Scientific Research Publishing

Open Journal of Ecology, 2021, 11, 407-436
https://www.scirp.org/journal/oje
ISSN Online: 2162-1993
ISSN Print: 2162-1985

Simple and Cost-Effective Biomonitoring Method for Assessing Pollution in Tropical African Rivers

Julius D. Elias

Tanzania Industrial Research and Development Organization (TIRDO), Dar es Salaam, Tanzania
Email: julius.zeanimo@gmail.com, animoj@yahoo.com

How to cite this paper: Elias, J.D. (2021) Simple and Cost-Effective Biomonitoring Method for Assessing Pollution in Tropical African Rivers. *Open Journal of Ecology*, **11**, 407-436.
https://doi.org/10.4236/oje.2021.114027

Received: November 20, 2020
Accepted: April 24, 2021
Published: April 27, 2021

Abstract

Adoption rule whereby tropical African regions rely on non-tropical biomonitoring methods to assess water pollution in their rivers has been hindered by geographical incompatibility due to environmental variations between the regions that affect the capability and reliability of adopted method. Likewise, inclusion of all identified macroinvertebrate assemblages in developing the existing biomonitoring methods (*i.e.*, South African Scoring System (SASS5) and Tanzania Riverine Scoring System (TARISS)) has made them complex taxonomically as their use requires users of greater expertise and much time during taxa identification. Such taxonomic complications and conflicting aspects regarding the adoption or modification of non-tropical biomonitoring methods in developing tropical biomonitoring methods have therefore necessitated the design of this study in order to develop simple and cost-effective tropical African biomonitoring methods, for initial application in Tanzanian rivers. Six pairwise screening criteria were employed to select orders with distinctive potential for inclusion in developing simple and cost-effective biomonitoring method. Only Ephemeroptera, Diptera, Odonata and Trichoptera (EDOT) orders met all six inclusion criteria after showing their ability to discern reference sites from monitoring sites and correlating strongly with environmental variables. Being developed using only four diverse orders with the wider range of occurrences and sensitivity to pollution, the EDOT method will minimize data variability, the need for greater expertise, cost, and time during taxa identification. The novelty of the present approach lies on the simplification of the taxonomic complication that is inherent in existing indices for four decades and modelling application to simulate sensitivity weightings for taxa with unknown sensitivity score ratings.

Keywords

Assessment, Biomonitoring Method, Pollution, Ecosystem, Macroinvertebrate

1. Introduction

Despite the worldwide popularity and longevity of the biomonitoring concept, the regional share and application of the biomonitoring methods between tropical and non-tropical regions are not rational. Tropical African rivers are known to be more speciose than other regions but their taxonomical and ecological knowledge on macroinvertebrates is still incomplete [1] [2] [3]. Such knowledge gap has hindered the potential use of tropical macroinvertebrate species in developing biomonitoring method(s) that can accurately evaluate the integrity of riverine ecosystems [2]. As a result, Rapid Biomonitoring Methods (RBMs), which have been developed for the past four decades in non-tropical regions using local macroinvertebrates [4]-[13], were adopted and/or modified to develop the Namibia Scoring System (NASS) in Namibia [14], Okavango Assessment System (OKAS) in the Okavango Delta [15], the Zambia Invertebrate Scoring System (ZISS) in Zambia [16], the Tanzania Riverine Scoring System (TARISS) [17] and Ethiopian Biomonitoring Scoring System (ETHbios) [18] for use in assessing pollution in tropical African rivers. Presence of geographical differences between tropical and non-tropical regions may, however, affect the capability, functioning, compatibility and reliability of the existing non-tropical biomonitoring methods when opted and applied on tropical rivers [1] [2] [19] [20]. As such, there is a risk of having unreliable findings when non-tropical biomonitoring methods are adopted, refined and used to assess water pollution in tropical African rivers.

Ecological and taxonomical variations between the regions can as well lead to variation in macroinvertebrate taxa composition, and their sensitivity levels to disturbance and general ecosystem impairment [21]. For instance, one ephemeropteran family (Teloganodidae) and five trichopteran families (Barbarochthonidae, Glossosomatidae, Hydrosalpingidae, Petrothrincidae and Sericosostomatidae) are prevalent in the southwest cape of South Africa representing the Mediterranean regions, as opposed to ephemeropterans (Ephemerythidae and Dicercomyzidae) which are endemically widespread in both afro-tropical and tropical regions [12] [14] [17]. Moreover, macroinvertebrate taxa in Temperate, Mediterranean, Arid and Semi-Arid regions do not necessarily correspond with those in tropics to confirm the existence of general adoption rules among macroinvertebrate-based methods from other regions [1] [3] [22] [23] [24]. Such varying regional complexities have increased recognition among ecologists on the new demands for having regional specific methods to render data accuracy in biomonitoring programmes [2]. Similarly, the inclusion of all identified macroinvertebrate assemblages in the existing biomonitoring methods (*i.e.*, ETHbios, SASS5 and TARISS) has made their use more complex taxonomically and less cost-effective [2] [12]. Such taxonomic complications and conflicting aspects regarding the adoption of non-tropical biomonitoring methods in developing tropical biomonitoring methods have therefore necessitated the design of this study.

Novelty of the approach lies in the simplification of the taxonomic complication that has characterized the existing biomonitoring methods for more than four decades. Being developed using only four diverse orders (**Appendix 1**) with wider range of occurrences and sensitivity to pollution, EDOT method minimizes data variability, the need for greater expertise, cost, and time during taxa identification, and the aspects that are not hitherto considered by existing biomonitoring methods. Therefore, the simplified method will provide guidelines and directions to meet current and anticipated future status of water pollution along the tropical African rivers towards the achievement of at least a good ecological status for all surface waters.

2. Materials and Methods

2.1. Description of Study Areas

Eighty-five (85) sampling sites of varying degradation levels along Pangani and Wami-Ruvu river basins were selected for sampling to ensure the characterization of macroinvertebrate taxa and determination of physico-chemical parameters (**Figure 1**). Pangani river basin is found within the north and north-eastern coastal Tanzania's ecoregion, whereas the Wami-Ruvu basin occupies the central and eastern coastal Tanzania's ecoregion. However, the basins provide a wide range of riverine systems, climate, geology, topography and human disturbance within different hydro-geological patterns. The mean annual rainfall between 1100 and 3000 mm per annum, with a maximum mean temperature of 28˚C to 35˚C in the dry season, and lowest of 14˚C to 18˚C during the wet season.

The Pangani river basin is located in the north-eastern mainland Tanzania, 36˚23'E to 39˚13'E and 03˚03'S to 05˚59'S with an altitude ranging from 0 to 4500 m. The basin has an estimated area of about 43,650 km^2 that covers Arusha (2369.76 km^2), Manyara (17,911.35 km^2), Kilimanjaro (10,346.76 km^2), and Tanga (10,223.17 km^2) regions. Land use practices along the Pangani basin range from small-scale farming to large-scale mechanized agriculture, overexploitation of riparian vegetation, construction of dams and hydropower projects, grazing, bathing and washing, dumping of industrial and domestic wastes and human settlement.

The Wami-Ruvu river basin is elongated and extends from the central part of Tanzania towards the eastern part between 36˚00'E and 39˚00'E and 05˚00'S to 07˚00'S with an altitude of between 0 and 2500 m before draining into the Indian Ocean at Saadani village. It extends from Dodoma, through Morogoro, Coast, and Dar es Salaam regions, covering a total area of 72,930 km^2 of wide plains and mountain ranges. Human activities that are impacting the Wami-Ruvu river basin include mining, brick making, poor agricultural practices involving application of agrochemicals, saline water intrusion, uncontrolled and illegal water obstruction for irrigation, bathing and washing along the river basin, fauna droppings, and disposal of untreated industrial and domestic wastes.

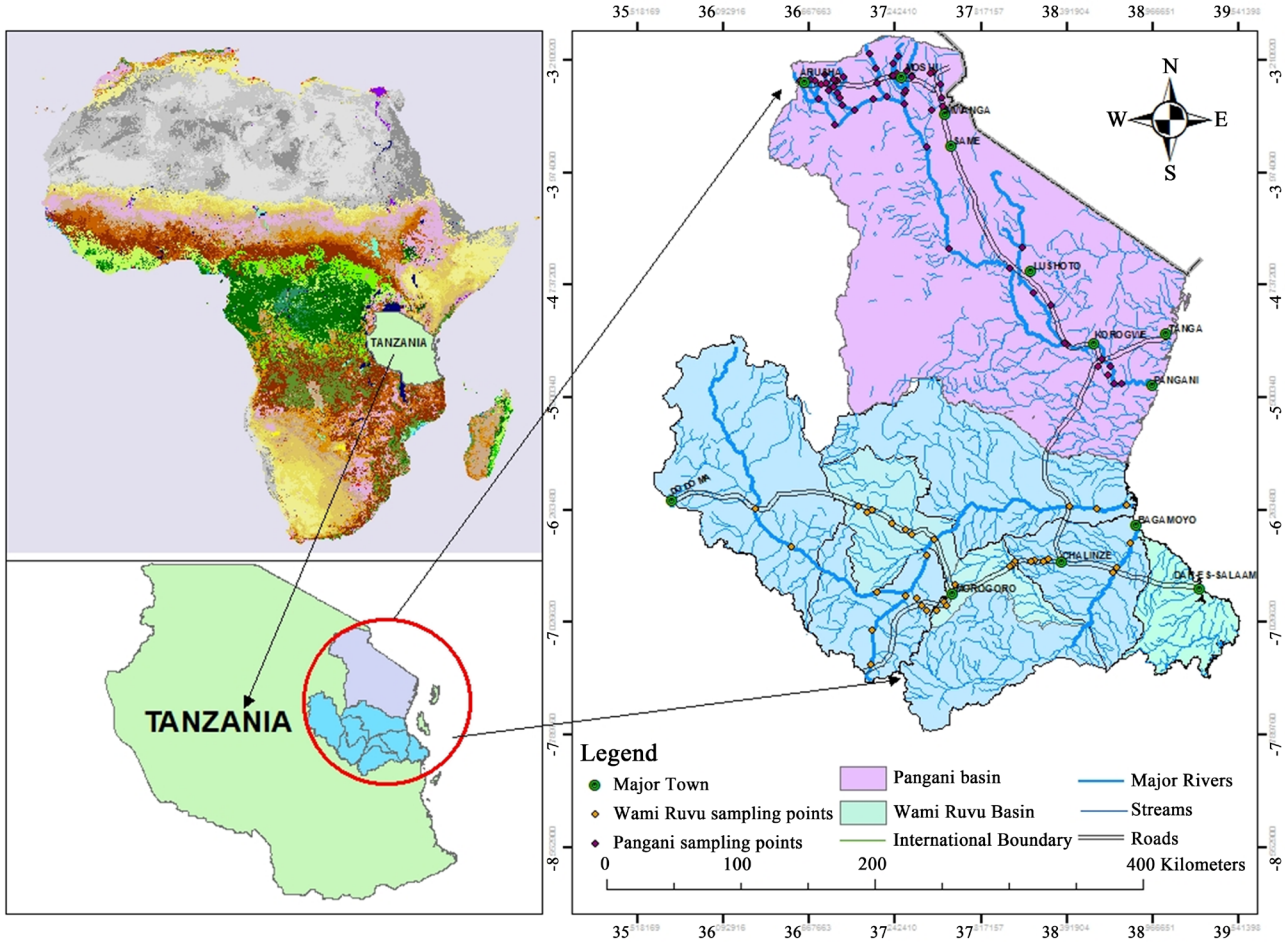


Figure 1. Tanzanian map showing sampling sites along Pangani and Wami-Ruvu basins.

2.2. Sampling Design

The two basins were divided into two site categories representing reference (least impacted) and monitoring (impacted) sites in accordance with Barbour *et al.* [8]. Water and macroinvertebrate assemblage samples were collected at each site near the end of dry, short and long-rain seasons to capture the effect of respective seasons. The sampling sites were selected on the basis of habitat score selection criteria: presence and/or absence of sustained anthropogenic disturbances, pools, riffles and runs, and degree of water physico-chemical, and habitat degradation.

Selection of Reference and Monitoring Sites

Habitat features were scored with the EPA Rapid Bioassessment Protocol (RBP) Habitat Assessment procedure prescribed by Barbour *et al.* [8]. This numerical scoring procedure qualitatively evaluates 200 meters reach for both spatial and longitudinal scales in order to distinguish reference sites from monitoring sites. Twenty-five habitat components described in **Appendix 2** were assessed to categorize reference and monitoring sites. These include epifaunal substrate quantity and quality, embeddedness/siltation, velocity/depth regimes, sediment

deposition, channel flow status, channel sinuosity, channel alteration, hydrological modifications, frequency of riffles or beds, in-stream flow modification, large and small scale farming, direct domestic pollution (washing, bathing, discharge and disposal), direct industrial pollution, livestock keeping, informal settlements, stream bank stability, nutrient enrichment, water quality and appearance, bank grass cover (graze), presence of exotic vegetation, canopy cover, bank vegetation protection, pool variability, pool substrate characterization, and riparian zone width. Each habitat component was scored on a 20-point scoring system to make the maximum summation of 500 points. Habitat score was calculated in each site by summing all rated screening criteria at a site (to get a total habitat score) and divided it by the highest possible score, before expressing it in percentage. The percentage habitat scores were then used to classify sites into two groups based on their degree of disturbance expressed as percentage. The first class having ≥ 90% degree of "naturalness" were considered as reference sites, whereas those with less than 90% naturalness are categorized as monitoring sites (**Appendix 3**).

2.3. Physico-Chemical Data Collection and Analysis

Water physico-chemical parameters *i.e.*, pH, dissolved oxygen (DO), temperature, turbidity, conductivity, total dissolved solids (TDS), ammonia (NH_4^+-N), potassium (K^+), sulphate (SO_4^{2-}), soluble reactive phosphorus (SRP), nitrate (NO_3^--N) and nitrite (NO_2^--N) plus Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were measured. Water temperature, conductivity, DO, TDS, and pH were measured and recorded *in situ* at each site using a multi-sensor probe device (Water Quality Instrument [Model 6,050,000]) while turbidity was measured using a turbidity meter. Laboratory analysis of water chemistry variables involved the filtering of collected water samples using 0.45 μm glass fibre filters and placing them in hydrochloric acid washed polythene bottles before being preserved in a cool box at ≤10˚C. The samples were then taken to the Department of Aquatic Sciences and Fisheries Laboratory of the University of Dar es Salaam for analysis of chemical parameters.

Nitrate (NO_3^--N), nitrite (NO_2^--N), ammonia (NH_4^+-N) and SRP (PO_4^{3-}-P) were analyzed using standard spectrophotometric methods described in APHA [25]. Ammonia was determined using the phenate method, nitrate and nitrite concentrations by cadmium reduction method, SRP analyzed using molybdate ascorbic acid method, SO_4^{2-} by turbid-metric method, BOD by instrumental (BOD track) method and COD using instrumental (semi-automated) calorimetric method [25] [26].

2.4. Macroinvertebrate Samples

Macroinvertebrates were sampled according to sampling methods developed by Barbour *et al.* [8], Dickens and Graham [12] and Lowe *et al.* [16], which were refined prior to use in order to match the study objectives and reflect tropical

aquatic environment. The refinement/modification includes the use of tighter sampling technique, analytical methods and procedures. Macroinvertebrate samples were collected near the end of long-rain, short-rain and dry seasons in order to capture the effect of the respective seasons on macroinvertebrates and the recovery of the riverine ecosystems. Samples were collected throughout the study period by the same operator using a 30 cm × 30 cm kick-net with a 250-µm mesh size.

To avoid bias due to spatial and longitudinal variations and/or heterogeneity impact, samples were collected separately from different biotopes found available at each site. The biotopes include; stone (comprised stone in current—SIC and stone out of current—SOOC), vegetation (marginal and in-water vegetation) and GSM (gravel-sand-mud/silt). Each biotope was disturbed (by hand scooping, feet stirring and kicking, or net sweeping) while moving the kick-net that was placed closely downstream towards upstream for one minute to trap the detached macroinvertebrates. Apart from the available biotopes, one-minute visual observation and hand picking of macroinvertebrates were also done to increase site sample accuracy. All samples were then pooled as one composite sample and sorted grossly in the field to order level before preservation in 10% formaldehyde solution for subsequent laboratory processing, identification and recording. In the laboratory, macroinvertebrate specimens were identified to the lowest possible taxonomic level (family level) with the help of a dissecting microscope (10 × 45 magnifications for detailed observation) and Day *et al.* [27] [28], Thorp and Covich [29], Day and De Moor [30] [31], Day *et al.* [32], De Moor *et al.* [33] [34] and Stals and De Moor [35] identification keys, followed by listing and counting of individuals.

2.5. Selection Criteria for Potential Orders for Use in Biomonitoring Methods

Numerical and statistical redundant criteria were employed to select key bio-indicator families with the potential of separating reference sites from monitoring sites for use in developing a simple and cost-effective method (**Figure 2**). The selection was done by performing numerical truncate test, a non-parametric Mann-Whitney U test ($p < 0.05$), the degree of inter-quartile (IQ) overlap in Box-and-Whisker plots, Spearman's rank (r_s) correlation analysis, more diverse orders (with >10 taxa) criterion and validation test using CAP and Spearman's rank correlation (r_s). Orders with abundances of >0.5%, a p-value < 0.05 in a Mann-Whitney U test, a sensitivity score of 3, $r_s < 75\%$ and higher numbers of taxa were considered as potential orders for inclusion in developing biomonitoring methods [9] [36].

1) ***Truncate test***

Numerically, orders were truncated in order to eliminate the rare taxa (with <0.5% of total macroinvertebrate abundance) that would contribute only noise to other statistical analyses. To reduce variability in the data set, only dominant taxa (with total macroinvertebrate abundance of >0.5%) were retained for further

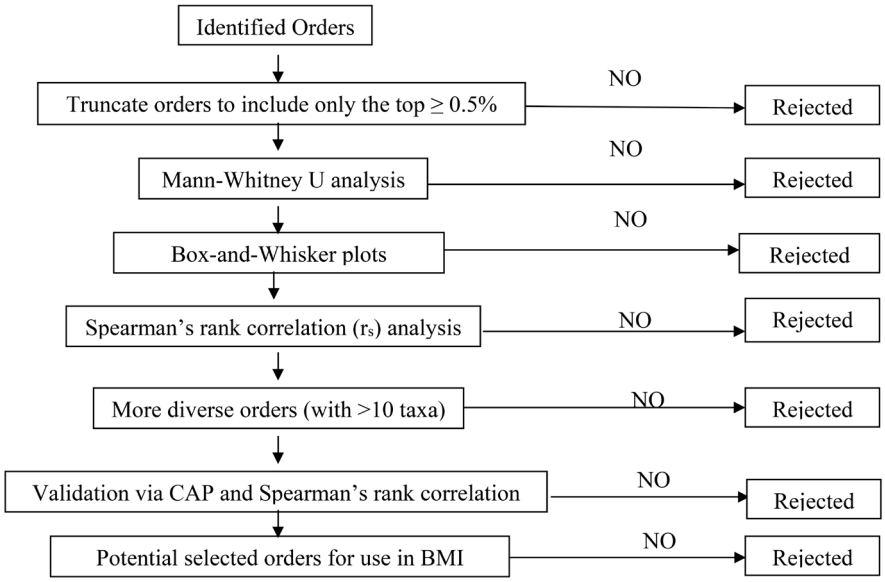


Figure 2. Selection criteria for potential BMI's orders.

statistical analysis [37].

2) ***Mann-Whitney U test with p value*** **< 0.05**

A non-parametric Mann-Whitney U test was used as a statistical testing criterion for eliminating the resulting orders that exhibited no significant differences ($p > 0.05$) after pairwise comparison of abundances for orders observed in reference sites with those in monitoring sites. In that regard, orders found to have a p-value of <0.05 in the test were considered to be strong discriminators of reference and monitoring conditions [9] [36] [38].

3) ***Box-and-Whisker test***

Sensitivity scores of the orders were based on the levels of overlapping interquartile ranges of Box-and-Whisker plots (**Figure 3**) according to the modified procedures prescribed by Barbour *et al.* [8], Baptista *et al.* [9], and Ferreira *et al.* [38]. Box-and-Whisker plots of reference and monitoring sites were examined in order to determine if there was a significant vertical separation between their interquartile ranges of the corresponding conditions. For each order, sensitivity scores of three, two and less than two, with the thresholds of median ranges between 25th and 75th percentiles of the reference site were used as selection criteria for potential orders representing a pivotal assessment tool. A sensitivity score of three (which meets the reference condition) was given if there was no overlap in the interquartile range (IQ) of Box-and-Whisker plots [9] [36] [38]. A score of two (that represents an intermediate condition) was scored if there was a partial overlap of the IQ range with both medians being outside of the overlap [9] [36] [38]. Likewise, a sensitivity score of less than two was given if the orders' abundances were below the 25th percentile. These scores were attained if: a) there was a moderate overlap of IQ range but one median appeared outside the IQ range overlap; b) one range completely overlapped the other IQ range but one median is outside the IQ range overlap; and c) both medians were inside the IQ range overlap.

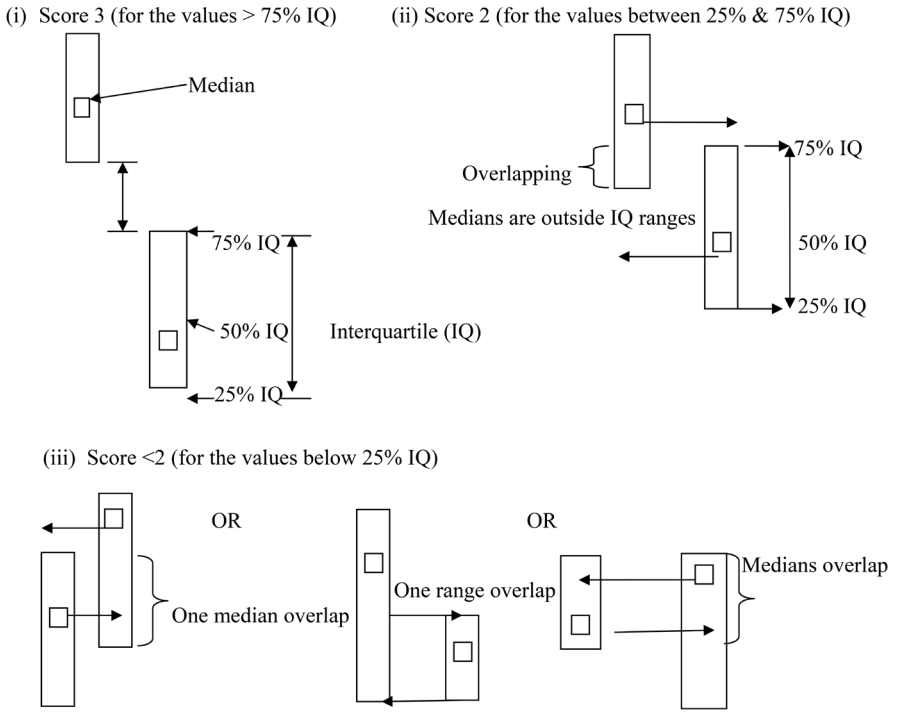


Figure 3. Sensitivity scores of Box-and-Whisker plots according to modified procedures prescribed by Barbour *et al.* [36], Baptista *et al.* [9] and Ferreira *et al.* [38].

4) *Spearman's rank correlation test*

For more simplification of the index, a Spearman's rank correlation was drawn with paired orders to eliminate any order if more than 75% of its values were identical. Orders with Spearman's correlation (r_s) > 0.75 were considered redundant in which the least abundant order was eliminated [38].

5) *More diverse orders* (*n* > 10 *taxa*) *criterion*

More diverse orders showing the highest representativeness of organisms distinguishing the reference sites from monitoring sites were chosen and used as potential candidates for developing the BMI. However, the orders were selected to establish the BMI if they had more than 10 families representing a wide range of occurrences and pollution sensitivity.

6) *Validation of selected more diverse orders* (*n* > 10 *taxa*)

A constrained CAP discriminatory analysis and Spearman's rank correlation analysis were used for validating the selected bioindicator orders that showed a wide representativeness of taxa in all sites and ability of distinguishing reference sites from monitoring sites. Spearman's rank correlation analysis was performed by correlating the selected more diverse orders with environmental variables.

Pollution Sensitivity Scoring of Selected Taxa of Bioindicator Orders

Each identified taxa of the selected macroinvertebrate orders with a potential of developing a biomonitoring method was assigned a pollution sensitivity weighting after an extensive literature review. The sensitivity scores of the reported taxa were assigned based on:

1) Known scores of taxa extracted from closely related existing indices which have been extensively tested and their capability and reliability for assessing water quality have been proven *i.e.*, SASS5.

2) Autecological knowledge of macroinvertebrate taxa;

3) Association of taxa occurrences or abundances with environmental variables;

4) Simulated results for taxa with unknown sensitive scores to stressors using Canonical Analysis of Principal coordinates (CAP) predictive model.

The CAP predictive model was firstly calibrated by simulating only the abundance of taxa with known scores and their respective scores to facilitate the interpretation of unknown scores. The model was then re-simulated while including all abundances of taxa with their known and unknown sensitivity scores.

2.6. Data Analysis

MS Excel, PRIMER® version 7 (with PERMANOVA add-on), OriginPro® version 8.5, Community Analysis Package® version 4 (CAP IV), Species Richness and Diversity IV (SDR IV), and Instat® version 3 (GraphPad®) software packages were used for analysing the data. Prior to the analysis, all the data were transformed where appropriate and those with different S.I. unit were normalized into unit-less according to Barbour *et al.* [36], and Baptista *et al.* [9] in order to maintain uniformity among the values. Significance tests were performed with PRIMER version 7 after the biotic data had undergone transformation (to either log (x + 1), square root, or absent and present), with p value set at 0.05 to determine the differences among basins and site categories. Mann-Whitney U test and Non-Parametric Spearman's rank correlation were performed by Instat® version 3 (GraphPad®) and Box-and-Whisker plots by OriginPro 8.5 used for revealing the discrimination power of the order among the site categories. Canonical Analysis of Principal coordinates (CAP) predictive model was simulated using PERMANOVA+ software package, which is an add-on to PRIMER® version 7 to calculate sensitivity weightings for taxa with unknown sensitivity ratings according to Anderson *et al.* [39]. Moreover, CAP and non-parametric Spearman's rank correlation analysis were used for validating the ability of taxa to discriminate reference sites from monitoring sites.

3. Results

Approximately 97 freshwater macroinvertebrate families belonging to 17 orders were identified collectively to summarize macroinvertebrate data set for Tanzanian rivers (**Appendix 4**). Six validation criteria (**Figure 2**) were used for selecting potential orders for use in the biomonitoring index (BMI). The selection criteria included numerical and statistical tests that have been successfully applied in other regions to identify the potential candidates for inclusion during the development of their BMIs. Out of the 17 orders, Ephemeroptera, Diptera, Odonata and Trichoptera (EDOT) were found with significant discriminating power

separating the reference from impaired sites according to truncate numerical test, Mann-Whitney U test ($p < 0.05$), Box-and-Whisker plot test, RDA and more diverse orders ($n > 10$ taxa) criterion. The rationale for the usefulness of each order is numerically and statistically tested in section 3.1 to 3.9.

3.1. Truncate Test

To reduce unusual variability of the data set [37], orders with ≤0.5% of the total macroinvertebrate abundance were numerically exempted for the next screening. Of the 17 macroinvertebrate orders, 10 had abundances of ≥0.5% and thus, passed the truncate numerical test and consequently were retained for the next screening test, with Arhynchobdellida, Rhynchobdellida, Hydroida, Pelecypoda, Megaloptera, Lepidoptera and Turbellaria orders, considered redundant.

3.2. Mann-Whitney U Test

Mann-Whitney U test was used for demonstrating the ability of orders to discern the difference between references and monitoring sites of the river basins. Orders were considered strong discriminators of impairment if the difference between monitoring and reference sites was significant (Mann-Whitney U, with $p < 0.05$). All the tested orders were found to be non-redundant (with $p < 0.05$) and thus considered for the next test (**Table 1**).

3.3. Box-and-Whisker Plot Test

Box-and-Whisker plots were used for evaluating how well each order could discriminate between the site categories, with a sensitivity score of three considered as a selection criterion [36]. The test showed that only six orders were highly sensitive (score = 3) and were consequently retained for non-parametric Spearman's rank correlation selection test. These included: Diptera, Decapoda, Odonata, Ephemeroptera, Coleoptera, and Trichoptera (**Table 2** and **Figure 4**).

Table 1. Results of Mann-Whitney U test for 10 Tanzanian orders of Tanzanian rivers.

ORDER	Mann-Whitney U test, p-value	Test remarks	Meets the test criteria
Tubificida	0.0186	Significant*	Yes
Coleoptera	<0.0001	Extremely significant***	Yes
Decapoda	0.0049	Very significant**	Yes
Diptera	<0.0001	Extremely significant***	Yes
Ephemeroptera	<0.0001	Extremely significant***	Yes
Gastropoda	<0.0001	Extremely significant***	Yes
Hemiptera	0.0001	Extremely significant***	Yes
Odonata	0.0372	Significant*	Yes
Plecoptera	0.0049	Very significant**	Yes
Trichoptera	<0.0001	Extremely significant***	Yes

Table 2. Results of Box-and-Whisker Plot tests for 10 tested orders.

ORDER	Response to pollution	Sensitivity score	Meets the test criteria
Tubicifida	Decrease	<2	No
Coleoptera	Decrease	3	Yes
Decapoda	Variable	3	Yes
Gastropoda	Decrease	<2	No
Diptera	Increase	3	Yes
Ephemeroptera	Decrease	3	Yes
Hemiptera	Decrease	2	No
Odonata	Increase	3	Yes
Plecoptera	Decrease	<2	No
Trichoptera	Decrease	3	Yes

3.4. Spearman's Rank Correlation

Non-parametric Spearman's rank correlation was used in order to avoid repeating information already summarized by other orders and for ensuring an accurate depiction of patterns by separating reference sites from monitoring sites. Orders with poor range are unlikely to differentiate monitoring and reference sites because the response gradient is highly compressed. Six orders that passed Box-and-Whisker plot tests were tested for redundancy amongst them using Spearman rank correlation analysis. Orders were considered redundant if the Spearman rank correlation coefficient (r_s) was higher than 0.75 with a p-value of <0.05 [40]. However, all the tested Diptera, Decapoda, Odonata, Ephemeroptera, Coleoptera, and Trichoptera orders were unique with r_s of <0.75 and p of <0.05 and thus, considered non-redundant and were retained for further selection tests.

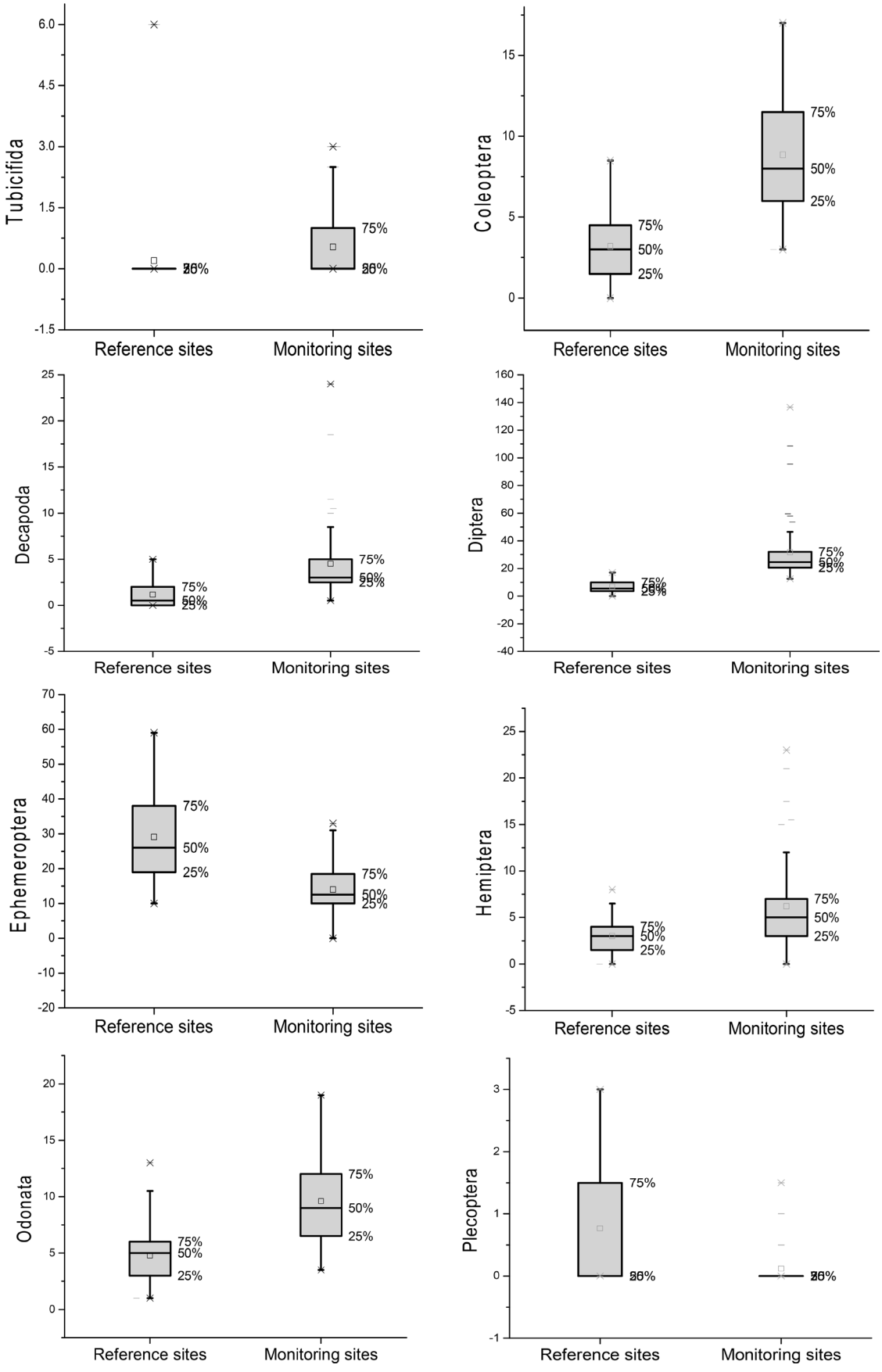
3.5. More Diverse Orders (n > 10 Taxa) Criterion

More diverse orders showing the wide representativeness of families in all sites were chosen and used as potential candidates for developing BMI. Ephemeroptera (E), Diptera (D), Odonata (O) and Trichoptera (T) were the only four orders containing large numbers of different taxa (n > 10) at all levels of pollution tolerance. Odonata was represented by 12 instances, ephemeropterans by 13, whereas, dipterans and trichopterans contained 14 instances each, making a total of 53 instances, representing about 55% (N = 97) of all Tanzanian taxa.

3.6. Validation of EDOT Taxa

A constrained CAP discrimination analysis was performed to analyse macroinvertebrate assemblages for their ability to discern the reference sites from monitoring sites along Tanzanian river basins (Figure 5).

In developing biomonitoring method, it is also important to understand how the selected bio-indicator orders or taxa are correlated with environmental variables.


Tubicifida
6.0
4.5
3.0
1.5
0.0
-1.5
75%
25%
Reference sites
Monitoring sites
Coleoptera
15
10
5
0
75%
50%
25%
Reference sites
Monitoring sites
Decapoda
25
20
15
10
5
0
-5
75%
50%
25%
Reference sites
Monitoring sites
Diptera
160
140
120
100
80
60
40
20
0
-20
-40
75%
50%
25%
Reference sites
Monitoring sites
Ephemeroptera
70
60
50
40
30
20
10
0
-10
-20
75%
50%
25%
Reference sites
Monitoring sites
Hemiptera
25
20
15
10
5
0
-5
75%
50%
25%
Reference sites
Monitoring sites
Odonata
20
15
10
5
0
75%
50%
25%
Reference sites
Monitoring sites
Plecoptera
3
2
1
0
-1
75%
Reference sites
Monitoring sites

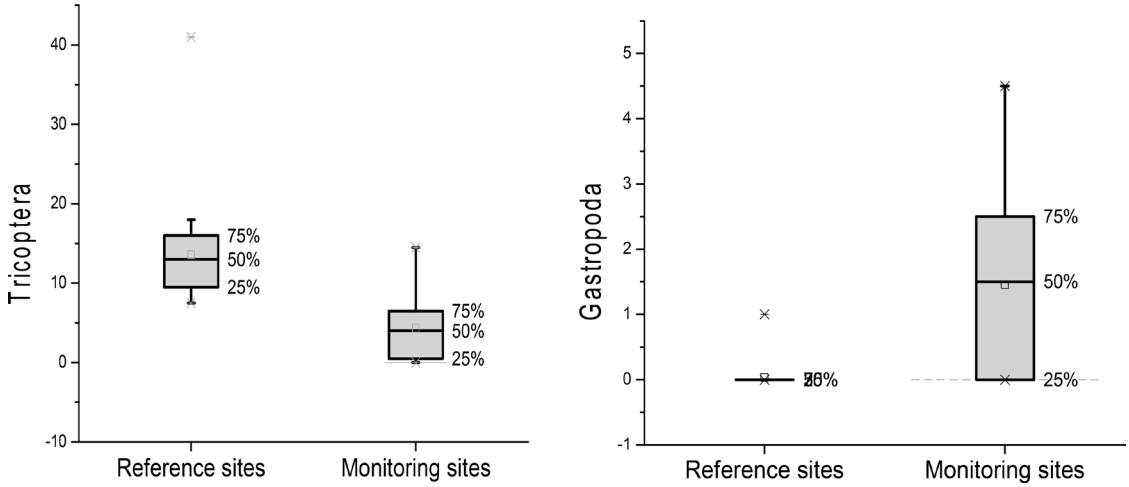


Figure 4. Box-and-Whisker plots for the orders distinguishing reference sites from monitoring sites of Tanzanian river basins.

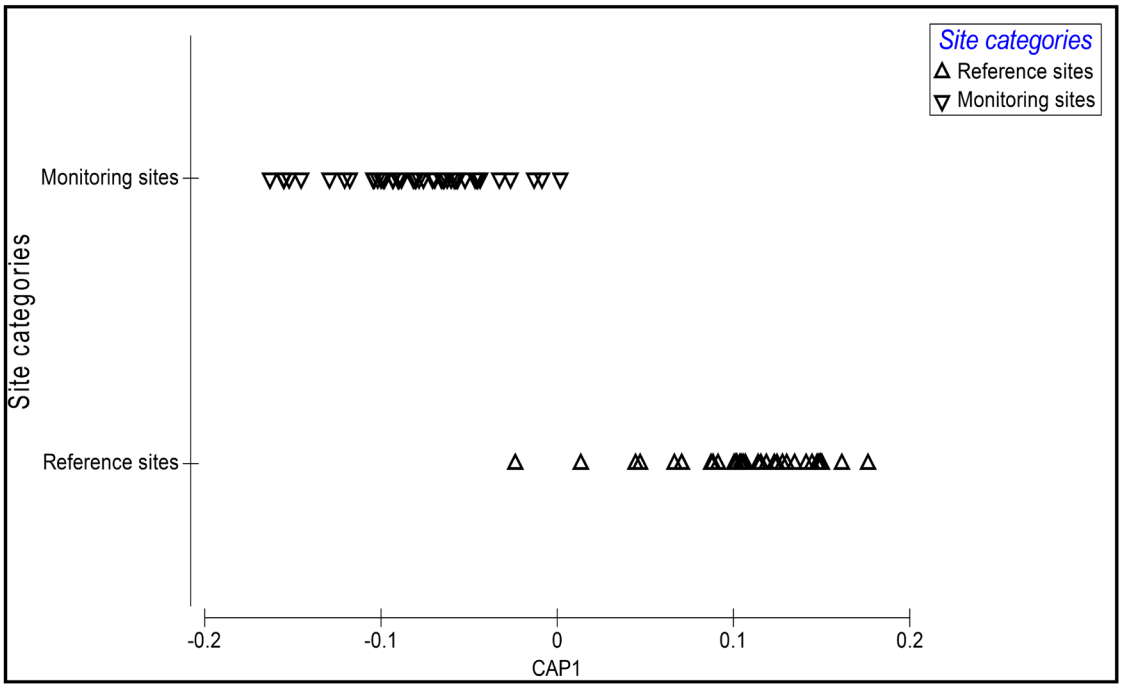


Figure 5. Macroinvertebrate taxa of four selected orders showing their discriminatory power separating reference from monitoring sites using canonical discrimination analysis in Tanzanian river basins ($\delta_2 = 0.8479$, $p < 0.001$): r = reference sites; m = monitoring sites.

EDOT taxa were therefore assessed together with various factors that may influence the scores. The results from non-parametric Spearman's rank correlation analysis showed strong significant correlation (with p of <0.0001) among the four orders and most environmental variables structuring macroinvertebrate assemblages. Ephemeroptera abundances showed strong correlation with conductivity ($r_s = -0.4330$) and temperature ($r_s = -0.4235$); dipterans with conductivity ($r_s = -0.4117$), temperature ($r_s = 0.5023$), NH_4^+-N ($r_s = 0.6544$), BOD ($r_s = 0.5434$), COD ($r_s = 0.6005$), NO_3^--N ($r_s = 0.7399$), SO_4^{2-} ($r_s = 0.4914$) and

potassium (r_s = 0.5734); Odonata with conductivity (r_s = 0.4098) and pH (r_s = 4152), and trichopterans with BOD (r_s = −0.5229), COD (r_s = −0.5492), NO_3^--N (r_s = 0.6278), NH_4^+-N (r_s = −0.5324) and potassium (r_s = −0.4530). Correlation strength reflects the reliability of the EDOT orders or taxa in detecting changes and/or discriminating the reference sites from monitoring sites along the rivers in the two basins. Since EDOT taxa have demonstrated their ability to discern the reference sites from monitoring sites via CAP and Spearman's rank correlation analysis, they can therefore be used as potential bio-indicators in developing EDOT method.

3.7. Scoring of Selected Bioindicator Taxa

Generally, the sensitivity scores ranged from zero to 15, representing three categories of macroinvertebrate groups. Sensitivity scores for most tolerant taxa to stressors ranged from 11 to 15, whereas, six to 10 is for moderately tolerant taxa, and one to five for the least tolerant taxa [41]. If the species vary within taxa (*i.e.*, Baetidae or Hydropsychidae) their sensitivity scores were assigned under the descending assumption that the more the species available at a site the less disturbed the site is, as such, a sensitivity rating of four is given to Baetidae 1 species, six to Baetidae 2 species and 12 to Baetidae > 2 species [42]. Of the 53 taxa, 50 were assigned scores based on related scoring systems [12] [14] [17] [42] while the sensitivity scores for the remaining three taxa were simulated by the CAP predictive model (**Figure 6**). The CAP predictive model with a correlation of 0.8543 and/or correlation square (δ^2 = 0.7299) calculated sensitivity scores for Dicercomyzidae, Ephemerythidae and Macromiidae as 9.7246 ≈ 10; 8.8258 ≈ 9 and 3.1 ≈ 3 respectively (**Figure 6**).

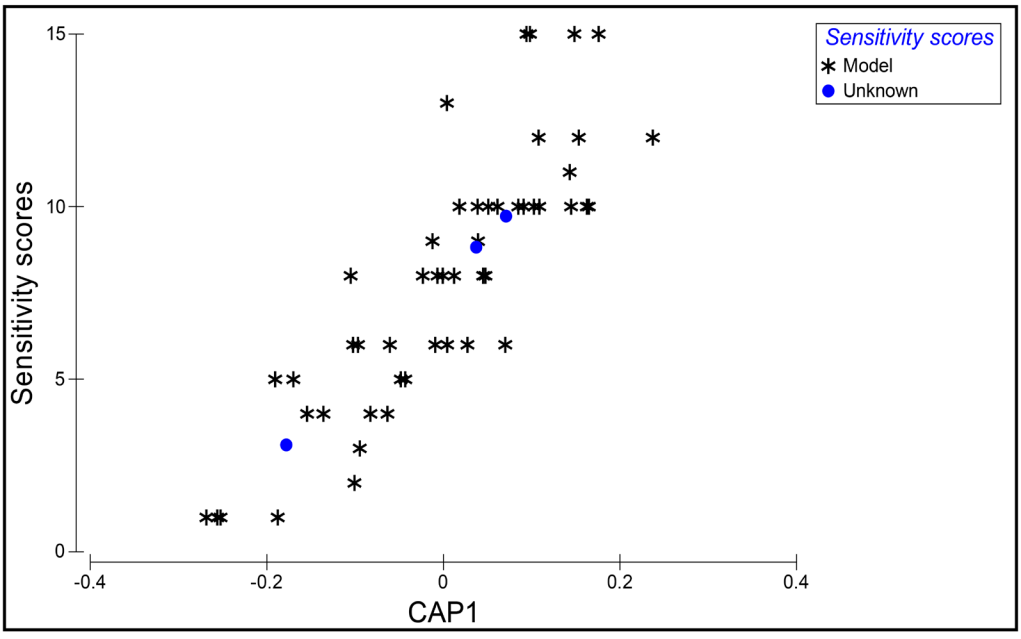


Figure 6. Canonical Analysis of Principal coordinates (CAP) predictive model showing the position of taxa in relation to their sensitivity scores.

3.8. Application of EDOT Index (Calculating EDOT Index)

EDOT index (**Appendix 1**) is a field based rapid scoring system entailing *in situ* observation (with the help of 10 × 45 magnifying stereo microscope were deemed necessary), in which taxa are identified up to family level. Regardless of its abundance, each observed taxon is estimated upon observation and tallied in their respective biotope (stone, vegetation and GSM) and the combined column one of EDOT scoring sheet. A single macroinvertebrate is estimated as one organism whereas, less than one to 10 organisms as two, >10 to 100 as three, >100 to 1000 as four and >1000 as five in order to minimize scoring time. Time less than 10 minutes per site is recommended to end the fieldwork but if there is not any observed taxon in the duration of one minute.

The ticked sensitivity score of each taxon in the combined column is summed up to provide an EDOT(f) score, whereas, the total number of taxa is obtained by counting the recorded taxa. ASPT, on the other hand, is calculated by dividing the EDOT(f) scores by the number of taxa. EDOT Index can be calculated as EDOT(f) Score, Number of Taxa (No. Taxa) and Average Score per Taxa (ASPT) but only the result calculated from the combined column will represent the EDOT result for that particular site.

Mathematically, EDOT(f) is calculated as:

$$\mathrm{EDOT}(\mathrm{f}) = \sum_{i=1}^{n} \mathrm{Score}_i$$

The Average Score Per Taxon (ASPT) is calculated by dividing EDOT(f) scores by the total number of taxa found as follows.

$$\mathrm{ASPT} = \frac{\sum_{i=1}^{n} \mathrm{Score}_i}{n}$$

where: Score_i stands for the score of taxon i and n for the number of taxa.

Moreover, separate results may be achieved for each biotope and used in various investigations, only the result calculated from the total column will represent the EDOT(f) result for a site. Since this new index is designed to describe the degree at which tropical African riverine systems are impacted by human induced pollution, the scores towards zero represent stressed river while towards 100 refers to unstressed river. However, Dickens and Graham [12] have cautioned on the implication of combining the scores from the three biotopes by adding the score of any index, the number of taxa and ASPT and dividing the total by three. The resulting EDOT(f) score and ASPT score are interpreted using modified threshold values in **Table 3**.

4. Discussion

A 15-sensitivity scoring range following SASS5 [12], ETHbios [42] and TARISS [17] was used for all identified families of selected orders. Out of the total 97 taxa recorded from all sites, scores were assigned to only 53 taxa that showed clear water quality preferences, using either closely related earlier indices (50 taxa) or

Table 3. The suggested EDOT(f) threshold limits for assessing river health status.

BAND BOUNDARY		BIOLOGICAL BAND	DESCRIPTION Impairment Level	WATER STATUS Water Quality
EDOT Score	ASPT Score			
≤50	≤5.0	Seriously Modified	Serious ecological impairment	Very poor water quality
51 to 75	5.1 to 6.0	Largely Modified	Large ecological impairment	Poor water quality
76 to 150	6.1 to 7.0	Moderately Modified	Moderate ecological impairment	Moderate water quality
151 to 225	7.1 to 8.0	Largely Natural	Slight ecological impairment	Good water quality
>225	>8.0	Natural	Little ecological impairment	High water quality

CAP predictive model (three taxa). However, a flexible consideration was applied to assigning sensitivity scores for specific taxa groups with a number of types *i.e.*, Baetidae (with 1 sp., 2 spp. and >2 spp.), and Hydropsychidae (1 sp., 2 spp. and >2 spp.) that cover wide pollution gradients [42] in order to increase the discrimination efficiency of these taxa among site categories.

The sensitivity scores for the taxa obtained from earlier indices strongly support the simulated CAP predictive model results with some families of the same order found matching the scores. For instance, the calculated score of 10 for Dicercomyzidae concurs with that of Polymitarcyidae whereas the score of nine awarded to Leptophlebiidae and Tricorythidae by earlier studies was at par with that simulated for Ephemerythidae (nine). Contrary to Dicercomyzidae and Ephemerythidae, Macromiidae was the least sensitive taxon (with a score of three) compared to the other Odonata families but close to the sensitive score of four, which was reported for Coenagrionidae and Libellulidae by existing biomonitoring methods. According to Gerber and Gabriel [41], the simulated sensitivity scores for Dicercomyzidae (10) and Ephemerythidae (nine) fall well within the range of moderately sensitive taxa while the Macromiidae (three) is grouped with the least sensitive taxa along the y-axis in **Figure 6**. The varied sensitivity levels to human stressors allow families of the EDOT orders to function as bio-indicators for assessing freshwater health status with strong relevance on conservation and management aspects [43].

Validation criteria, which included six selection criteria, were also set during the selection of orders to be involved in developing the index for the sake of simplifying taxonomic complications and improving the accuracy and efficiency of the index while minimizing the data collection time and cost. Indeed, EDOT orders are well known as more diverse and abundant orders in freshwater ecosystems with a large number of taxa and species [44] [45] [46], varied degrees of sensitivity to a wide range of anthropogenic stressors [47] and a recognizable contribution in the biomonitoring programmes [48]. In the presence of various environmental stress types *i.e.*, organic pollution [49], heavy metals [50], hydro-morphological degradation [51], nutrient enrichment [52], acidification [53] and general stressors [8], their families can collectively reflect short and long-term health status of aquatic ecosystems [9]. However, 13 other orders were eliminated because they either failed to reflect the different features of freshwater

macroinvertebrates communities or discriminating reference sites from monitoring sites according to truncate numerical test, the Mann-Whitney U test (with $p < 0.05$), Box-and-Whisker plot test, Non-parametric Spearman's rank correlation test and more diverse taxa ($n > 10$) criteria. Similarly, the presence of cryptic species (e.g., chironomids) with varied responses towards pollution, and some being rarely identified to the species level [46] has restricted the development of species level EDOT index. Moreover, the lowest taxonomical unit identification has cost and time bound implications, and also requires more specialized knowledge and expertise [2] [42] [54]. However, the sensitivity variation for some families of the same order might contradict the biomonitoring efforts. For example, the Odonata family Gomphidae, has been classified among the most sensitive taxa whereas Coenagrionidae is far less sensitive to pollution [55].

Ephemeropterans are considered as ecologically an important order in biomonitoring programmes all over the world due to their least tolerant character against low dissolved oxygen, higher levels of nutrients, and toxicant chemical elements and compounds [56]. The order is abundantly found in sites with good water quality at interstitial spaces between rocks, rock surfaces, sediments, submerged underwater and marginal vegetation, with high amount of dissolved oxygen [56].

Contrary to Ephemeroptera, Trichoptera are somewhat more tolerant to pollution, but do not persist as a diverse community in the presence of significant impairment [57]. Trichopterans on the other hand inhabit a wide variety of habitats, ranging from fast flowing riffles to slow moving water type of sparsely vegetated pools. Being diverse, abundant and able to thrive in lentic conditions of both slow and fast-moving rivers makes them excellent indicators of habitat quality [57]. Regardless of their reported inconsistent nature in detecting impacts [48], the inclusion of trichopterans in biomonitoring programmes is not only virtual in evaluating the long-term interaction of several environmental conditions, but also in detecting short-term impact.

The strong significant correlation (with $p < 0.0001$) shown between the EDOT orders and most of the environmental variables structuring macroinvertebrate assemblages indicates better performance of the orders to organic pollution. In polluted rivers, abundance and diversity of more sensitive orders (ephemeropterans and trichopterans) are strongly reduced due to direct and indirect impact of pollutants where dipterans commonly possess the dominant status. The ability of dipterans to survive well in highly polluted freshwater environment and in slow moving water than most of the ephemeropterans, trichopterans and Odonata, render them good indicators for assessment of aquatic health status [58]. EDOT has ensured response of overall ecological status in river basins by segregating reference sites from monitoring sites and thus, concurring with other studies in the U.S.A [36], Europe [59], Brazil [9], and Tanzania [17].

5. Conclusion

The study has provided the first simplified biomonitoring method comprised of

local based macroinvertebrate taxa with a wide range of occurrences, trophic levels and sensitivity to pollution as a tool for assessing water pollution in tropical African rivers. Being developed using only a few (four) and more diverse orders (with >10 taxa), minimizes data variability, needs for greater expertise and time in the field and thus makes it a less complex method than existing biomonitoring methods. A high EDOT method score describes an ecosystem containing diversified physical habitats, good water quality with conducive physicochemical conditions and adequate food resources for sustaining the lives of many species. This method is also in line with the interest shown by African and non-African environmental and water quality monitoring institutions and/or authorities in the application of biomonitoring methods, which tend to be lower cost and more effective than physical-chemical methods [18] [46], with emphasis on regionally or country-based water quality biomonitoring programmes. Upon validation, the resulting EDOT index can therefore be regarded as simple and cost-effective tool for assessing the ecological condition in Tanzanian rivers and other related watersheds in tropical African regions, where freshwater resources are under high pressure as a result of anthropogenic activities.

Acknowledgements

The authors would like to thank the Nelson Mandela African Institute of Science and Technology (NM-AIST) for their support during the field work and the Department of Aquatic Sciences and Fisheries Laboratory of the University of Dar es Salaam for space during the analysis of chemical parameters.

Funding

This study received financial support from Nelson Mandela Foundation through Tanzania Commission for Science and Technology (COSTECH).

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Umar, D.M., Harding, J.S. and Winterbourn, M.J. (2013) Freshwater Invertebrates of the Mambilla Plateau, Nigeria. Gombe State University and University of Canterbury, Christchurch, 88 p.

[2] Elias, J.D., Ijumba, J.N. and Mamboya, F.A. (2014) Effectiveness and Compatibility of Non-Tropical Biomonitoring Indices for Assessing Pollution in Tropical Rivers—A Review. *International Journal of Ecosystem*, **4**, 128-134. https://doi.org/10.1155/2014/985389

[3] Elias, J.D., Ijumba, J.N., Mgaya, Y.D. and Mamboya, F. (2014) Study on Freshwater Macroinvertebrates of Some Tanzanian Rivers as a Basis for Developing Biomonitoring Index for Assessing Pollution in Tropical African Regions. *Journal of Ecosystems*, **2014**, Article ID: 985389. https://doi.org/10.1155/2014/985389

[4] Chutter, F.M. (1972) An Empirical Biotic Index of the Quality of Water in South

African Streams and Rivers. *Water Research*, **6**, 19-30. https://doi.org/10.1016/0043-1354(72)90170-4

[5] Wright, J.F., Moss, D., Armitage, P.D. and Furse, M.T. (1984) A Preliminary Classification of Running-Water Sites in Great Britain Based on Macroinvertebrate Species and Prediction of Community Type Using Environmental Data. *Freshwater Biology*, **14**, 221-256. https://doi.org/10.1111/j.1365-2427.1984.tb00039.x

[6] Hawks, H.A. (1997) Origin and Development of the Biological Monitoring Working Party System. *Water Research*, **32**, 964-968. https://doi.org/10.1016/S0043-1354(97)00275-3

[7] Chutter, F.M. (1998) Research on the Rapid Biological Assessment of Water Quality Impacts in Streams and Rivers. Water Research Commission Report No. 422/1/98. Water Research Commission, Pretoria.

[8] Barbour, C.D.M.T., Gerritsen, J., Snyder, B.D. and Stribling, J.B. (1999) Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish; 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington DC.

[9] 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. https://doi.org/10.1007/s10750-006-0286-x

[10] Jacobsen, D. and Marın, R. (2007) Bolivian Altiplano Streams with Low Richness of Macroinvertebrates and Large Diel Fluctuations in Temperature and Dissolved Oxygen. *Aquatic Ecology*, **42**, 643-656. https://doi.org/10.1007/s10452-007-9127-x

[11] Day, J.A. (2000) Biomonitoring: Appropriate Technology for the 21st Century. 1*st WARFSA/WaterNet Symposium*: *Sustainable Use of Water Resources*, Maputo, 7 p.

[12] 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. https://doi.org/10.2989/16085914.2002.9626569

[13] Dallas, H.F., Kennedy, M., Taylor, J., Lowe, S. and Murphy, S. (2010) SAFRASS. South African Rivers Assessment Scheme, WP4. Review Paper. 39 p.

[14] Palmer, R.W. and Taylor, E.D. (2004) The Namibian Scoring System (NASS) Version 2 Rapid Bioassessment Method for Rivers. *African Journal of Aquatic Science*, **29**, 229-234. https://doi.org/10.2989/16085910409503814

[15] Dallas, H.F. (2009) Wetland Monitoring Using Aquatic Macroinvertebrates. Technical Report. Report 5/2009 Prepared for the Biokavango Project, Harry Oppenheimer Okavango Research Centre, University of Botswana, The Freshwater Consulting Group, University of Cape Town, Cape Town, 27 p.

[16] Lowe, S., Dallas, H., Kennedy, M., Taylor, J.C., Gibbins, C., Lang, P., Day, J., Sichingabula, H., Saili, K., Willems, F., Briggs, J.A. and Murphy, K. (2013) The SAFRASS Biomonitoring Scheme: General Aspects, Macrophytes (ZMTR) and Benthic Macroinvertebrates (ZISS) Protocols. Produced for the ACP Science and Technology Programme. 16 p.

[17] Kaaya, L.T. (2015) Towards a Classification of Tanzanian Rivers: A Bioassessment and Ecological Management Tool. A Case Study of the Pangani, Rufiji and Wami-Ruvu River Basins. *African Journal of Aquatic Science*, **40**, 37-45. https://doi.org/10.2989/16085914.2015.1008970

[18] Aschalew, L. (2014) Development of Biological Monitoring Systems Using Benthic Invertebrates to Assess the Ecological Status of Central and South-East Highland Rivers of Ethiopia. Unpublished Thesis for Award of PhD Degree at University of

Natural Resources and Life Sciences, Vienna, 163 p.

[19] Boulton, A.J., Boyero, L., Covich, A.P., Dobson, M., Lake, S. and Pearson, R. (2008) Are Tropical Streams Ecologically Different from Temperate Streams? In: Dudgeon, D., Ed., *Tropical Stream Ecology*, Elsevier Inc., London, 257-284. https://doi.org/10.1016/B978-012088449-0.50011-X

[20] Pearson, R.G. and Boyero, L. (2009) Gradients in Regional Diversity of Freshwater Taxa. *Journal of the North American Benthological Society*, **28**, 504-514. https://doi.org/10.1899/08-118.1

[21] Jacobsen, D., Cressa, C., Mathooko, J.M. and Dudgeon, D. (2008) Macroinvertebrates: Composition, Life Histories and Production. In: Dudgeon, D., Ed., *Tropical Stream Ecology*, Academic Press, Cambridge, 66-96. https://doi.org/10.1016/B978-012088449-0.50006-6

[22] Masese, F.O., Muchiri, M. and Raburu, P.O. (2010) A Preliminary Benthic Macroinvertebrate Index of Biotic Integrity (B-IBI) for Monitoring the Moiben River, Lake Victoria, Kenya. *African Journal of Aquatic Science*, **34**, 1-14. https://doi.org/10.2989/AJAS.2009.34.1.1.726

[23] Blakely, T.J., Harding, J.S., Clews, E. and Winterbourn, M.J. (2010) An Illustrated Guide to the Freshwater Macroinvertebrates of Singapore. School of Biological Sciences, University of Canterbury, Christchurch, 74 p.

[24] Ngupula, G.W. and Kayanda, R. (2010) Benthic Macrofauna Community Composition, Abundance and Distribution in the Tanzania and Uganda Inshore and Offshore Waters of Lake Victoria. *African Journal of Aquatic Science*, **35**, 185-192. https://doi.org/10.2989/16085914.2010.490978

[25] APHA (2000) Standard Methods for the Analysis of Water and Wastewater. 15th Edition, American Public Health Association and Water Pollution Control Federation, Washington DC, 12-56.

[26] Wetzel, R.G. and Linkens, G. (2000) Limnological Analyses. Springer (India) Publisher Private Limited, New Delhi, 426 p. https://doi.org/10.1007/978-1-4757-3250-4

[27] Day, J.A., de Moor, I.J., Stewart, B.A. and Louw, A.E. (2001) Guides to the Freshwater Invertebrates of Southern Africa: Volume 3 Crustacea II—Ostracoda, Copepoda and Branchiura. WRC Report No. TT 148/01. Water Research Commission, Pretoria, 177 p.

[28] Day, J.A., de Moor, I.J., Stewart, B.A. and Louw, A.E. (2001) Guides to the Freshwater Invertebrates of Southern Africa: Volume 4 Crustacea III—Bathynellacea, Amphipoda, Isopoda, Spelaeogriphea, Tanaidacea and Decapoda. WRC Report No. TT 141/01. Water Research Commission, Pretoria, 126 p.

[29] Thorn, J.H. and Covich, A.P. (1991) Ecology and Classification of North American Freshwater Invertebrates. Academic Press, San Diego, 1056 p.

[30] Day, J.A. and De Moor, I.J. (2002) Guides to the Freshwater Invertebrates of Southern Africa: Volume 6 Arachnida and Mollusca—Araneae, Water Mites and Mollusca. WRC Report No. TT 182/02. Water Research Commission, Pretoria, 141 p.

[31] Day, J.A. and De Moor, I.J. (2002) Guides to the Freshwater Invertebrates of Southern Africa: Volume 5 Non-Arthropods—The Protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and Hirudinea. WRC Report No. TT 167/02. Water Research Commission, Pretoria, 293 p.

[32] Day, J.A., Harrison, A.D. and de Moor, I.J. (2003) Guides to the Freshwater Invertebrates of Southern Africa: Volume 9 Diptera. WRC Report No. TT 201/02. Water Research Commission, Pretoria, 288 p.

[33] 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. WRC Report No. TT 207/03. Water Research Commission, Pretoria, 288 p.

[34] De Moor, I.J., Day, J.A. and De Moor, F.C. (2003) Guides to the Freshwater Invertebrates of Southern Africa: Volume 8 Insecta II—Hemiptera, Megaloptera, Neuroptera, Trichoptera and Lepidoptera. WRC Report No. TT 214/03. Water Research Commission, Pretoria, 209 p.

[35] Stals, R. and De Moor, I.J. (2007) Guides to the Freshwater Invertebrates of Southern Africa: Volume 7 Insecta I—Ephemeroptera, Odonata and Plecoptera. WRC Report No. TT 320/07, Water Research Commission, Pretoria, 263 p.

[36] Barbour, M.T. and Gerritsen, J. (1996) Sub Sampling of Benthic Samples: A Defense of the Fixed-Count Method. *Journal of the North American Benthological Society*, **15**, 386-391. https://doi.org/10.2307/1467285

[37] Gauch, H.G. (1982) Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge, 307 p. https://doi.org/10.1017/CBO9780511623332

[38] Ferreira, W.R., Paiva, L.T. and Callisto, M. (2011) Development of a Benthic Multimetric Index for Biomonitoring of a Neotropical Watershed. *Brazil Journal of Biology*, **71**, 15-25. https://doi.org/10.1590/S1519-69842011000100005

[39] Anderson, M.J., Gorley, R.N. and Clarke, K.R. (2008) PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. The University of Auckland, Plymouth, 214 p.

[40] Whittier, T.R., Stoddard, J.L., Larsen, D.P. and Herlihy, A.T. (2007) Selecting Reference Sites for Stream Biological Assessments: Best Professional Judgment or Objective Criteria. *Journal of the North American Benthological Society*, **26**, 349-360. https://doi.org/10.1899/0887-3593(2007)26[349:SRSFSB]2.0.CO;2

[41] Gerber, A. and Gabriel, M.J.M. (2002) Aquatic Invertebrates of South African Rivers. Field Guide. Institute for Water Quality Studies, Vol. I and II, 150 p.

[42] Aschalew, L. and Moog, O. (2015) A Multimetric Index Based on Benthic Macroinvertebrates for Assessing the Ecological Status of Streams and Rivers in Central and Southeast Highlands of Ethiopia. *Hydrobiologia*, **751**, 229-242. https://doi.org/10.1007/s10750-015-2189-1

[43] Hornung, J.P. and Rice, C.L. (2003) Odonata and Wetland Quality in Southern Alberta, Canada: A Preliminary Study. *Odonata*, **32**, 119-129.

[44] Hofmann, T.A. and Mason, C.F. (2005) Habitat Characteristics and the Distribution of Odonata in a Lowland River Catchment in Eastern England. *Hydrobiologia*, **539**, 137-147. https://doi.org/10.1007/s10750-004-3916-1

[45] Hughes, S.J. (2006) Temporal and Spatial Distribution Patterns of Larval Trichoptera in Madeiran Streams. *Hydrobiologia*, **553**, 27-41. https://doi.org/10.1007/s10750-005-0627-1

[46] Mereta, S., Boetsa, P., De Meesterc, L. and Goethalsa, P.L.M. (2013) Development of a Multimetric Index Based on Benthic Macroinvertebrates for the Assessment of Natural Wetlands in Southwest Ethiopia. *Ecological Indicators*, **29**, 510-521. https://doi.org/10.1016/j.ecolind.2013.01.026

[47] Verdonschot, R.C.M., Keizer-Vlek, H.E. and Verdonschot, P.F.M. (2012) Development of a Multimetric Index Based on Macroinvertebrates for Drainage Ditch Networks in Agricultural Areas. *Ecological Indicators*, **13**, 232-242. https://doi.org/10.1016/j.ecolind.2011.06.007

[48] Kashian, D.R. and Burton, T.M. (2000) A Comparison of Macroinvertebrates of

Two Great Lakes Coastal Wetlands: Testing Potential Metrics for an Index of Ecological Integrity. *Journal of Great Lakes Research*, **26**, 460-548. https://doi.org/10.1016/S0380-1330(00)70708-8

[49] Zamora-Muñoz, C. and Alba-Tercedor, J. (1996) Bioassessment of Organically Polluted Spanish Rivers, Using a Biotic Index and Multivariate Methods. *Journal of the North American Benthological Society*, **15**, 332-352. https://doi.org/10.2307/1467281

[50] Smolders, A.J.P., Lock, R.A.C, Van der Velde, G., Medina Hoyos, R.I. and Roelofs, J.G.M. (2003) Effects of Mining Activities on Heavy Metal Concentrations in Water, Sediment and Macroinvertebrates in Different Reaches of the Pilcomayo River, South America. *Archives of Environmental Contamination and Toxicology*, **44**, 314-323. https://doi.org/10.1007/s00244-002-2042-1

[51] Lorenz, A., Hering, D., Feld, C.K. and Rolauffs, P. (2004) A New Method for Assessing the Impact of Hydromorphological Degradation on the Macroinvertebrate Fauna of Five German Stream Types. *Hydrobiologia*, **516**, 107-127. https://doi.org/10.1007/978-94-007-0993-5_7

[52] Johnson, R.K., Hering, D., Furse, M.T. and Verdonschot, P.F.M. (2006) Indicators of Ecological Change: Comparison of the Early Response of Four Organism Groups to Stress Gradients. *Hydrobiologia*, **566**, 139-152. https://doi.org/10.1007/s10750-006-0100-9

[53] Sandin, L. and Johnson, R.K. (2000) The Statistical Power of Selected Indicator Metrics Using Macroinvertebrates for Assessing Acidification and Eutrophication of Running Waters. *Hydrobiologia*, **422-423**, 233-243. https://doi.org/10.1007/978-94-011-4164-2_19

[54] Schmidt-Kloiber, A. and Nijboer, R.C. (2004) The Effect of Taxonomic Resolution on the Assessment of Ecological Water Quality Classes. *Hydrobiologia*, **516**, 269-283. https://doi.org/10.1007/978-94-007-0993-5_16

[55] Foote, A.L. and Hornung, C.L.R. (2005) Odonates as Biological Indicators of Grazing Effects on Canadian Prairie Wetlands. *Ecological Entomology*, **30**, 273-283. https://doi.org/10.1111/j.0307-6946.2005.00701.x

[56] Arimoro, F.O. and Muller, W.J. (2010) Mayfly (Insecta: Ephemeroptera) Community Structure as an Indicator of the Ecological Status of a Stream in the Niger Delta Area of Nigeria. *Environmental Monitoring and Assessment*, **166**, 581-594. https://doi.org/10.1007/s10661-009-1025-3

[57] Houghton, D.C. (2004) Biodiversity of Minnesota Caddisflies (Insecta: Trichoptera): Delineation and Characterization of Regions. *Environmental Monitoring and Assessment*, **95**, 153-181. https://doi.org/10.1023/B:EMAS.0000029890.07995.90

[58] Shelly, S.Y., Mirza, Z.B. and Bashir, S. (2011) Comparative Ecological Study of Aquatic Macroinvertebrates of Mangla Dam and Chashma Barrage Wetland Areas. *Journal of Animal and Plant Science*, **21**, 340-350.

[59] Pinto, P., Rosado, J., Morais, M. and Antunes, I. (2004) Assessment Methodology for Southern Siliceous Basins in Portugal. *Hydrobiologia*, **516**, 191-214. https://doi.org/10.1007/978-94-007-0993-5_12

Appendix

Appendix 1: The new EDOT Index developed under Tanzanian riverine conditions

DESCRIPTION OF PHYSICAL ENVIRONMENT	EDOT(f) INDEX						
EDOT Index Version 1 Score Sheet @2015	Order	Family	Scores	S	MV	GSM	TOT
Date:...../...../20.....; Time:.....	Ephemeroptera	Baetidae 1 sp.	4				
Operator:.....		Baetidae 2 spp.	6				
Tittle:.....		Baetidae > 2spp	12				
Ecoregion:.....		Caenidae	6				
River:.....		Dicercormyzidae	10				
Site Code:.....		Ephemeridae	15				
Latitudes: S:.....°.....',.....''		Ephemerythidae	9				
Longitudes: E:.....°,.....',....."		Heptageniidae	13				
Altitude:.....m a.s.l		Leptophlebiidae	9				
Slope @ Left bank:.....%; Right bank:.....%		Oligoneuridae	15				
Landform:.....		Polymitarcyidae	10				
Flow:.....m/s		Potomanthidae	10				
Temp:.....°C		Prosopistomatidae	15				
pH:.....		Tricorythidae	9				
DO:.....mg/l	Diptera	Athericidae	10				
Conductivity:.....mS/m		Blephariceridae	15				
Turbidity:.....NTU		Ceratopogonidae	5				
Site Description:		Chironomidae	2				
		Culicidae	1				
		Dixidae	10				
		Empididae	6				
Instream Disturbance:		Ephydridae	3				
		Muscidae	1				
		Psychodidae	1				
		Simuliidae	5				
Riparian Land Use:		Syrphidae	1				
		Tabanidae	5				
		Tipulidae	5				
	Odonata	Aeshnidae	8				
		Calopterygidae	10				
		Chlorocyphidae	10				
Stone In Currenct (SIC) sampling time (min):.....		Chlorolestidae	8				
Stone Out Of Current (SOOC) sampling time (min):....		Coenagrionidae	4				
Aquatic vegetation dominant sp.:.....		Corduliidae	8				
Marginal Vegetation In Current Dominant sp:.....		Gomphidae	6				
Marginal Vegetation Out Of Current Dominant sp:.....		Lestidae	8				
Gravel:.....		Libellulidae	4				
Sand:.....		Macroiidae	3				
Mud:.....		Platycnemidae	10				
Average size of Stones:.....cm		Protoneuridae	8				
Average size of Bedrock:.....cm	Trichoptera	Calamoceratidae	11				
Hand Picking/Vissual Observation:		Ecnomidae	8				
		Dipseudopsidae	10				
		Hydroptilidae	6				
Other Observations:		Hydropsychidae 1 sp.	4				
		Hydropsychidae 2 spp	6				
		Hydropsychidae >2 sp	12				
		Lepidostomatidae	10				
		Leptoceridae	6				
		Philopotamidae	10				
		Phryganeidae	10				
		Polycentropodidae	12				
		Psychomyiidae	8				
		EDO(f) SCORE					
		NUMBER OF TAXA					
		ASPT					

Appendix 2: Modified EDOT Habitat Scores Criteria

RIVER NAME:			**LOCATION:**
STATION:			**STREAM CLASS:**
LATITUDES:			**RIVER BASIN:**
LONGITUDES:			**DATE & TIME:**
INVESTIGATOR(S):			**PURPOSE FOR SURVEY:**
	Habitat Parameter	**Physical habitat condition or criteria**	
S/N.		**Optimal to Sub-ptimal**	**Marginal to Poor**
1	Hydrological modification	Little or absence of water abstraction for irrigation and hydroelectic power project.	Presence of water abstraction for irrigation, water intake and supply, dams and hydroeletric power project.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
2	Channel alteration	Stream with normal pattern; Absent or present of less than 50% channelization or dredging; Evidence of past channelization i.e., dredging may be present. Absence of sand, gravel and mining extractions, animals trampling, and construction of roads and bridges.	Instream habitat greatly alterd or removed entirely. Channelization might be extensive with over 50% of stream reach channelized and disrupted plus the evidence of sand, gravel and mining extractions, animals trampling, and construction of bridges.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
3	Channel sinuosity	Presence of stream bends that increases the length of a stream by 2-4 times longer if it was in straight stream.	Presence of stream bends that increases the length of a stream to less than 2 times longer if it was in straight stream.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
4	Channel flow status	Water fill both lower banks and only <25% of channel substrates is exposed.	Very little water in the channel with >25% of channel substrates is exposed.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
5	Water quality and appearances	Relatively high water clarity, DO & EC with relatively low turbidity, water surface oils and water odours.	Relatively low water clarity, DO & EC with relatively high turbidity, water surface oils and water odours.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
6	Direct domestic pollution	Very little or absence of discaharge, disposal, washing and bathing activities.	Prensence of little, moderate and maximum discaharge, disposal, washing and bathing activities.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
7	Direct industrial pollution	Very little or absence of point sources and diffused industrial discaharge and disposal.	Prensence of moderate and maximum point sources and diffused industrial discaharge and disposal.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
8	Dumping of solid wastes	Very minimal or absence of any evidence regarding solid wastes dumping.	Presence of clear evidence regarding solid wastes dumping.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
9	Velocity/ depth regime	Present of 3 to 4 velocity or depth regimes (slow-shallow, fast-shallow, slow-deep of fast-deep); slow is <0.3m/s and deep is >0.5m.	Present of <3 velocity or depth regimes; usually with slow deep (if slow & fast - shallow regimes are absent, scores low).
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
10	Frequency of riffles (or beds)	Frequent riffles or beds occurances; distance between riffles divide by width of the stream is between 1 to 15.	Presence of shallow pools or occasionally occured riffles or beds; distance between riffles divide by stream width is >15.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
11	Embeddedness/siltation	Gravel, cobbles and boulder particles are less than 50% surrounded by fine sediments.	Gravel, cobbles and boulder particles are >50% surrounded by fine sediments.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1

12	Pool variability	Presence of mix of large-shallow & deep, and small-shallow & deep pools; majority of large-deep pools and very few shallow pools.	Presence of small and shallow pools.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
13	Pool substrate charactarization	Presence of substrate materials, with mixture of firm & soft sand, gravel, mud, clay, root mats and submerged vegetations.	Presence of mud or clay or sand or bedrock botom with no root mats or submerged vegetations.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
14	Nurient enrichment	Good levels of nutrients favourable organisms survival.	High or very low levels of nutrients unavourable for organisms survival.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
15	Informal settlements	Absence of informal settlements and/or industries or presence of very scartered	Presence of some informal settlements and industries.
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
16	Small and large scale farming	Very little or absence of small and/or large scale agricultural activities.	Presence of large and/or small scale agricultural activities.
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
17	Bank stability/erosion	Presence of stable to moderate bank stability; absent or presence of minimal evidence of erosion or bank failure.	Presence of moderately unstable to unstable bank; >20% of bank in reach has eroded areas.
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
18	Riparian vegetations zone width	Presence of riparian zone with a width of more than 10 meters; human activities have only impacted riparian zone minimally.	Presence of riparian zone with a width of less than 10 meters; little or no riparian vegetation due to impact associated with
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
19	Bank vegetative protection	More than 70% of the streambank surfaces are protected by native vegetations.	Less than 70% of the streambank surfaces are protected by native vegetations.
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
20	Graze/ bank grass cover	More than 70% of the banks are covered by	Less than 70% of the banks are covered by
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
21	Presence of exotic vegetation	Absence of exotic vegetations or presence of very few and little exotic vegetations.	Presence of some or large number of exotic vegetations.
	SCORE: RIGHT BANK	10 9 8 7 6	5 4 3 2 1
	SCORE: LEFT BANK	10 9 8 7 6	5 4 3 2 1
22	Canopy cover	Presence of partially (50-75%) to fully (75-100%) shaded (overhead canopy cover) throughout the sream reach.	Presence of fully (0-25%) to partially (25-50%) exposed (overhead canopy cover) throughout the sream reach.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
23	Epifaunal substrate	More than 50% of substrate (mix of stable habitat) favourable for epifauna colonization; presence of new substrata (submerged logs undercut banks, cobbles or other stable habitat)	Less than 50% mix of stable habitat; lack of habitat is obvious; lack or less than desirable habitat; unstable substrate, frequently disturbed, removed or lacking.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
24	Livestock keeping	Absence or very little signs of livestock trampling and droppings.	Presence of livestock or signs of livestock trampling and droppings.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1
25	Sediment deposition	Pesence of little or slightly deposition of sediments with <30% (<50% of low gradient) of the bottom affected by deposition.	Pesence of moderate to severe sediments deposition with >30-50% (>50% of low gradient) of the bottom affected by deposition.
	SCORE	20 19 18 17 16 15 14 13 12 11	10 9 8 7 6 5 4 3 2 1

Appendix 3.

(a) List of sampling sites with their respective geomorphological and biotopes (S = stone; MV = marginal vegetation; GSM = gravel-sand-mud) along Pangani basin.

SAMPLING STATIONS			Habitat Score		GPS Readings		Biotopes			Geomorphology	
Code	River name	Site name	%	Site category	Latitude	Longitude	S	MV	GSM	Landform	Ecoregion
P1	Themi	Olosha at AUWSA	98	Reference	3.20311	36.43261	S			Mountains	PH
P2	Themi	Arusha-Moshi road	94	Reference	3.21821	36.42147	S			Mountains	PH
P3	Themi	Lokii	41	Monitoring	3.30349	36.46308	S		GSM	Foot slopes	PH
P4	Themi	Sekei	48	Monitoring	3.35101	36.70629	S	MV	GSM	Hills	PH
P5	Themi	Darajani polisi	55	Monitoring	3.37299	36.69609	S			Plains	PH
P6	Themi	Daraja mbili	58	Monitoring	3.38877	36.70003	S			Plains	PH
P7	Themi	Kijenge	43	Monitoring	3.37909	36.69989	S			Plains	PH
P8	Malala	Nkoamaala	99	Reference	3.19992	36.45367	S			Mountains	PH
P9	Nduruma	Deker Bruins	59	Monitoring	3.24324	36.46941	S			Hills	PH
P10	Tengeru	Tengeru	63	Monitoring	3.39491	36.82803	S		GSM	Mountains	PH
P11	Ngarasero	NAIC	92	Reference	3.35287	36.84014	S		GSM	Hills	PH
P12	Themi	Naura	78	Monitoring	3.37295	36.70106	S			Plains	PH
P13	Kikuletwa	Malala	82	Monitoring	3.40123	36.77929	S	MV	GSM	Hills	PH
P14	Kikuletwa	Mbembe	86	Monitoring	3.39508	36.82842	S	MV	GSM	Plains	PH
P15	Kikuletwa	Karangai	40	Monitoring	3.26889	36.51506	S	MV		Plains	PH
P16	USA river	Old Moshi-Arusha road	62	Monitoring	3.22367	36.51787	S		GSM	Hills	PH
P17	Maji ya chai	Darajani	92	Reference	3.29883	36.89038	S			Hills	PH
P18	Maji ya chai	Mpakani	92	Reference	3.31707	36.89245	S			Mountains	PH
P19	Tululusia	Campsite two	93	Reference	3.23301	36.84428	S			Mountains	PH
P20	Ngarenanyuki	Campsite three	91	Reference	3.24503	36.84304	S			Mountains	PH
P21	Maio	Maio	93	Reference	3.24627	36.80967	S			Mountains	PH
P22	Mue	Mue bridge	92	Reference	3.31033	37.48365		MV	GSM	Mountains	PH
P23	Magdarisho	Magdarisho	95	Reference	3.35301	36.85289	S	MV	GSM	Hills	PH
P24	Kikafu	Moshi -Arusha road	59	Monitoring	3.19119	37.13074	S		GSM	Foot slopes	PH
P25	Kikafu	TPC	42	Monitoring	3.43598	37.30309	S	MV		Alluvial plains	PH
P26	Ona	Ona bridge	97	Reference	3.31491	37.49507	S		GSM	Mountains	PH
P27	Karanga	Kibo match	56	Monitoring	3.20697	37.19028	S			Foot slopes	PH
P28	Rau	Msaranga	54	Monitoring	3.20236	37.21379	S		GSM	Foot slopes	PH
P29	Himo	Himo Bridge	58	Monitoring	3.23454	37.32715	S		GSM	Foot slopes	PH
P30	Ruvu	Kifaru bridge	55	Monitoring	3.31732	37.33744	S		GSM	Foot slopes	PH
P31	Pangani	Nyumba ya Mungu	49	Monitoring	3.49859	37.28031		MV		Alluvial plains	PH
P32	Pangani	Gunge, shimanjiro	60	Monitoring	4.35198	37.52696	S	MV	GSM	Alluvial plains	PH
P33	Mkomazi	Mbuta	44	Monitoring	4.39766	38.04593		MV	GSM	Alluvial plains	PH

Continued

P34	Soni	Mombo	57	Monitoring	4.53194	38.17307		MV	GSM	Alluvial plains	PH
P35	Pangani	Maurui	66	Monitoring	5.13615	38.38979		MV		Foot slopes	PH
P36	Luegera	Korongwe	59	Monitoring	5.10091	38.27738		MV	GSM	Alluvial plains	PH
P37	Pangani	Hale Bridge	51	Monitoring	5.17745	38.36138		MV	GSM	Plains	PC
P38	Pangani	Mgombani	42	Monitoring	5.20774	38.38741		MV		Plains	PC
P39	Pangani	Mwakinyumbi	74	Monitoring	5.30141	38.59648		MV		Alluvial plains	PC
P40	Pangani	Nkhole	70	Monitoring	5.51672	38.55349		MV	GSM	Alluvial plains	PH
P41	Pangani	Kwamkoro	67	Monitoring	5.13284	38.61917	S	MV	GSM	Mountains	PH
P42	Pangani	Tundulu	71	Monitoring	5.10342	38.64055	S	MV		Mountains	PC
P43	Pangani	Bulwa bridge	91	Reference	5.09059	38.64201	S		GSM	Mountains	PC
P44	Pangani	Mkwajuni	94	Reference	5.01101	38.78646	S		GSM	Hills	PC
P45	Pangani	Longuza	92	Reference	5.05016	38.69997	S			Foot slopes	PC
P46	Pangani	Sega	74	Monitoring	5.05398	39.04626		MV		Plains	PC

(b) List of sampling sites with their respective geomorphological and biotopes (S = stone; MV = marginal vegetation; GSM = gravel-sand-mud) along Wami-Ruvu basin.

SAMPLING STATIONS			Habitat Score		GPS Readings		Boitopes			Geomorphology	
Code	**River name**	**Site name**	**%**	**Site category**	**Latitude**	**Longitude**	**S**	**MV**	**GSM**	**Landform**	**Ecoregion**
W01	Wami	Dikurura	91	Reference	6.11213	37.58025	S			Mountains	CEA
W02	Chazi	Magole	69	Monitoring	6.10604	37.56956		MV	GSM	Foot slopes	CEA
W03	Wami	Mkindo	66	Monitoring	6.23606	37.54913		MV		Plains	CEA
W04	Wami	Matipwili	91	Reference	6.24245	38.69144	S	MV	GSM	Plains	CCEA
W05	Wami	Dakawa	88	Monitoring	6.26876	37.32009		MV	GSM	Plains	CEA
W06	Msowero	Msowero	42	Monitoring	6.31891	37.12826		MV	GSM	Plains	CEA
W07	Wami	Tana	96	Reference	6.47197	37.11995		MV		Mountains	CEA
W08	Wami	Tami	92	Reference	6.50112	37.12124		MV		Mountains	CEA
W09	Kisangata	Mvumi	64	Monitoring	6.58806	37.11997		MV		Plains	CEA
W10	Wami	Mkondoa	70	Monitoring	6.82929	36.98098		MV	GSM	Foot slopes	CEA
W11	Wami	Miyombo	58	Monitoring	6.90895	36.97134		MV	GSM	Foot slopes	CEA
R01	Ngerengere	Dar-Morogoro bridge	92	Reference	6.39082	38.02255		MV	GSM	Foot slopes	CEA
R02	Ruvu	Dar-Chalinze road bridge	41	Monitoring	6.41431	38.41664		MV	GSM	Plains	CEA
R03	Morogoro	Morogoro water intake	97	Reference	6.86157	37.00451	S			Mountains	CEA
R04	Mangwe	Chumbi	95	Reference	6.94191	37.61718	S			Mountains	CEA
R05	Ngerengere	Tangeni	93	Reference	6.94902	37.60588	S			Mountains	CEA
R06	Ngerengere	Konga	77	Monitoring	6.90902	37.61153	S			Hill slopes	CEA
R07	Ngerengere	Mission	79	Monitoring	6.89859	37.59915	S			Mountains	CEA
R08	Manga	Tawa	96	Reference	7.01205	37.73188	S			Plains	CEA

Continued

R09	Ruvu	Kibungo	94	Reference	7.02812	37.81102	S			Plains	CEA
R10	Ruvu	Mzinga	74	Monitoring	7.05103	37.52424		MV	GSM	Hill slopes	CEA
R11	Mgeta	Kibaoni	63	Monitoring	7.03538	37.56901		MV	GSM	Plains	CEA
R12	Mzinga	Mzinga bridge	58	Monitoring	6.88901	37.61199		MV	GSM	Plains	CEA
R13	Mzinga	Luhungo	92	Reference	6.90482	37.63701	S	MV	GSM	Plains	CEA
R14	Morogoro	Morogoro industrial area	41	Monitoring	6.76961	37.67264			GSM	Plains	CEA
R15	Morogoro	Morogoro bridge	52	Monitoring	6.84557	37.67232		MV	GSM	Plains	CEA
R16	Ruvu	Kinole intake	97	Reference	6.92495	37.76934	S			Mountains	CEA
R17	Ruvu	Mji mpya @ Kikundi	58	Monitoring	6.82301	37.66792			GSM	Plains	CEA
R18	Mgeta	@ Mgeta	42	Monitoring	7.03333	37.56673	S	MV	GSM	Foot slopes	CEA
R19	Mgeta	Duthumi	94	Reference	7.41171	37.77663	S			Mountains	CEA
R20	Mvuha	Tulo primary school	92	Reference	7.24034	37.91775	S	MV		Hill slopes	CEA
R21	Mzumbe	Mlali	84	Monitoring	6.90127	37.56162			GSM	Foot slopes	CEA
R22	Ngerengere	Kingolwira	44	Monitoring	6.75184	37.75761	S		GSM	Foot slopes	CEA
R23	Ngerengere	Mgude	47	Monitoring	6.76376	38.14456		MV	GSM	Foot slopes	CEA
R24	Ngerengere	Bwawani	86	Monitoring	6.65139	38.03811			GSM	Foot slopes	CEA
R25	Ruvu	Mindu	50	Monitoring	6.85548	37.61399		MV	GSM	Plains	CEA
R26	Ruvu	Kidunda	76	Monitoring	7.26963	38.21723		MV	GSM	Mountains	CEA
R27	Ruvu	Kongo	82	Monitoring	6.53912	38.83		MV	GSM	Mountains	CEA
R28	Ruvu	Ruvu near estuary	53	Monitoring	6.39714	38.8698		MV	GSM	Hill slopes	CCEA

Appendix 4: Macroinvertebrate Species Collected Based on Major Site Categories

TAXA	PANGANI SITES				WAMI-RUVU SITES				TOTAL	
	REFERENCE		MONITORING		REFERENCE		MONITORING		ALL SITES	
	Abundance	%	Abundance	%	Abundance	%	Abundance	%	Abundance	%
Hirudinidae	0	0.0	8	0.15	0	0.0	6	0.17	14	0.11
Glossiphoniidae	0	0.0	14	0.27	0	0.0	5	0.14	19	0.15
Naididae/Tubificidae	0	0.0	50	0.96	0	0.0	9	0.25	59	0.47
Dytiscidae	31	1.26	82	1.57	17	1.23	99	2.41	229**	1.81
Dryopidae/Elmidae	9	0.36	115	2.20	12	0.87	96	2.71	232**	1.84
Gyrinidae	64	2.59	50	0.96	0	0.0	47	1.33	161*	1.27
Haliplidae	2	0.08	30	0.57	0	0.0	52	1.47	84	0.67
Hydraenidae	11	0.45	28	0.54	0	0.0	30	0.85	69	0.55
Hydrophilidae	30	1.2	43	0.82	0	0.0	58	1.64	131*	1.04
Limnichidae	12	0.49	22	0.42	0	0.0	0	0.0	34	0.27
Psephenidae	28	1.13	55	1.05	26	1.88	45	1.27	154*	1.22
Scirtidae	5	0.20	34	0.65	0	0.0	22	0.62	61	0.48

Continued

Amphipoda	0	0.0	5	0.10	2	0.14	22	0.62	29	0.23
Atyidae	0	0.0	17	0.33	0	0.0	16	0.45	33	0.26
Palaemonidae	0	0.0	15	0.29	2	0.14	29	0.82	46	0.36
Potamonautidae	59	2.39	209	4.00	19	1.37	45	1.27	332**	2.63
Athericidae	55	2.23	132	2.53	31	2.24	22	0.62	240**	1.90
Ceratopogonidae	14	0.57	183	3.50	0	0.0	43	1.21	240**	1.90
Chironomidae	68	2.75	1455	27.84	45	3.25	639	18.02	2207***	17.48
Culicidae	0	0.0	57	1.09	0	0.0	52	1.47	109*	0.86
Dixidae	62	2.51	31	0.59	7	0.51	45	1.27	145*	1.15
Ephydridae	3	0.12	14	0.27	0	0.0	5	0.14	22	0.17
Muscidae	0	0.0	31	0.59	0	0.0	11	0.31	42	0.33
Simuliidae	17	0.69	384	7.35	0	0.0	137	3.86	538**	4.26
Tabanidae	34	1.38	84	1.61	17	1.23	50	1.41	185*	1.46
Tipulidae	38	1.54	51	0.98	22	1.59	59	1.66	170*	1.35
Baetidae	751	30.40	470	8.99	235	16.96	227	6.40	1683***	13.33
Caenidae	115	4.66	266	5.09	26	1.88	128	3.61	535**	4.24
Dicercormyzidae	19	0.77	28	0.54	36	2.60	30	0.85	113*	0.89
Ephemerythidae	8	0.32	15	0.29	22	0.62	52	1.47	97	0.77
Heptageniidae	41	1.66	45	0.86	29	2.09	79	2.23	194*	1.54
Leptophlebiidae	29	1.17	64	1.22	34	2.45	66	1.86	193*	1.53
Oligoneuridae	62	2.51	18	0.34	82	5.92	12	0.34	174*	1.38
Polymitarcyidae	38	1.54	6	0.11	26	1.88	0	0.0	70	0.55
Prosopistomatidae	57	2.31	16	0.31	45	3.25	41	1.16	159*	1.26
Tricorythidae	10	0.40	5	0.10	23	1.66	32	0.90	70	0.55
Lymnaeidae	0	0.0	12	0.23	0	0.0	25	0.71	37	0.29
Physidae	0	0.0	22	0.42	0	0.0	19	0.54	41	0.32
Planorbidae	0	0.0	11	0.21	0	0.0	18	0.51	29	0.23
Thiaridae	0	0.0	9	0.17	0	0.0	28	0.79	37	0.29
Belastomatidae	17	0.69	0	0.0	24	1.73	8	0.23	49	0.39
Corixidae	6	0.24	152	2.39	10	0.72	0	0.0	168*	1.33
Gerridae	9	0.36	43	0.82	0	0.0	19	0.54	71	0.56
Hydrometridae	6	0.24	14	0.27	0	0.0	10	0.28	30	0.24
Naucoridae	14	0.57	65	1.24	22	1.59	90	2.54	191*	1.51
Nepidae	9	0.36	13	0.25	0	0.0	0	0.0	22	0.17
Notonectidae	13	0.53	113	2.16	0	0.0	0	0.0	126*	1.0
Pleidae	5	0.20	5	0.10	0	0.0	4	0.11	14	0.11
Veliidae	20	0.81	64	1.22	12	0.87	71	2.00	167*	1.32

Continued

Hydridae	0	0.0	4	0.08	0	0.0	8	0.23	12	0.10
Pyralidae	5	0.20	1	0.02	2	0.14	3	0.08	11	0.09
Aeshnidae	43	1.74	102	1.95	33	2.38	80	2.26	258**	2.04
Calopterygidae	57	2.31	31	0.59	37	2.67	83	2.34	208**	1.65
Chlorocyphidae	28	1.13	13	0.25	22	1.59	71	2.00	134*	1.06
Chlorolestidae	0	0.0	7	0.13	6	0.43	11	0.31	24	0.19
Coenagrionidae	32	1.30	92	1.76	20	1.44	86	2.43	230**	1.82
Corduliidae	5	0.20	38	0.73	13	0.94	56	1.58	112*	0.89
Gomphidae	33	1.34	68	1.30	12	0.87	42	1.18	155*	1.23
Lestidae	0	0.0	12	0.23	15	1.08	47	1.33	74	0.59
Libellulidae	0	0.0	28	0.54	29	2.09	52	1.47	109*	0.86
Macromiidae	0	0.0	18	0.34	0	0.0	8	0.23	26	0.21
Perlidae	19	0.77	0	0.0	27	1.95	13	0.37	59	0.47
Turbellaria	0	0.0	13	0.25	0	0.0	14	0.39	27	0.21
Corbiculidae	0	0.0	3	0.04	0	0.0	1	0.03	4	0.03
Sphaeriidae	0	0.0	4	0.08	0	0.0	1	0.03	5	0.04
Unionidae	0	0.0	1	0.02	0	0.0	1	0.03	2	0.02
Corydalidae	4	0.16	2	0.04	0	0.0	6	0.17	12	0.10
Sialidae	3	0.12	1	0.02	6	0.43	3	0.08	13	0.10
Calamoceratidae	112	4.53	5	0.01	58	4.18	38	1.07	213**	1.74
Dipseudopsidae	16	0.65	4	0.08	34	2.45	32	0.90	86	0.68
Ecnomidae	21	0.85	11	0.21	6	0.43	32	0.90	70	0.55
Hydroptilidae	13	0.53	9	0.17	3	0.22	23	0.65	48	0.38
Hydropsychidae	55	0.22	32	0.61	56	0.40	31	0.87	174*	1.38
Lepidostomatidae	20	0.81	0	0.0	40	2.89	35	0.99	95	0.75
Leptoceridae	59	2.39	19	0.36	6	0.43	27	0.76	111*	0.88
Philopotamidae	69	2.79	19	0.36	52	3.75	32	0.90	172*	1.36
Phryganeidae	25	1.01	4	0.08	20	1.44	45	1.27	94	0.74
Polycentropodidae	58	2.35	4	0.08	54	3.90	34	0.96	150*	1.19
Psychomyiidae	22	0.89	27	0.52	9	0.65	28	0.79	86	0.68
TOTAL	**2470**		**5227**		**1386**		**3546**		**12629**	**100.0**