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How does Urban Pollution Influence Macroinvertebrate Traits in Forested Riverine Systems?

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Abstract: The influence of urbanization on macroinvertebrate traits was explored in forested rivers in the Niger Delta area of Nigeria. Physico-chemical variables were sampled on a monthly basis alongside macroinvertebrates in 20 sites of 11 rivers spanning 2008–2012. Physico-chemical variables were used to classify the 20 sites into three ecological classes, namely: least impacted sites (LIS), moderately impacted sites (MIS) and highly impacted sites (HIS) using principal component analysis. Our results based on RLQ (R = physico-chemical variables, L = macroinvertebrate taxa and Q = macroinvertebrate traits) and fourth-corner analyses revealed that large body size, grazing and hardshell were positively significantly associated with LIS on the RLQ. They were also either negatively correlated with any two of water temperature, nutrients, BOD_5 and flow velocity or positively significantly correlated with increasing DO. Thus, these traits were considered sensitive to urban pollution in forested rivers. Burrowing, predation and pupa aquatic stage, which were positively associated with HIS, were also significantly negatively correlated with increasing DO, and were deemed tolerant of urban pollution in forested rivers. Box plots and a Kruskal–Wallis test revealed that the three sensitive traits were significantly highest at LIS ($p < 0.05$) except grazing; while the three tolerant traits were significantly highest at MIS ($p < 0.05$) except burrowing. Overall, this study revealed that urban pollution influences macroinvertebrate traits differently in forested rivers.

Keywords: macroinvertebrate traits; large and small body sizes; potential biological indicator traits; ecological classes; forested riverine systems; urban pollution; RLQ and fourth-corner tests; Niger Delta; Nigeria

1. Introduction

Increasing urbanization and other human activities such as urban storm water return flow poses serious threats to riverine systems within forested catchments [1,2]. Riverine systems draining urban catchments have been reported to display poor water quality conditions, depleted biodiversity, river channel modification and loss of habitat complexity [2,3]. In the Afro tropics (e.g., Nigeria) rural-urban migration is on the rise, and there is a high probability that forested riverine systems would be gravely disturbed due to the increasing population growth. Rural–urban migration has caused forested rivers within the Afro tropics to be draining urban landscapes as developmental activities are increasing exponentially [4,5]. The Niger Delta area of Nigeria is not exempt from this avoidable ecological risk, as the area which houses a number of forested rivers is now urbanizing precipitously because of the activities of oil exploration firms and other industrial undertakings.

The Niger Delta area sits within the tropical rainforest belt of Nigeria [6] and most riverine systems within the area are forested and as such depend mainly on coarse particulate organic materials (CPOM) and fine particulate organic materials (FPOM) arising from allochthonous materials from nearby forest [7]. Additionally, studies conducted with regard to functional ecology are of the viewpoint that macroinvertebrates that are shredders and collector-gatherers are usually common in forested rivers, which break down CPOM, and accelerate their conversion into FPOM for efficient transfer of energy and carbon along the food web [2,8–10]. Consequently, the functional feeding groups (FFGs), particularly shredders within forested catchments, are critical to the functioning and delivery of the required ecosystem services to the society that depends on forested rivers [9]. The majority of the forested riverine systems in the Niger Delta are shaded, making them consistently cooler than non-forested rivers. However, due to increasing urbanization and other anthropogenic activities, natural patterns observed in the forested rivers of the Niger Delta region of Nigeria are being altered [8]. For instance, temperature increase due to influx of floodwaters from nearby unplanned urban settlements within forested riverine systems has been reported in an earlier study [6], and this has also been reported to impact the structural and functional dynamics of riverine systems—e.g., excessive algal growth and high nutrient concentration [8,9]. Judging from the potential negative impact that follow nutrient influx from urban pollution sources into forested riverine systems in the Niger Delta area of Nigeria [4], the question was thus asked: "how does urban pollution influence macroinvertebrate traits in forested riverine systems in the Niger Delta area of Nigeria?" This question was addressed in this study by exploring the influences of pollution on traits distribution in the studied riverine systems. The trait-based approach is important because it gives an indirect evaluation of ecosystem function through trait-mediated ecosystem processes such as top-down control and energy transfer [2,8,9].

Macroinvertebrates traits are among the most explored aquatic biota traits for biomonitoring the health of aquatic systems [2,11]. Urban pollution has been reported to negatively affect the composition, diversity, richness and abundance of macroinvertebrate community structure in riverine systems within forested catchments [11,12]. Through the effects of the so-called urban stream syndrome [2,13,14], certain sensitive macroinvertebrate taxa such as Ephemeroptera, Plecoptera and Trichoptera (EPT) have been reported to be negatively affected [2,13,15]. On the other hand, pollution tolerant macroinvertebrate taxa such as some genera of Oligochaetes and Diptera—e.g., the syrphids and culicids—have been reported to be favoured by the effects of urban-stream syndrome [8]. However, in spite of the increasing studies on urban pollution effects on the community structure of forested systems, trait-based studies in such systems, particularly in the Afrotropical region, remain scarce [5,16,17]. Thus, the present study which investigates the effect of urban pollution on macroinvertebrate traits in forested riverine systems is crucial, because organisms use their inherent traits to adapt to prevailing environmental alteration in the face of ecosystem alteration [13]. Therefore, the overall goal of the present study is to assess the responses of macroinvertebrate traits to urban pollution in forested riverine systems in the Niger Delta area of Nigeria. Then, we addressed the following two specific objectives from the overall goal: (i) identify and classify potential indicator macroinvertebrate traits that would be suitable for assessing the effect of urban pollution in forested riverine systems in the Niger Delta area of Nigeria; (ii) explore the pattern of distribution of macroinvertebrate traits along urban pollution gradient in forested riverine systems in the Niger Delta area of Nigeria.

2. Materials and Methods

2.1. Study Sites

The study area comprises Edo and Delta States within the Niger Delta region of Nigeria. It is an approximate area of about 70,000 km^2 in the southern part of Nigeria [13]. Wetlands, inland waters and mangrove swamps are the major aquatic ecosystem types within the region [4,18]. Most aquatic systems in the area are mainly forested, with patches of agricultural and urban catchments. Fishing, wood logging and crop farming are the major occupations of local people in the area [8,19].

The area is characterized by two seasons (dry and wet seasons) [6]. The dry season spans from October to March, while the wet season is between late March to the end of September [4]. The Niger Delta is the oil rich region of Nigeria, and as such is the nation's economic backbone. Exploitation and exploration of crude oil are the major industrial activities in the region, and this is what is attracting people to the region leading to increasing rural–urban migration. Towns and cities in the region have very poor drainage systems, and riverine systems in the area usually suffer the impact of untreated wastewater, storm water return flow and run-offs from nearby fish mongers and other farmers' settlements [8].

Twenty (20) sites in 11 forested riverine systems draining urban catchments in Edo and Delta States within the Niger Delta region were selected for the present study (see Supplementary Table S1) for geographical locations of the twenty sites sampled. The 11 riverine systems include Adofi, Ase, Benin, Eriora, Iyiukwu, Orogodo, Ossiomo, Owan, Umakuku, Umomi and Warri Rivers (Figure 1), and samples were collected across the 20 sites in these 11 rivers for a period of five years (2008–2012).

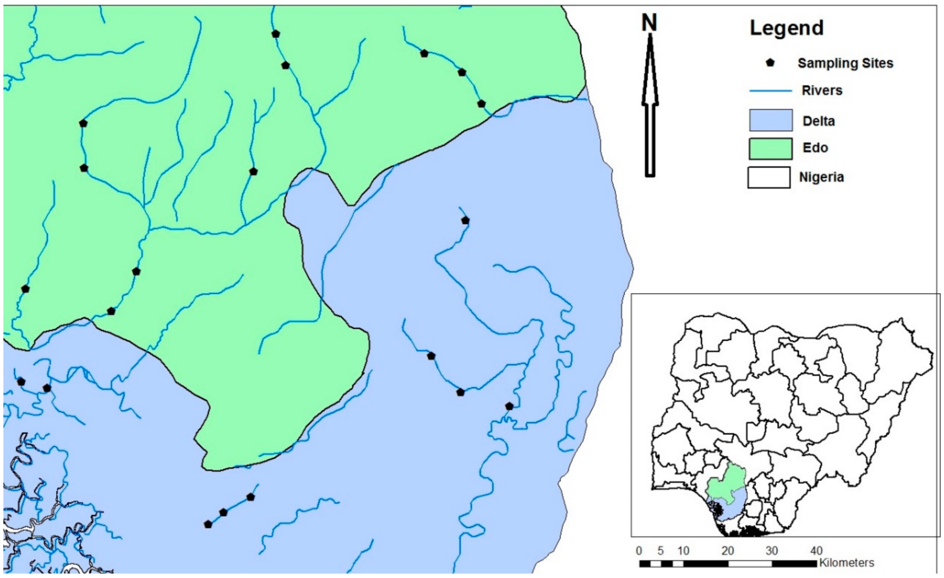

Figure 1. Study area map showing the sampling sites and the map of Nigeria, indicating the location of study area within the southern part of Nigeria.

The sampled sites' land use types were determined using Google Earth satellite imagery. A particular land use type was considered dominant in a site if it covered more than 70% of the adjacent catchment area within the sampling site as per the site catchment sizes indicated in the Supplementary Table S1. A similar method was employed by Pena-Cortes et al. [20] and Fierro et al. [21] to characterise land use types. Supplementary Table S1 shows the sites dominant and not-dominant land use types, and catchment sizes.

2.2. *Sampling and Analysing of Macroinvertebrates and Physico-Chemical Variables*

In the five years from 2008 to 2012, samples of macroinvertebrates were collected during the wet and dry seasons on a monthly basis using a modified D-frame kick-net (500 µm mesh size) [22] at each sampling site. Sampling at each site per biotope takes place for three minutes and covers all biotopes in each site. The biotopes include leaf litters, sand, mud, silt, stones and vegetation, and sampling was performed equally across the sampled sites per biotope. Collected macroinvertebrates samples from leaf litters, sand, mud, silt, stones and vegetation were pooled together to represent a composite sample, and afterward the samples were preserved in 70% alcohol, before transferring to the laboratory for sorting, identification and enumeration. Identification of collected macroinvertebrates was performed to the family level using a stereoscopic microscope at X10 magnification with the aid of available taxonomic keys [23–25].

Physico-chemical variables were also analysed for a period of five years (2008–2012). Physico-chemical variables analysed in the present study include water temperature (°C), flow velocity (ms^{-1}), depth (m), electrical conductivity (EC-µScm^{-1}), dissolved oxygen (DO-mgL^{-1}), five-day biochemical oxygen demand (BOD_5-mgL^{-1}), pH, phosphate (mgL^{-1}) and nitrate (mgL^{-1}) (see Supplementary Table S2). Water temperature was measured using a mercury-in-glass thermometer, and water depth was measured using a rod calibrated in metres. Flow velocity was measured by floating a timed table tennis ball in the mid-channel of each sampling site over a distance of ten metres according to the Gordon et al. [26] method. Dissolved oxygen, EC and pH were determined using a portable HANNA HI9829 multi-probe meter manufactured by HANNA instruments. For the analyses of BOD_5, phosphate and nitrate, three replicates of water samples were collected in 500 mL glass bottles at each sampling site and fixed with appropriate reagents, and thereafter analysed in the laboratory following APHA [27] methods.

2.3. *Data/Statistical Analyses*

Physico-Chemically-Based Classification of Sites into Ecological Classes

The 20 sites in 11 rivers of the forested riverine systems within urban catchments were physico-chemically classified into three potential ecological classes using Principal Component Analysis (PCA—Figure S1 on Supplementary material). The ecological classes were least impacted sites (LIS), moderately impacted sites (MIS) and highly impacted sites (HIS), as shown in Table 1. Initially, the classification of the 20 sites was performed by visual examination of the physico-chemical variables analysed for each site [8]. Sites with pollution indicating physico-chemical variables such as increasing concentration of nutrients, BOD_5 and EC were classified as either moderately or highly impacted sites, while sites with increasing concentration of physico-chemical variables indicating good water quality such DO were classified as least impacted sites [8]. The three ecological classes (LIS, MIS and HIS) were employed for this study because the sampled forested rivers and sites are within urban catchment. The actual ecological classification of the sampled sites was performed by extracting coordinate values of each of the sites from Axis 1 of the PCA biplot, and thereafter inter-site distance of the first occurring site in the PCA first axis was computed by subtracting the site with the lowest coordinate value from the site with the highest coordinate value on the PCA, and subsequently the coordinate values of the remaining sites were computed by subtracting their values from the site with the highest coordinate value on the PCA to obtain their corresponding inter-site distances [4,17,28]. The inter-site distances computed for the 20 sites were converted to percentage inter-site distances, and the obtained percentage inter-site distances were interpreted as percentile distribution which was used to classify the sites into one of the three ecological classes: LIS, MIS and HIS. A percentile distribution of 100–90th, <90–50th and <50–0th was used to classify sites into LIS, MIS and HIS, respectively [4,17,28]. Vegan package version 2.5.4 within the R-programming language was used in developing the PCA biplot in this study [29,30].

Table 1. Coordinate values of sites on the PCA first axis, inter-site distances, percentage inter-site distances and ecological categories of the sampled sites for the forested riverine systems within urban catchments. Abbreviations: Site Codes: Ad (Adfofi River), Wa1 (Warri River Site 1),Wa2 (Warri River Site 2), As1 (Ase River Site 1), As2 (Ase River Site 2), Iy1 (Iyiukwu River Site 1), Iy2 (Iyiukwu River Site 2), Iy3 (Iyiukwu River Site 3), Or (Orogodo River), Be1 (Benin River Site 1), Be2 (Benin River Site 2), Be3 (Benin River Site 3), Er (Eriora River), Os1 (Ossiomo River Site 1), Os2 (Ossiomo River Site 2), Oa (Owan River), Um1 (Umaluku River Site 1), Um2 (Umaluku River Site 2), Ui1 (Umomi River Site 1) and Ui2 (Umomi River Site 2). Ecological classes: LIS = least impacted sites, MIS = moderately impacted sites, HIS = highly impacted sites.

Site Codes	Coordinate Values of Sampled Sites on the PCA Axis 1	Inter-Site Distances	% Inter-Site Distances	Site Ecological Classes
Wa2	−28.3	189	100	LIS
Wa1	−28	188.6	99.8	LIS
Ad	−26.1	186.7	98.8	LIS
Or	−24.2	184.8	97.8	MIS
As2	−22.5	183.1	96.9	MIS
Iy3	−22.4	183.0	96.9	MIS
Iy1	−21.2	181.8	96.2	MIS
As1	−20.7	181.4	96.0	MIS
Iy2	−20.3	181	95.8	MIS
Be3	−17.1	177.7	94.0	MIS
Os2	−14.8	173.5	91.8	HIS
Be1	−12.9	173.5	91.8	HIS
Os1	−12.2	172.9	91.5	HIS
Oa	−8.6	169.3	89.6	HIS
Um2	−2.3	163	86.2	HIS
Er	19.0	142	75.0	HIS
Ui2	24.7	136	71.9	HIS
Um1	32.9	127.8	67.6	HIS
Ui1	44.1	116.6	61.7	HIS
Be2	160.7	0.0	0.0	HIS

2.4. Trait Selection for this Study

Traits were selected following a stressor-based approach. Firstly, the literature was reviewed to identify stressors linked to urban pollution in forested systems, and the identified stressors were nutrient enrichments, organic pollution, increased sedimentation and storm water return-flow, pesticide pollution and potential heavy metal pollutions [13,31–38]. Most of the outlined stressors are evident in the sampled forested riverine systems draining urban catchments in the Niger Delta area of Nigeria. Secondly, based on the outlined stressors, traits that were potentially mechanistically linked to the forested riverine systems draining urban catchments were then selected. In the present study, 12 categories of traits which include 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 [17]. The traits categories were resolved into 53 trait attributes. Trait information was obtained primarily from available trait literature in Nigeria [17], and supplemented and confirmed by literature information on traits from elsewhere [2,14,39]. Trait information was retrieved at the family level because species level information is sparse in the Afro tropics. The trait family level information comes with its limitations, as species within a given family may exhibit plasticity traits. Based on the limitations of using family level traits information, a fuzzy coding method was used to compensate trait variation among species within a family. The fuzzy coding method takes into account the variability, plasticity and life history stages of macroinvertebrate species within a given family [40]. A fuzzy coding system of 0–3 was used to award affinity scores to macroinvertebrate taxa, with a score of 0 awarded to macroinvertebrate taxa with no affinity to a given trait(s), while 1, 2 and 3 were awarded to macroinvertebrate taxa with low affinity, moderate affinity and high affinity, respectively, to a given trait(s) [40]. Each trait score was multiplied by the relative abundance of macroinvertebrate taxa.

2.5. Assessing Macroinvertebrate Traits and Taxa Responses, as Well as Identifying and Classifying Potential Indicator Macroinvertebrate Traits along Urban Pollution Gradient in Forested Riverine Systems

In assessing macroinvertebrate traits and taxa responses, and identifying and classifying potential indicator traits in relation to urban pollution in forested riverine systems, an RLQ analysis was performed. RLQ is a multivariate analysis that was developed by Dolédec et al. [41], and it performs an ordination on three datasets, namely environmental variables—e.g., physico-chemical variables (R), taxa (L) and traits (Q). In the present study, we used the RLQ ordination test to relate physico-chemical variables (R), macroinvertebrates taxa (L) and the traits (Q) in relation to urban pollution gradient in forested riverine systems. The function dudi.COA (i.e., dudi.Correspondence Ordination Analysis) was applied to the macroinvertebrate taxa table [42], and the function dudi.PCA (i.e., Principal Component Analysis) was applied to the trait table [42]. Finally, the Hillsmith transformation function (dudi.Hillsmith) was applied to the physico-chemical variables [42].

In identifying and classifying potential indicator urban pollution traits in forested riverine systems, two criteria were adopted. Firstly, on the RLQ ordination plots, trait attributes that were associated with least impacted sites (LIS) were considered urban pollution sensitive traits in forested riverine systems, and traits associated with highly impacted sites (HIS) were deemed urban pollution tolerant traits in forested riverine systems. The two axes of the RLQ ordination plot were tested for statistical significance using the Monte Carlo permutation test at 999 permutation arguments ($p = 0.05$). Secondly, to further confirm urban pollution potential indicator traits in forested riverine systems, a fourth-corner test was computed. The fourth-corner test is a multivariate analysis which expounds the relationships between multiple traits and environmental variables—e.g., physico-chemical variables. The test shows traits that either positively or negatively correlates with a given physico-chemical variable.

The final identification and classification of trait attributes into potential urban pollution indicators in forested riverine systems were confirmed by both the RLQ and fourth-corner tests results. Thus, a trait associated with the LIS based on the RLQ plot, and either significantly positively correlated with increasing concentration of DO, or negatively correlated with any two of increasing nutrients, five-day Biochemical Oxygen Demand (BOD_5), water temperature and increased flow velocity on the fourth-corner test were finally considered urban pollution sensitive traits in forested riverine systems. On the other hand, traits associated with HIS on the RLQ plot and also either significantly negatively correlated with increasing concentration of DO or positively correlated with any two of increasing nutrients, BOD_5, water temperature and increased flow velocity on the fourth-corner test were considered urban pollution tolerant traits in forested riverine systems. The RLQ, fourth-corner and other analyses (COA, PCA and Hillsmith) were computed using the ade4 package for R-statistics version 2.5.4 within the R programming environment [29,42]. The approach adapted in the present study has recently been used to identify and classify urban pollution indicator signature and sensitive traits and ecological preferences in riverine systems draining urban and agricultural catchments [5,17].

2.6. Exploring the Distribution Patterns of Macroinvertebrate Traits along Urban Pollution Gradient in Forested Riverine Systems

The distribution patterns of macroinvertebrate traits identified and classified as sensitive to and tolerant of urban pollution in forested riverine systems were plotted using box plots, and they were further tested for statistical significance across the ecological classes using the Kruskal–Wallis multiple comparison test. The box plots and Kruskal–Wallis tests were computed using Statistica version 13.4.0.14 (TIBCO Software Inc., CA, USA, 2018).

3. Results

3.1. Assessing Macroinvertebrate Traits and Taxa Responses, as Well as Identifying and Classifying Potential Indicator Macroinvertebrate Traits along Urban Pollution Gradient in Forested Riverine Systems

Of the 20 sites sampled in the forested riverine systems draining urban catchments in the present study, three sites were classified as LIS, seven were classified as MIS and 10 were classified as HIS (Table 1). On the RLQ ordination, two of the three LIS (Sites 1 and 2 of Warri River) were positioned on the first axis of the RLQ ordination along with MIS (Site 2 of Ase River, and Site 3 of Benin River) (Figure 2). Two of the classified MIS sites (Site 1 of Ase River, and Orogodo River) were positioned at the centre of the RLQ ordination plot. All sites classified as HIS were positioned on Axis 2 of the RLQ ordination plot, except Sites 1 and 2 of Umomi River and Owan River, which were positioned at the centre of the RLQ ordination plot (Figure 2). One site classified as LIS (Adofi River) and three sites classified as MIS (Sites 1, 2 and 3 of Iyiukwu River) were also positioned on the second axis of the RLQ ordination plot.

The first axis of the RLQ ordination explained a variance of 93%, and the second axis explained a variance of 6%. The eigenvalues of the first and second axes of the RLQ were 10.70 and 0.70, respectively, and a total inertia of 11.50. The RLQ ordination first two axes showed no significant differences between macroinvertebrate traits and physico-chemical variables ($p > 0.05$) as revealed by the Monte Carlo test at 999 permutation arguments. Increasing water temperature, flow velocity, nutrients, EC and BOD_5 were positively strongly correlated with sites classified as HIS, and increasing DO was positively associated with sites classified as LIS (Figure 2).

Traits such as temporary attachment, grazing, hardshell and large body size (>20–40 mm) were positively correlated with sites classified as LIS, and traits which include burrowing, predation, free living, small body size (>5–10 mm) and pupa aquatic stage were positively correlated with sites classified as HIS (Figure 2). Conversely, traits such as gills, 2 years (bivoltine), cylindrical/tubular and filter feeding were positively correlated with sites classified as LIS and MIS (Figure 2).

A total of 32,816 macroinvertebrate individuals in 54 families were collected and recorded during the entire sampling period (see Supplementary Table S3). Site 2 of Warri River had the highest absolute abundance of macroinvertebrate individuals (10,468) followed by Site 1 of Umaluku River (5402), while the least absolute abundance of macroinvertebrate individuals was recorded in Site 1 of Umomi River (109), followed by Site 3 of Benin River (126) (Supplementary Table S3). The dominant macroinvertebrate taxa were Thiaridae represented by 5286 individuals; this was immediately followed by Chironomidae with 4624 individuals (Supplementary Table S3). On the other hand, Lymnaidae was the least represented macroinvertebrate taxa with only two individuals recorded in the entire sampling period, and it was only collected in Orogodo River; this taxon was immediately followed by Tubificidae with eight individuals (Supplementary Table S3).

From the RLQ analysis and further correlation of traits with physico-chemical variables using fourth-corner test, it was revealed that traits such as large body size (>20–40 mm), grazing and hardshell, which were positively significantly associated with the sites classified as LIS on the RLQ ordination (Figure 2), were also either negatively significantly correlated with water temperature, nutrients, BOD_5 and flow velocity or positively significantly correlated with increasing DO concentration (Table 2). Thus, these traits were considered sensitive to urban pollution in forested riverine systems. Furthermore, burrowing, predation and pupa aquatic stages that were positively significantly associated with sites classified as HIS on the RLQ ordination (Figure 2) were also significantly negatively significantly correlated with increasing DO concentration (Table 2). These traits were considered tolerant of urban pollution in forested riverine systems draining urban landscapes.

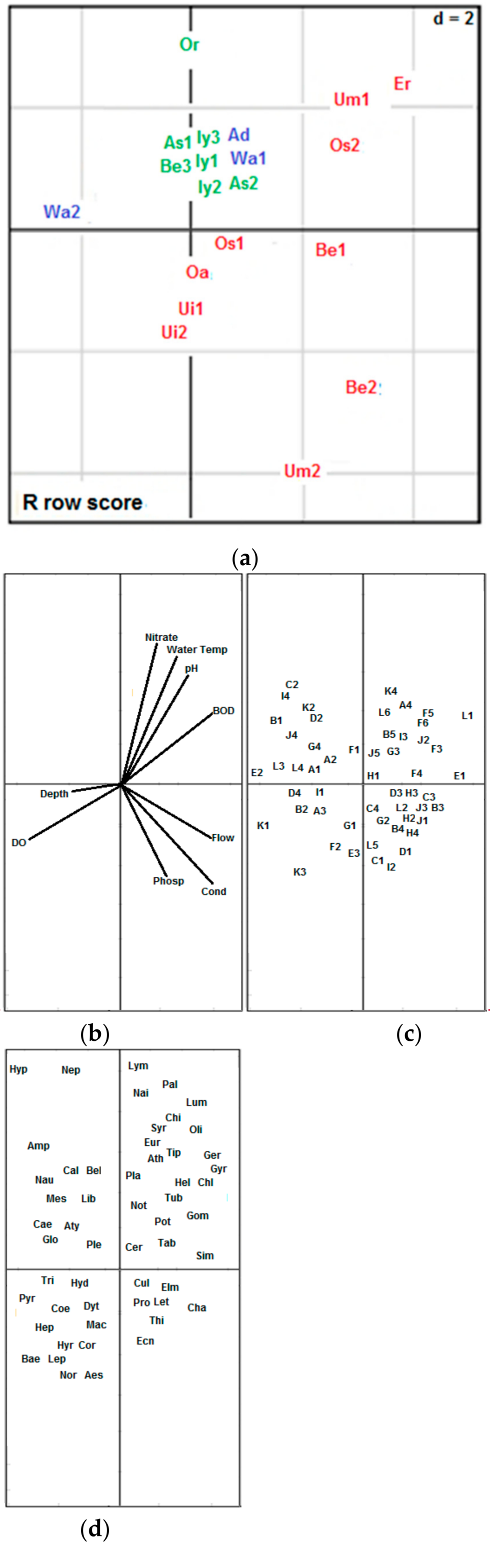

Figure 2. RLQ analysis showing the co-variation of the 20 river sites sampled in the present study (see Table 1 for site codes); sites in blue = LIS, sites in green = MIS and sites in red = HIS) (**a**), physico-chemical variables (**b**), macroinvertebrate traits (**c**), and macroinvertebrate taxa (**d**) in relation to the first two axes of the RLQ. **Abbreviations:** Water Temp = Water Temperature, Cond = Electrical conductivity, Flow = Flow velocity, Phosp = Phosphate, DO = Dissolved oxygen, BOD = Five-day biochemical oxygen demand (BOD_5). **Site ecological classes:** LIS = least impacted sites, MIS = moderately impacted sites, HIS = highly impacted sites. **Traits:** "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 = >2 years (Multivoltine), D4 = longer than one year (Semivoltine), E1 = Free-living, E2 = Temporary attachment, E3 = Permanent attachment, F1 = Climbing, F2 = Crawling, F3 = Sprawling, F4 = Swimming, F5 = Skating, F6 = Burrowing, 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 body size (>20–40 mm), J5 = Very large body size (>40–80 mm), K1 = Egg, K2 = Larva aquatic stage, K3 = Nymph aquatic stage, K4 = Pupa aquatic stage, L1 = Predation, L2 = Scraping, L3 = Grazing, L4 = Filter feeding, L5 = Deposit feeding, L6 = Shredding" [17]. **Taxa:** Nai = Naididae, Tub = Tubificidae, Lum = Lumbricidae, Lym = Lymnaidae, Pla = Planorbidae, Thi = Thiaridae, Amp = Amphullariidae, Aty = Atyidae, Eur = Euryrhynchidae Pal = Palaemonidae, Bae = Baetidae, Lep = Leptophlebiidae, Cae = Caenidae, Hep = Heptageniidae, Tri = Tricorythidae, Oli = Oligoneuridae, Pot = Potamanthidae, Pro = Prosopistomatidae, Glo = Glossosomatidae, Hyd = Hydroptilidae, Hyr = Hydropsychidae, Ecn = Ecnomidae, Hel = Helicopsychidae, Let = Leptoceridae, Pyr = Pyraustidae, Not = Notonectidae, Cor = Corixidae, Ple = Pleidae, Mes = Mesoviliidae, Nep = Nepidae, Nau = Naucoridae, Bel = Belostomatidae, Ger = Gerridae, Dyt = Dytiscidae, Hyp = Hydrophilidae, Elm = Elmidae, Gyr = Gyrinidae, Nor = Noteridae, Aes = Aeschnidae, Gom = Gomphidae, Coe = Coenagrionidae, Lib = Libellulidae, Cal = Calopterygidae, Mac = Macromidae, Chl = Chlorocyphidae, Cul = Culicidae, Sim = Simulidae, Tab = Tabanidae, Cer = Ceratopogonidae, Ath = Athericidae, Cha = Chaoboridae, Tip = Tipulidae, Syr = Sryhidae, Chi = Chironomidae.

Table 2. Fourth-corner test performed for macroinvertebrate traits and physico-chemical variables in the selected forested riverine systems draining urban landscapes in the present study area. + shows significant positive correlations, and - shows significant negative correlations correlations.

Traits	Physico-Chemical Variables								
	Water Temp	Depth	Flow	Cond	DO	BOD_5	pH	Nitrate	Phosp
Gills									
Tegument/cutaneous									+
Aerial: spiracle									
Aerial/vegetation: breathing tube, strap/other apparatus									
Hardshell				-	+				-
Completely sclerotized									
Partly sclerotized									
Soft and exposed									
Cased/tubed		-							
Clear and transparent waters									
Silty waters		-	+						
Turbid waters									
No preference for turbid waters									
1 year (Univoltine)		+							
2 years (Bivoltine)	+	-	+						
D3 = >2 years (Multivoltine)									
D4 = longer than one year (Semivoltine)									
Free-living									
Temporary attachment									

Table 2. *Cont.*

Traits	Physico-Chemical Variables								
	Water Temp	Depth	Flow	Cond	DO	BOD_5	pH	Nitrate	Phosp
Permanent attachment									
Climbing									
Crawling		+							
Sprawling				+			+		
Swimming	-	+	-						-
Skating									
Burrowing	+		+		-				
Streamlined	-								
Flattened			-						
Spherical									
Cylindrical/tubular									
Detritus (FPOM)									
Detritus (CPOM)									
Macrophytes/algae									
Animal materials									
Highly sensitive to oxygen depletion									
Moderately sensitive to oxygen depletion	-		-						
Moderately tolerant of oxygen depletion									
Highly tolerant of oxygen depletion	+	-		+					
Very small body size (<5 mm)									
Small body size (>5–10 mm)									
Medium body size (>10–20 mm									
Large body size (>20–40 mm)					+				
Very large body size (>40–80 mm)									
Egg aquatic stage									
Larva aquatic stage									
Nymph aquatic stage									
Pupa aquatic stage					-			+	
Predation					-				
Scraping									
Grazing						-			-
Filter feeding									
Deposit feeding									
Shredding									

3.2. Exploring the Distribution Patterns of Macroinvertebrate Traits along Urban Pollution Gradient in Forested Riverine Systems

The relative abundance of the three identified and classified traits sensitive to urban pollution in forested systems were highest at LIS except grazing as a feeding preference (Figure 3). The Kruskal–Wallis test revealed that large body size (>20–40 mm) and the possession of hardshell were significantly lowest at MIS and HIS compared to LIS ($p < 0.05$), and they showed no significant difference between the MIS and HIS ($p > 0.05$). Grazing as a feeding preference showed no significant difference between LIS, MIS and HIS ($p > 0.05$) (Figure 3).

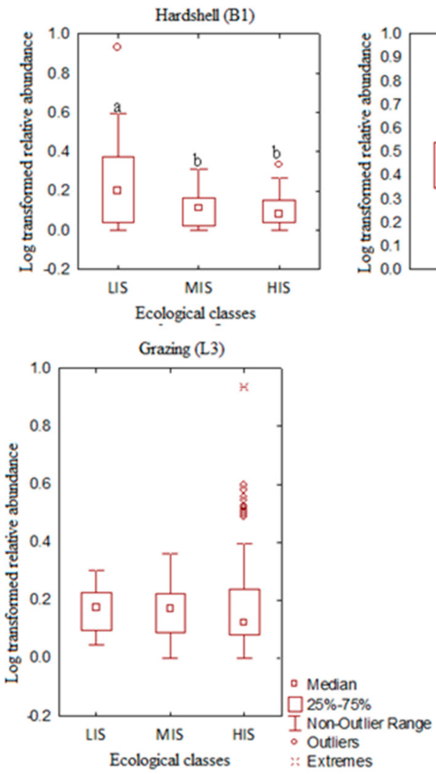

Figure 3. Pattern of distribution of sensitive traits across the three ecological classes in the present study.

The relative abundance of the three identified and classified traits tolerant of urban pollution in forested riverine systems was highest at MIS (Figure 4). The Kruskal–Wallis test showed that predation and the pupa aquatic stage were statistically significantly lowest at LIS and HIS compared MIS ($p < 0.05$) (Figure 4).

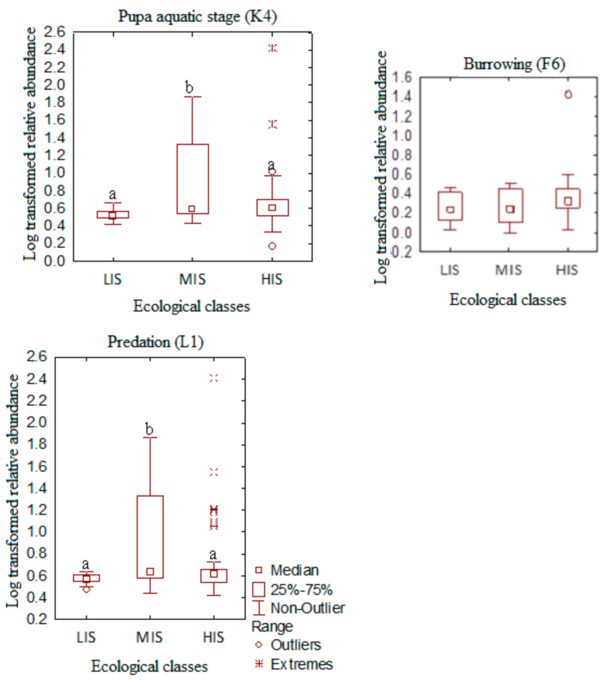

Figure 4. Pattern of distribution of tolerant traits across the three ecological classes in the present study.

4. Discussion

In this study, we explore how urban pollution influences macroinvertebrates traits in forested riverine systems. Macroinvertebrates traits were found to respond differently to urban pollution with regard to the classified ecological classes. In the West Africa sub-region where this study was conducted, most of the existing studies on biomonitoring have been centred on macroinvertebrate taxonomic structures [4,6,15]: only a very few study have explored the use of trait-based approaches in assessing the ecological health of riverine systems in the sub-region [17]. While taxonomy accounts for biota structural diversity, composition and abundance, traits explore the functional characteristics of aquatic biota [8]. Furthermore, traits unlike taxonomy are less constrained by geographical differences, making trait-based tools likely to be more widely applicable on a broad spatial scale.

Trait-based ecology is gaining popularity because of its potential to turn community ecology into a predictive science, enabling the prediction of the responses of biota to a given stress because of the stressor mediation role of traits [2]. It has also been argued that the use of traits may be less costly because of potential reduction in species identification time [8]. Thus, identifying trait-based indicators can add value to the practice of freshwater biomonitoring particularly in a region where taxonomic expertise is sparse. In this study, we explore trait distribution in relation to urban pollution in forested riverine systems with the aim of identifying indicator traits using RLQ and fourth-corner analyses.

The RLQ ordination in recent time has been consistently used to explain how environmental variables (R) relate with taxa (L), and their inherent trait attributes (Q) in a bid to understand trait patterns of distribution in ecological sites [43,44]. The concept has been used to develop biomonitoring criteria in conjunction with the fourth-corner test to assess the level of disturbances riverine systems are undergoing [44]. In this present study, the LIS were associated with the first axis of RLQ ordination which harbours macroinvertebrate taxa that have been reported to be sensitive to pollution—e.g., Plecoptera, Ephemeroptera and Trichoptera. This association was explained by increased dissolved oxygen concentration and water depth [8,15]. This study corroborates the study by Kuzmanovic et al. [13], who recorded sensitive taxa of macroinvertebrates in less disturbed sites. Taxa in the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) have been shown to disappear in impacted sites due to their high sensitivity to degradation and poor water condition [15]. This was noted in this study, as most of the pollution sensitive species were sharply separated from the HIS compared to the LIS and MIS. Furthermore, the second RLQ axis which harbours more of the HIS and aggravated pollution indicating physico-chemical variables such as EC, nutrients and BOD_5 favours the assemblages of most pollution tolerant Dipterans (e.g., Chironomidae, Culicidae), Mollusca (e.g., Thiaridae, Planorbidae) and Annelids, most especially the Oligochaetes (e.g., Naididae, Tubificidae). A similar study conducted elsewhere reported very high proportions of Oligochaetes and Dipterans in urban dominated sites with high concentrations of nutrients and heavy metals [13].

Following our results for traits, it was revealed that macroinvertebrates possessing large body sizes such as Gomphidae, Dytiscidae, Atyidae and Amphullaridae were particularly sensitive to urban pollution in forested riverine systems as they were dominant in the LIS compared to MIS and HIS. Urban pollution can introduce elevated dissolved solids into forested riverine systems, thereby increasing the risk of absorbing dissolved materials—e.g., metals that are potentially toxic [13,14,37,38]. Organisms with a large body size have been reported to have a large surface area to volume ratio, which increases their likelihood of increased exposure and adsorption to chemicals due to their increased surface area to volume ratio compared to organisms possessing a small body size [45,46]. Organisms with a large body size have also been predicted to be particularly sensitive to pollution because they are often associated with the production of fewer offspring per reproductive cycle [47]. This prediction is in congruence with our result in the present study, as the relative abundance of macroinvertebrates with large body size was highest in the least impacted sites, which showed that body size can serve as an indicator of urban pollution in forested riverine systems. A study on macroinvertebrate body size distribution in an effluent-impacted system in a South African river also found large-bodied macroinvertebrate being less dominant at the impacted sites compared with the less impacted sites [5].

Small body sized organisms—e.g., Chironomidae and Culicidae—have been confirmed by earlier studies to be resilient in environments subjected to pollution [48,49]. Serra et al. [48] and Castro et al. [49] in their studies attributed the preponderance of small body sized organisms in disturbed sites to their relatively short life cycles, which enables them to recover quickly after disturbance activities.

The relative abundance of macroinvertebrates such as Gomphidae, Calopterygidae, Dysticidae, Hydrophilidae, Elmidae, Gyrinidae and Hydraenidae, which grazed as a primary feeding mode, was significantly lower in the MIS and HIS compared to the LIS, and they were also negatively significantly correlated with urban pollution indicating physico-chemical variables on the RLQ ordination plot. It was unexpected for grazing as a feeding mode to be positively associated with the LIS instead of the HIS and MIS, as grazers are often expected to prefer sites with potential for algal growth due to the increased concentration of nutrients which characterised HIS and MIS in the present studied rivers. However, the results of the present study are in congruence with the result reported elsewhere by Odume [5] who reported grazing as a feeding mode to be associated with a less impaired site in an industrially impacted river in South Africa.

Predation as a feeding mode was expected to be sensitive to urban pollution in forested riverine systems as most predators are specialist feeders [2]. Nevertheless, in this study, predating macroinvertebrates were associated with the MIS and HIS, and thus proved to be tolerant to urban pollution. Odume [5] recently reported the majority of the predating macroinvertebrates collected in the impacted sites to be coleopterans and hemipterans, which possess special apparatus for taking in atmospheric oxygen in the face of depleting dissolved oxygen concentration in a river. In the present study, the majority of the predators such as Nepidae, Notonectidae, Belostomatidae, Macromidae, Aeschnidae, Libellulidae, Cordulidae, Platycnemididae, Calopterygidae, Coenagrionidae and Chlorocyphidae were also distributed in sites classified as HIS. Similar distribution of some predating Hemipterans has been documented in the neotropics [50]. Thus, it is possible that the possession of traits which enable intake of atmospheric oxygen in the face of depleting dissolved oxygen concentration may confer resilience on these groups of macroinvertebrates, thereby enabling them to survive and thrive in impacted sites as noticed in the HIS of the present study.

Organisms possessing hardshell body armouring—e.g., Thiaridae and Planorbidae—have been reported to harbour sites with high concentration of nutrients and heavy metals pollution [2]. This is in consonance with the result of the present study, as organisms possessing hardshell body armouring such as Thiaridae, Planorbidae and Hydrobiidae were highly abundant in sites classified as HIS, except for Amphullariidae that was abundant in sites classified as LIS. The inconsistency observed in the present study with regard to the relationship between organisms possessing hardshell body armouring and sites ecological classes may not be unconnected to the concept of "trait syndromes" [1]. Phylogenetic constraints have been attributed to the relationship between the diversity of trait attributes and level of pollution in an ecosystem [51]. Poff et al. [52] and [46] pointed to the fact that the response of a trait to a given indirect stressor(s) may be the cause of an inter-correlation in the response of traits to different stressors. Furthermore, random diffusion or unfinished migration may be attributed to the presence of some of the pollution tolerant trait attributes noted in the LIS relating with the pollution sensitive taxa [53].

Traits that are a full reflection of perturbation were associated with the second axis of RLQ ordination in this study. The pupa aquatic stage which has been reported to associate with increased concentration nutrients and EC were highly distributed in sites classified as HIS [2]. This is as expected as Mollusca families—e.g., Thiaridae—which was one of the dominant groups of macroinvertebrates in the HIS have been reported to be tolerant of pollution due to their pupa aquatic stage [1]. This is a confirmation of the association of the Mollusca with the HIS in this study area. Additionally, organisms exhibiting burrowing mobility mode were associated with sites classified as HIS and pollution indicating physico-chemical variables such as EC and BOD_5. Organisms exhibiting burrowing mobility mode have been reported to thrive in disturbed sites with high sedimentation [54]. This also confirmed some of our predicted key stressors of urban pollution—storm water return flow and increased suspended solids—which were evident in the present study area.

5. Conclusions

The present study elucidates the influence of urban pollution on macroinvertebrate traits in forested riverine systems draining urban landscapes. The study highlighted that macroinvertebrate traits responded differently to urban pollution in forested riverine systems. We identified and classified sensitive and tolerant traits which were suggested as potential biological indicators for assessing forested riverine systems health draining urban landscapes in the Niger Delta area of Nigeria. The present study identified and classified large body size (>20–40 mm), grazing and hardshell as traits potentially sensitive to urban pollution in forested riverine systems, while traits such as burrowing, predation and pupa aquatic stage were deemed tolerant of urban pollution in forested riverine systems. On the other hand, our earlier study explored the influence of agricultural and urban pollution on the distribution patterns of macroinvertebrate traits [17]. In that study, we identified traits such as large body size, permanent attachment, moderate and high sensitivity to oxygen depletion to be sensitive to agricultural and urban pollution, while traits such as small body size and CPOM were identified as traits tolerant of agricultural and urban pollution. The present study confirms that large body sized organisms are sensitive to urban pollution, as earlier reported by us. Furthermore, the identification and classification of predation as tolerant trait, in this study is not unconnected to the high concentration of nutrients which usually characterised river systems draining urban catchment. Overall, the present study is an important step in the field of community ecology and predictive science, and the study serves as a roadmap in using macroinvertebrates TBA for biomonitoring riverine systems most especially in the Afrotropical region where study of this kind is still scarce. This study can be improved upon by developing more sophisticated potential trait-based biomonitoring indicators globally by using macroinvertebrate genera or species as against the family levels used in the present study.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/11/3111/s1, Figure S1: PCA biplot correlating physico-chemical variables with forested riverine system sites within urban catchment in the study area, Table S1: Geographical locations, land use types and catchment sizes of the sampled sites in the present study, Table S2: Mean values of physico-chemical variables analyzed between 2008 and 2012 in forested riverine systems draining urban catchments in the Niger Delta area of Nigeria, Table S3: Absolute abundance of macroinvertebrate taxa collected during the sampling period spanning from 2008–2012 in forested riverine systems draining urban catchments in the Niger Delta area of Nigeria.

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