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Water quality related macroinvertebrate community responses to environmental gradients in the Portoviejo River (Ecuador)

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Abstract – The Portoviejo River, located in the central western part of Ecuador, has been heavily impacted by damming, intensive agriculture and untreated wastewater discharge. Unfortunately, detailed information on the water quality and the ecological status of the Portoviejo River is not available, inhibiting decision-making and the development of water management plans. Therefore, the aims of this study were (1) to assess the ecological water quality, (2) to investigate the point along the environmental gradient where the most significant change in macroinvertebrate community occurs and (3) to find potential macroinvertebrate taxa that significantly change in abundance and frequency of occurrence along the Portoviejo River. To this end, macroinvertebrate and physico-chemical data were collected and hydro-morphological conditions were recorded at 31 locations during the dry season of 2015. The results showed that the ecological water quality of the sampling sites ranged from good to bad. In addition, the Threshold Indicator Taxa ANalysis was used to examine changes in macroinvertebrate communities and revealed significant community change points for sensitive taxa declining at a conductivity value of 930 ($\mu S.cm^{-1}$) and nitrate-nitrogen concentrations of 0.6 $mg.L^{-1}$. In addition, the thresholds estimated for tolerant taxa were set at a conductivity value of 1430 $\mu S.cm^{-1}$ and nitrate-nitrogen concentration of 2.3 mg.L. Atyidae, Corbiculidae, Thiaridae, Acari, Baetidae and Leptohyphidae can be considered indicator taxa, showing shifts in the community. This study suggests that values of conductivity and nitrate-nitrogen concentrations should not exceed the threshold levels in order to protect macroinvertebrate biodiversity in the Portoviejo River.

Key words: Macroinvertebrates / ecological water quality / threshold indicator taxa analysis / portoviejo river

Introduction

Rivers are one of the most important freshwater resources for human life (Chapman, 1996). They provide many ecosystem services such as a source of drinking water, irrigation of croplands, industrial and municipal water supply, waste disposal, fishing, sightseeing, shipping and an aesthetic value (Chapman, 1996; Pan *et al.*, 2012). However, the increase in population and human activities often leads to habitat degradation, poor water quality (Kibena *et al.*, 2014) and reduced ecosystem services (Pan *et al.*, 2012) as rivers are highly vulnerable to anthropogenic activities (*e.g.*, urbanization, changes in land use, intensification of agriculture) (Bredenhand and Samways, 2008). For example, the construction of a dam changes the hydrological conditions, and modifies the flow regime and

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sediment transportation (Takao *et al*., 2008), thus strongly influencing aquatic ecosystems (Zhang *et al*., 2010).

Water pollution, erosion, alterations in stream hydrology and changing habitat structure are known to affect freshwater organisms (Allan, 2004). Various types of aquatic organisms (*e.g.,* fish and macroinvertebrates) have been used as indicators of water quality and biological integrity. Among them, macroinvertebrates are considered to be good indicators of overall ecosystem health (Water Framework Directive, 2002). Their presence, abundance and activities are a representation of water quality and may effectively reveal the ecological status of the ecosystem (Bredenhand and Samways, 2008). Moreover, macroinvertebrates reflect stream conditions and integrate human and natural stressors over a long period of time, thus giving a good representation of the quality of their surroundings (Cairns and Pratt, 1993). Therefore, macroinvertebrates have been used for freshwater monitoring and assessment for several decades (Smith *et al*., 2007). The information about the ecological sensitivities of each macroinvertebrate taxon has been used to develop biological indices (*e.g.,* biological monitoring working party (BMWP) (Armitage *et al*., 1983)) for the assessment of water quality. Afterwards, the English BMWP index was adapted to specific countries or regions such as the BMWP for Colombia (Pérez, 2003a). The BMWP-Colombia index has already been applied in Ecuador to study the ecological water quality in the Guayas river basin (Damanik-Ambarita *et al*., 2016) and Chaguana river basin (Dominguez-Granda *et al*., 2011).

In Ecuador, water quality issues, aquatic ecosystems and ecosystem services received limited attention. To our knowledge, standardized sampling procedures and environmental monitoring programs are not available to assess water quality, resulting in limited availability of information on the physical, chemical and ecological status of Ecuadorian rivers (Andres, 2009; Nolivos *et al*., 2015). However, water managers and political leaders need to manage water to meet human requirements, to identify and protect endangered species and to support freshwater ecosystems (Richter *et al*., 2003). Therefore, threshold identification is needed for the development of environmental standards for river water quality. However, when reviewing the criteria for preservation of aquatic life established for the Ecuadorian environment, most of the criteria were found to be defined only for heavy metals (*e.g.,* Ag, Pb) and water quality standards for important variables (*e.g.,* conductivity) were missing.

Portoviejo city is considered as the center of economic, political and cultural events in the province of Manabí. During recent years, high pressure has been exerted on water quality and natural ecosystems in the Portoviejo River basin driven by the growing population and increasing anthropogenic activities such as intensive agriculture and damming. Pollution of the Portoviejo River causes scarcity of clean water for domestic consumption and irrigation, loss of fishing grounds (Párraga and Aguirre, 2010) and strongly affects biodiversity and mangrove ecosystems in the Portoviejo River Estuary (ACBIO, 2012). However, to our knowledge, no research has been carried out on the assessment of water quality and macroinvertebrate communities in the Portoviejo River. Therefore, the main objective of this study was to assess the ecological water quality of the Portoviejo River based on benthic macroinvertebrate communities. More specifically, we investigated shifts in macroinvertebrate communities along measured physico-chemical gradients and identified potential macroinvertebrate indicator taxa that significantly change in abundance and frequency of occurrence due to water quality degradation. The thresholds relevant to the macroinvertebrate communities' response in the Portoviejo River will be particularly useful for early warning in Ecuadorian rivers. This information could be used to establish priorities for conservation efforts for the Portoviejo River and other similar river basins, where water resources are facing multiple threats.

Materials and methods

Study area

Portoviejo is the capital of the Province of Manabí (Ecuador) and is situated 30 km from the Pacific coast. The Portoviejo River basin, with a total area of 2231 km^2 and a river length of 132 km (Pérez, 2003b), is located along the coast in the western part of Ecuador (US Army Corps of Engineers, 1998). The river provides water to 700 000 inhabitants for domestic use, agriculture, recreation and other purposes (Pérez, 2003b). The Portoviejo River basin is one of the most productive farming regions in Ecuador, with production of bananas, mangoes and other tropical fruits, tomatoes, onions, peppers, coffee and especially cattle and fish (http://www.gutenberg.us 2016). The Poza Honda dam is located 30 km upstream from the city of Portoviejo and started operating in 1971. The Poza Honda reservoir has a water storage capacity of 100 million m^3 (US Army Corps of Engineers, 1998), a maximum surface area of approximately 607.5 ha and a maximum depth of 37.3 m. The reservoir faces eutrophication problems due to intensive agriculture and livestock (Perez, 2004) in the surrounding area.

Portoviejo is located at low latitude and has a semi-arid, hot climate. The seasons are well defined; rainfall is concentrated in the period December–May, in which 90% of the annual rainfall occurs and the dry season months are June–November. The mean temperature and monthly rainfall of the region vary between 24 and 29 °C and 2–115 mm, respectively (http://www.portoviejo.climatemps.com "http://www.gutenberg.us, 2016 Portoviejo.http://www.gutenberg.us/article/WHEBN0001827357/Portoviejo (in Spanish, assessed date 12.02.2016"). Land use in the basin consists mostly of arable land and plantations (onions, bananas and other tropical fruits), with urban and semi-urban areas.

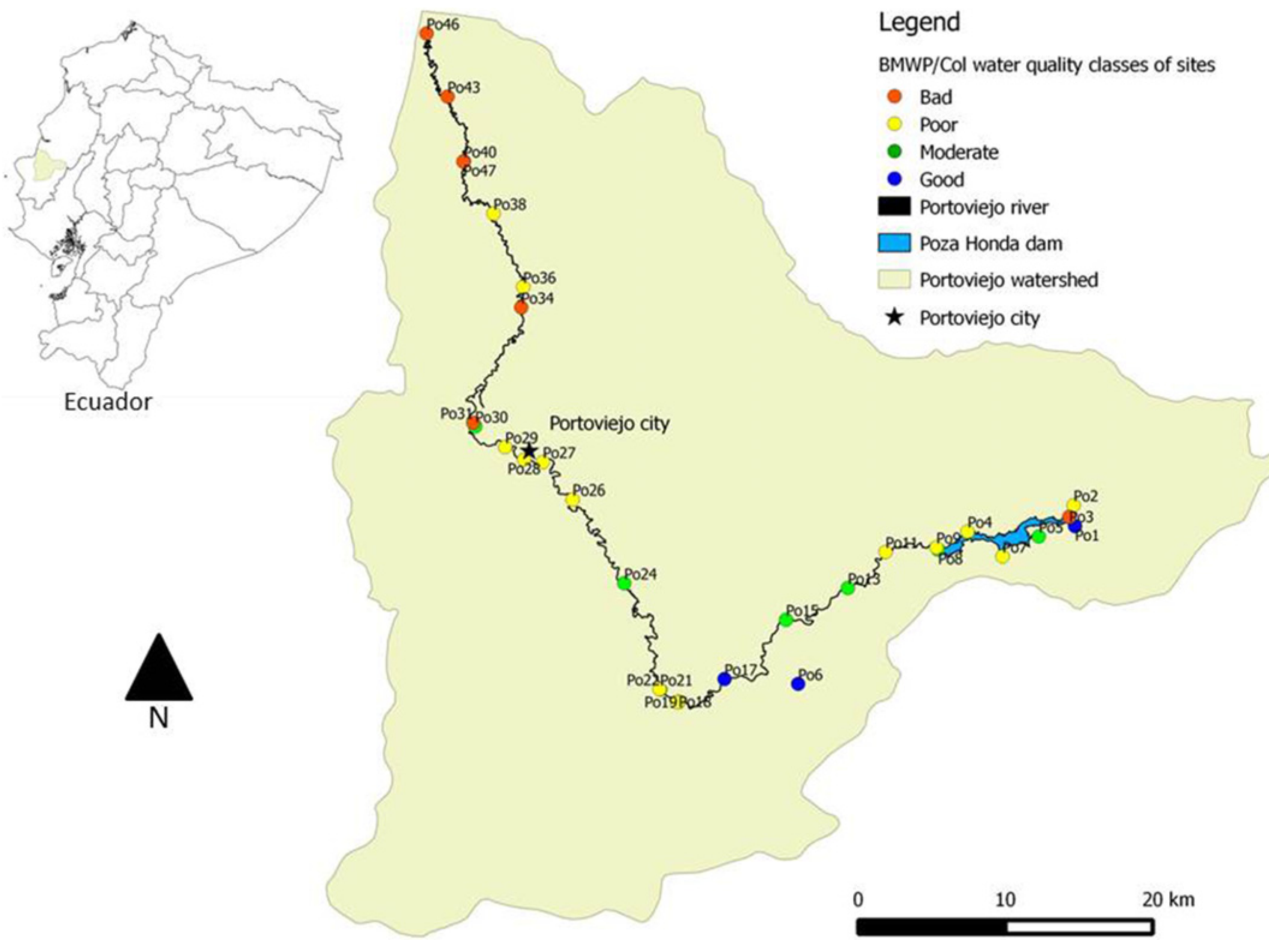

Fig. 1. Map of the study area of the Portoviejo River with indication of the ecological water quality based on the BMWP-Colombia for each sampling site.

Sampling and assessment methods

Description of sampling sites

The physico-chemical water quality variables and macroinvertebrate samples were collected from 31 locations along the Portoviejo River in Ecuador (Fig. 1). We assumed worst-case conditions in terms of water quality (*e.g.,* conductivity) during the dry season (low dilution due to rain), possibly indicating severe water quality problems. Therefore, each sampling site was sampled once during the dry season (August) of 2015 in order to assess the water quality and the ecological status of the river. In the Portoviejo River, it was expected that the ecological water quality would decrease from source to mouth due to increasing human pressure. Main sources of disturbance are residential areas, wastewater treatment plants and agricultural activities. The sites were selected based on accessibility and presence of point sources of pollution. In total 18 sites were sampled starting from 10 km upstream of Portoviejo city to the mouth of the river. These sites are located near visual sources of disturbance (*e.g.,* densely populated areas, downstream of wastewater treatment plants, downstream of hydropower dam and intensive agriculture activities) and were considered affected sites. In addition, 13 sites from 20 km upstream of Portoviejo city to the source of the river were chosen as less affected sites and served as reference locations. Differentiation between affected and reference sites was based on population density and surrounding land use.

Recording of environmental variables

At each sampling site, the values of temperature (°C), pH, dissolved oxygen (DO) (mg.L^{-1}), chlorophyll (μg.L^{-1}), turbidity (FTU) and electrical conductivity (EC) (μS.cm^{-1}) were measured using a multiprobe (model YSI 6600 V2, YSI manufacturer). The multiprobe contains different sensors and was placed directly underneath the water surface to measure water quality. The values of each reading were saved after the reading was stable. Moreover, water samples from each sampling location were collected and stored in plastic bottles (1 L), kept cool and in the dark immediately after collection and subsequently transported to the laboratory for further analysis. At the laboratory, Hach-Lange DR 3900 spectrophotometer kits were used to determine biological oxygen demand (BOD_5) ($mg.L^{-1}$), total phosphorus (TP) ($mg.L^{-1}$), orthophosphate-phosphorus (oPO_4^{3-}) ($mg.L^{-1}$), ammonium-nitrogen (NH_4^+) ($mg.L^{-1}$), nitrate-nitrogen (NO_3^-) ($mg.L^{-1}$), nitrite-nitrogen (NO_2^-) ($mg.L^{-1}$), total nitrogen (TN) ($mg.L^{-1}$) and total organic carbon (TOC) ($mg.L^{-1}$).

The elevation of sampling sites was measured using Global Positioning System (GPS) equipment (Garmin GPS). Stream velocity was measured with a handheld flow meter (HFA, Höntzsch, Waiblingen, Germany). The surrounding land use was visually estimated at both banks for a stretch of 100×10 m (the point where the water sample was taken was considered as the centre). The surrounding land use was divided into five classes (shrubs/grasses, orchard, residential/urban areas, arable land (suitable for farming) and forest). The type of dominant substrate was visually assessed at each site and divided into five classes (silt or clay, sand, gravel, cobble and boulder). The sludge layer was classified into absent, < 5, 5–20 and > 20 cm. Six classes of pool-riffle pattern were distinguished (structural changes, absent, poorly developed, moderately developed, well developed and pristine). The percentage of water hyacinth cover was visually estimated from the bank along a transect of 100 m. The site where water samples were taken was considered as the centre. The vegetation cover classes were divided as follows (based on the Braun-Blanquet cover/abundance scale): 0 = non-vegetated/absent, 1 = 1–5% (rare), 2 = 5–25% (occasional), 3 = 25–50% (frequent), 4 = 50–75% (common) and 5 = 75–100% (abundant). Classes rather than exact values for cover of vegetation were used as this yields more reliable measures (Ellenberg and Mueller-Dombois, 1974). The hydro-morphological characteristics of the sampling sites were determined based on field inspection and completed per sampling location by a standard field protocol. The field protocol was modified from the Australian River Assessment System (AUSRIVAS) physical assessment protocol (AUSRIVAS, 1994) and UK and Isle of Man River Habitat Quality (Raven *et al.*, 1998).

Sampling of macroinvertebrates

Samples of macroinvertebrates were collected from each sampling site immediately after determining the physico-chemical water quality variables. Macroinvertebrates were collected with a standard hand net consisting of a metal frame holding a conical net (mesh-size 300 μm) at the same sites where water quality was measured. Macroinvertebrates were collected over 5 min active sampling, including all different microhabitats present at the sampling site (Gabriels *et al.*, 2010). Samples were sieved (500 μm mesh size) in the laboratory and sorted in white trays. Macroinvertebrates from each location were placed in separate small plastic vials containing 80% ethanol for preservation. After sorting, organisms were counted and identified under a stereo-microscope. Macroinvertebrates were identified to family level using the identification keys developed by Domínguez and Fernández (2009) for two reasons. Firstly, previous research has shown that using biotic indices based on family level provides sufficient information to assess biological water quality (Dominguez-Granda *et al.*, 2011; Mereta *et al.*, 2013; Everaert *et al.*, 2014). Secondly, because of practical implications, we could only identify up to family level as there are no detailed keys available to lower taxonomic levels.

Calculation of macroinvertebrate metrics

The biotic macroinvertebrate index BMWP-Colombia was calculated according to the method proposed by Zuniga and Cardona (2009). Each macroinvertebrate taxon received a score that reflects its susceptibility to pollution, where pollution-intolerant taxa receive high scores, whereas pollution-tolerant taxa were given low scores (Zuniga and Cardona, 2009). Eight taxa did not appear in the original index and were considered in the BMWP-Colombia index calculation, being Acari (Actinotrichida), Cambaridae (Decapoda), Corbiculidae (Veneroidea), Gerridae (Hemiptera), Littorinidae (Neotaenioglossa), Mysidae (Mysida), Ochteridae (Hemiptera) and Spionidae (Polychaeta). The BMWP-Colombia was calculated per site based on a summation of all tolerance scores of the macroinvertebrate taxa present. The total score for each site indicated ecological water quality, with categories ranging from very bad (0–15), bad (16–35), poor (36–60), moderate (61–100) to good (> 100). To obtain a more complete understanding of the community structure, species abundances were calculated and taxonomic richness (number of taxa) and Shannon–Wiener Diversity Index (Shannon and Wiener, 1949) were computed using the Vegan package (Oksanen *et al.*, 2016) for each sampling site.

Data analysis

All statistical analyses were done using R software (version 3.2.3) (R Core Team, 2015). The protocol for data exploration as described by Zuur *et al.* (2010) was used to avoid common statistical problems related to outliers and correlated variables. Prior to the actual data analysis, the initial data set was tested for outliers and correlations between explanatory variables. One sampling location with five extremely high and low values compared to the majority of observations (*e.g.*, conductivity = 49384 $\mu S.cm^{-1}$, TN = below detection limit, elevation = -4 m a.s.l.), was discarded from the analysis as high conductivity indicated saline conditions.

Spearman's rank correlation coefficient was used to explore the relationship among physico-chemical variables and the BMWP-Colombia ecological water quality index. A Mann–Whitney *U*-test was used to compare physico-chemical variables between more affected and less affected sampling sites in order to test for significant differences in environmental variables between these sites. Scatter plots were made to visualize the relationship between the

Table 1. Mean, median, maximum, minimum values and standard deviation of continuous environmental variables measured in the Portoviejo River and their Spearman's Rank correlation coefficients with the BMWP-Colombia index (*$P < 0.05$, **$P < 0.01$).

Variable	Mean	Median	Max.	Min.	SD	*r*
Velocity ($m.s^{-1}$)	0.38	0.45	0.88	0.00	0.29	0.48**
Temperature (°C)	27.71	27.91	31.33	25.56	1.40	− 0.38*
Conductivity ($\mu S.cm^{-1}$)	880	385	2447	164	722	− 0.39*
pH	7.87	7.85	8.81	6.50	0.41	− 0.05
Dissolved oxygen ($mg.L^{-1}$)	8.05	7.72	18.29	2.22	2.50	0.08
Chlorophyll ($\mu g.L^{-1}$)	13.50	7.21	55.16	1.86	15.18	− 0.47**
Turbidity (FTU)	14.46	12.40	34.54	0.00	11.20	0.09
BOD_5 ($mg.L^{-1}$)	3.02	2.94	5.86	0.79	1.47	− 0.21
Nitrate-nitrogen ($mg.L^{-1}$)	1.05	0.54	2.81	0.23	0.89	− 0.27
Nitrite-nitrogen ($mg.L^{-1}$)	0.04	0.03	0.14	0.00	0.04	− 0.33
Ammonium-nitrogen (mg. L^{-1})	0.09	0.08	0.19	0.04	0.04	0.30
Total nitrogen ($mg.L^{-1}$)	1.81	1.20	5.70	0.50	1.49	− 0.18
Orthophosphate ($mg.L^{-1}$)	0.20	0.21	0.33	0.05	0.08	− 0.17
Total phosphorus ($mg.L^{-1}$)	0.23	0.21	0.53	0.05	0.11	− 0.39*
Total organic carbon ($mg.L^{-1}$)	15.78	16.85	37.70	3.00	10.38	− 0.35
Elevation (m a.s.l.)	61	59	121	0	37	0.39*

BMWP-Colombia water quality index and all measured variables. A Kruskal–Wallis test followed by *post hoc* multiple pairwise comparisons was performed to test whether significant differences in ecological indices could be found between different types of land use, dominant substrate, sludge layer and pool-riffle pattern.

Threshold Indicator Taxa ANalysis (TITAN) was used to detect community shifts along each measured physico-chemical variable in the Portoviejo River. TITAN is a non-parametric technique that integrates occurrence, abundance and directionality of taxa responses along a physico-chemical gradient. For each value within this gradient, the data were split into two groups: one group consisting of taxa present at lower values (the so-called negative side of the partition, *i.e.,* the (z −)-group) and one group consisting of taxa present at higher values (the so-called positive side of the partition, *i.e.,* the (z +)-group). For each taxon, an optimal change point was determined as the value that maximizes the association of taxa within both groups. When passing this change point from low to high values, the abundance and frequency of occurrence of the (z −)-group will decrease, while an increase will be observed for the (z +)-group. To determine the accuracy of the change point value, bootstrapping (1000 repetitions) was implemented. This allowed us to derive two important diagnostic indices for evaluating the quality of the response for each taxon: purity and reliability. Purity is defined as the proportion of response directions (increasing or decreasing) when passing the change point that agree with the observed response. Pure indicators are consistently assigned the same response direction. Reliability is estimated by the proportion of change points that consistently result in the significant grouping of a taxon. Only taxa with high reliability (≥ 0.95) can be considered as indicator taxa. Graphical representation of the change point identification is supported by the summation of the standardized association values for each indicator taxon along the physico-chemical gradient, resulting in fsum(z −) and fsum(z +) scores. Both scores will obtain a maximum value along the physico-chemical gradient, representing the change points for both the (z −)-group and the (z +)-group separately, and can be defined as the community change point (of the considered taxa). TITAN was performed in the package TITAN 2 (Baker *et al.*, 2015) in R software (version R.3.2.3). Only taxa occurring in at least five sites were included in the TITAN (Baker *et al.*, 2015). Abundance data were not transformed because transformation is unnecessary in TITAN 2. 1000 permutations were used to determine species specific z-scores and related fsum(z)-scores, as this calculation is based on a small dataset, thus a higher number of permutations are recommended for more precise z-scores (Baker *et al.*, 2015). Further information and details of the TITAN method can be found in Baker and King (2010), King and Baker (2014) and Baker *et al.* (2015).

Results

Physico-chemical water quality

High oxygen levels were observed for most sampling sites. The lowest water velocity (0 $m.s^{-1}$) was measured at the reservoir. There was a significant negative correlation between elevation and conductivity, NO_3^-, NO_2^-, oPO_4^{3-}, TP and TOC ($r = -0.84$, $r = -0.81$, $r = -0.78$, $r = -0.76$, $r = -0.73$, $r = -0.86$, respectively) (Appendix 1). The lower conductivity values (< 880 $\mu S.cm^{-1}$) were observed at non-affected sites, while higher conductivity values (980–2447 $\mu S.cm^{-1}$) were measured at the affected and more downstream sites. The values of every variable measured at 30 sampling locations (one site was discarded from the analysis) within the Portoviejo River are presented in Table 1. Based on the Mann–Whitney *U*-test ($P < 0.05$), it was found that

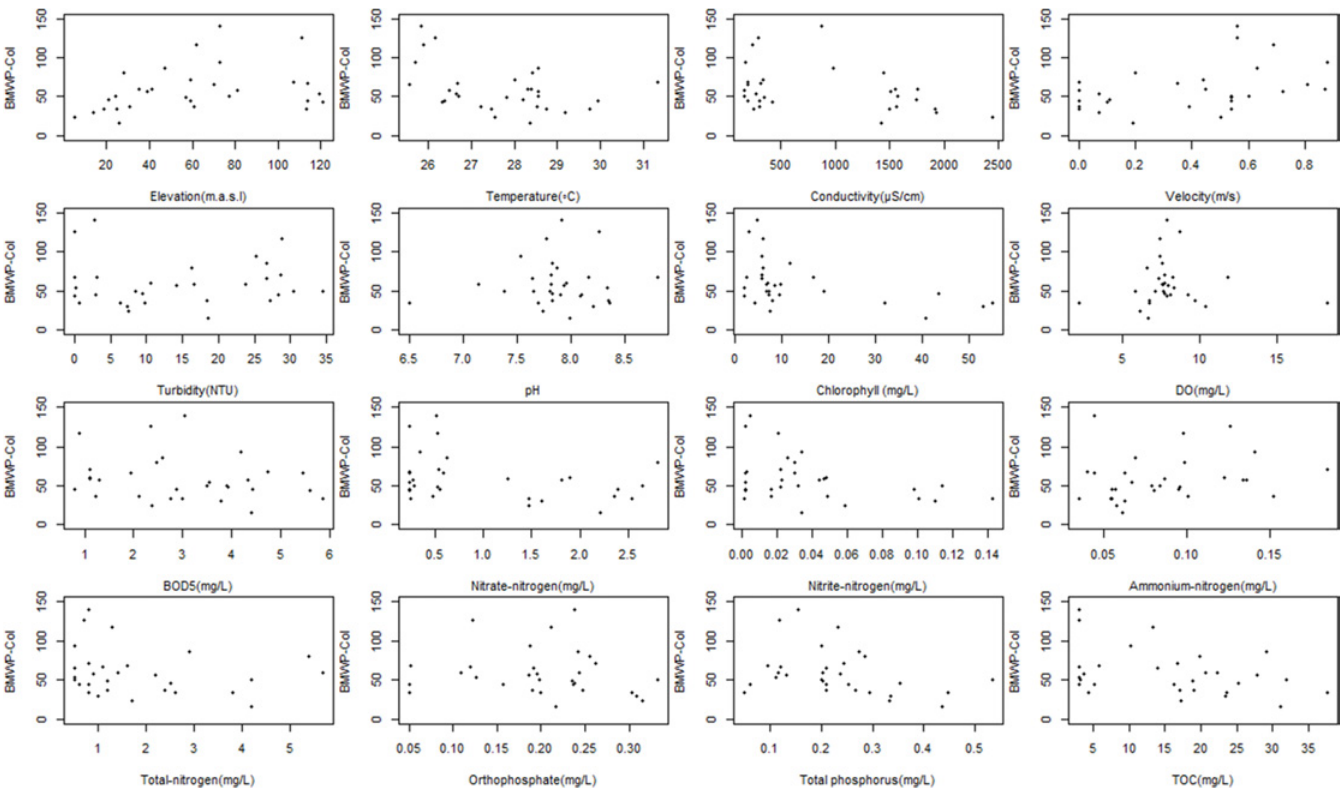

Fig. 2. Plots of physico-chemical variables in relation to BMWP-Colombia for sampling sites in the Portoviejo River.

conductivity and concentrations of NO_3^-, NO_2^-, oPO_4^{3-}, TN, TP, TOC and chlorophyll were significantly higher for more affected sites, compared with reference (less affected) sites.

Relationship between macroinvertebrate communities, physico-chemical conditions and habitat characteristics

In total, more than 8300 individuals belonging to 53 macroinvertebrate families were found (Appendix 2). The taxon richness varied from 4 to 22 taxa per sampling site. Chironomidae (Diptera) was the most frequently encountered taxon, followed by Coenagrionidae (Odonata) and Libellulidae (Odonata) (29, 21 and 20 sites, respectively). Thiaridae (Pectinibranchia) was the most abundant taxon, followed by Chironomidae (5231 and 805 individuals, respectively). Based on the BMWP-Colombia scores, the sampling sites of the Portoviejo River were categorized into four water quality classes: good, moderate, poor and bad (Fig. 1). The Shannon–Wiener index ranged from 0.23 to 2.58, representing from very low to intermediate community diversity. There was a strong positive correlation between the BMWP-Colombia scores and taxonomic richness (S) (Spearman's rank correlation coefficient = 0.94). The Spearman's rank correlation coefficient between BMWP-Colombia and Shannon's diversity index (H) was 0.58. The Spearman's rank correlation coefficient between the BMWP-Colombia and taxa abundance was 0.30. The highest BMWP-Colombia value (140) was recorded at one sampling site where the taxonomic richness was also the highest (22 taxa). This location is surrounded by forest, has gravel substrate and a moderately developed pool-riffle pattern.

The Spearman's rank correlation coefficients between the biological water quality index (BMWP-Colombia) and the physico-chemical variables indicated that the BMWP-Colombia scores were positively correlated with elevation and stream velocity (Table 1, Fig. 2). In addition, BMWP-Colombia values showed a negative association with temperature, conductivity, chlorophyll and TP (Table 1, Fig. 2). Higher BMWP-Colombia values were observed at sampling sites surrounded by arable land, gravel sediment, absence of a sludge layer and at least a moderately developed pool-riffle pattern (Fig. 3). However, the statistical analysis did not reveal any significant difference.

Threshold change points and indicator taxa

TITAN was used to detect the variation in taxonomic composition of macroinvertebrate communities in response to all physico-chemical variables. However, due to the low number of reliable indicator taxa, TITAN did not find strong evidence of a threshold for temperature, pH, DO, chlorophyll, turbidity, BOD_5, TP, oPO_4^{3-},

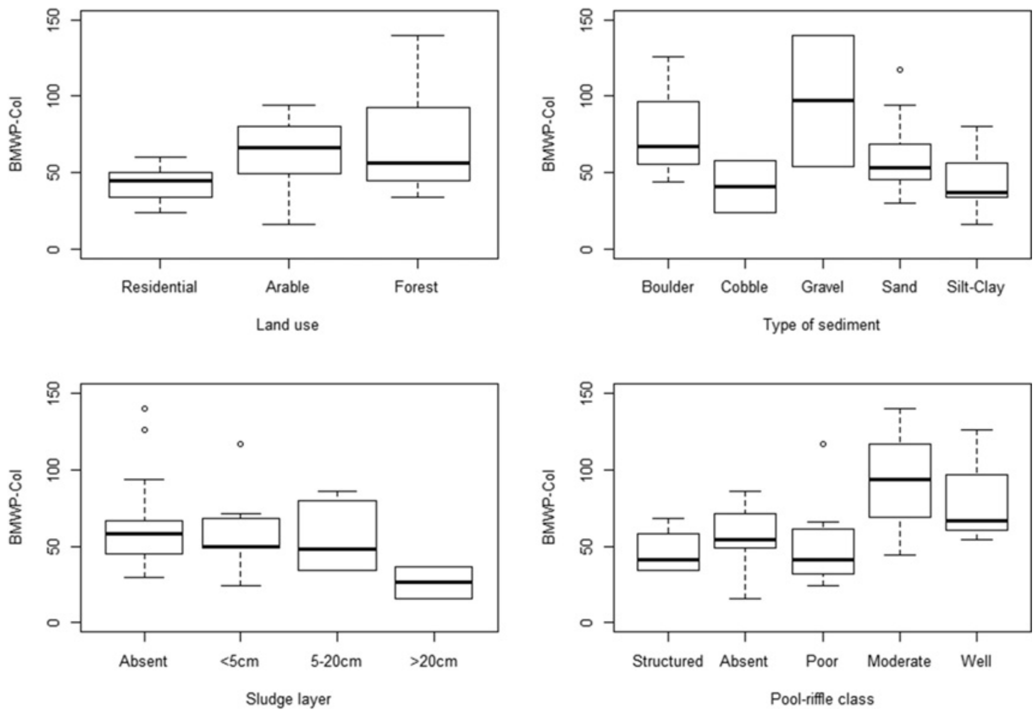

Fig. 3. Boxplots of the different classes of land use, type of sediment, sludge layer and pool-riffle class in relation to BMWP-Colombia for sampling sites in the Portoviejo River. Bold horizontal lines represent medians, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values; small black dots show outliers.

Table 2. Threshold Indicator Analysis (TITAN) community response results for observed changes points (Obs.) and selected quantiles (5, 50 and 95%) correspond to change points from 1000 bootstrap replicates of the observed data. Thresholds are given for only those taxa that are determined to be pure and reliable indicators.

Environmental gradient		Change points			
		Obs.	5%	50%	95%
Elevation (m.a.s.l.)	fsumz −	30	26	32	53
	fsumz +	58	44	58	66
Conductivity (μS.cm^{-1})	fsumz −	930	330	930	1160
	fsumz +	1430	663	982	1433
Nitrate-Nitrogen (mg.L^{-1})	fsumz −	0.6	0.2	0.6	1.5
	fsumz +	2.3	0.6	1.7	2.5

NH_4^+, NO_2, TN, TOC and stream velocity. TITAN could only reveal the community change along the gradient of elevation, conductivity and nitrate-nitrogen concentration (Table 3).

Two groups of indicator taxa were identified: (1) taxa preferring low altitude (z −), but high conductivity and nitrate concentrations (z +) and (2) taxa preferring high altitude (z +), but low conductivity and nitrate concentration (z −). The first group is characterized by a sharp increase in either abundance or frequency of occurrence when conductivity values go beyond 1430 μS.cm^{-1} and nitrate concentrations pass the 2.3 mg.L^{-1} change point. The second group will show a sharp decrease in either abundance or frequency of occurrence when conductivity values go beyond 930 μS.cm^{-1} and nitrate concentrations pass the 0.6 mg.L^{-1} change point (Table 2, Fig. 4(C) and (E)). In contrast, the first group is negatively influenced by increasing elevation (change point at 30 m a.s.l.), while the second group is positively influenced (change point at 58 m a.s.l.) (Table 2, Fig. 4(A)).

The threshold values were obtained for different taxa such as Atyidae (Decapoda), Thiaridae (Gastropoda) and Corbiculidae (Bivalvia), which displayed a negative response to increasing elevation (z −), while they showed a positive response to the increase of conductivity and nitrate-nitrogen (z +). In contrast to those taxa, Acari, Baetidae (Ephemeroptera) and Leptohyphidae (Ephemeroptera) showed a positive response to increasing elevation (z +, Fig. 4(B)) and displayed a negative response to increasing conductivity (z −, Fig. 4(D)) and increasing nitrate-nitrogen values (z −, Fig. 4(F)). Naucoridae (Hemiptera) showed a positive response to an increase in elevation (z +, Fig. 4(B)), while Veliidae (Hemiptera) and Libellulidae (Odonata) showed a negative response to an increase of conductivity and nitrate-nitrogen, respectively (z −, Fig. 4(D) and (F)).

Table 3. Threshold Indicator Taxa Analysis of individual taxa in response to (a) elevation, (b) conductivity and (c) Nitrate-nitrogen in the Portoviejo River. Taxa are listed in alphabetic order.

Taxa	Shortcode	ienv.cp	zenv.cp	freq	maxgrp	IndVal	obsiv.prob	zscore	5 (%)	50 (%)	95 (%)	Purity	reliability	z.median	filter
a. Elevation (m a.s.l.)															
Acari	**ACARI**	**94**	**94**	**6**	**2**	**71.3**	**0.00**	**7.01**	**72**	**94**	**112**	**1.00**	**0.99**	**6.64**	**2**
Atyidae	**ATYIDAE**	**26**	**26**	**6**	**1**	**83.0**	**0.00**	**7.59**	**25**	**28**	**35**	**1.00**	**0.99**	**7.54**	**1**
Baetidae	**BAETIDAE**	**40**	**52**	**19**	**2**	**77.7**	**0.00**	**5.06**	**38**	**44**	**58**	**1.00**	**1.00**	**4.97**	**2**
Belostomatidae	BELOSTOM	27	27	9	2	39.1	0.09	1.60	27	58	109	0.86	0.55	2.34	0
Calopterygidae	CALOPTER	75	75	11	1	52.4	0.02	2.80	27	75	90	0.78	0.86	2.89	0
Cambaridae	CAMBARID	33	33	6	1	27.9	0.19	1.20	26	41	94	0.80	0.43	1.91	0
Ceratopogonidae	CERATOPO	109	109	8	2	29.4	0.25	0.59	29	61	114	0.71	0.38	1.70	0
Chironomidae	CHIRONOM	25	25	29	1	70.9	0.23	0.84	23	59	114	0.70	0.45	1.76	0
Coenagrionidae	COENAGRI	75	75	21	1	69.9	0.01	3.14	25	75	112	0.85	0.87	3.29	0
Corbiculidae	**CORBICUL**	**52**	**52**	**9**	**1**	**61.2**	**0.00**	**3.60**	**30**	**49**	**53**	**1.00**	**0.97**	**4.36**	**1**
Corydalidae	CORYDALI	62	62	3	2	23.1	0.07	2.12	59	62	109	0.94	0.41	2.35	0
Dryopidae	DRYOPIDA	72	72	3	2	27.3	0.00	2.85	66	73	94	0.96	0.57	3.24	0
Elmidae	ELMIDAE	33	33	6	2	28.6	0.14	1.42	32	72	109	0.90	0.50	2.42	0
Gerridae	GERRIDAE	112	44	5	2	18.3	0.46	0.40	21	56	107	0.46	0.18	1.47	0
Gomphidae	GOMPHIDA	26	26	17	2	48.0	0.24	0.71	20	62	113	0.61	0.50	1.96	0
Haliplidae	HALIPLID	44	44	9	2	50.0	0.01	3.85	41	48	79	0.99	0.90	3.87	0
Hydrophilidae	HYDROPHI	27	27	5	2	21.7	0.15	0.48	28	58	94	0.48	0.24	−0.99	0
Hydropsychidae	HYDROPSY	112	112	9	1	36.0	0.25	0.77	33	72	112	0.71	0.37	1.67	0
Hydroptilidae	HYDROPTI	109	109	4	2	30.3	0.09	2.08	30	73	111	0.85	0.47	2.65	0
Leptoceridae	LEPTOCER	59	59	9	2	42.4	0.04	2.07	30	59	114	0.92	0.71	2.72	0
Leptohyphidae	**LEPTOHYP**	**58**	**58**	**11**	**2**	**68.8**	**0.00**	**4.30**	**50**	**58**	**66**	**1.00**	**1.00**	**4.85**	**2**
Leptophlebiidae	LEPTOPHL	62	62	8	2	46.8	0.01	4.02	38	62	109	1.00	0.90	4.32	0
Libellulidae	LIBELLUL	94	44	20	2	63.9	0.06	1.72	22	37	94	0.87	0.63	2.42	0
Limoniidae	LIMONIID	62	62	7	2	34.4	0.04	2.14	27	62	98	0.88	0.62	2.62	0
Naucoridae	**NAUCORID**	**62**	**62**	**12**	**2**	**60.1**	**0.01**	**3.44**	**35**	**58**	**66**	**1.00**	**0.98**	**4.19**	**2**
Nepidae	NEPIDAE	94	94	3	2	25.9	0.09	2.31	33	92	113	0.79	0.40	2.26	0
Palaemonidae	PALAEMON	25	25	4	1	59.6	0.00	6.00	23	26	35	0.99	0.92	6.17	0
Perlidae	PLEIDAE	52	52	6	2	35.3	0.05	2.64	49	53	112	0.97	0.67	3.01	0
Pyralidae	PYRALIDA	72	72	4	2	20.5	0.26	0.97	35	68	109	0.74	0.34	1.78	0
Scirtidae	SCIRTIDA	40	72	3	1	15.8	0.29	0.84	38	62	72	0.39	0.12	1.46	0
Stratiomyidae	STRATIOM	44	44	5	2	27.8	0.12	1.74	43	58	94	0.86	0.37	1.98	0
Tabanidae	TABANIDA	60	60	3	1	18.8	0.22	1.51	30	59	61	0.80	0.19	1.73	0
Thiaridae	**THIARIDA**	**52**	**44**	**17**	**1**	**89.1**	**0.00**	**3.79**	**36**	**49**	**66**	**0.99**	**0.97**	**3.91**	**1**
Tubificidae	TUBIFICI	27	94	6	1	26.1	0.27	0.71	27	44	94	0.58	0.22	1.48	0
Veliidae	VELIIDAE	62	62	11	2	57.3	0.01	3.60	44	62	79	0.98	0.92	3.95	0
b. Conductivity (μS.cm^{-1})															
Acari	**ACARI**	**198**	**198**	**6**	**1**	**59.1**	**0.01**	**4.83**	**198**	**282**	**331**	**1.00**	**0.96**	**4.95**	**1**
Atyidae	**ATYIDAE**	**1434**	**1434**	**6**	**2**	**54.6**	**0.00**	**4.60**	**1164**	**1434**	**1751**	**1.00**	**0.98**	**5.27**	**2**
Baetidae	**BAETIDAE**	**1200**	**1200**	**19**	**1**	**78.6**	**0.00**	**4.93**	**344**	**930**	**1475**	**1.00**	**1.00**	**5.19**	**1**
Belostomatidae	BELOSTOM	219	219	9	1	66.7	0.01	4.14	218	226	1555	0.98	0.84	4.14	0
Calopterygidae	CALOPTER	1662	1555	11	1	36.5	0.20	0.77	198	612	1628	0.46	0.42	1.80	0
Cambaridae	CAMBARID	1568	930	6	2	24.5	0.21	0.95	218	930	1555	0.60	0.45	2.02	0
Ceratopogonidae	CERATOPO	1662	1662	8	1	32.0	0.34	0.67	255	982	1662	0.51	0.24	1.49	0

Table 3. (*Contd.*)

Taxa	Shortcode	ienv.cp	zenv.cp	freq	maxgrp	IndVal	obsiv.prob	zscore	5 (%)	50 (%)	95 (%)	Purity	reliability	z.median	filter
Chironomidae	CHIRONOM	302	302	29	2	67.5	0.20	0.92	198	328	1751	0.68	0.50	1.85	0
Coenagrionidae	COENAGRI	1434	1434	21	2	46.7	0.33	0.27	186	310	1837	0.65	0.39	1.60	0
Corbiculidae	**CORBICUL**	**930**	**930**	**9**	**2**	**61.2**	**0.00**	**3.65**	**664**	**930**	**1189**	**0.98**	**0.95**	**3.87**	**2**
Corydalidae	CORYDALI	302	302	3	1	18.3	0.09	1.21	246	302	1148	0.80	0.22	1.68	0
Dryopidae	DRYOPIDA	930	302	3	1	17.6	0.16	1.12	186	302	1148	0.84	0.27	1.84	0
Elmidae	ELMIDAE	302	302	6	1	20.0	0.31	0.28	196	652	1750	0.57	0.29	1.62	0
Gerridae	GERRIDAE	1200	1200	5	1	18.3	0.48	0.34	196	425	1751	0.56	0.22	1.53	0
Gomphidae	GOMPHIDA	1662	1662	17	1	55.9	0.12	1.31	219	1200	1750	0.60	0.58	2.19	0
Haliplidae	HALIPLID	198	1200	9	1	50.0	0.01	3.74	198	385	1216	1.00	0.93	4.46	0
Hydrophilidae	HYDROPHI	1475	1475	5	1	25.0	0.22	1.07	262	930	1477	0.56	0.24	1.34	0
Hydropsychidae	HYDROPSY	219	652	9	2	33.3	0.15	1.07	219	652	1750	0.85	0.55	2.16	0
Hydroptilidae	HYDROPTI	1475	1475	4	1	20.0	0.27	0.96	282	371	1477	0.54	0.14	1.54	0
Leptoceridae	LEPTOCER	1200	1200	9	1	41.5	0.05	2.09	246	1200	1568	0.85	0.59	2.27	0
Leptohyphidae	**LEPTOHYP**	**930**	**930**	**11**	**1**	**64.7**	**0.00**	**3.78**	**302**	**930**	**1164**	**1.00**	**0.99**	**4.19**	**1**
Leptophlebiidae	LEPTOPHL	302	302	8	1	37.8	0.06	2.27	196	330	1568	0.93	0.71	3.02	0
Libellulidae	LIBELLUL	1200	1200	20	1	63.9	0.06	1.71	195	1148	1751	0.84	0.62	2.39	0
Limoniidae	LIMONIID	198	302	7	1	46.4	0.01	3.76	186	270	1475	0.98	0.87	4.48	0
Naucoridae	NAUCORID	1200	1200	12	1	59.0	0.01	3.37	246	1200	1526	1.00	0.95	3.73	0
Nepidae	NEPIDAE	198	198	3	1	37.5	0.04	3.50	197	219	1526	0.80	0.52	2.90	0
Palaemonidae	PALAEMON	1662	1662	4	2	59.6	0.01	5.76	1216	1625	1837	0.99	0.87	5.30	0
Perlidae	PLEIDAE	385	385	6	1	40.0	0.02	3.16	262	385	664	1.00	0.84	3.58	0
Pyralidae	PYRALIDA	1526	1526	4	1	18.2	0.44	0.51	196	612	1526	0.57	0.21	1.34	0
Scirtidae	SCIRTIDA	246	246	3	1	25.9	0.08	2.36	226	255	1750	0.66	0.42	2.68	0
Stratiomyidae	STRATIOM	1200	1200	5	1	27.8	0.11	1.76	270	1200	1241	0.78	0.34	1.96	0
Tabanidae	TABANIDA	330	330	3	2	17.7	0.08	1.25	319	365	1475	0.58	0.14	1.52	0
Thiaridae	**THIARIDA**	**930**	**1200**	**17**	**2**	**89.1**	**0.00**	**3.64**	**330**	**930**	**1418**	**0.98**	**0.96**	**3.96**	**2**
Tubificidae	TUBIFICI	1568	1568	6	1	25.0	0.15	0.55	182	310	1555	0.63	0.42	1.90	0
Veliidae	**VELIIDAE**	**302**	**302**	**11**	**1**	**61.6**	**0.00**	**3.83**	**198**	**302**	**1200**	**0.99**	**0.95**	**4.32**	**1**
c. Nitrate-nitrogen (mg.L^{-1})															
Acari	**ACARI**	**0.25**	**0.25**	**6**	**1**	**71.3**	**0.00**	**6.94**	**0.23**	**0.25**	**0.43**	**1.00**	**0.98**	**6.47**	**1**
Atyidae	**ATYIDAE**	**2.29**	**2.29**	**6**	**2**	**73.4**	**0.00**	**6.17**	**1.04**	**1.54**	**2.45**	**1.00**	**0.97**	**6.35**	**2**
Baetidae	**BAETIDAE**	**1.36**	**0.60**	**19**	**1**	**77.7**	**0.00**	**4.91**	**0.56**	**1.03**	**1.36**	**1.00**	**0.99**	**4.94**	**1**
Belostomatidae	BELOSTOM	0.53	0.53	9	1	29.8	0.19	1.05	0.23	0.54	2.38	0.72	0.46	1.98	0
Calopterygidae	CALOPTER	0.32	0.32	11	2	52.4	0.02	2.79	0.31	0.32	1.36	0.91	0.86	3.06	0
Cambaridae	CAMBARID	2.29	2.29	6	2	55.0	0.02	3.53	0.48	2.06	2.47	0.93	0.73	3.53	0
Ceratopogonidae	CERATOPO	0.23	0.23	8	1	38.5	0.09	1.36	0.23	0.53	2.29	0.63	0.46	1.92	0
Chironomidae	CHIRONOM	1.47	1.47	29	1	67.5	0.20	0.94	0.25	0.57	2.29	0.56	0.47	1.81	0
Coenagrionidae	COENAGRI	0.24	0.32	21	2	69.9	0.01	2.96	0.24	0.32	2.22	0.93	0.88	3.49	0
Corbiculidae	**CORBICUL**	**1.86**	**1.86**	**9**	**2**	**69.9**	**0.01**	**4.33**	**0.57**	**1.66**	**2.47**	**1.00**	**0.98**	**5.06**	**2**
Corydalidae	CORYDALI	0.53	0.53	3	1	23.1	0.06	2.26	0.23	0.53	0.54	0.94	0.44	2.37	0
Dryopidae	DRYOPIDA	0.52	0.52	3	1	25.0	0.06	2.51	0.23	0.52	0.52	0.95	0.57	3.00	0
Elmidae	ELMIDAE	2.06	0.52	6	1	23.8	0.19	0.90	0.23	0.52	1.86	0.82	0.41	2.07	0
Gerridae	GERRIDAE	1.71	1.71	5	1	22.7	0.29	0.89	0.23	0.57	1.71	0.55	0.17	1.46	0
Gomphidae	GOMPHIDA	1.71	1.71	17	2	52.4	0.11	1.37	0.26	0.61	2.21	0.75	0.58	2.23	0
Haliplidae	HALIPLID	0.93	0.54	9	1	49.2	0.01	3.62	0.37	0.56	1.04	1.00	0.93	4.25	0

Table 3. (*Contd.*)

Taxa	Shortcode	ienv.cp	zenv.cp	freq	maxgrp	IndVal	obsiv.prob	zscore	5 (%)	50 (%)	95 (%)	Purity	reliability	z.median	filter
Hydrophilidae	HYDROPHI	2.29	2.29	5	2	19.2	0.54	0.11	0.25	0.56	2.47	0.58	0.38	1.76	0
Hydropsychidae	HYDROPSY	2.06	0.60	9	2	28.6	0.29	0.52	0.23	0.49	1.54	0.65	0.41	1.68	0
Hydroptilidae	HYDROPTI	0.23	0.23	4	1	37.0	0.07	2.74	0.23	0.28	2.39	0.79	0.53	2.88	0
Leptoceridae	LEPTOCER	0.93	0.93	9	1	41.5	0.09	1.79	0.24	0.93	1.07	0.82	0.58	2.25	0
Leptohyphidae	**LEPTOHYP**	**0.60**	**0.60**	**11**	**1**	**64.7**	**0.00**	**3.82**	**0.41**	**0.60**	**1.03**	**1.00**	**0.99**	**4.19**	**1**
Leptophlebiidae	LEPTOPHL	0.53	0.53	8	1	46.8	0.01	3.89	0.23	0.53	1.36	0.99	0.89	4.14	0
Libellulidae	**LIBELLUL**	**1.47**	**1.47**	**20**	**1**	**80.7**	**0.00**	**3.38**	**0.24**	**1.47**	**2.06**	**0.97**	**0.96**	**3.31**	**1**
Limoniidae	LIMONIID	0.53	0.53	7	1	34.4	0.04	2.17	0.24	0.53	1.25	0.86	0.66	2.76	0
Naucoridae	NAUCORID	0.93	0.93	12	1	59.0	0.01	3.18	0.52	0.93	1.26	0.98	0.90	3.48	0
Nepidae	NEPIDAE	0.23	0.23	3	1	37.5	0.04	3.87	0.23	0.24	1.86	0.76	0.53	3.05	0
Palaemonidae	PALAEMON	1.36	1.36	4	2	36.4	0.01	3.46	1.03	1.43	2.36	0.99	0.81	4.02	0
Perlidae	PLEIDAE	0.54	0.54	6	1	40.0	0.01	3.46	0.25	0.54	0.57	0.99	0.83	3.67	0
Pyralidae	PYRALIDA	0.52	0.52	4	1	18.1	0.22	0.80	0.23	0.52	1.71	0.67	0.38	1.85	0
Scirtidae	SCIRTIDA	0.52	0.52	3	2	16.7	0.27	0.99	0.44	0.56	1.36	0.63	0.12	1.53	0
Stratiomyidae	STRATIOM	0.93	0.93	5	1	27.8	0.12	1.74	0.23	0.54	1.04	0.85	0.44	2.14	0
Tabanidae	TABANIDA	0.53	0.53	3	2	17.7	0.08	1.20	0.50	0.54	2.47	0.66	0.21	1.81	0
Thiaridae	**THIARIDA**	**1.86**	**1.54**	**17**	**2**	**98.0**	**0.00**	**4.09**	**0.58**	**1.54**	**2.21**	**0.98**	**0.98**	**4.49**	**2**
Tubificidae	TUBIFICI	2.29	2.29	6	2	36.4	0.11	1.60	0.25	1.04	2.39	0.72	0.37	1.87	0
Veliidae	VELIIDAE	0.53	0.53	11	1	57.3	0.00	3.74	0.26	0.53	1.71	0.99	0.93	4.00	0

Ienv.cp – environmental change point for each taxon based on IndVal maximum; zenv.cp – environmental change point for each taxon based on z maximum; freq – number of times each taxon occurred in the data set; maxgrp – 1 if z- (negative response); 2 if z+ (positive response); IndVal-Dufrene and Legendre 1997 IndVal statistic, scaled 0–100% (with 100 indicating a taxon that occurred in all of the samples above or below a change point value and in none of the samples on the other side of the change point); obsiv.prob – the probability of obtaining an equal or larger IndVal score from random data; (number of random IndVals $\geqq$ observed IndVal)/ numPerm); zscore – IndVal z score; 5%, 50%, 95% – change point quantiles among bootstrap replicates; purity – proportion of replicates matching observed maxgrp assignment; reliability – proportion of replicate obsiv.prob values $\leqq 0.05$; z.median – median score magnitude across all bootstrap replicates; filter – logical (if > 0) indicating whether each taxa met purity and reliability criteria, value indicates maxgrp assignment. Negatively and positively associated taxa are shown in bold.

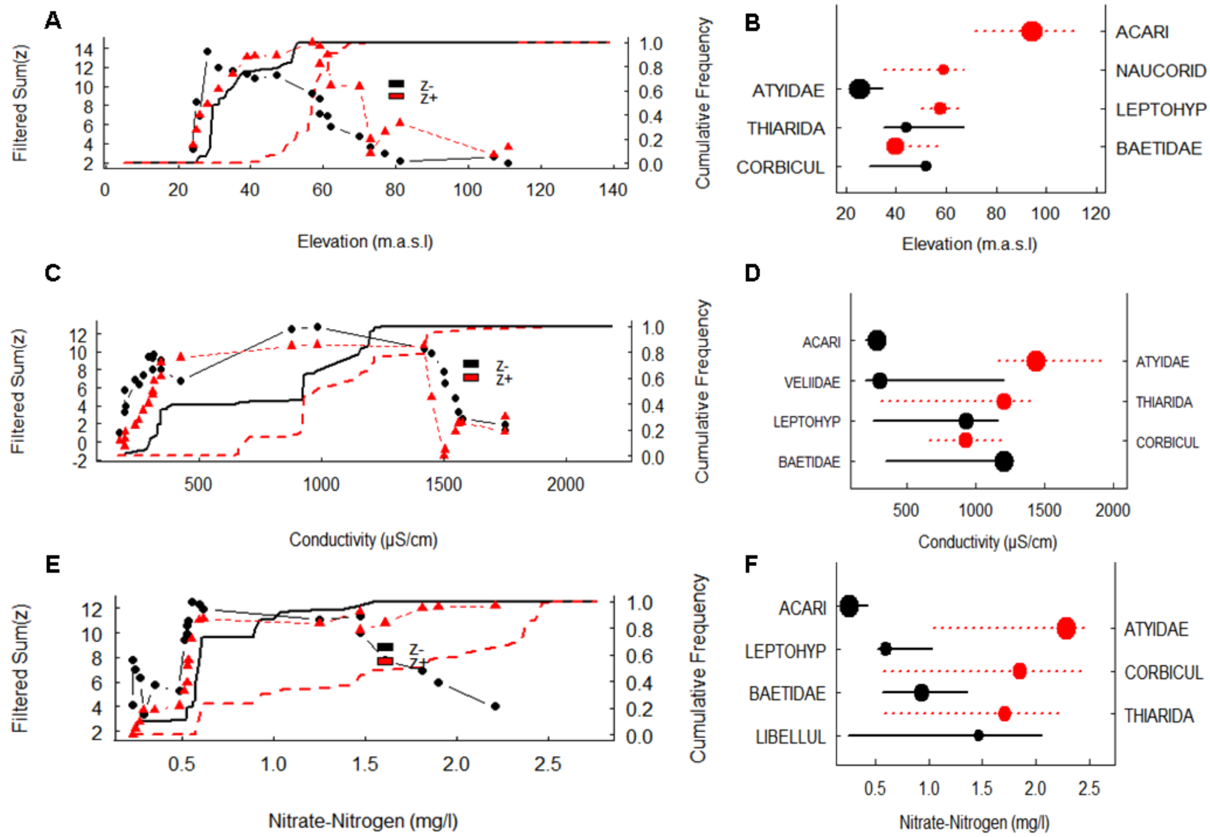

Fig. 4. Threshold Indicator Taxa ANalysis and change points (dots) for the pure and reliable indicator taxa response to elevation (A, B), conductivity (C,D) and nitrate-nitrogen (E, F) gradient ($P \leq 0.05$, purity = 0.95, reliability = 0.95 for 5 minimum number of observations, 1000 bootstrap and 1000 permutation replicates). Negatively associated taxa (z–) are indicated by black symbols and lines and positively associated taxa (z +) are indicated by red symbols and dashed lines. Solid and dashed lines are cumulative frequency distributions of sum(z–) and sum(z +) maxima (respectively) across bootstrap replicates. Size of change point symbol (dots) is proportional to the magnitude of the taxa response. Z − species responded negatively to an increase in the environmental gradient, Z + species responded positively to an increase in the gradient. Horizontal lines suggest 5–95% quantiles from the bootstrapped change point distribution. ACARI, Acari; ATYIDAE, Atyidae; BAETIDAE, Baetidae; CORBICUL, Corbiculidae, LEPTOHYP, Leptohyphidae; LIBELLUL, Libellulidae; NAUCORID, Naucoridae, THIARIDA, Thiaridae; VELIIDAE, Veliidae.

Discussion

Water quality of the portoviejo river

The majority of the sampling locations have DO concentrations ranging from 7 to 10 mg.L^{-1}. The lowest value of DO (2.2 mg.L^{-1}) was observed at the Poza Honda reservoir, which was characterized by the highest BOD_5 (5.9 mg.L^{-1}), a sludge layer between 5 and 20 cm, a water hyacinth coverage of 50–75% and stagnant water. Based on the ecological water quality index (BMWP-Colombia), this sampling site (site Po3, Fig. 1) had bad water quality with seven taxa present. This finding may be explained by a combination of unfavorable conditions (*e.g.,* low stream velocity, thick sludge layer) for rheophilic taxa and the fact that when the percentage of water hyacinth cover is higher than 50%, the cover might be too dense, which negatively affects the physico-chemical water quality (*e.g.,* DO, BOD_5) (Nguyen *et al.*, 2015). Flow velocity is often considered as one of the most important variables that influences macroinvertebrate communities (Forio *et al.*, 2015; Damanik-Ambarita *et al.*, 2016). In this respect, damming causes unfavorable changes in the riverine biota through changes in flow regime, sediment transport and habitat modification (Käiro *et al.*, 2011). As the Poza Honda dam started operating in 1971, there might be sediment accumulation. Sedimentation can negatively affect the macroinvertebrate community by changing the suitability of the substrate for some taxa, increasing drift due to sediment deposition, affecting respiration due to the deposition and affecting feeding activities (Wood and Armiage, 1997).

The negative correlation between elevation and conductivity, nutrient concentrations (*e.g.,* NO_3^-, NO_2^-, oPO_4^{3-}, TP) and TOC (Appendix 1) indicates the cumulative negative impacts of human disturbance on water quality from upstream to downstream in the Portoviejo River. The affected sites have higher conductivity, a higher chlorophyll concentration and higher concentrations of NO_3^-, NO_2^-, oPO_4^{3-}, TN, TP and TOC, compared with less affected sites. The Portoviejo River suffers from a high level of environmental deterioration caused by deforestation, burning of vegetation, drainage of agrochemicals and fertilizers, garbage disposal and discharge of sewage without adequate previous treatment (Párraga and Aguirre, 2010; ACBIO, 2012). Nutrients (*e.g.,* NO_3^-) enter the water as a result of domestic wastewater discharge, agricultural activities (*e.g.,* using manure and fertilizer containing NO_3^-) and as a result of oxidation of nitrogenous waste products in human and animal excreta (Singh and Sharma, 2014).

Lower BMWP-Colombia scores were reported for sites located right after the outlets of wastewater treatment plants (sites Po31 and Po34, Fig. 1), in front of a small weir (site Po47, Fig. 1) and right after the small weir (site Po40, Fig. 1); with chlorophyll concentrations higher than 30 μg.L^{-1}. It is estimated that each year roughly 20 million cubic meters of wastewater are discharged into the Portoviejo River (ACBIO, 2012). As such, the Portoviejo River continues to suffer from sewage pollution, as the related increase in nutrient concentrations supports phytoplankton growth. Our results indicate negative effects of anthropogenic disturbances on biological diversity and water quality in the Portoviejo River. Based on our results, we emphasize the need for management actions to control the diffuse pollution and future investments in wastewater treatment in order to reduce the nutrient load in the river.

Relevance of the TITAN method: indicator taxa of environmental change and threshold for macroinvertebrates

TITAN uncovered a clear community change along both human affected gradients (*e.g.,* conductivity and nitrate-nitrogen) and a natural gradient (*e.g.,* elevation). For instance, a community change point for tolerant taxa (z +) was observed at 1430 μS.cm^{-1}, which is close to the findings of Schröder *et al.* (2015), who determined a community threshold for tolerant taxa at 1464 μS.cm^{-1} in the Lippe River, Germany. The change point for sensitive indicator taxa (z −) at a conductivity value of 930 μS.cm^{-1} in the Portoviejo River is similar to the threshold identified by Schröder *et al.* (2015) at 926 μS.cm^{-1}. In this study, half of the sites (*i.e.,* less affected sites) had conductivity values lower than 500 μS.cm^{-1}, the other half (*i.e.,* affected sites) had values higher than 1400 μS.cm^{-1} and only two sites had conductivity values in between (Fig. 4(C)). Moreover, only four taxa (Acari, Veliidae, Leptohyphidae and Baetidae) showed a negative association (z −) with increasing conductivity. In this case, it is possible that the community change point was high due to the limited amount of samples in the middle section of the conductivity gradient (only two). Therefore, the results should be carefully considered before use.

Nitrate-nitrogen was found to influence community composition at a concentration of 0.6 mg.L^{-1} (for sensitive (z −) taxa) and 2.3 mg.L^{-1} (for tolerant (z +) taxa). There was a strong relationship between conductivity and NO_3^- ($r = 0.82$), NO_2^- ($r = 0.75$) and TOC ($r = 0.73$), suggesting that a similar effect could be observed for NO_2^- and TOC gradients. However, these were not observed when applying TITAN. Nevertheless, the observed influence of NO_3^- is consistent with the literature, as it has been shown that there is a strong relationship between nutrient concentrations and macroinvertebrate communities (Ashton *et al.*, 2014).

Combining the observed change points and the ecological water quality shows that the less affected sites had a conductivity lower than 880 μS.cm^{-1} and a nitrate-nitrogen concentration lower than 0.6 mg.L^{-1} (Appendix 3), while the ecological water quality ranged from poor to good. On the other hand, at the affected sites, conductivity was higher than 980 μS.cm^{-1} and the nitrate-nitrogen concentration was higher than 0.6 mg.L^{-1} (Appendix 3), while the ecological water quality ranged from bad to poor. This supports the assumption that macroinvertebrate communities respond predictably to the degradation of water quality in the Portoviejo River. However, biological communities are always affected by multiple factors, making it impossible to separate the effects of each factor when they co-vary (Berger *et al.*, 2016). Therefore, the identified thresholds for conductivity and nitrate-nitrogen should be seen as preliminary results.

Finally, elevation was found to influence the macroinvertebrate community with a change point at 30 m a.s.l. (fsumz −) and at 58 m a.s.l. (fsumz +). Yet, elevation is a natural gradient that was highly correlated with conductivity ($r = -0.84$) and nitrate-nitrogen ($r = 0.81$); thus, a similar pattern in community shift was expected. The elevation of the sampling sites in the Portoviejo River ranged between 0 and 121 m a.s.l., which is very low. As such, the reported change points for elevation may not represent the influence of altitude on the community, but indicate the cumulative negative impacts of human disturbance on water quality from upstream to downstream in the Portoviejo River.

Among the tolerant taxa, three widely distributed taxa were present: Atyidae, Thiaridae and Corbiculidae. Moreover, these taxa are also considered to be invasive, which highlights the importance of identifying change points so as to conserve sensitive species and predict local community composition (Schröder *et al.*, 2015). With increasing conductivity and nitrate-nitrogen, sensitive taxa like Baetidae and Leptohyphidae will show a decrease in abundance and frequency of occurrence, which will reduce the indigenous community composition and allow for invasive species to take over. Based on these observations, management related to aquatic conservation, biological invasions, ecosystem restoration and natural resources can be performed (King and Baker, 2010). Moreover, these change point values have valuable applications for detecting reference condition boundaries and selecting sites at greatest risk of significant change (Kovalenko *et al.*, 2014). Nevertheless, the obtained change point values should be considered as preliminary results, as this is only the first publication on macroinvertebrate communities in the

Portoviejo River and therefore require confirmation through future research.

Conclusions

In the present research, we provided baseline information about the physico-chemical water quality and the macroinvertebrate community composition in the Portoviejo River (Ecuador). The BMWP-Colombia scores showed that water quality of the sampling sites within the Portoviejo River ranged from good to bad. TITAN revealed clear tipping points in elevation, conductivity and nitrate-nitrogen concentrations and associated indicator taxa. Atyidae, Corbiculidae and Thiaridae showed a positive response to the increase in conductivity and nitrate-nitrogen, while they were assigned a negative response to increasing elevation. In contrast to these taxa, Acari, Baetidae and Leptohyphidae showed a negative response to the increase in conductivity and nitrate-nitrogen, while they were assigned a positive response to increasing elevation. Based on these correlations, these taxa can be considered as indicator taxa that significantly change in abundance and frequency of occurrence due to the change in water quality along the Portoviejo River. Based on the patterns that are characterized in this research, novel management approaches can be developed and implemented.

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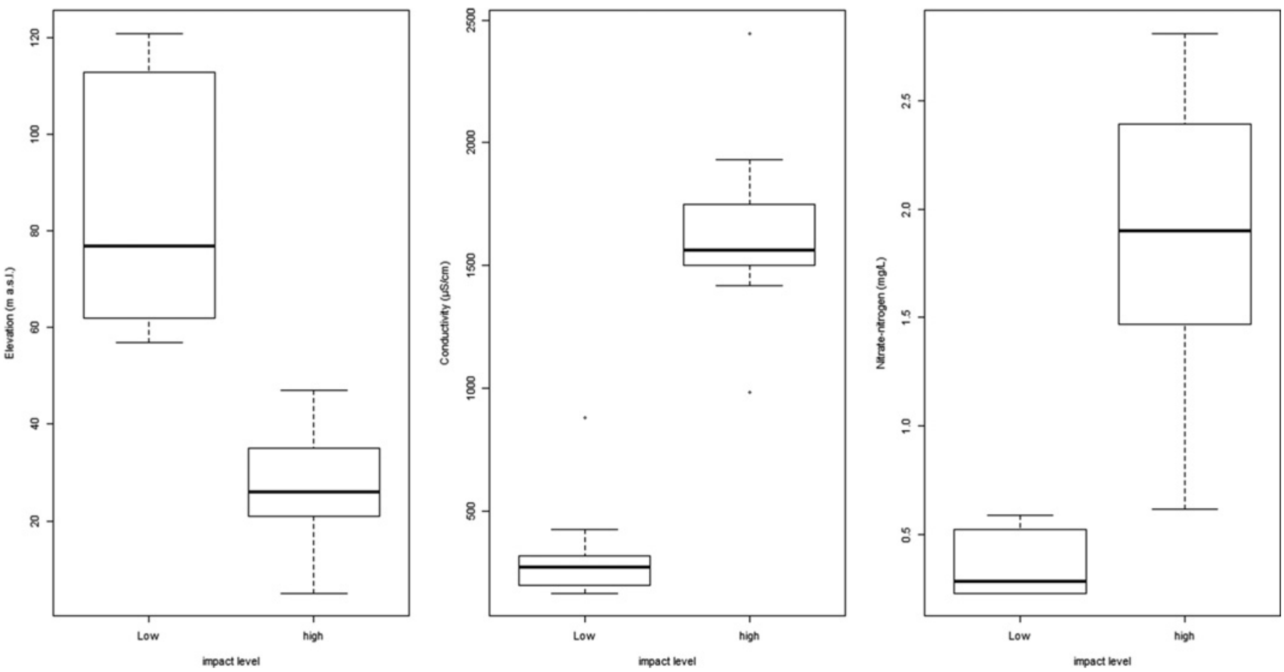
Appendix

Appendix 1. Spearman's rank correlation matrix of the 16 environmental variables of the Portoviejo River dataset ($n = 30$). Correlation coefficients with an absolute value of at least 0.70 are marked in bold.

	Elevation	Velocity	Temperature	Conductivity	pH	DO	Chlorophyll	Turbidity	BOD_5	NO_3^-	NO_2^-	NH_4^+	TN	oPO_4^{3-}	TP	TOC
Elevation	1															
Velocity	− 0.22	1														
Temperature	− 0.33	− 0.39	1													
Conductivity	− **0.84**	0.05	0.46	1												
pH	0.03	− 0.21	0.36	0.1	1											
DO	− 0.07	− 0.31	0.38	0.11	0.69	1										
Chlorophyll	− 0.57	− 0.33	0.53	0.58	0.23	0.49	1									
Turbidity	− 0.28	0.42	− 0.25	− 0.23	− 0.28	− 0.13	− 0.15	1								
BOD_5	0.51	− 0.51	0.05	− 0.3	− 0.12	− 0.05	0.08	− 0.43	1							
NO_3^-	− **0.81**	0.05	0.43	**0.82**	0	− 0.08	0.52	− 0.06	− 0.31	1						
NO_2^-	− **0.78**	0.01	0.41	**0.75**	0.03	0.34	**0.79**	− 0.05	− 0.18	**0.7**	1					
NH_4^+	− 0.08	0.23	− 0.16	− 0.08	− 0.16	− 0.22	− 0.32	0.37	− 0.37	0.08	− 0.1	1				
TN	− 0.61	− 0.06	0.47	0.63	0.1	0.07	0.36	− 0.07	− 0.21	**0.79**	0.48	0.03	1			
oPO_4^{3-}	− **0.76**	0.34	− 0.06	0.54	− 0.08	− 0.07	0.33	0.38	− 0.39	0.52	0.58	0.08	0.22	1		
TP	− **0.73**	0.09	0.14	0.55	− 0.04	− 0.02	0.58	0.21	− 0.13	0.66	**0.7**	− 0.07	0.44	0.83	1	
TOC	− **0.86**	0.15	0.48	**0.73**	0.11	0.23	0.65	0.21	− 0.41	**0.74**	**0.76**	0	0.68	0.58	0.68	1

Appendix 2. List of all families and their tolerance score of macroinvertebrate taxa collected in the Portoviejo river.

Taxa	Shortcode	BMWP-Colombia score	No. present	Frequency
Acari	ACARI	–	184	6
Atyidae	ATYIDAE	8	199	6
Baetidae	BAETIDAE	7	181	19
Belostomatidae	BELOSTOM	4	12	9
Calopterygidae	CALOPTER	7	93	11
Cambaridae	CAMBARID	–	16	6
Ceratopogonidae	CERATOPO	5	19	8
Chironomidae	CHIRONOM	2	805	29
Coenagrionidae	COENAGRI	7	124	21
Corbiculidae	CORBICUL	–	247	9
Corydalidae	CORYDALI	6	19	3
Culicidae	CULICIDA	2	1	1
Dryopidae	DRYOPIDA	6	28	3
Elmidae	ELMIDAE	6	8	6
Ephydridae	EPHYDRID	4	1	1
Gelastocoridae	GELASTOC	5	1	1
Gerridae	GERRIDA	–	8	5
Glossiphoniidae	GLOSSIPH	5	8	1
Gomphidae	GOMPHIDA	9	71	17
Haliplidae	HALIPLID	4	13	9
Hydrobiidae	HYDROBII	7	59	1
Hydrophilidae	HYDROPHI	3	29	5
Hydropsychidae	HYDROPSY	7	74	9
Hydroptilidae	HYDROPTI	8	7	4
Lampyridae	LAMPYRID	10	2	1
Leptoceridae	LEPTOCER	8	22	9
Leptohyphidae	LEPTOHYP	7	175	11
Leptophlebiidae	LEPTOPHL	9	61	8
Libellulidae	LIBELLUL	5	231	20
Limoniidae	LIMONIID	3	15	7
Littorinidae	LITTORIN	–	20	1
Lymnaeidae	LYMNAEID	8	1	1
Mysidae	MYSIDAE	–	1	1
Naucoridae	NAUCORID	8	42	12
Nepidae	NEPIDAE	5	8	3
Notonectidae	NOTONECT	5	135	2
Ochteridae	OCHTERID	–	1	1
Palaemonidae	PALAEMON	8	30	4
Perlidae	PERLIDAE	10	3	1
Philopotamidae	PHILOPOT	9	7	1
Physidae	PHYSIDAE	3	2	2
Pleidae	PLEIDAE	6	12	6
Polycentropodidae	POLYCENT	9	5	1
Ptilodactylidae	PTILODAC	10	1	1
Pyralidae	PYRALIDA	7	11	4
Scirtidae	SCIRTIDA	4	4	3
Simuliidae	SIMILIID	7	2	2
Spionidae	SPIONIDA	–	14	2
Stratiomyidae	STRATIOM	3	12	5
Tabanidae	TABANIDA	5	6	3
Thiaridae	THIARIDA	5	5231	17
Tubificidae	TUBIFICI	1	18	6
Veliidae	VELIIDAE	7	56	11



Appendix 3. Boxplots of the different elevation, conductivity and nitrate-nitrogen in relation to impact level. Bold horizontal lines represent median, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values, small black dots show outliers.