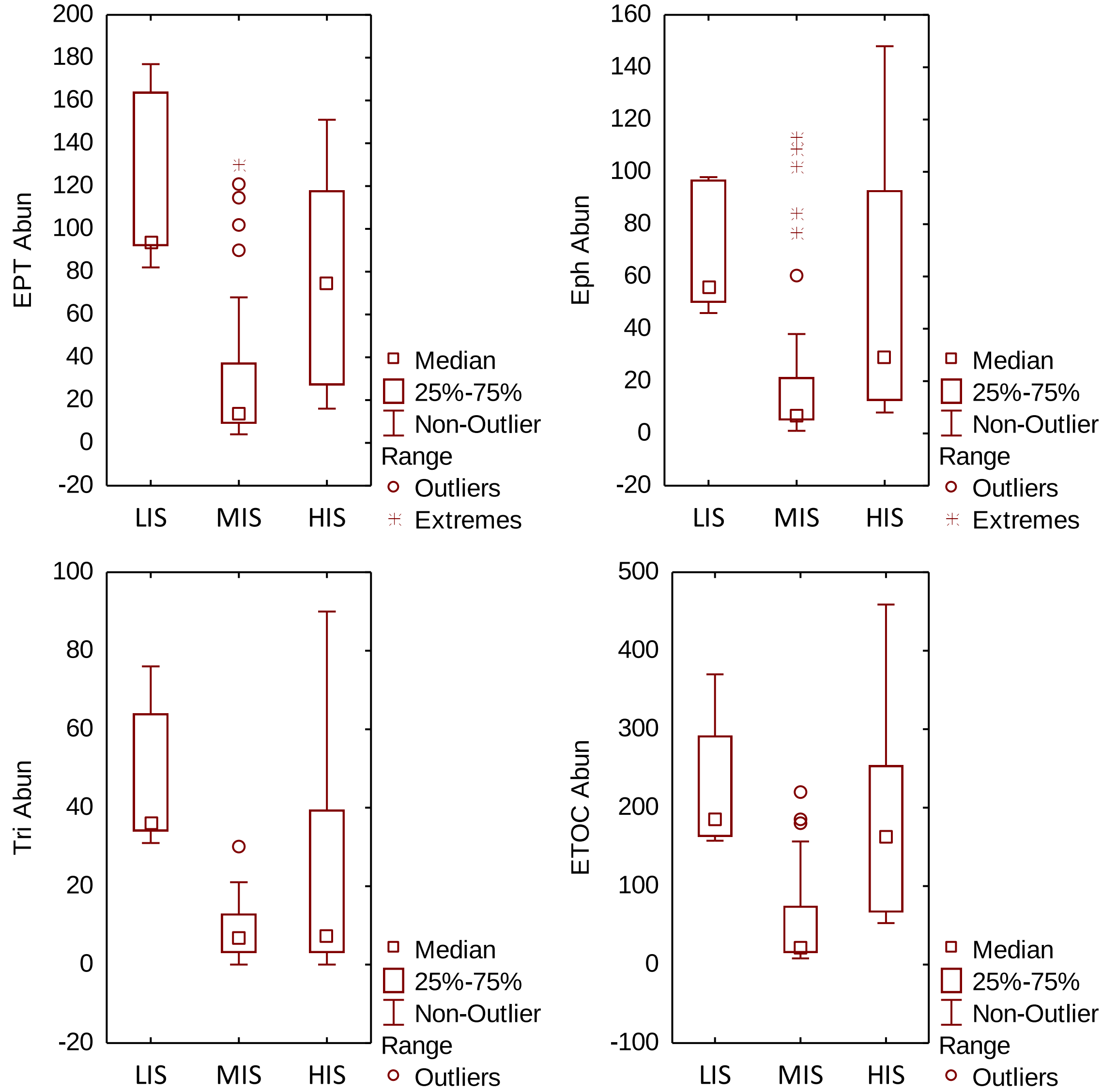
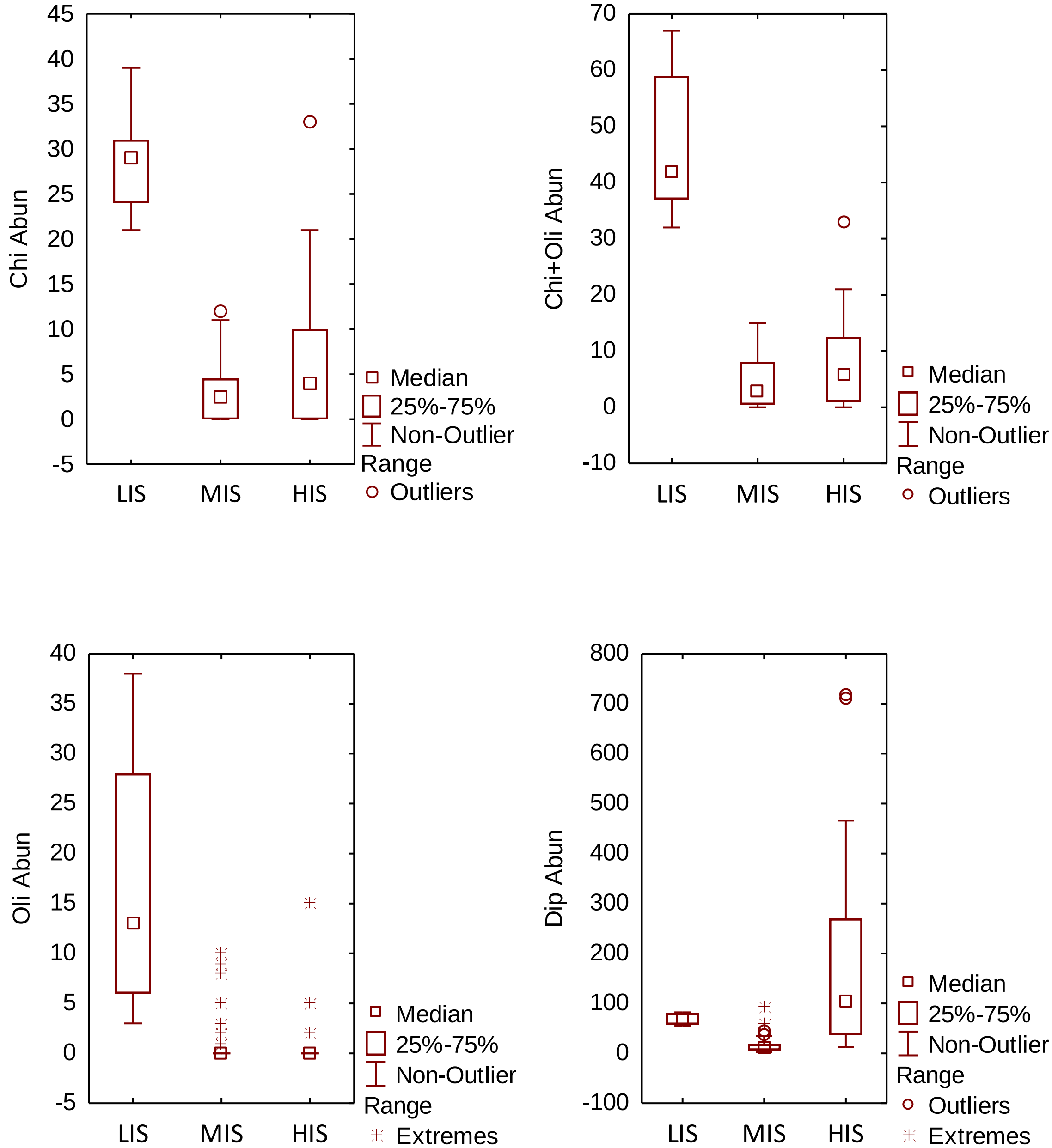
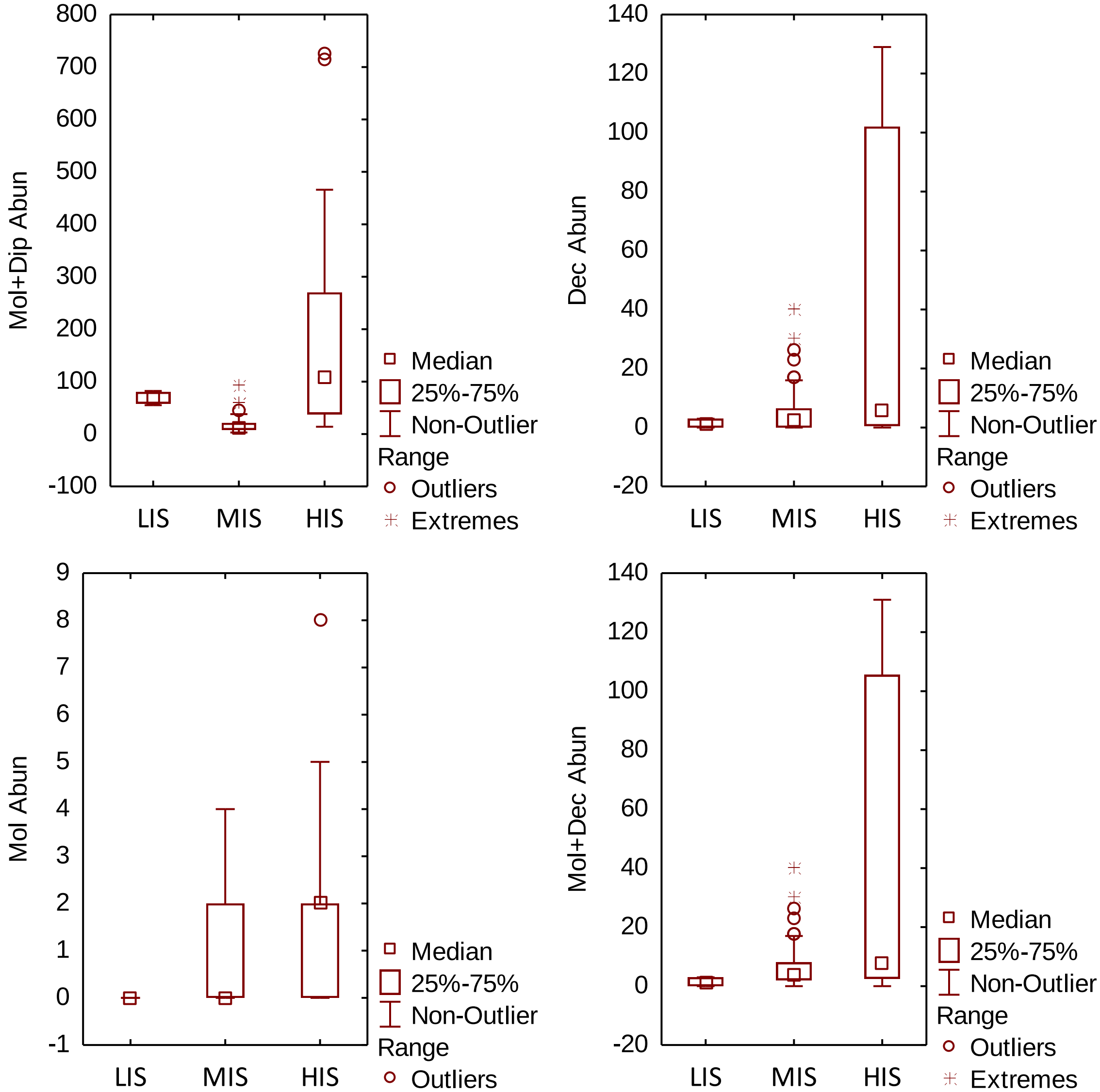



**Figure D8:** Non-seasonally stable metrics in the richness measures not integrated into the urban multimetric index (MMI-urban)

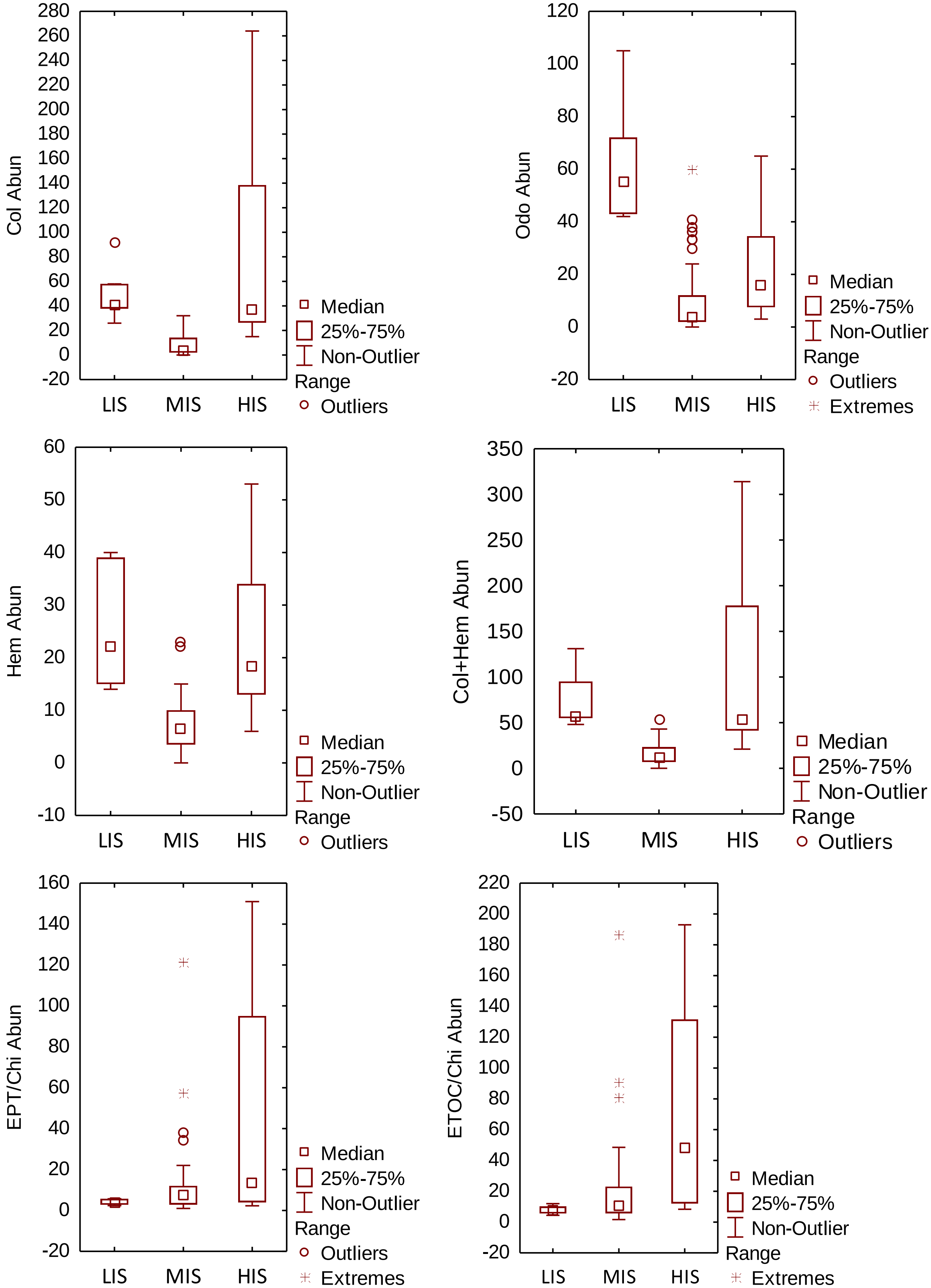
**Appendix E: Sensitive/non-sensitive and seasonally stable/non-seasonally stable metrics not integrated into the urban-agricultural multimetrc index (MMI-urban-agric)**




Chi Abun
45
40
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Chi+Oli Abun
70
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Oli Abun
40
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes
Dip Abun
800
700
600
500
400
300
200
100
0
-100
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes



Mol+Dip Abun
800
700
600
500
400
300
200
100
0
-100
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Dec Abun
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Mol Abun
9
8
7
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Mol+Dec Abun
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Col Abun
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Odo Abun
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Hem Abun
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Col+Hem Abun
350
300
250
200
150
100
50
0
-50
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
EPT/Chi Abun
160
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
ETOC/Chi Abun
220
200
180
160
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes

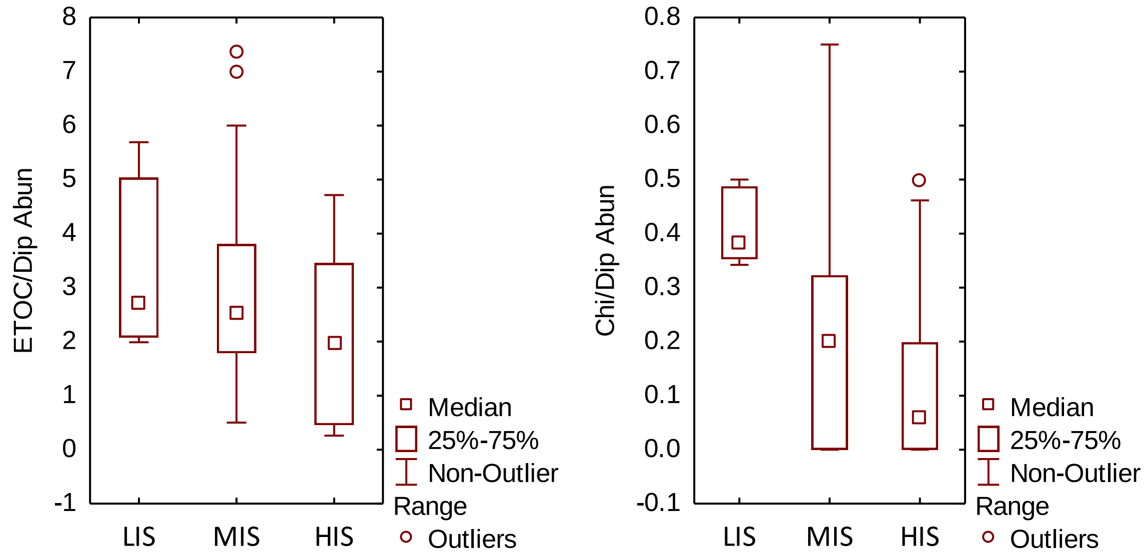
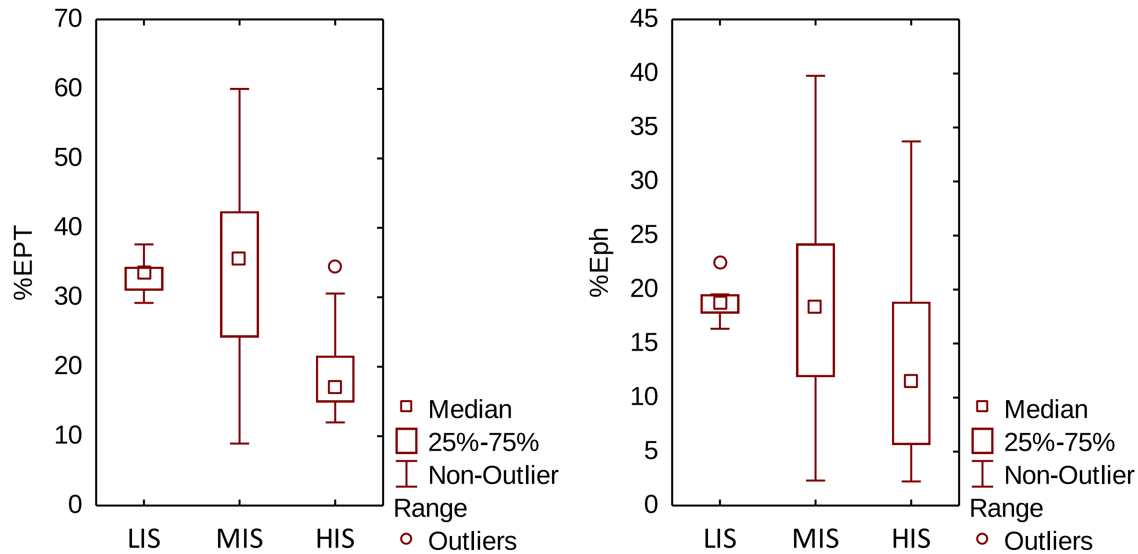
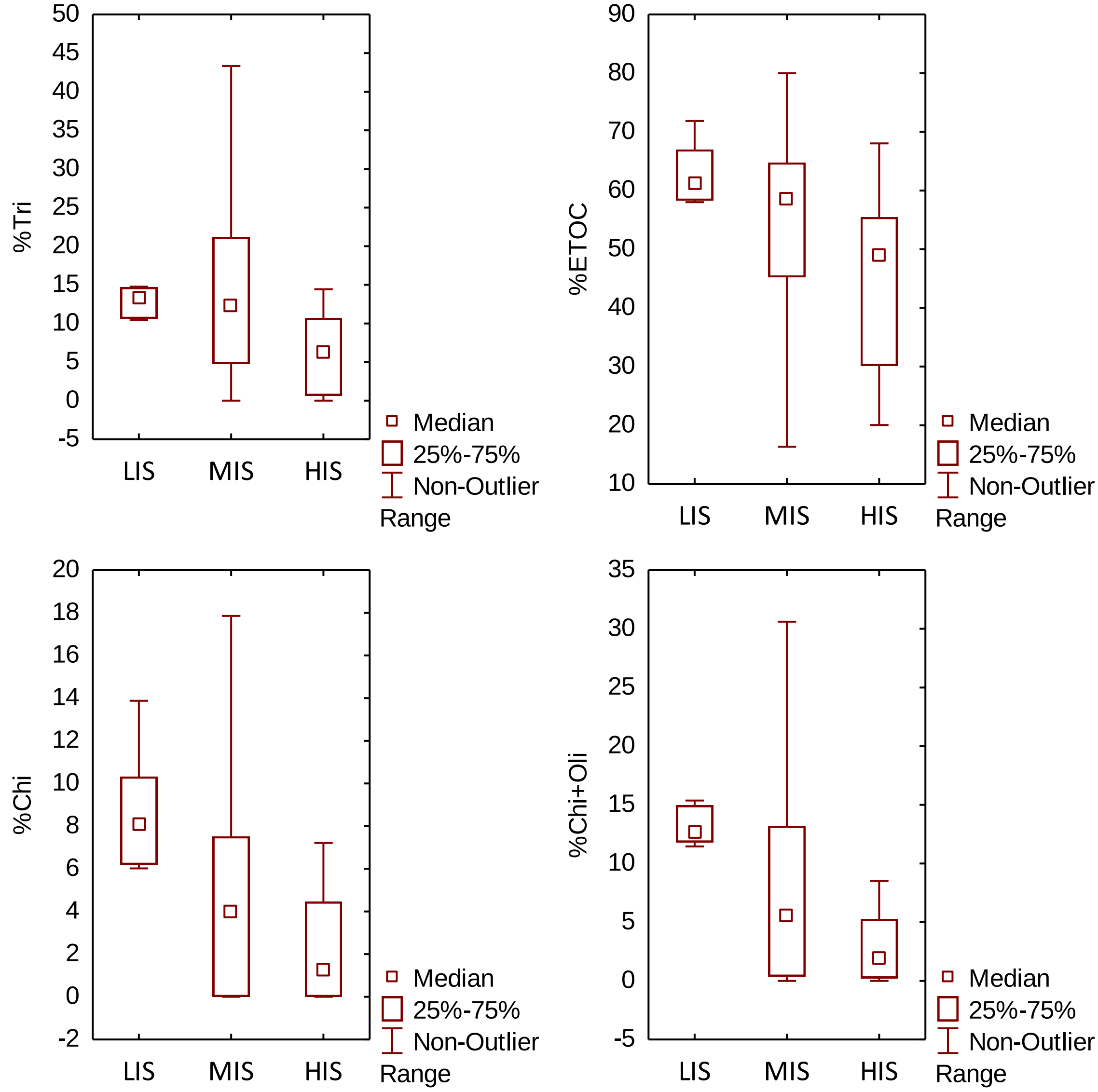
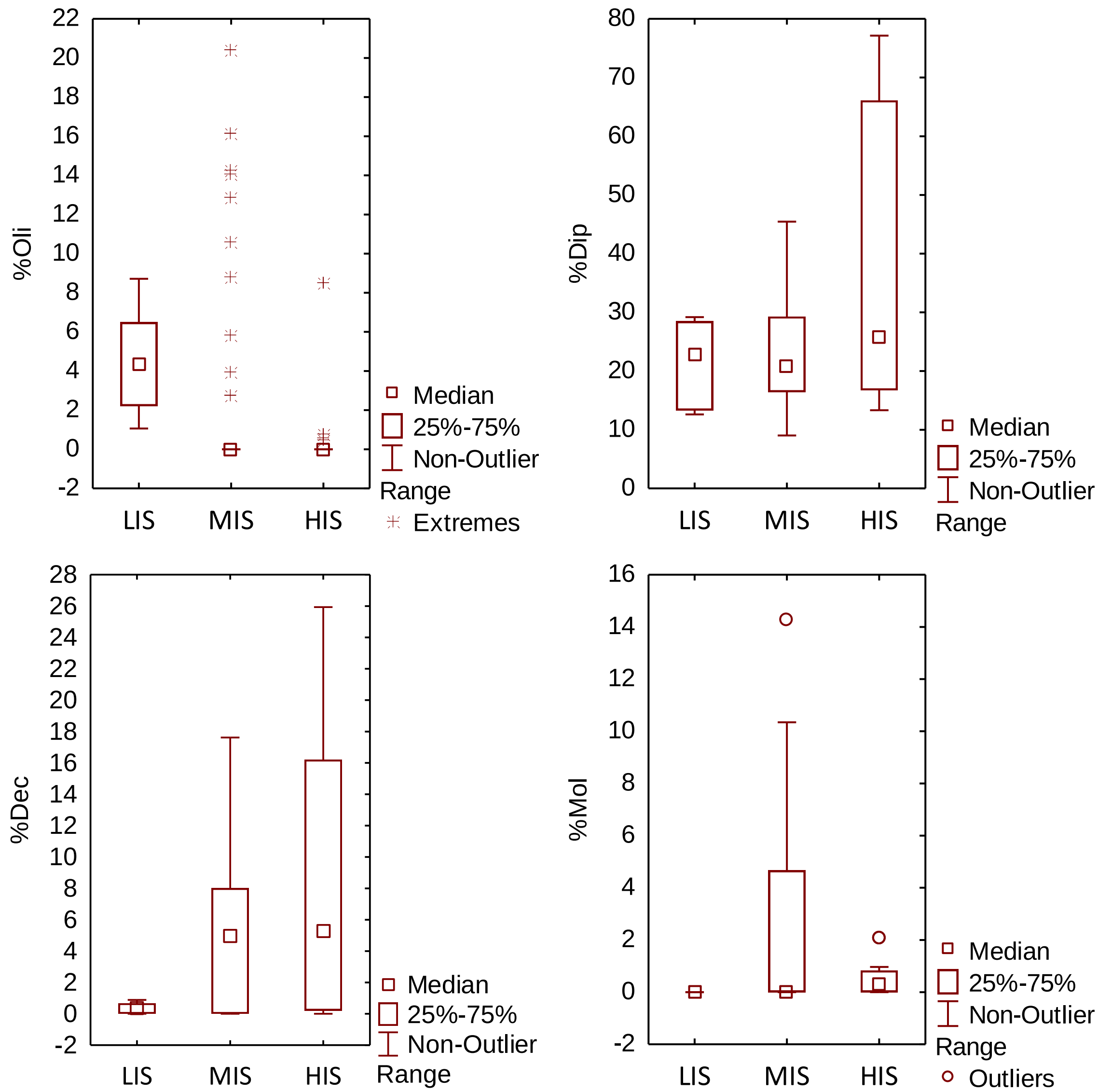
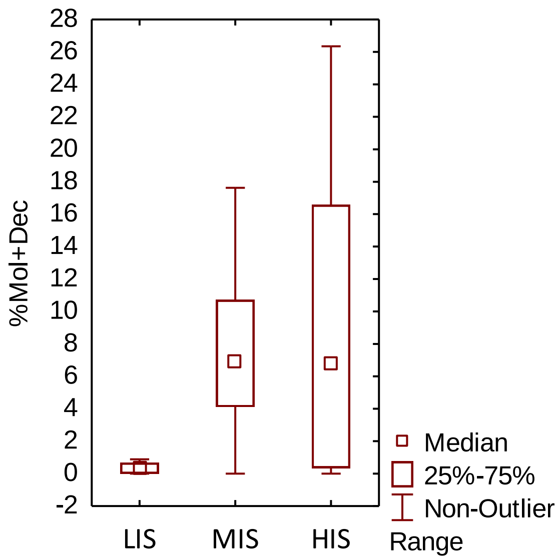


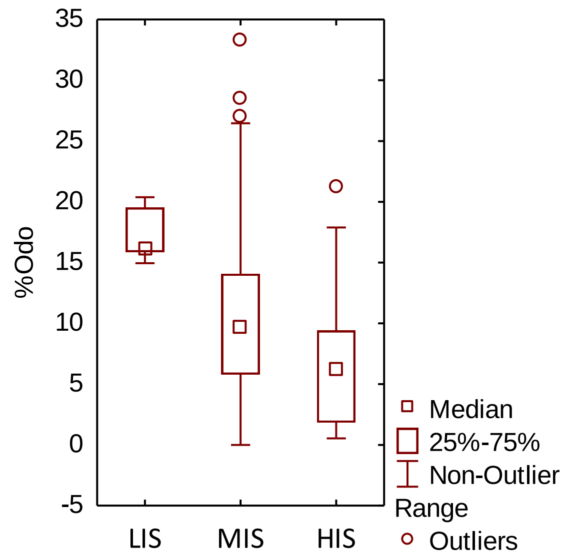

**Figure E1:** Sensitive and non sensitive metrics in the abundance measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)

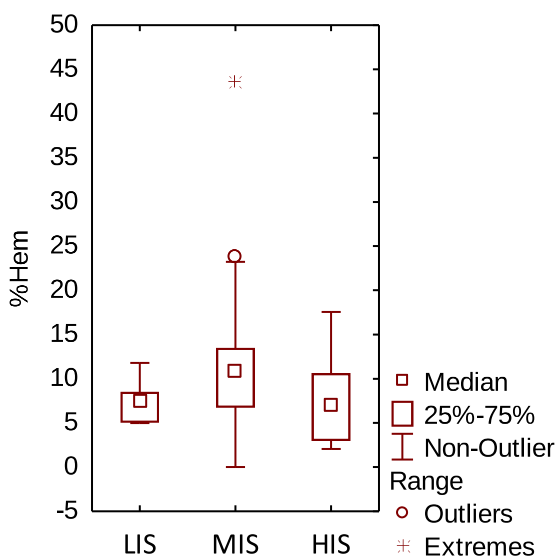


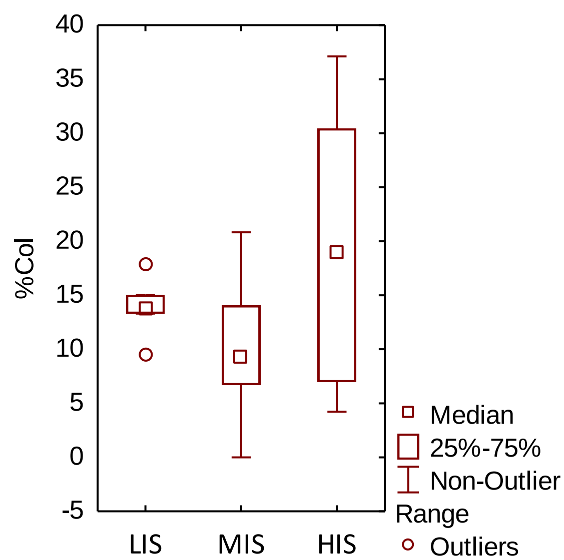

%Tri
50
45
40
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%ETOC
90
80
70
60
50
40
30
20
10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%Chi
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%Chi+Oli
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


%Oli
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes
%Dip
80
70
60
50
40
30
20
10
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%Dec
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%Mol
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers


%Mol+Dec
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


%Odo
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers


%Hem
50
45
40
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


%Col
40
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers

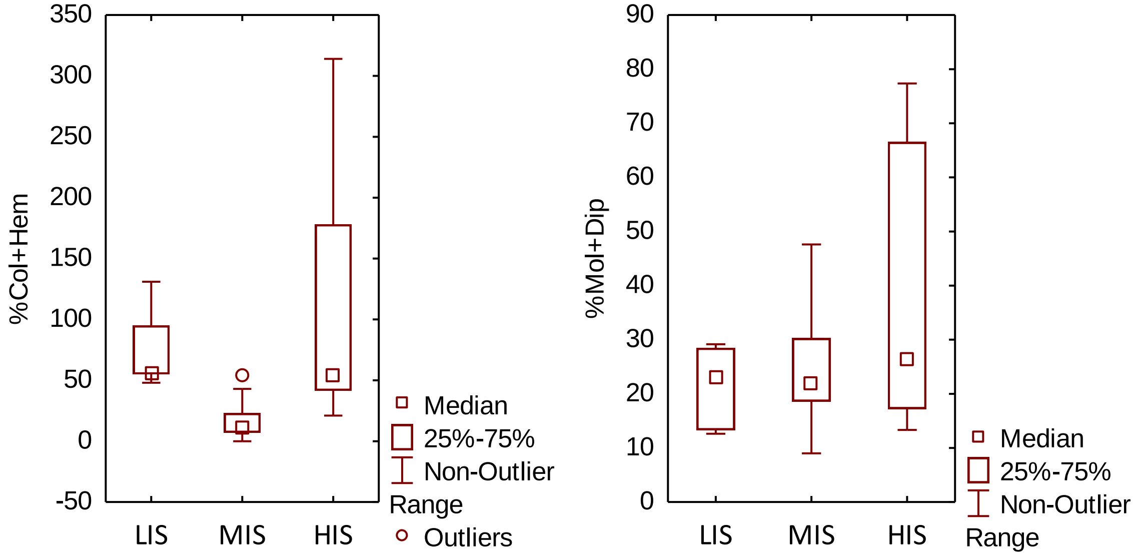
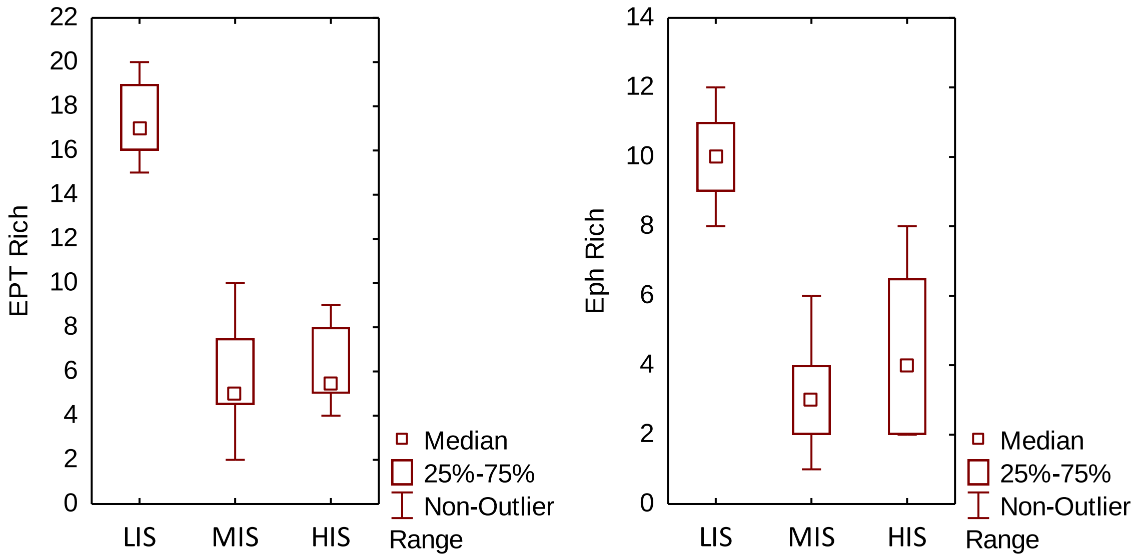
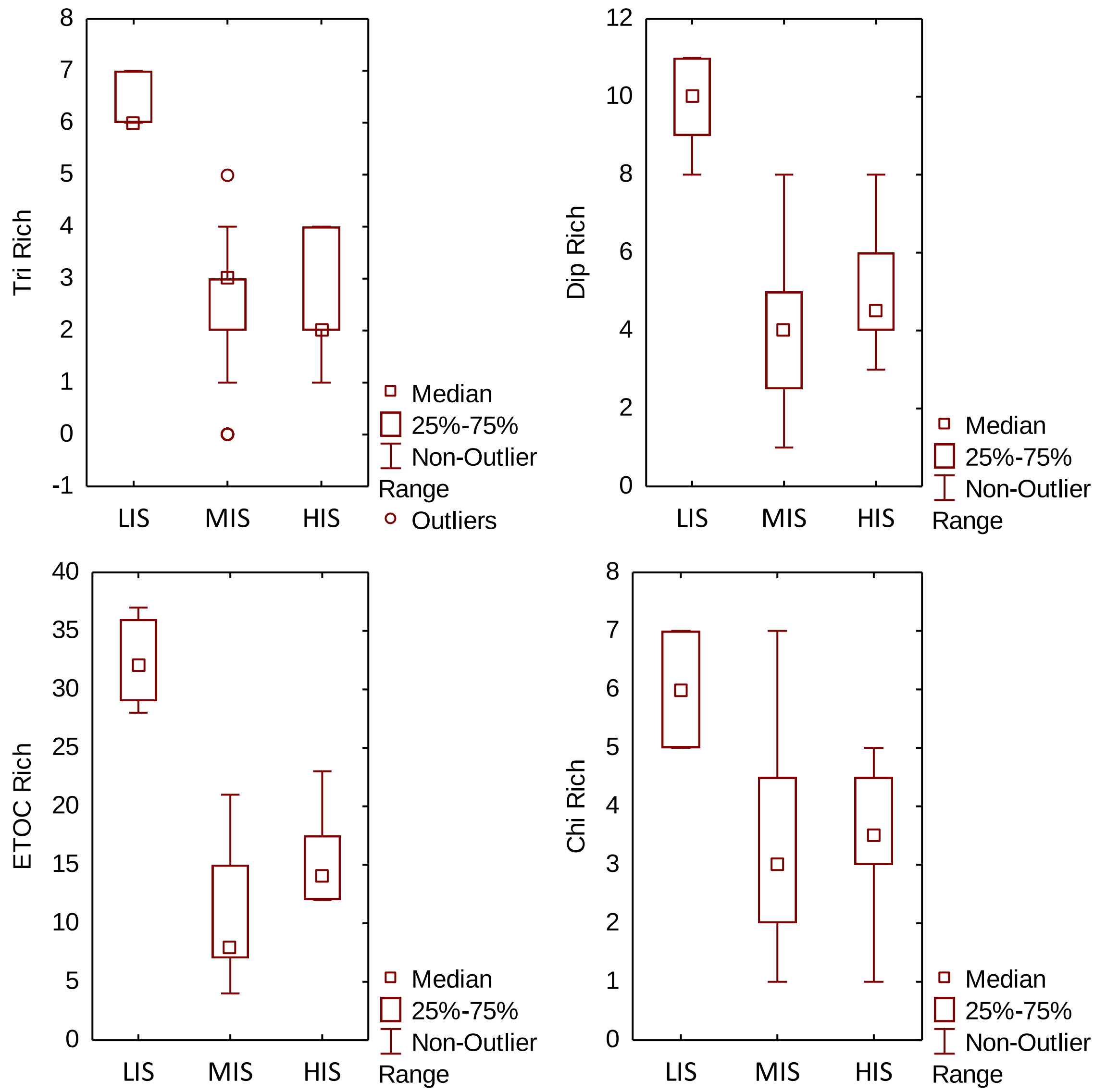
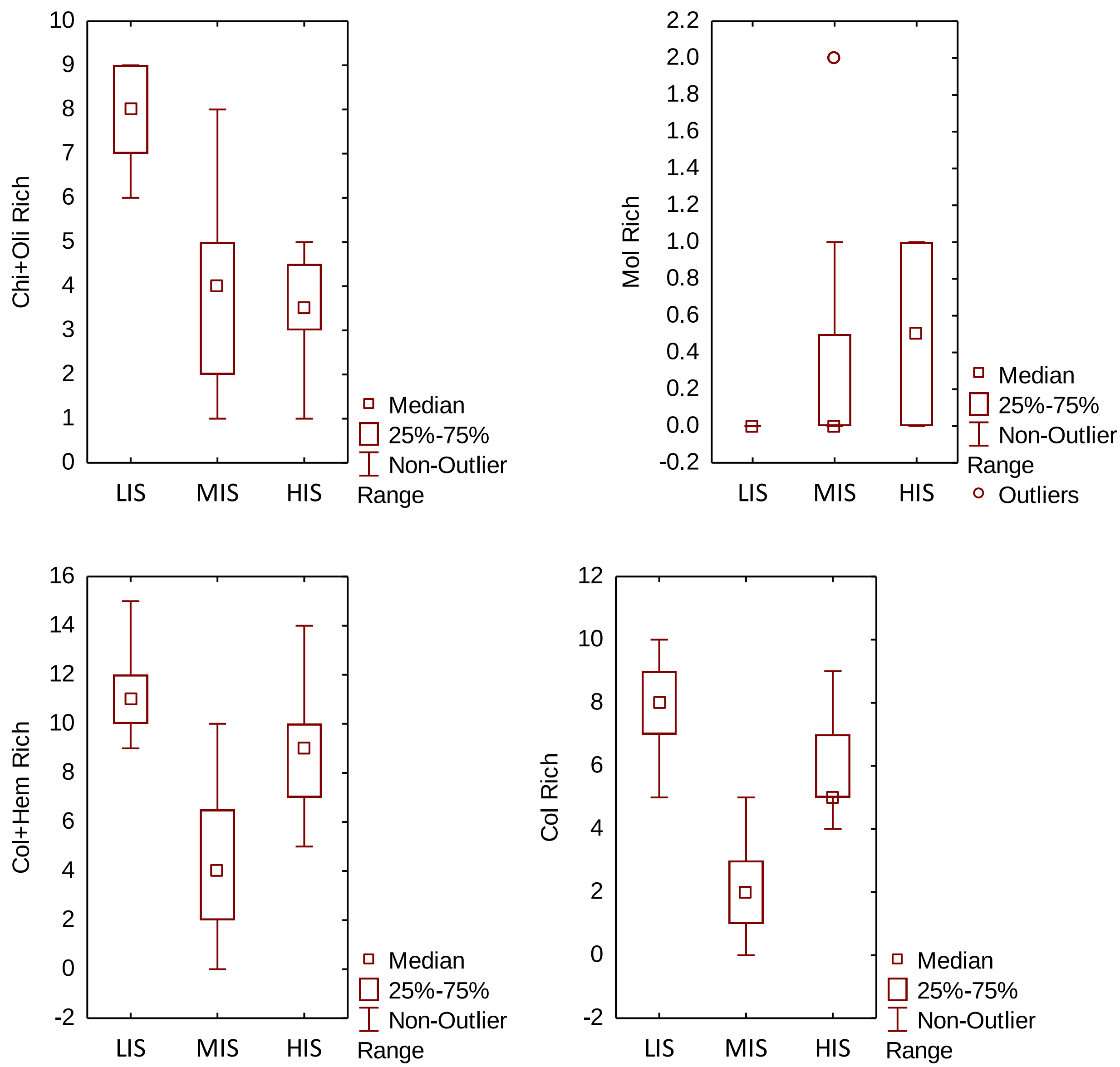



**Figure E2:** Sensitive and non sensitive metrics in the composition measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)




Tri Rich
8
7
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Dip Rich
12
10
8
6
4
2
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
ETOC Rich
40
35
30
25
20
15
10
5
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Chi Rich
8
7
6
5
4
3
2
1
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


Chi+Oli Rich
10
9
8
7
6
5
4
3
2
1
0
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Mol Rich
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Col+Hem Rich
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Col Rich
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range

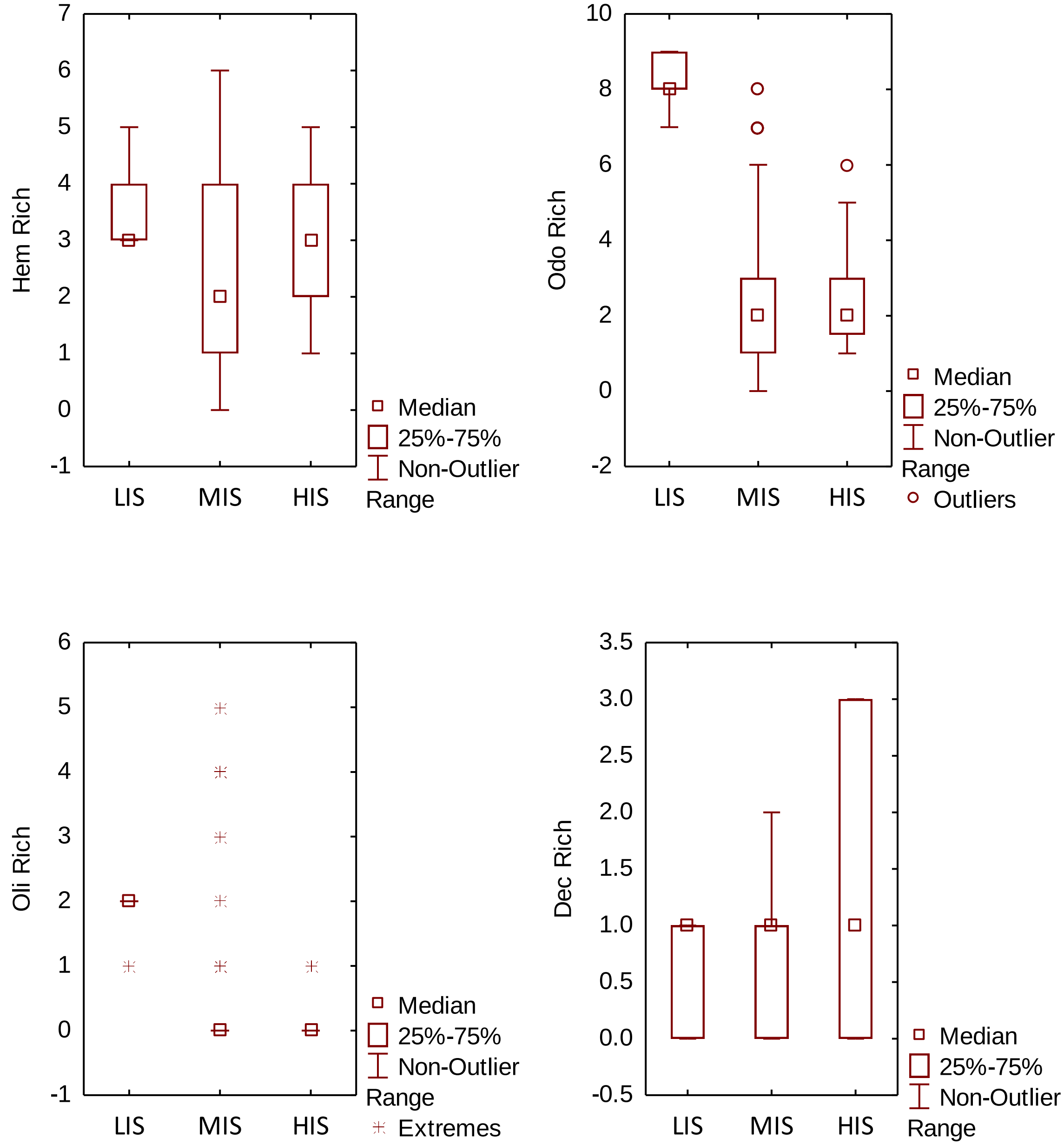



**Figure E3:** Sensitive and non sensitive metrics in the richness measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)

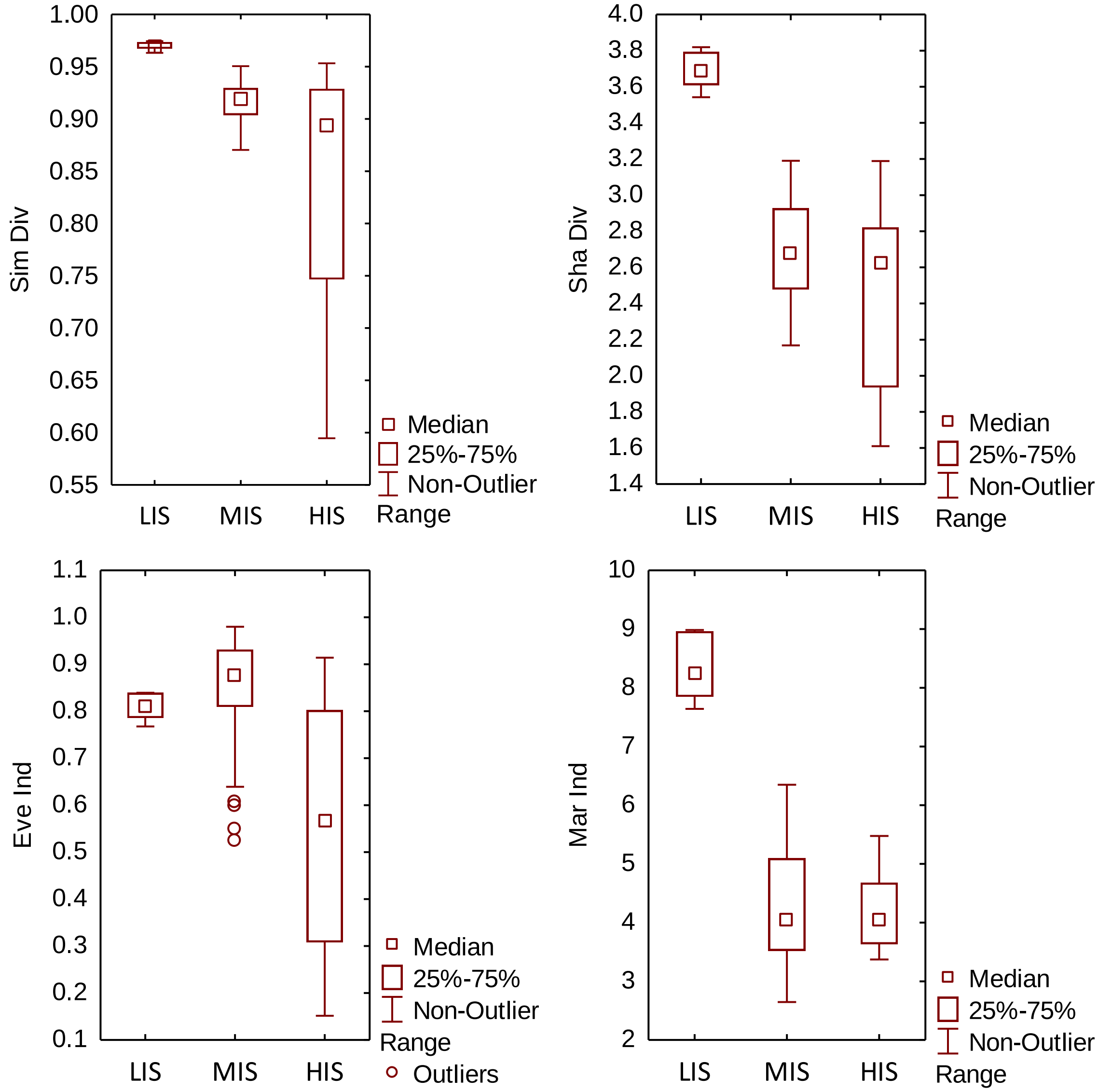
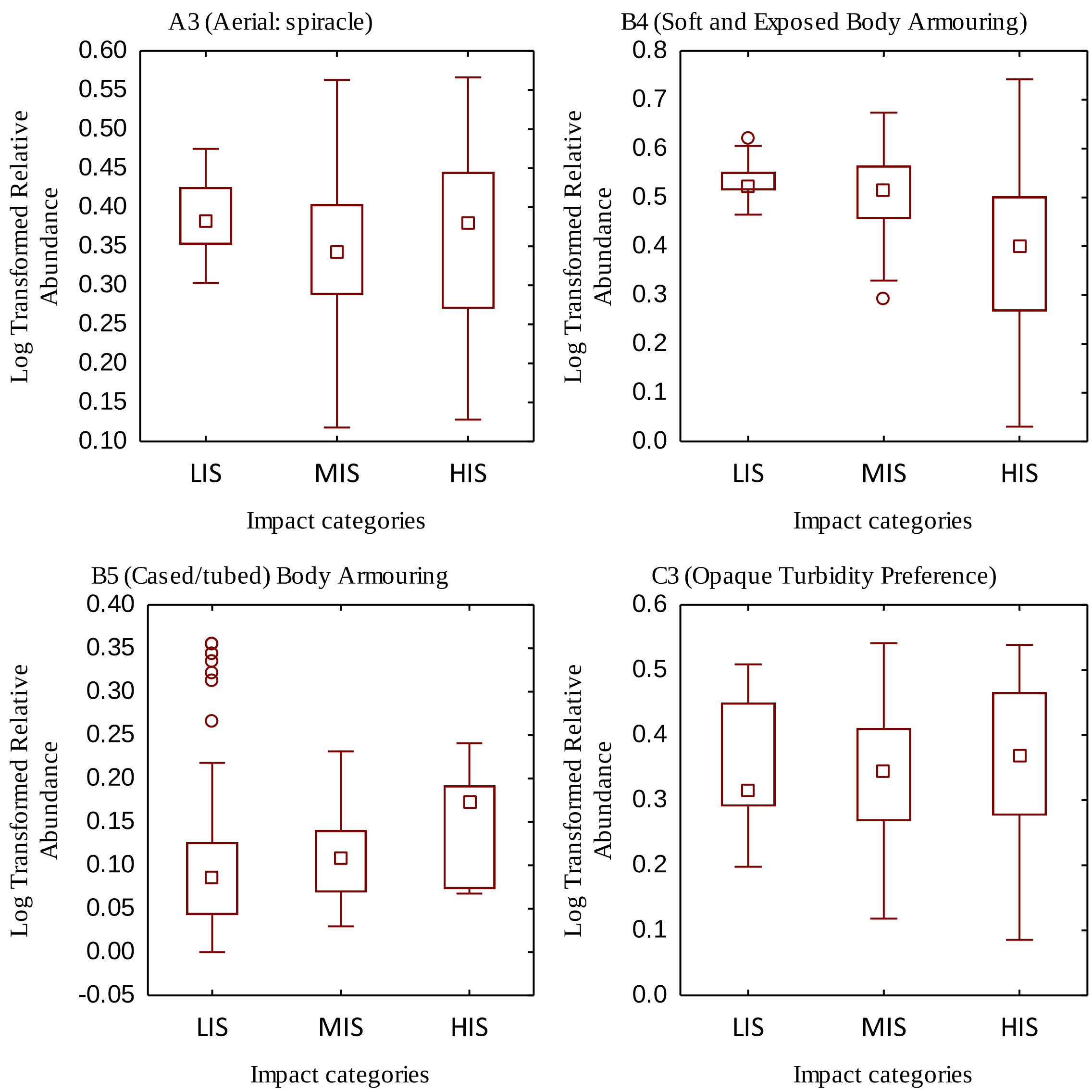
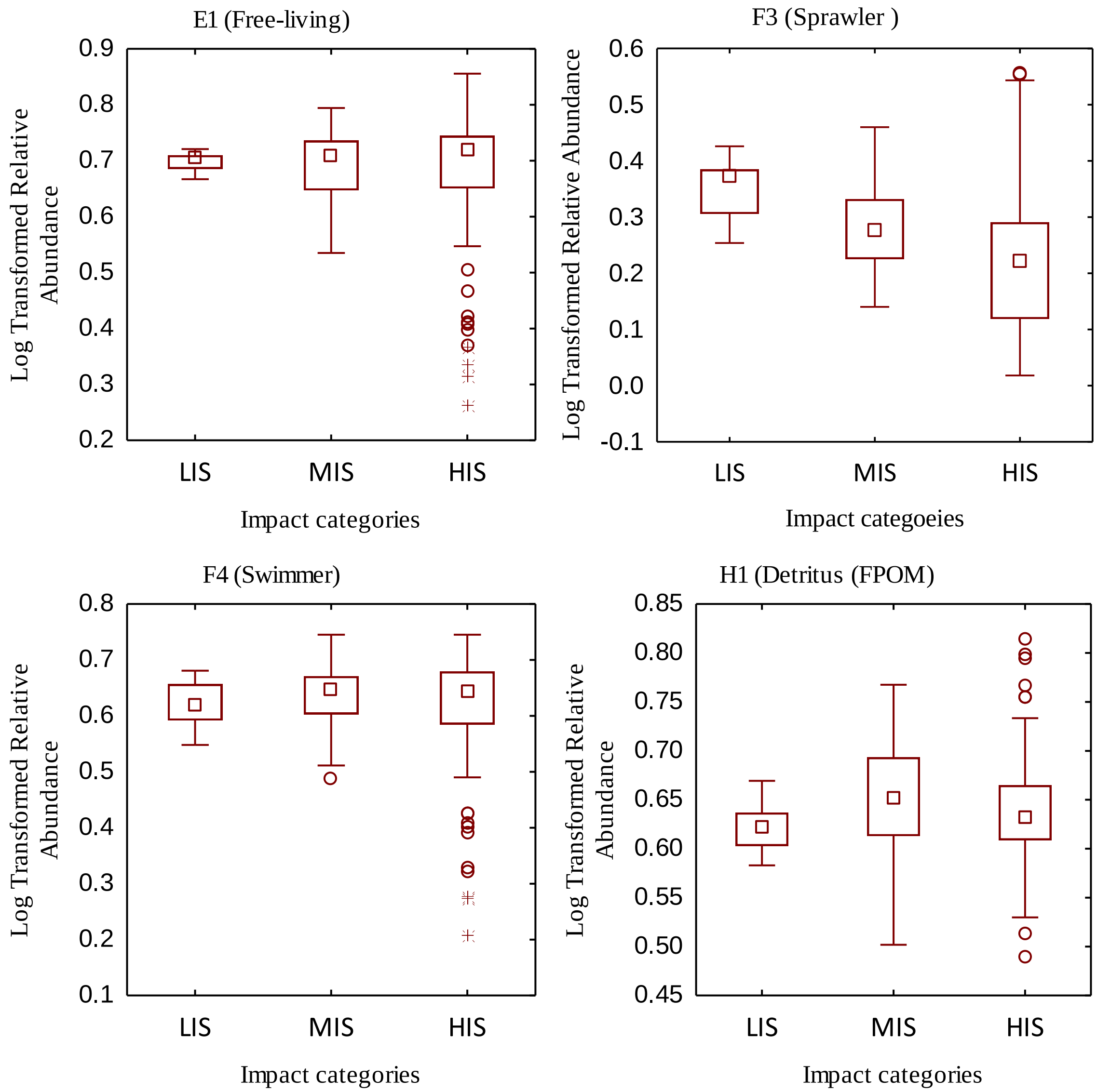
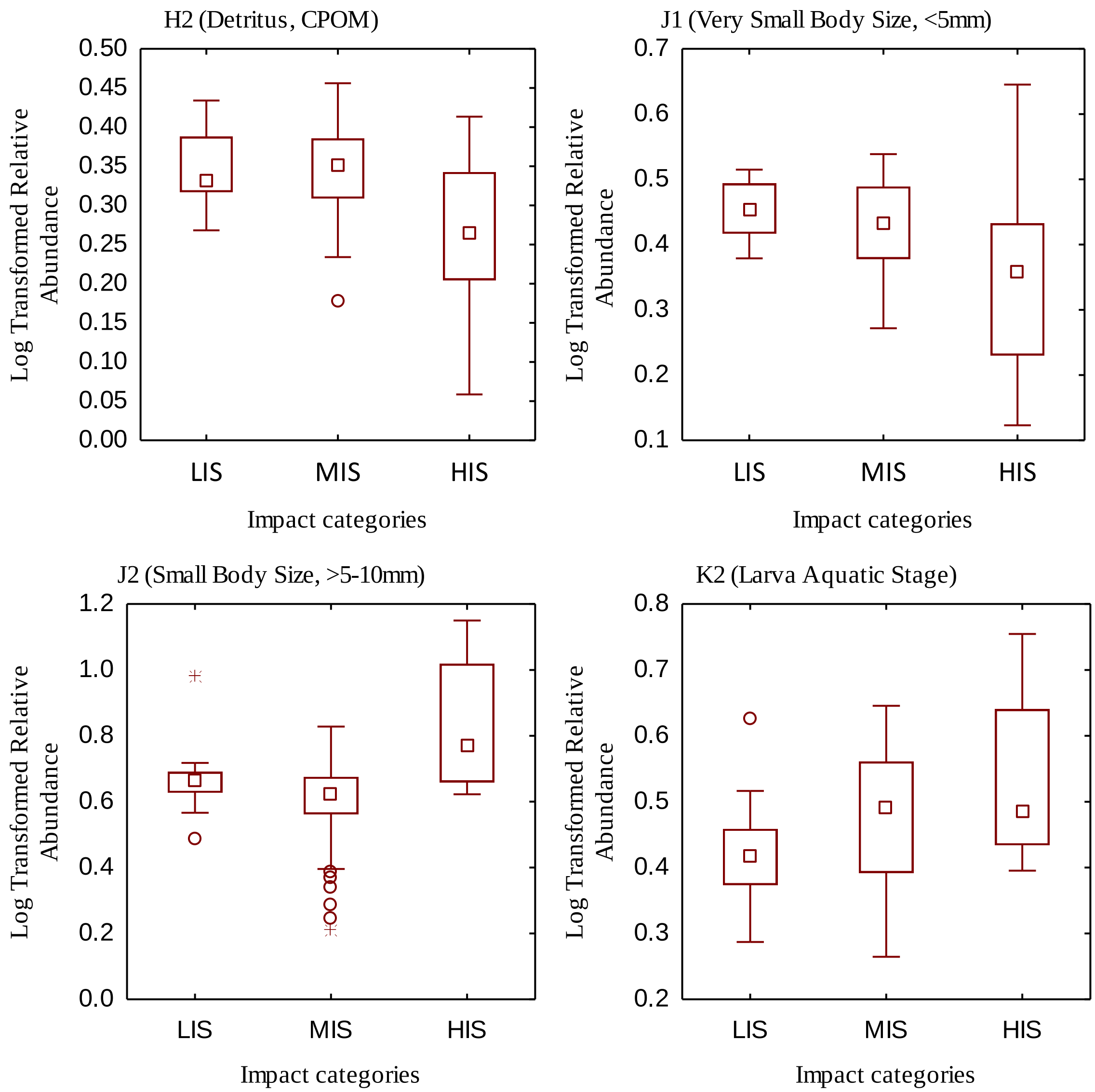



**Figure E4:** Sensitive and non sensitive metrics in the diversity measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)


A3 (Aerial: spiracle)
B4 (Soft and Exposed Body Armouring)
B5 (Cased/tubed) Body Armouring
C3 (Opaque Turbidity Preference)
Log Transformed Relative Abundance
Impact categories
LIS
MIS
HIS
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.05
0.00
-0.05


E1 (Free-living)
Log Transformed Relative Abundance
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
LIS
MIS
HIS
Impact categories
F3 (Sprawler )
Log Transformed Relative Abundance
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
LIS
MIS
HIS
Impact categoeies
F4 (Swimmer)
Log Transformed Relative Abundance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
LIS
MIS
HIS
Impact categories
H1 (Detritus (FPOM)
Log Transformed Relative Abundance
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
LIS
MIS
HIS
Impact categories


H2 (Detritus, CPOM)
Log Transformed Relative Abundance
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
LIS
MIS
HIS
Impact categories
J1 (Very Small Body Size, <5mm)
Log Transformed Relative Abundance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
LIS
MIS
HIS
Impact categories
J2 (Small Body Size, >5-10mm)
Log Transformed Relative Abundance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LIS
MIS
HIS
Impact categories
K2 (Larva Aquatic Stage)
Log Transformed Relative Abundance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
LIS
MIS
HIS
Impact categories

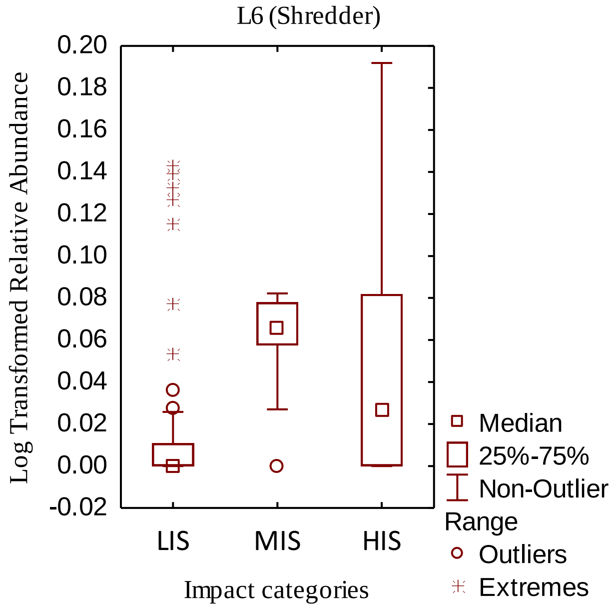
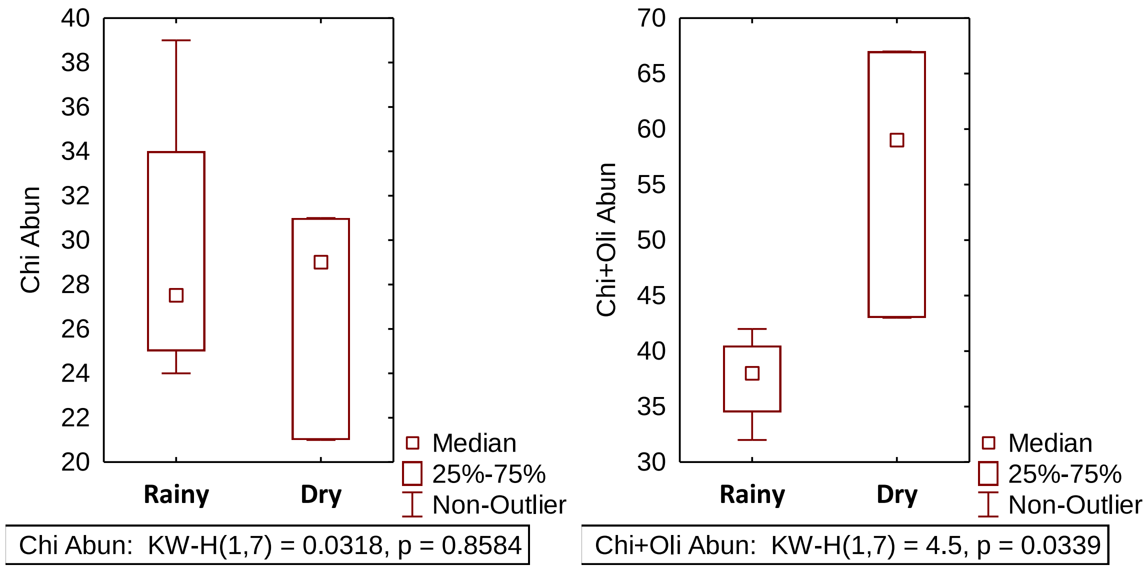



**Figure E5:** Sensitive and non sensitive metrics in the traits and ecological preferences measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)

**Table E1:** Sensitive metrics selection for urban-agricultural multimetric index (MMI-urban-agric). **Note: a metric sensitivity is confirmed if significant at *P*<0.05**

| Discriminatory metrics | Mann-Whitney test (U-test) | *P*-value | Sensitivity confirmed |
|---|---|---|---|
| **Abundance measures** | | | |
| EPT Abun | 471.5 | 0.09873 | No |
| Eph | 586 | 0.7598 | No |
| Tri Abun | 553 | 0.4872 | No |
| Chi Abun | 442.5 | 0.04318 | Yes |
| Chi+Oli Abun | 414.5 | 0.01954 | Yes |
| Oli Abun | 378.5 | 0.0007141 | Yes |
| Odo Abun | 597.5 | 0.8645 | No |
| Chi/Dip Abun | 325.5 | 0.0006454 | Yes |
| **Composition measures** | | | |
| %EPT | 538 | 0.3847 | No |
| %Chi | 317 | 0.0004432 | Yes |
| %Chi+Oli | 221 | 3.922E-06 | Yes |
| %Oli | 360 | 0.0002606 | Yes |
| %Dec | 589 | 0.7833 | No |
| %Mol+Dec | 486.5 | 0.1389 | No |
| %Odo | 436.5 | 0.03922 | Yes |
| %Col | 611.5 | 0.9953 | No |
| **Richness measures** | | | |
| EPT Rich | 504.5 | 0.2015 | No |

| | | | |
|---|---|---|---|
| Eph Rich | 453.5 | 0.05847 | No |
| Tri Rich | 604 | 0.9234 | No |
| Dip Rich | 412 | 0.01754 | Yes |
| ETOC Rich | 602 | 0.906 | No |
| Chi Rich | 427.5 | 0.02725 | Yes |
| Chi+Oli Rich | 267 | 4.113E-05 | Yes |
| Col+Hem Rich | 564.5 | 0.575 | No |
| Col Rich | 512.5 | 0.2362 | No |
| Hem Rich | 597 | 0.8576 | No |
| Odo Rich | 460 | 0.06882 | No |
| Oli Rich | 344 | 2.797E-05 | Yes |
| **Diversity measures** | | | |
| Sim Div | 250.5 | 2.17E-05 | Yes |
| Sha Div | 312.5 | 0.0004348 | Yes |
| Eve Div | 386 | 0.00794 | Yes |
| Mar Ind | 339 | 0.001342 | Yes |
| **Trait attributes measures** | | | |
| Log Spr | 319 | 0.000578 | Yes |
| LogLav | 565 | 0.5809 | No |
| LogShr | 418.5 | 0.007992 | Yes |



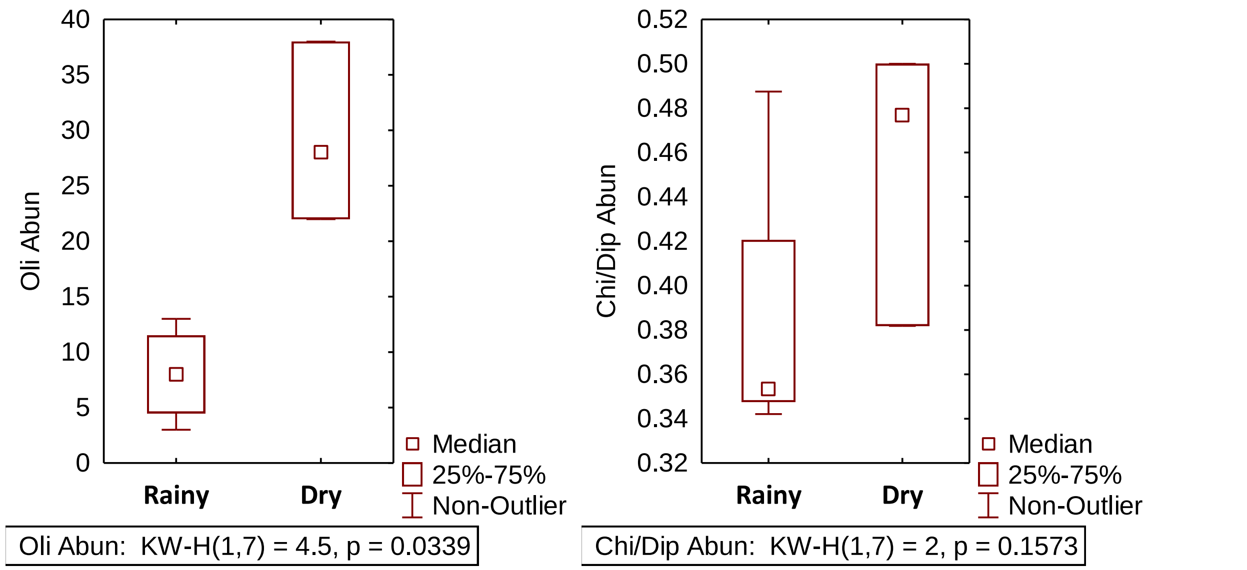
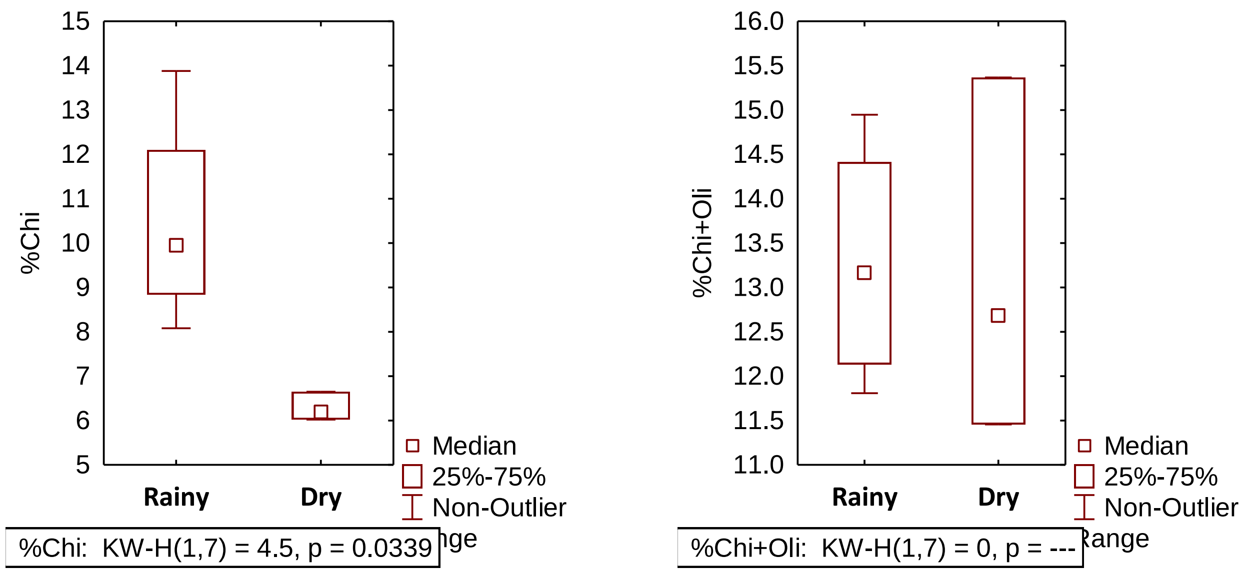
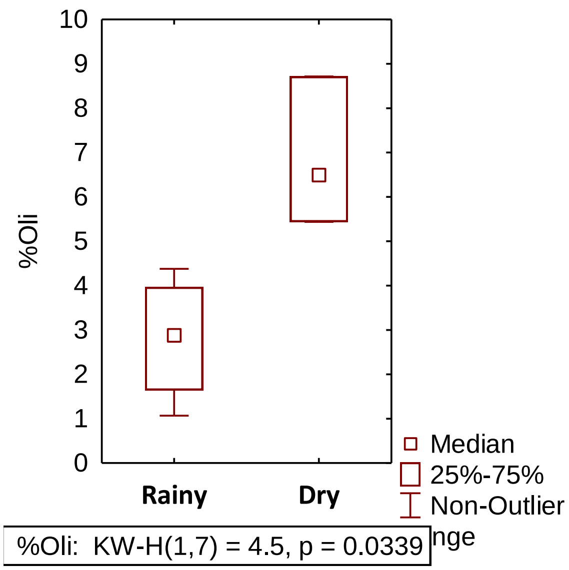



**Figure E6:** Seasonally and non-seasonally stable metrics in the abundance measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)





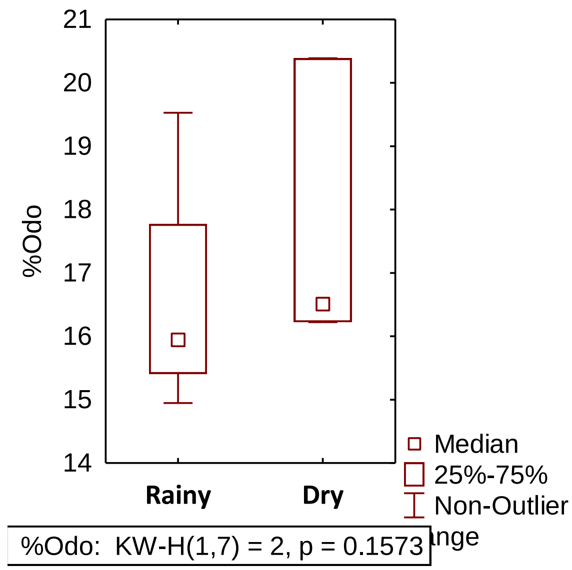
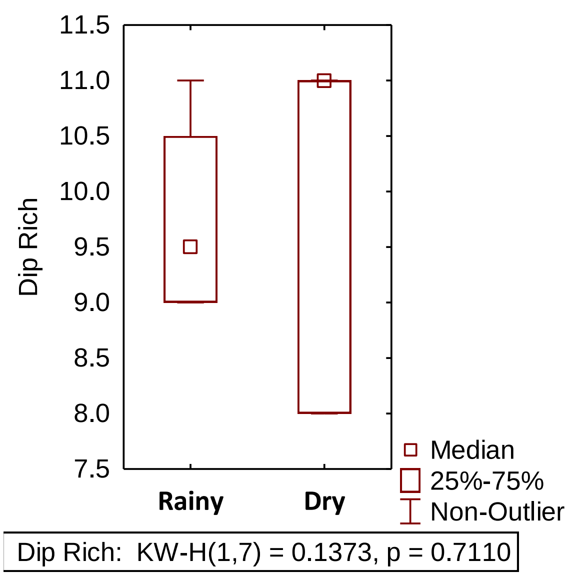
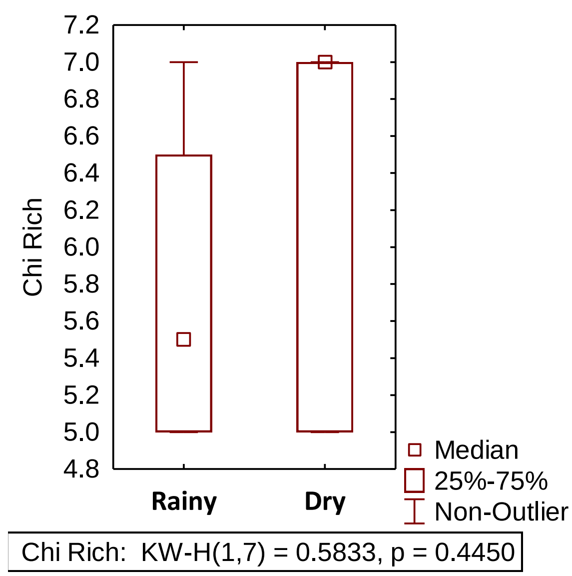




**Figure E7:** Seasonally and non-seasonally stable metrics in the composition measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)



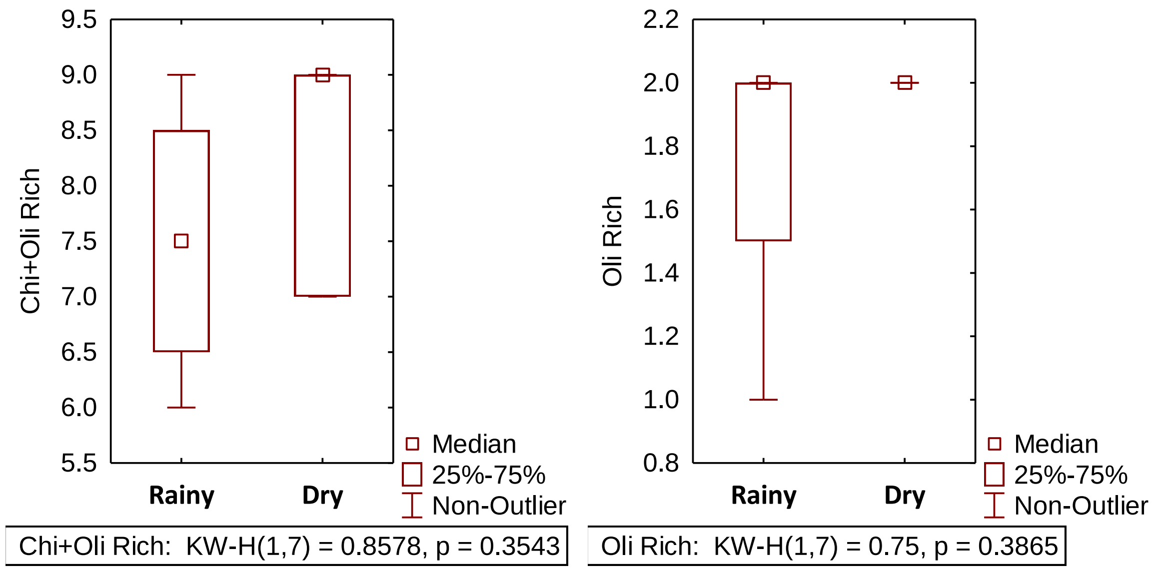
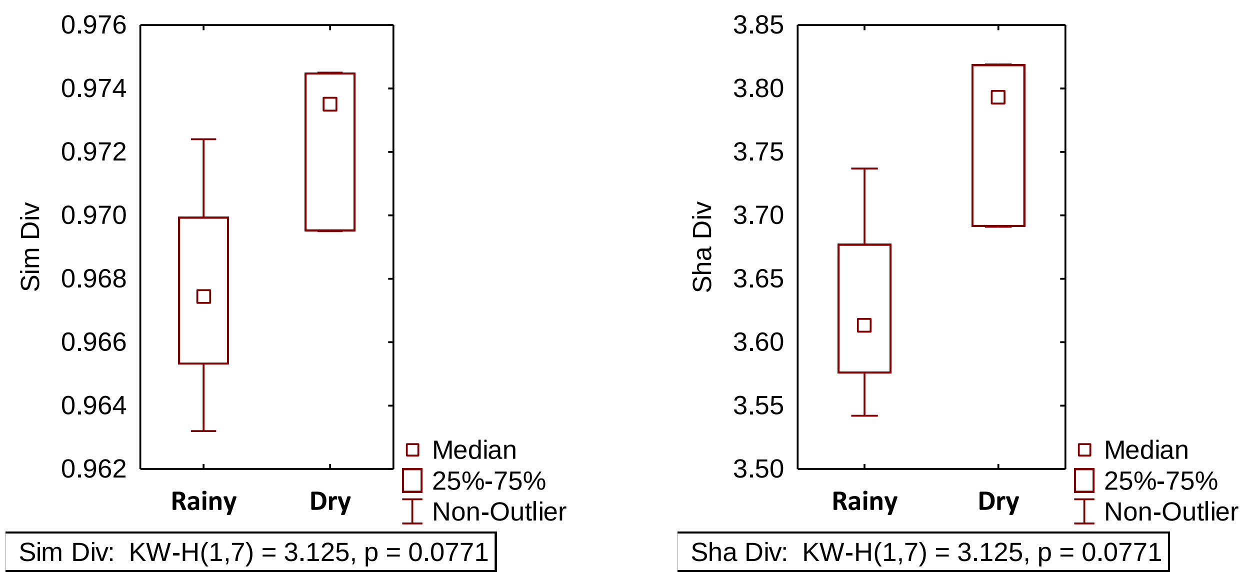



**Figure E8:** Seasonally stable metrics in the richness measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)



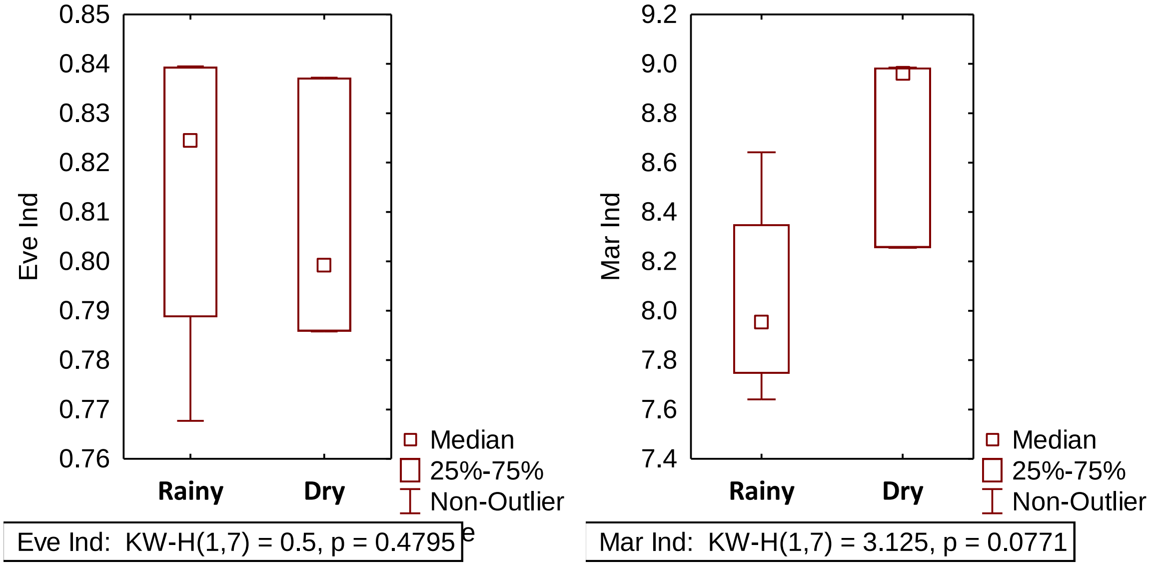



**Figure E9:** Seasonally stable metrics in the diversity measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)

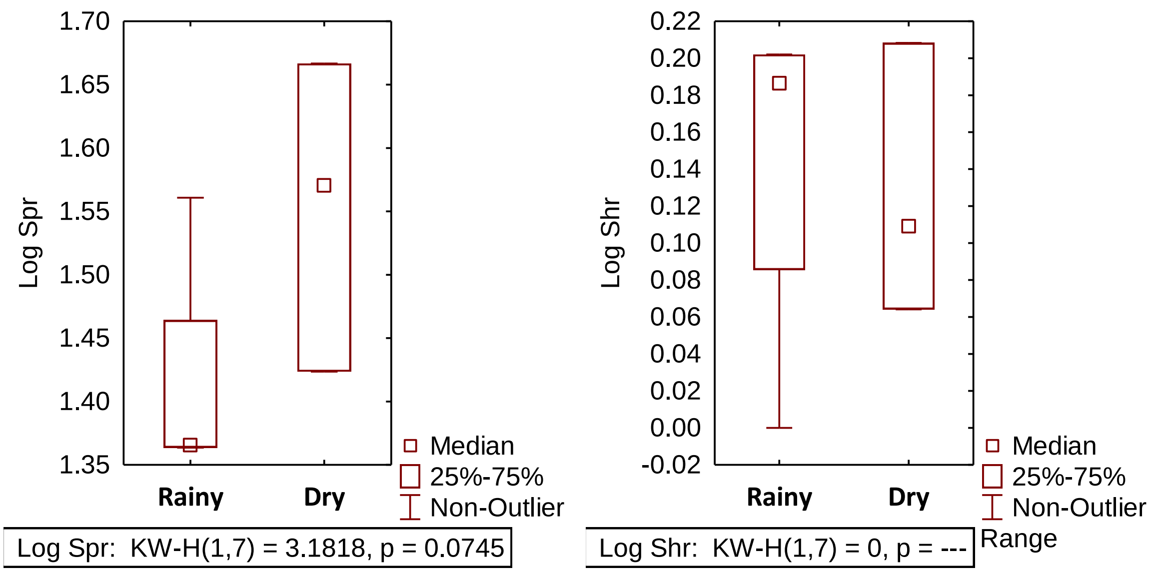
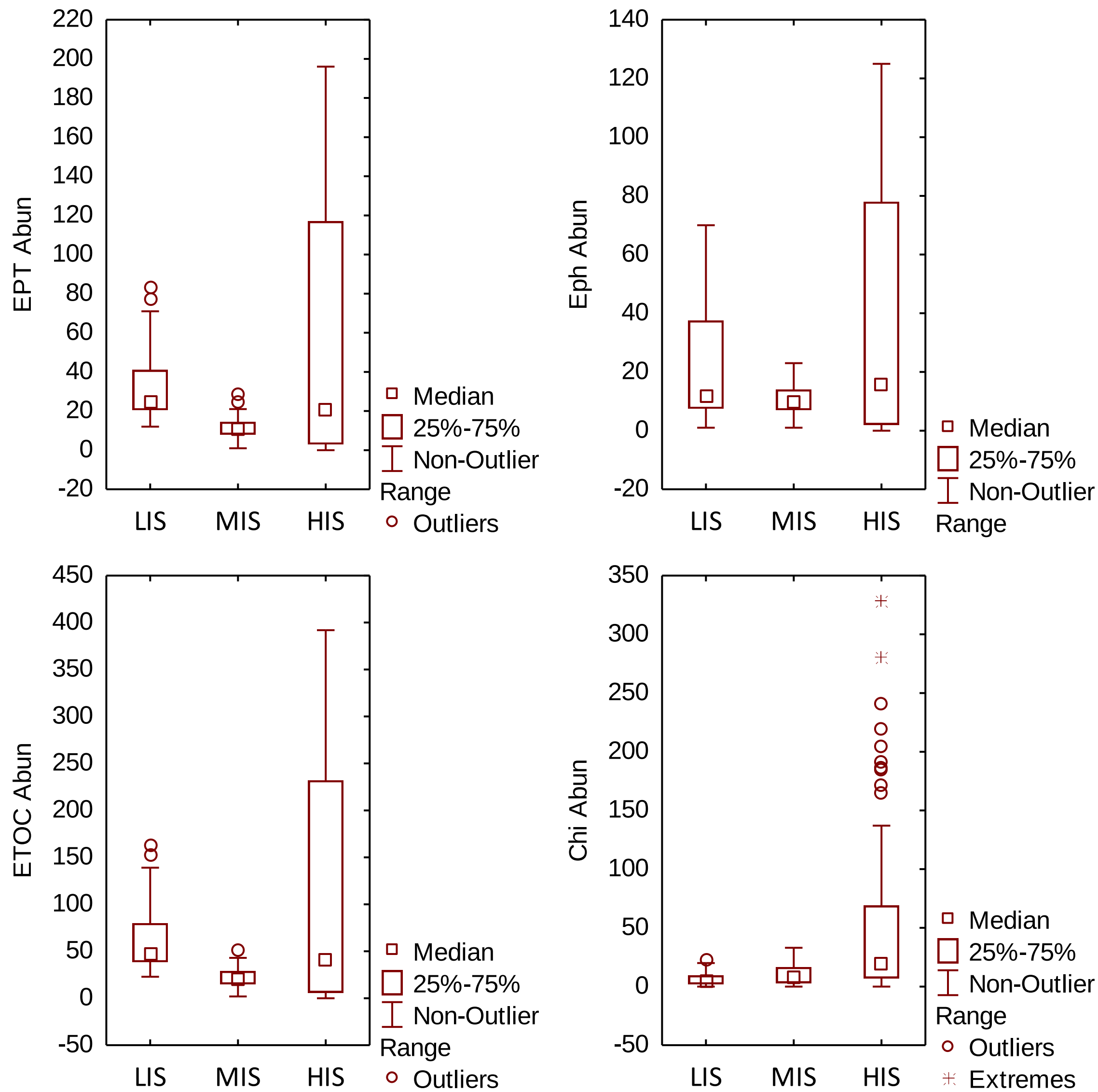
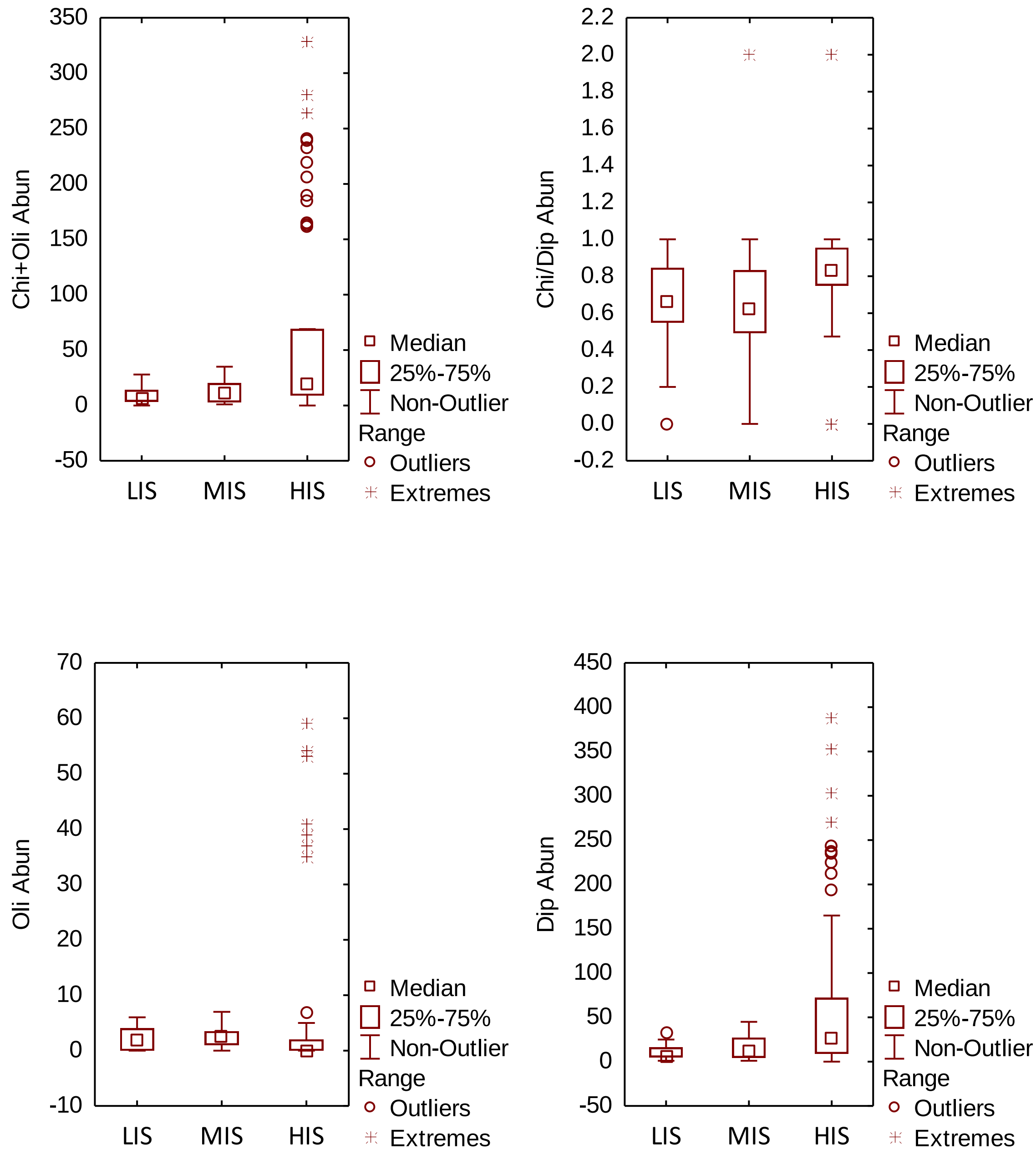
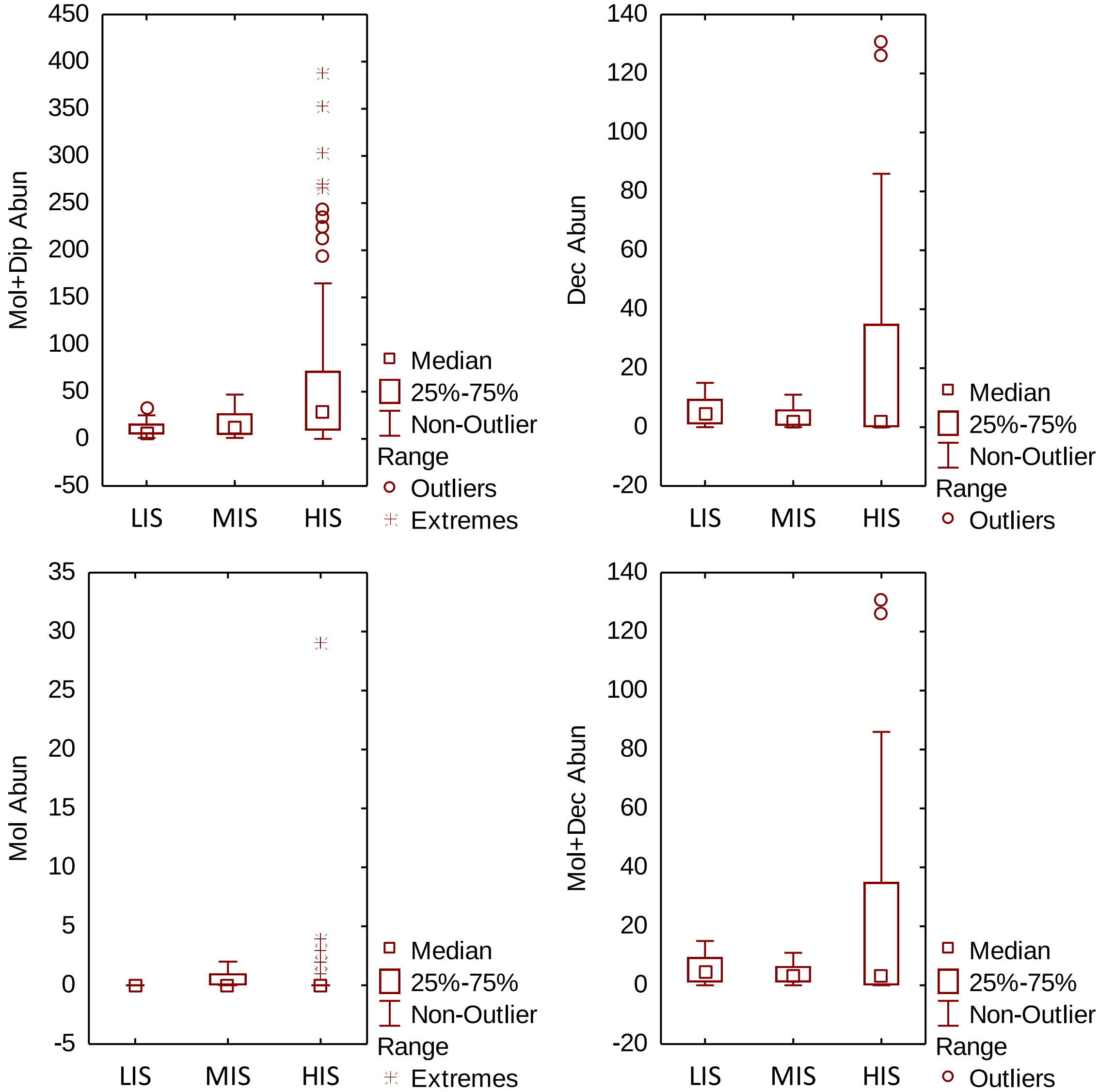


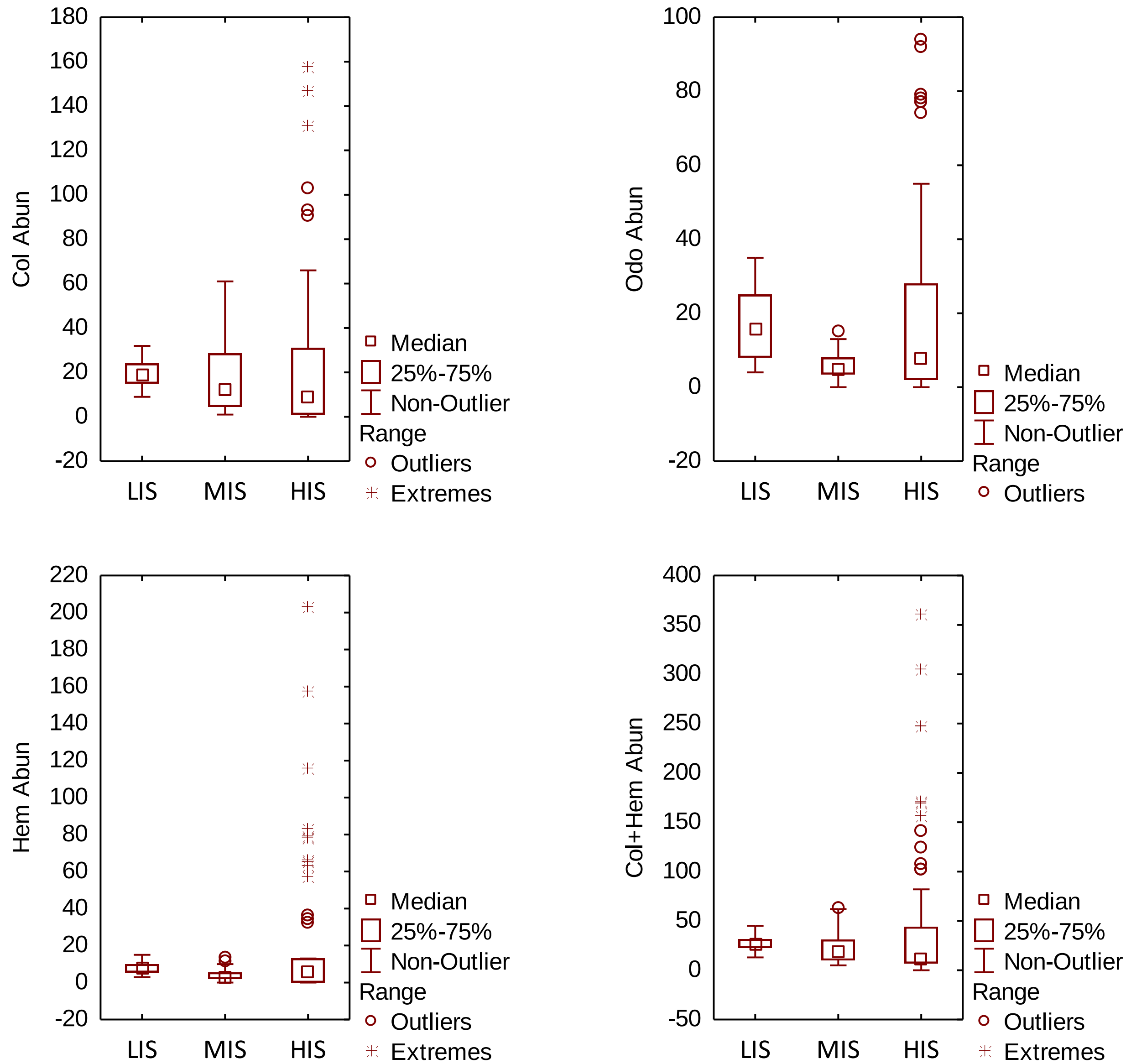

**Figure E10:** Seasonally and non-seasonally stable metrics in the traits and ecological preferences measures not integrated into the urban-agricultural multimetric index (MMI-urban-agric)

**Appendix F: Sensitive/non-sensitive and seasonally stable/non-seasonally stable metrics not integrated into the urban-forestry multimetrc index (MMI-urban-forest)**




Chi+Oli Abun
350
300
250
200
150
100
50
0
-50
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Chi/Dip Abun
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Oli Abun
70
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Dip Abun
450
400
350
300
250
200
150
100
50
0
-50
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Mol+Dip Abun
450
400
350
300
250
200
150
100
50
0
-50
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Dec Abun
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Mol Abun
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes
Mol+Dec Abun
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers


Col Abun
180
160
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Odo Abun
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Hem Abun
220
200
180
160
140
120
100
80
60
40
20
0
-20
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Col+Hem Abun
400
350
300
250
200
150
100
50
0
-50
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

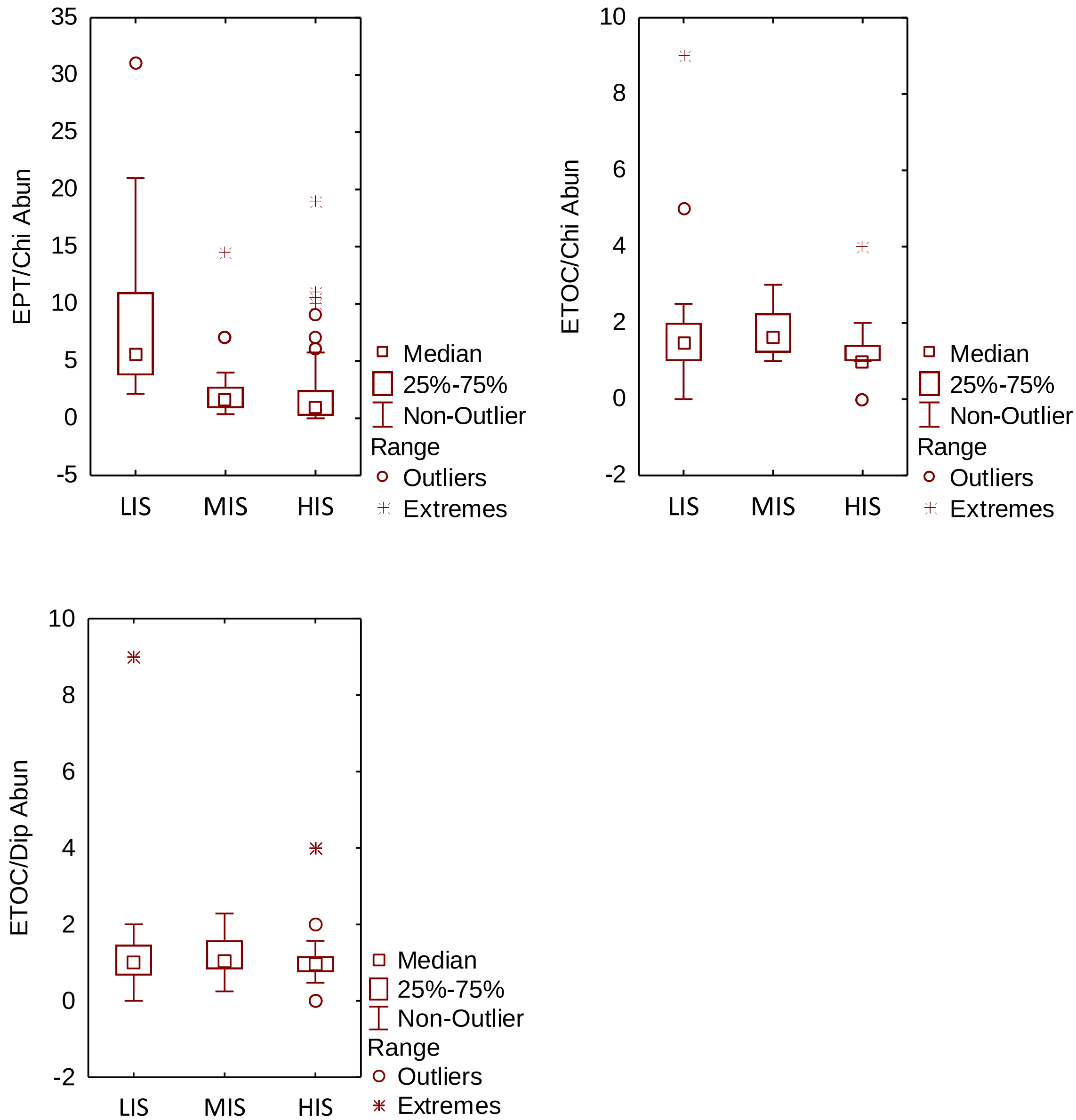
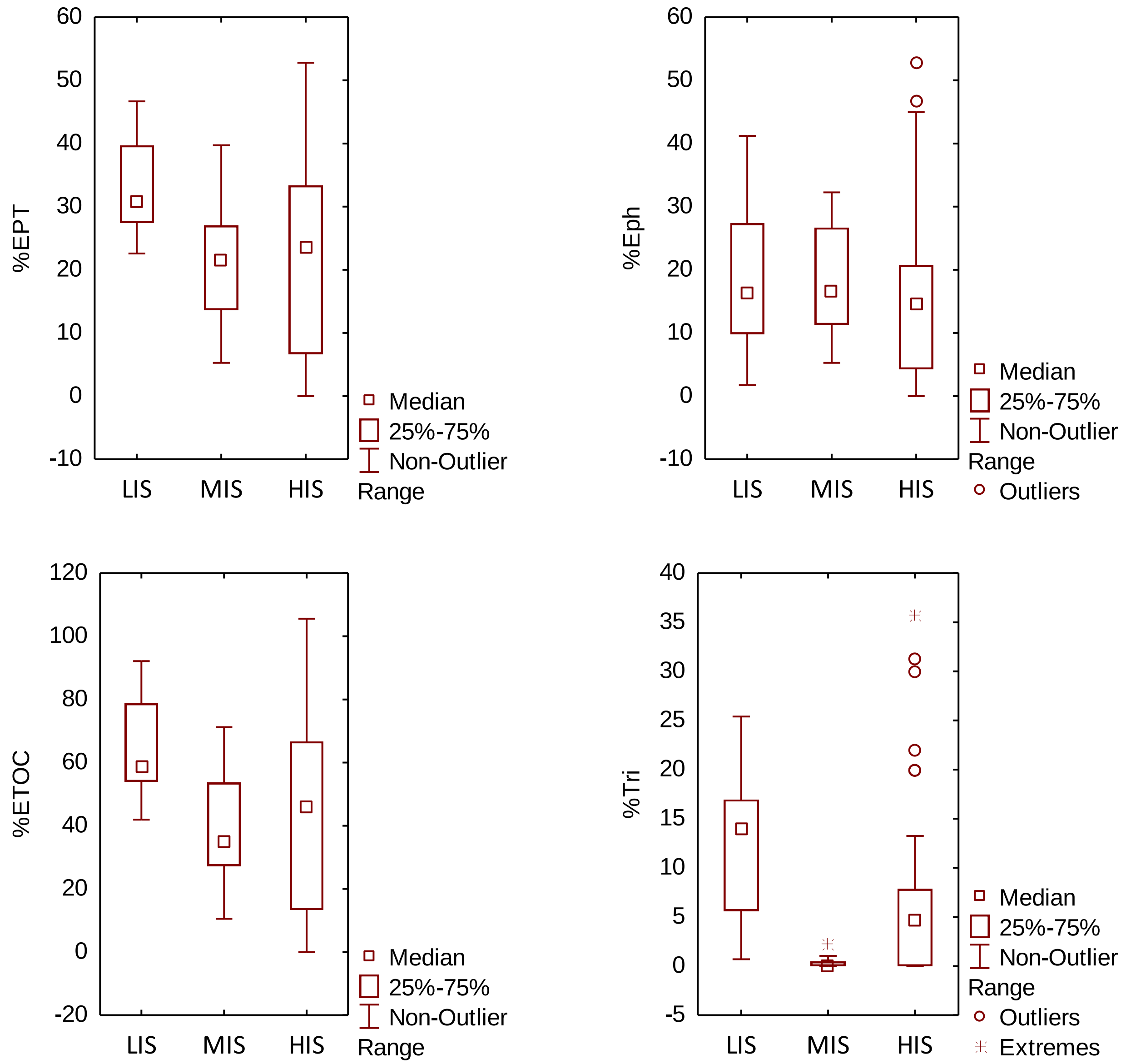
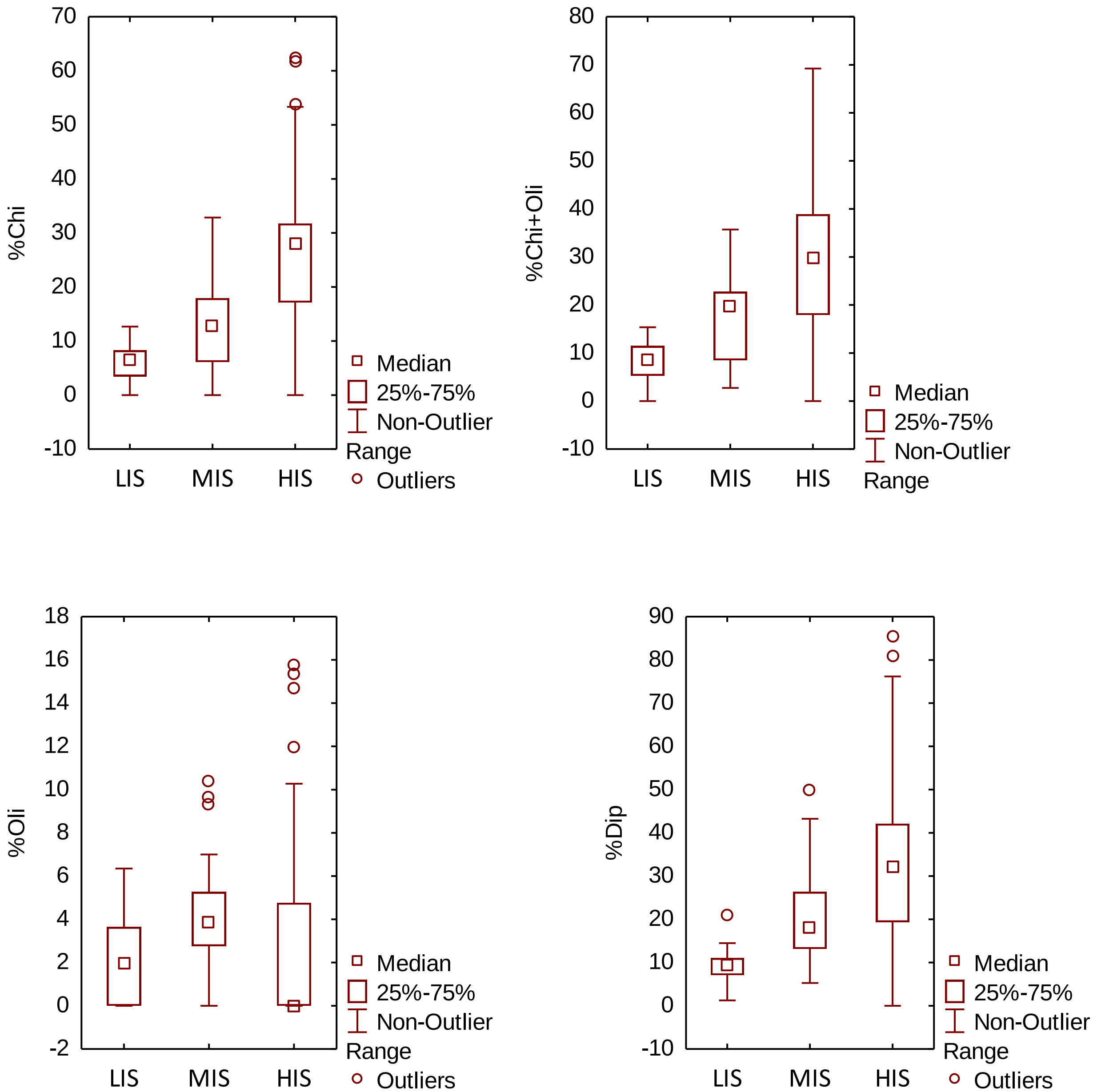
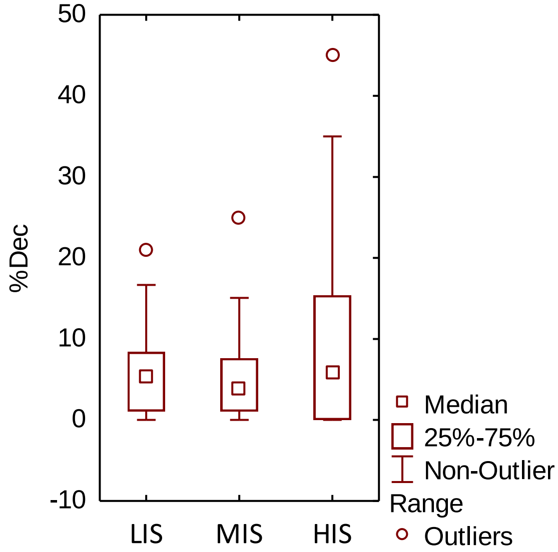
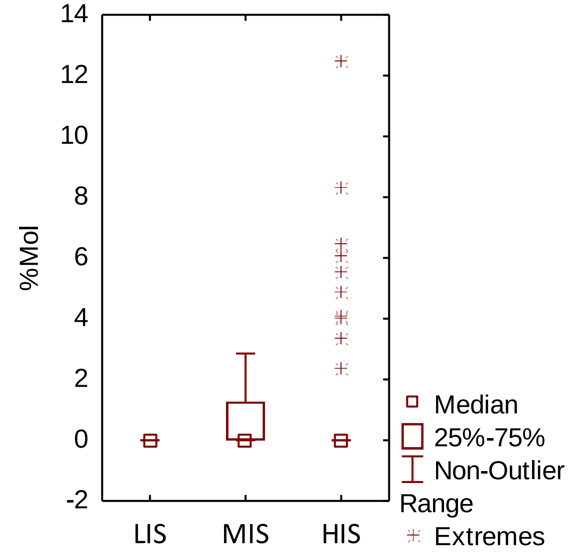


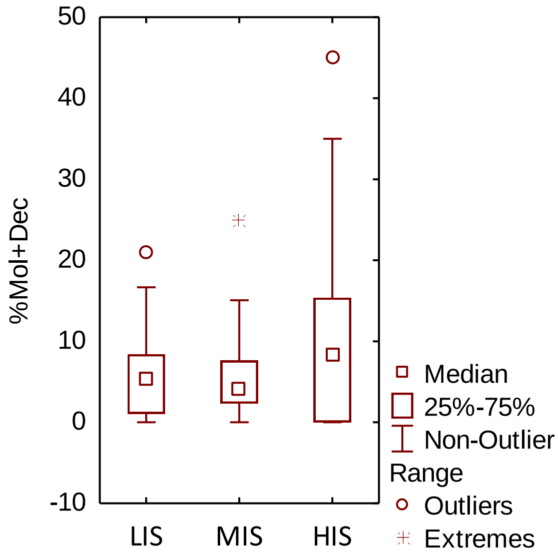

**Figure F1:** Sensitive and non sensitive metrics in the abundance measures not integrated into the urban-forestry multimetric index (MMI-urban- forest)

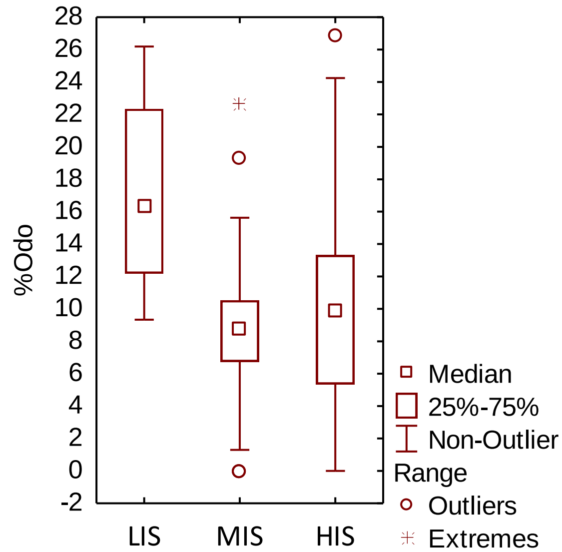

%EPT
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%Eph
Outliers
%ETOC
120
100
80
%Tri
Extremes


%Chi
70
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
%Chi+Oli
80
70
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
%Oli
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
%Dip
90
80
70
60
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers


%Dec
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers


%Mol
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes


%Mol+Dec
50
40
30
20
10
0
-10
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


%Odo
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

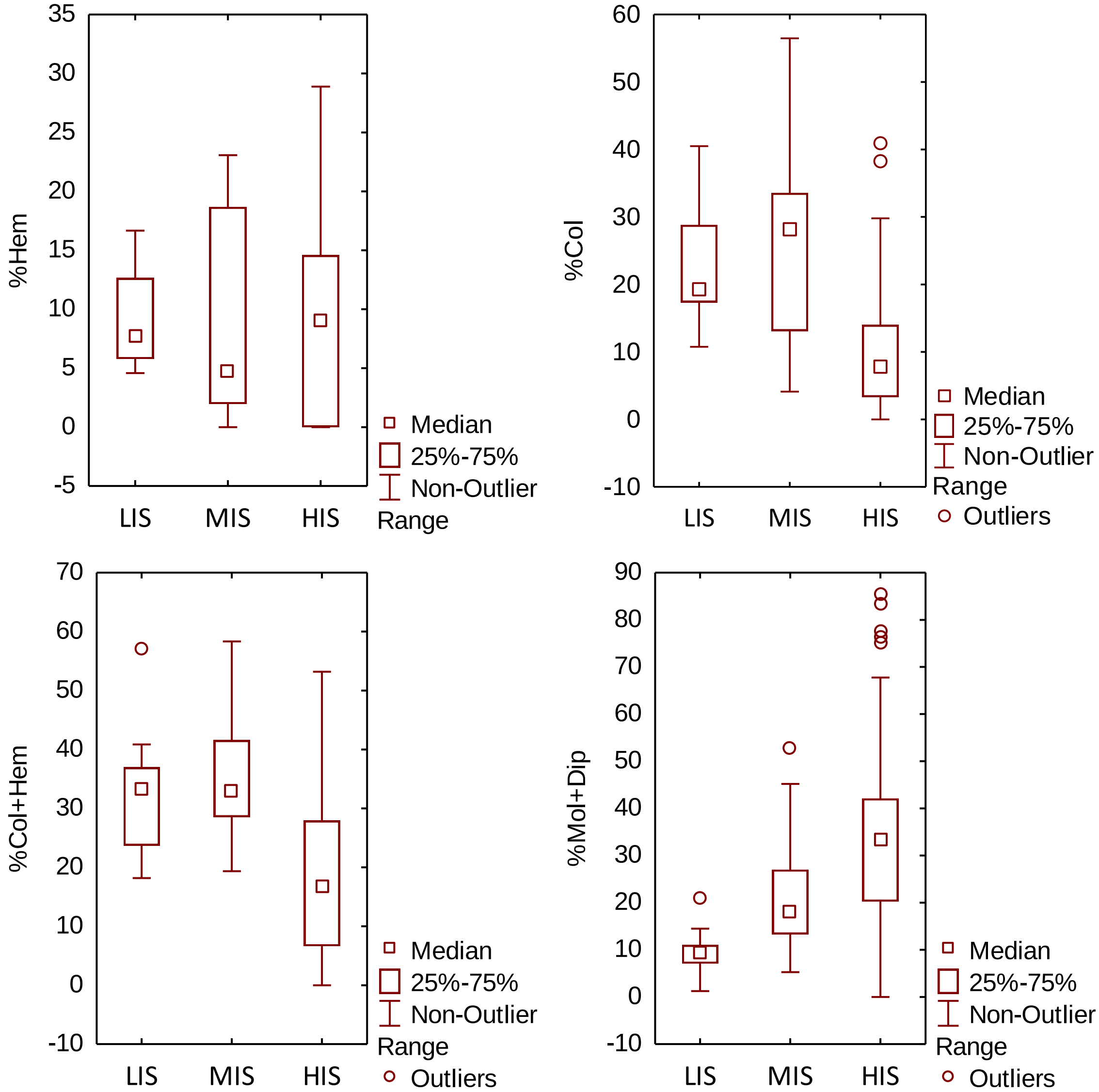
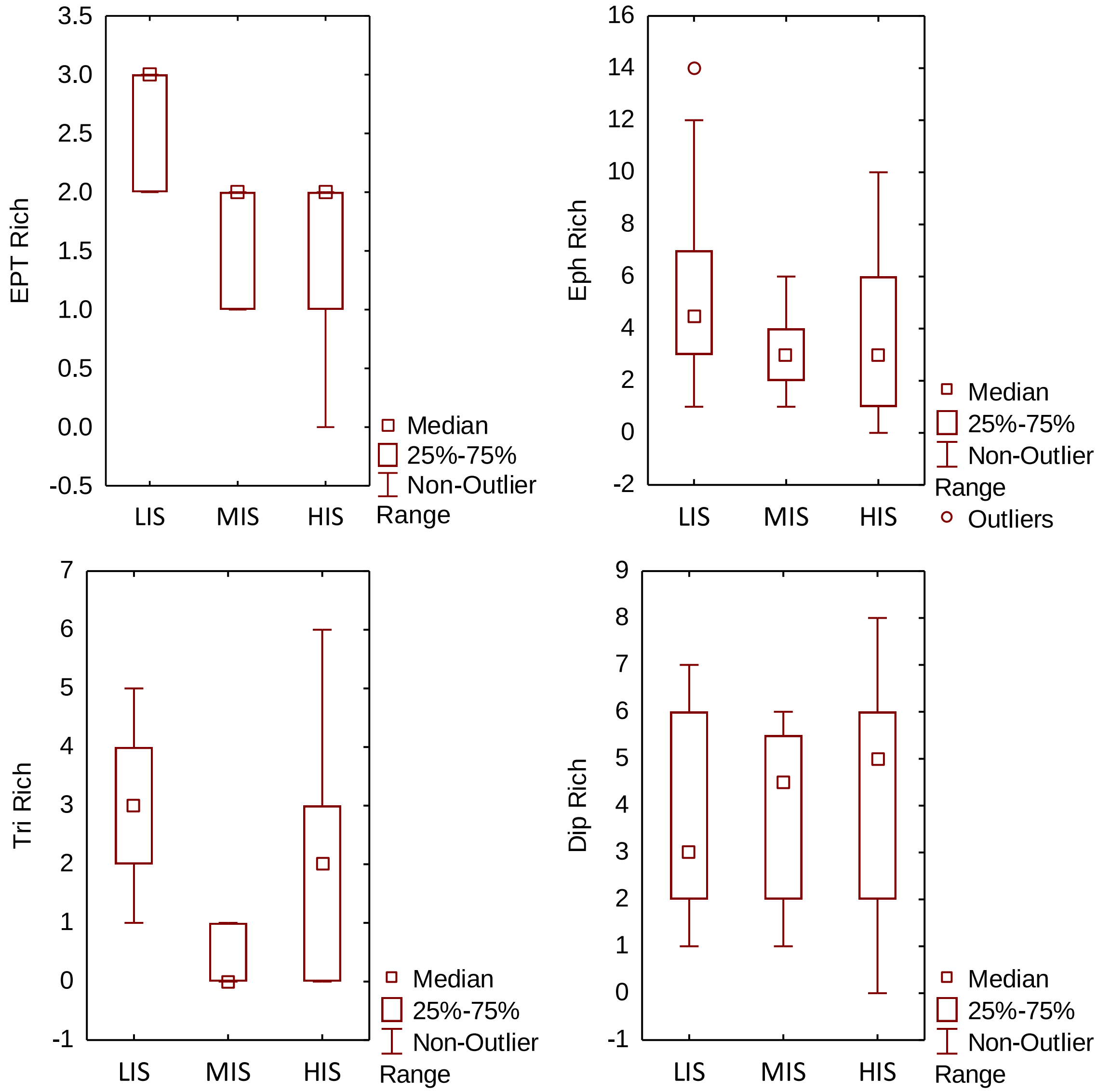
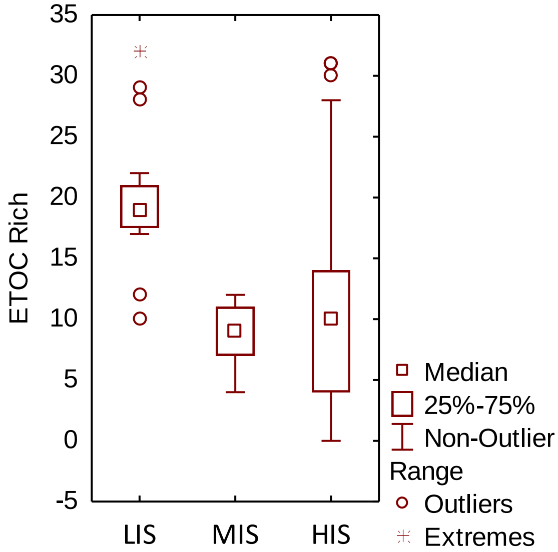
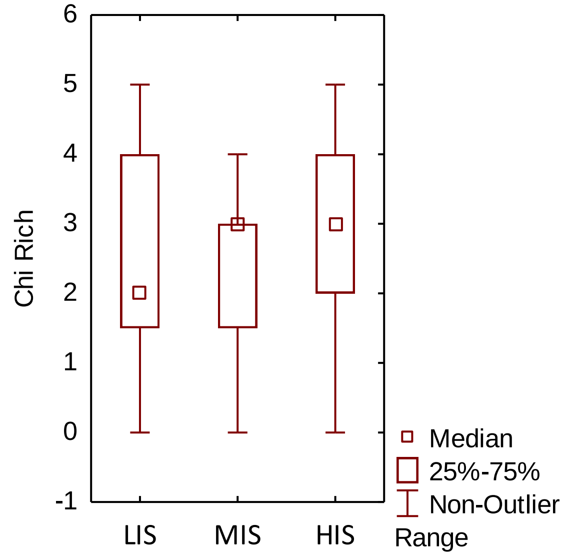
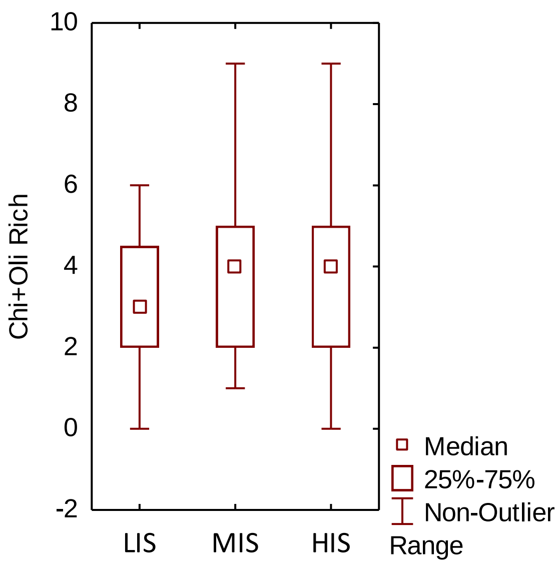


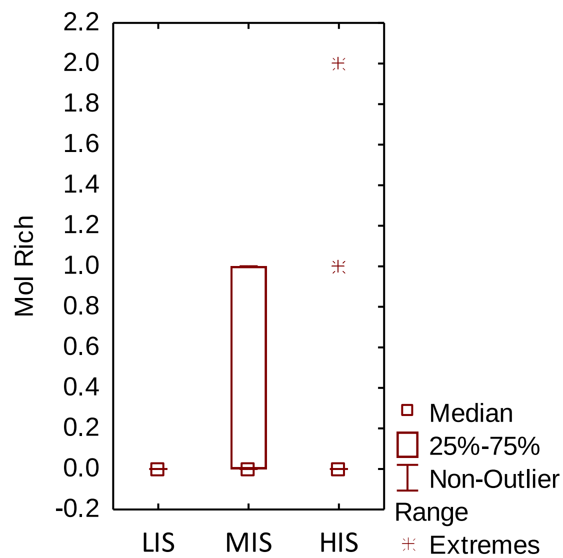

**Figure F2:** Sensitive and non sensitive metrics in the composition measures not integrated into the urban-forestry multimetric index (MMI-urban- forest)

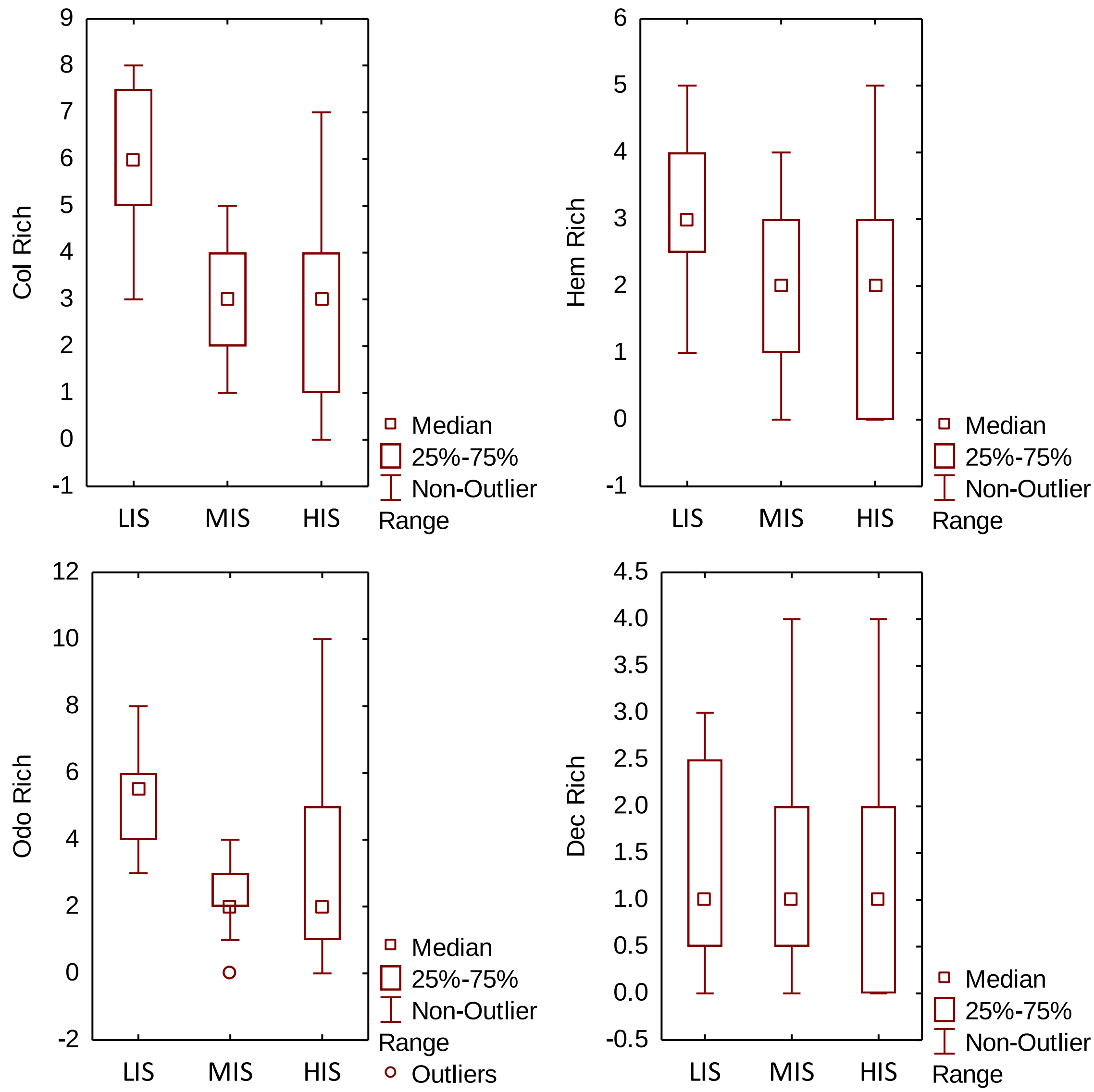

EPT Rich
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Eph Rich
16
14
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Tri Rich
7
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Dip Rich
9
8
7
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


ETOC Rich
35
30
25
20
15
10
5
0
-5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Chi Rich
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


Chi+Oli Rich
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range


Mol Rich
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Extremes


Col Rich
9
8
7
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Hem Rich
6
5
4
3
2
1
0
-1
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Odo Rich
12
10
8
6
4
2
0
-2
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range
Outliers
Dec Rich
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
LIS
MIS
HIS
Median
25%-75%
Non-Outlier Range

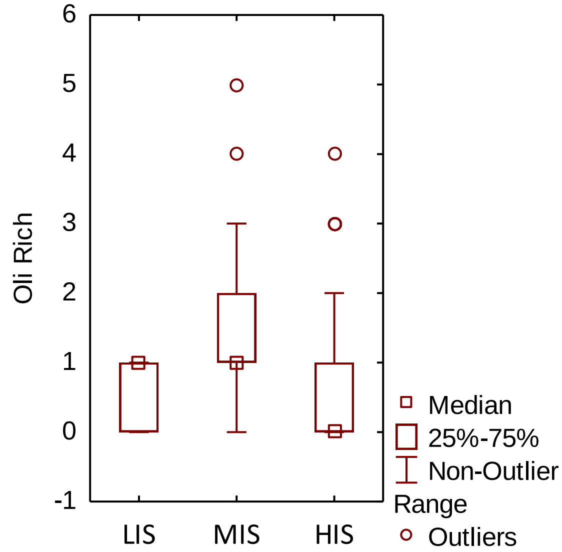
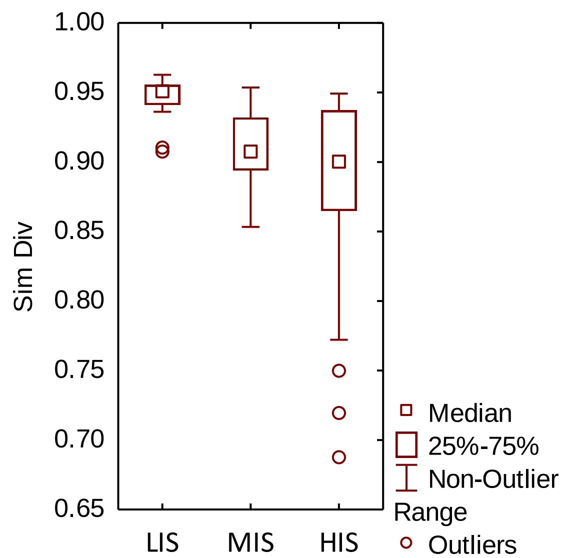
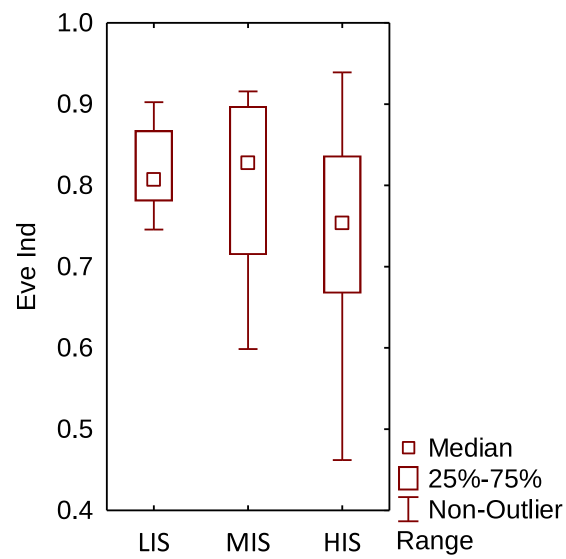



**Figure F3:** Sensitive and non sensitive metrics in the richness measures not integrated into the urban-forestry multimetric index (MMI-urban- forest)



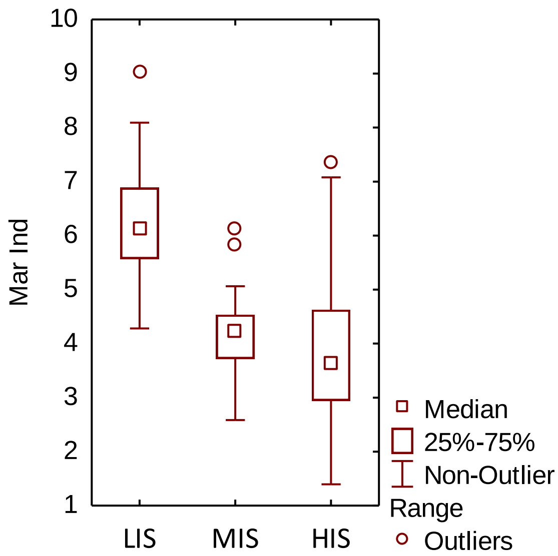



**Figure F4:** Sensitive and non sensitive metrics in the diversity measures not integrated into the urban-forestry multimetric index (MMI-urban- forest)

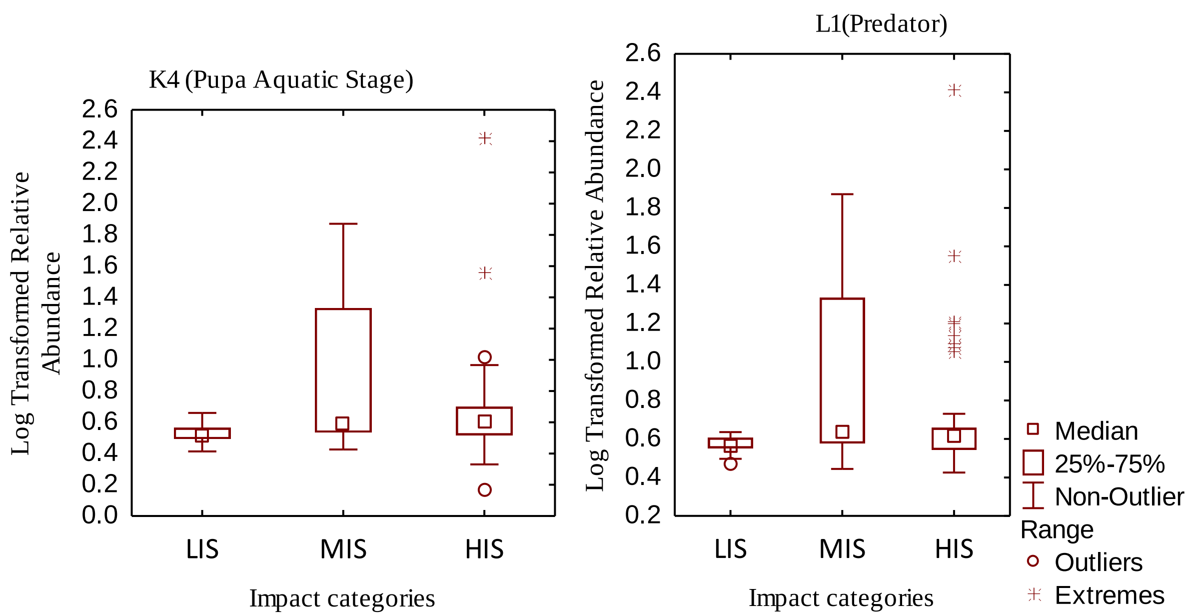



**Figure F5:** Sensitive and non sensitive metrics in the traits and ecological preferences measures not integrated into the urban-forestry multimetric index (MMI-urban- forest)

**Table F1:** Sensitive metrics selection for urban-forestry multimetric index (MMI-urban-forest). **Note: a metric sensitivity is confirmed if significant at *P*<0.05**

| Discriminatory metrics | Mann-Whitney test (U-test) | *P*-value | Sensitivity confirmed |
|---|---|---|---|
| **Abundance measures** | | | |
| Tri Abun | 749 | 0.00247 | Yes |
| Col Abun | 918.5 | 0.08745 | No |
| EPT/Chi Abun | 467.5 | 5.348E-07 | Yes |
| **Composition measures** | | | |
| %EPT | 967.5 | 0.1775 | No |
| %ETOC | 991.5 | 0.2409 | No |
| %Tri | 966.5 | 0.1646 | No |
| %Chi | 296 | 3.609E-10 | Yes |
| %Chi+Oli | 387.5 | 2.163E-08 | Yes |
| %Dip | 441 | 1.925E-07 | Yes |
| %Odo | 763 | 0.004386 | Yes |
| %Mol+Dip | 401 | 3.808E-08 | Yes |
| **Richness measures** | | | |
| ETOC Rich | 958.5 | 0.1565 | No |
| Col+Hem Rich | 601.5 | 5.168E-05 | Yes |
| Col Rich | 663 | 0.000295 | Yes |
| Hem Rich | 720.5 | 0.00128 | Yes |
| Odo Rich | 922.5 | 0.09012 | No |
| **Diversity measures** | | | |
| Sim Div | 662.5 | 0.000393 | Yes |
| Sha Div | 764 | 0.004519 | Yes |
| Mar Ind | 607.5 | 6.709E-05 | Yes |
| **Trait attributes measures** | | | |
| LogPup | 993 | 0.02455 | Yes |

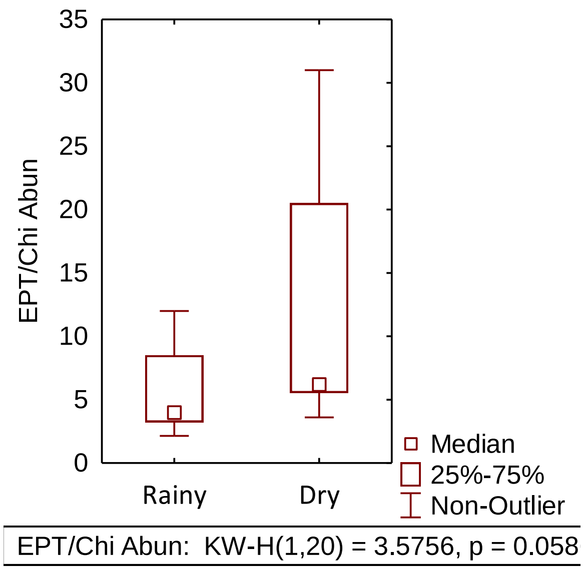
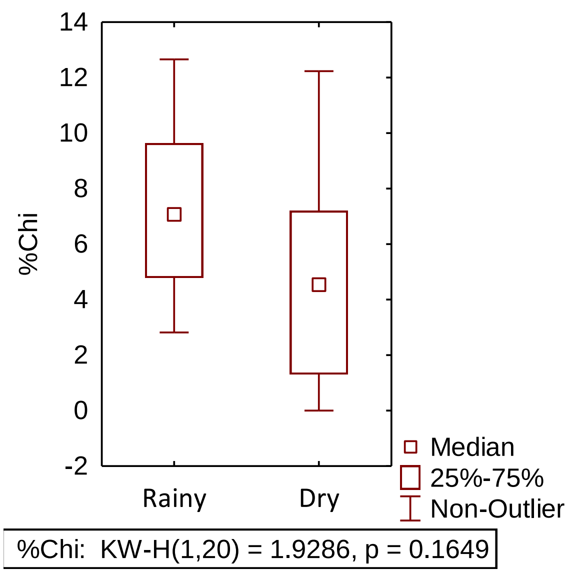
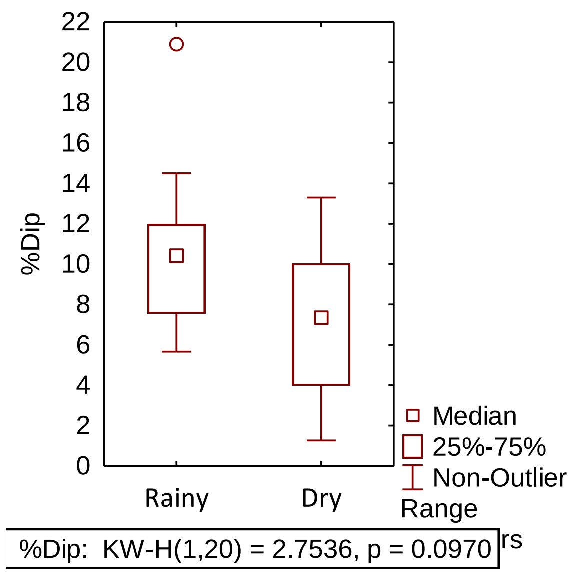



**Figure F6:** Seasonally stable metric in the abundance measures not integrated into the urban-forestry multimetric index (MMI-urban-forest)



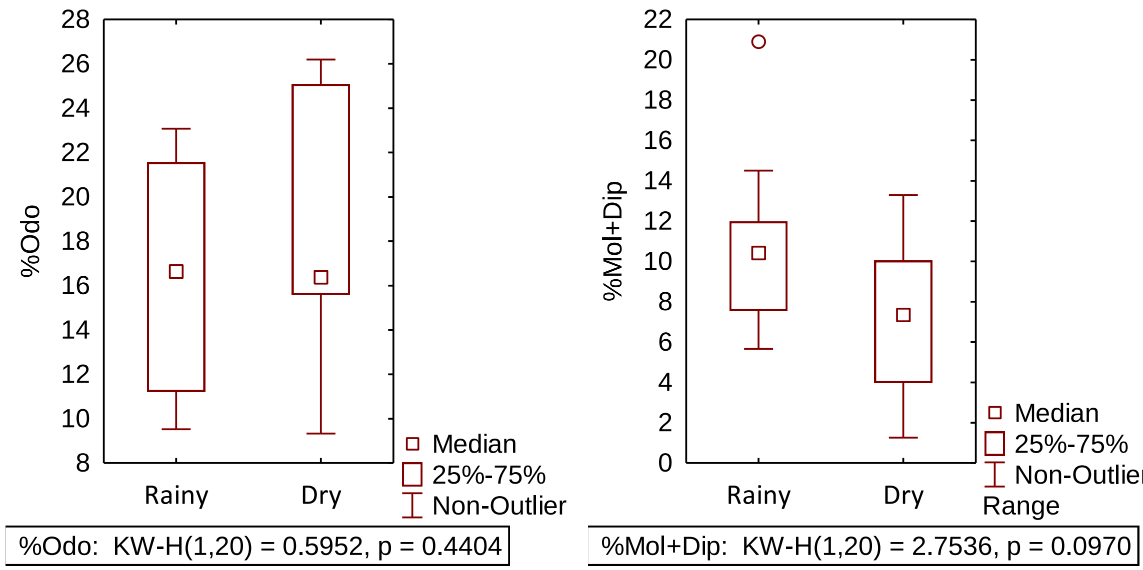
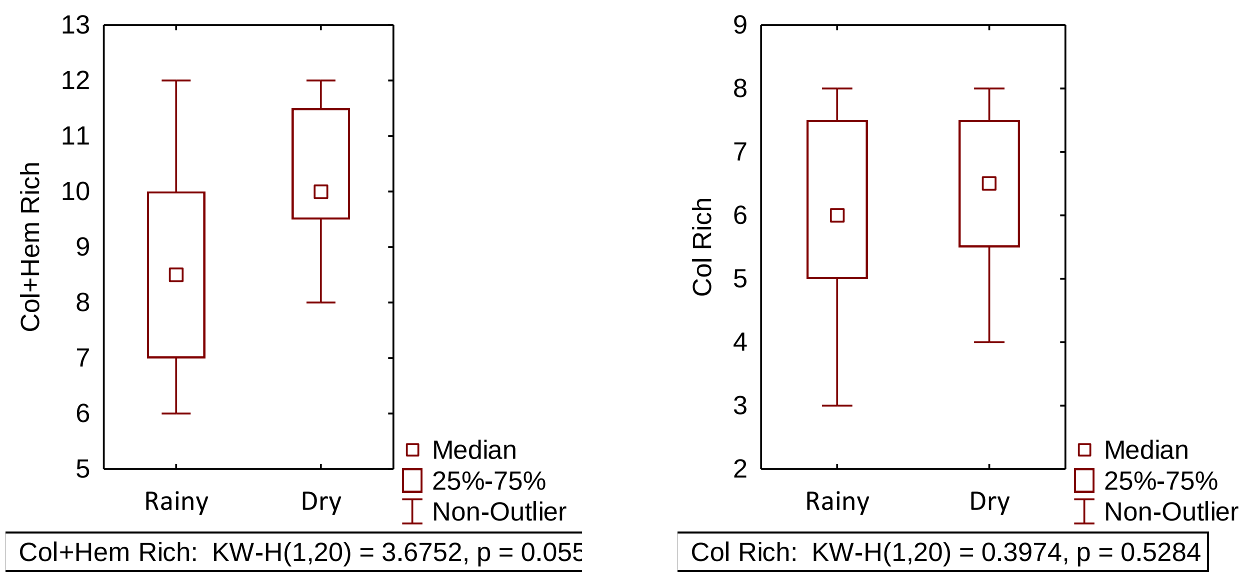
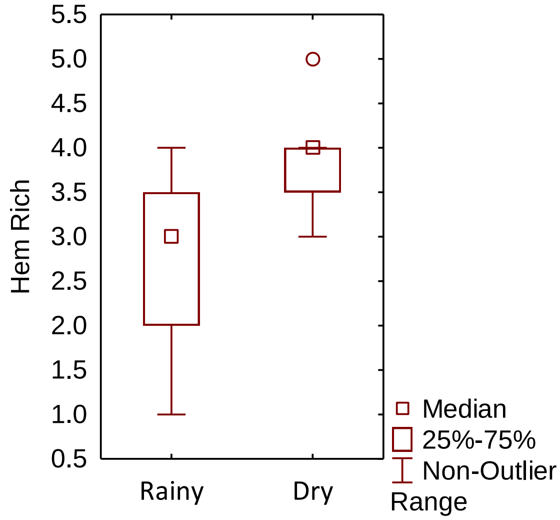


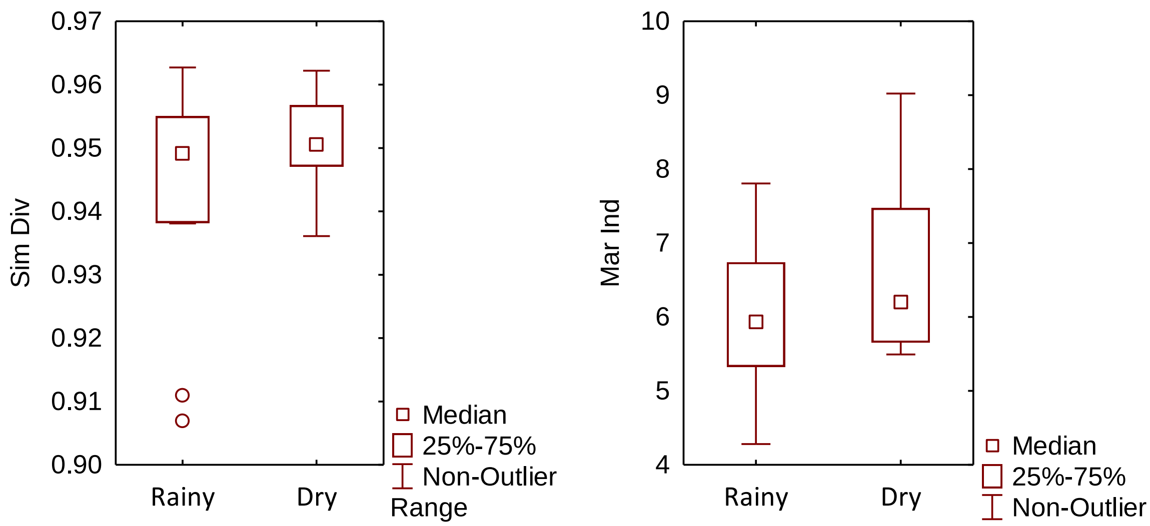

**Figure F7:** Seasonally and non-seasonally stable metrics in the composition measures not integrated into the urban-forestry multimetric index (MMI-urban-forest)






Hem Rich: KW-H(1,20) = 6.132, p = 0.0133

**Figure F8:** Seasonally and non-seasonally stable metrics in the richness measures not integrated into the urban-forestry multimetric index (MMI-urban-forest)




Sim Div: KW-H(1,20) = 0.2921, p = 0.5889

Mar Ind: KW-H(1,20) = 0.8578, p = 0.3544

**Figure F9:** Seasonally and non-seasonally stable metrics in the diversity measures not integrated into the urban-forestry multimetric index (MMI-urban-forest)

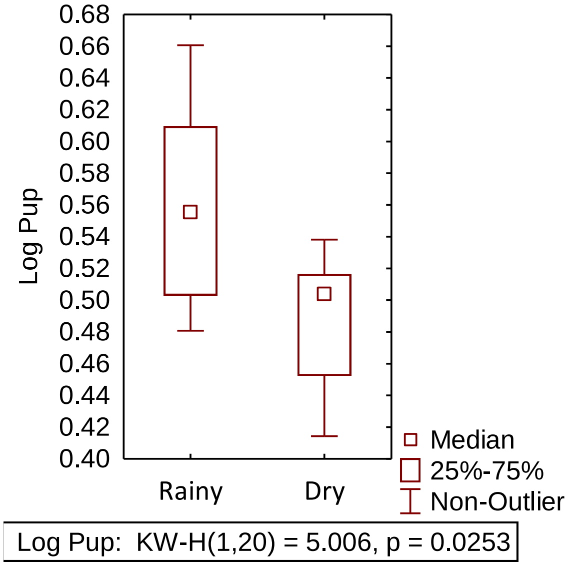



**Figure F10:** Non-seasonally stable metrics in the trait and ecological preference measure not integrated into the urban-forestry multimetric index (MMI-urban-forest)

**Appendix G: List of macroinvertebrate taxa (families) collected in selected rivers in the Niger Delta, Nigeria during the study period (2008 – 2012).**

| **Order** | **Taxa (families)** | **Taxa codes** | **River(s) where specimens were collected** |
|---|---|---|---|
| Oligochaeta | Naididae | Nai | Wa1,Wa2, Wa3, An1, An2, An3, Ad1, Ad2, Ad3, As1, As2, Be1, Be2, Be3, Iy1, Iy2, Iy3, Ob2 Ol2, Ed3, Ol1, Or1, Or2, Or3, Or4, Ob3, Et2, Et3, Et4, Og1, Og2,Og3, Og4,Ow1, Os1, Os2, Os3, Oa1, Ui1, Ui2, Ui3, Ut1, Ut2, Ut3, Uu1, Uu2 |
| | Tubificidae | Tub | Wa2, Wa3, An2, Ad1, Ad2, As1, As2, Be1, Be2, Be3, Iy1, Iy2, Iy3, Ob2 Ol2, Ed3, Ol1, Or1, Or2, Or3, Or4, Et3, Et4, Og2,Og3, Ol1, Og4,Ow1, Ow3, Or1, Or2, Os1, Os2, Os3, Oa1, Ui1, Ui2, Ui3, Ut1, Ut2, Ut3, Uu1, Uu2 |
| | Lumbricidae | Lum | Wa2, An2, Ad1, As1, As2, Be1, Be2, Be3, Iy1, Iy2, Iy3, Ob2 Ol2, Ed3, Ol1, Or1, Or2, Or3, Or4, Et3, Et4, Og2,Og3, Ol1, Og4,Ow1, Ow3, Or1, Or2, Os1, Oa1, Ui1, Ui2, Ui3, Ut1, Ut2, Ut3, Uu1 |
| Gastropoda | Lymnaidae | Lym | Ad3, An1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ad1, As1, As2, Be1, Iy1, Iy3, Or1, Os1, Os2, Oa2, Um3, Ui1, Ui2, Wa2, Wa3 |
| | Planorbidae | Pla | AAn2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Thiaridae | Thi | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, |

| | | | |
|---|---|---|---|
| | | | Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Amphullariidae | Amp | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |
| Decapoda | Atyidae | Aty | An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Desmocarididae | Des | An1, An3, Et1, Et2, Ob3, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3 |
| | Euryrhynchidae | Eur | Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1 |
| | Palaemonidae | Pal | An1, An3, Et1, Et2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Um3, Ui3, |

| | | | |
|---|---|---|---|
| | | | Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| Ephemeroptera | Baetidae | Bae | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Ed1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2 |
| | Leptophlebiidae | Lep | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Caenidae | Cae | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Heptageniidae | Hep | An1, An3, Et1, Et2, Ob3, Og1, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Um3, Um4, Ui1, Wa2, Wa3 |
| | Tricorythidae | Tri | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, |

| | | | |
|---|---|---|---|
| | | | Os3, Os4, Oa3, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Oligoneuridae | Oli | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Potamanthidae | Pot | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Prosopistomatidae | Pro | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |
| | Polymitarcyidae | Pol | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| Plecoptera | Perlidae | Per | Wa1, Et1, Wa3, Ad1, Or1, Oa2, Ui1, Ob2, Et3, Er3, Or4, Oa1, Oa4, Er1 |
| Trichoptera | Glossosomatidae | Glo | An1, An3, Et1, Et2, Ob3, Og1, Og2, |

| | | | |
|---|---|---|---|
| | | | Ol2, Or3, Wa1, An2, Ed2, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Ut2, Ut3, Ad1, As1, As2, Be1, Er4, Iy1, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa3 |
| | Hydroptilidae | Hyd | An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Hydropsychidae | Hyr | An1, An3, Et1, Ob3, Og1, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Wa2, Wa3 |
| | Ecnomidae | Ecn | An1, An3, Et1, Et2, Ob3, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Helicopsychidae | Hel | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Leptoceridae | Let | An1, Et1, Et2, Ob3, Og2, Ol2, Or3, |

| | | | |
|---|---|---|---|
| | | | Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| Lepidoptera | Pyraustidae | Pyr | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Wa3 |
| Hemiptera | Notonectidae | Not | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, |
| | Corixidae | Cor | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Pleidae | Ple | An1, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Wa2, Wa3 |

| | | | |
|---|---|---|---|
| | Mesoviliidae | Mes | An1, An3, Et1, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |
| | Nepidae | Nep | An1, An3, Et1, Et2, Ob3, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Ui1, Ui2, Wa2, Wa3 |
| | Naucoridae | Nau | An1, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui2, Wa3 |
| | Belostomatidae | Bel | An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Ow2, Ow3, Or4, Um3, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Gerridae | Ger | An1, An3, Et1, Et2, Ob3, Og1, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Ui2, Wa2, Wa3 |
| Coleoptera | Aspidytidae | Asp | An1, An3, Et1, Et2, Ob3, Og1, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, |

| | | | |
|---|---|---|---|
| | | | Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Dytiscidae | Dyt | An1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Hydrophilidae_ | Hyp | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1 |
| | Elmidae | Elm | An1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |
| | Gyrinidae | Gyr | An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Noteridae | Nor | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Uu2, Ad2, Ed1, |

| | | | |
|---|---|---|---|
| | | | Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |
| | Hydraenidae | Hya | An1, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, As1, As2, Be1, Be2, Be3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| Odonata | Aeschnidae | Aes | An1, An3, Et1, Et2, Ob3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Gomphidae | Gom | An3, Et1, Et2, Og2, Ol2, Or3, Wa1, An2, Ed2, Er3, Et3, Et4, Ob1, Ob2, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, Be2, Be3, Er4, Iy1, Iy2, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Coenagrionidae | Coe | An1, An3, Et1, Et2, Ob3, Og1, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1 |
| | Libellulidae | Lib | An1, An3, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Or4, Um3, Ui3, Uu1, Ut1, Ut2, |

| | | | |
|---|---|---|---|
| | | | Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Calopterygidae | Cal | Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, , Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Cordulidae | Cod | An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ad1, As1, As2, Be1, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1 |
| | Macromidae | Mac | An1, An3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ol1, Ow1, Ow2, Ow3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2 |
| | Chlorocyphidae | Chl | An1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3 |
| Diptera | Culicidae | Cul | An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be3, Er4, Iy1, Iy2, Iy3, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |

| | | | |
|---|---|---|---|
| | Simulidae | Sim | An1, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, r1, Er2, Ol1, Ow1, Ow2, Um3, Ui3, Uu1, Ut1, Ut2, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Tabanidae | Tab | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, Ed4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ad1, As1, As2, Be1, Er4, Iy1, Iy2, Iy3, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2, Wa3 |
| | Ceratopogonidae | Cer | An1, An2, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Er2, Ol1, Ow1, Or4, Um3, Ui3, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa2 |
| | Athericidae | Ath | An1, Et2, Ob3, Og1, Og2, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Uu2, Ad2, Ed1, Er2, Ol1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Os1, Os2, Oa2, Um3, Um4, Wa2, Wa3 |
| | Chaoboridae | Cha | Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Os3, Os4, Oa3, Um1, Um2, Uu2, Ed3, Er1, Ow1, Ow2, Ow3, Or4, Um3, Ui3, Uu1, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4 |
| | Tipulidae | Tip | An1, Og1, Og2, Ol2, Or3, Wa1, |

| | | | |
|---|---|---|---|
| | | | An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Um2, Uu2, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ui3, Uu1, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Or1, Os1, Os2, Oa2, Um3, Ui2, Wa2, Wa3 |
| | Sryphidae | Sry | An1, An3, Et1, Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Os4, Oa3, Um1, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow2, Ui3, Uu1, Ut1, Ut2, Ut3 Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1 |
| | Chironomidae | Chi | Et2, Ob3, Og1, Og2, Ol2, Or3, Wa1, An2, Ed2, Ed4, Er3, Et3, Et4, Ob1, Ob2, Og3, Og4, Or2, Os3, Um1, Ad2, Ed1, Ed3, Er1, Er2, Ol1, Ow1, Ow3, Or4, Um3, Ut1, Ut2, Ut3, Ad1, As1, As2, Be1, Be2, Be3, Er4, Iy1, Iy2, Iy3, Or1, Os1, Os2, Oa2, Um3, Um4, Ui1, Ui2, Wa3 |