

Article

Odonata, Coleoptera, and Heteroptera (OCH) Trait-Based Biomonitoring of Rivers within the Northwestern Rif of Morocco: Exploring the Responses of Traits to Prevailing Environmental Gradients

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Abstract: This study aimed to determine the impact of various pressures on the functional composition of OCH (Odonata, Coleoptera, and Heteroptera) in streams within the northwest Rif region of Morocco. We examined how OCH traits respond to human-induced pressures in selected stream sites in Morocco's northwestern Rif region. OCH specimens were collected from 36 sites using a Surber sampler with dimension of 20 × 20 cm and mesh size of 500 µm over the course of two years, from 2021 to 2023. We measured physico-chemical and hydraulic parameters such as temperature, pH, DO, and NO-3. Sixty-seven trait attributes from 11 trait classes were assigned to the collected OCH taxa at the family level. Following the delineation of sites along the gradient of impacts in the study area, we categorized 7 sites as slightly impacted sites (SISs), 19 sites as moderately impacted sites (MISs), and 10 sites as heavily impacted sites (HISs). We successfully identified and categorized the traits as either vulnerable or tolerant based on RLQ models. Traits such as reproductive cycles per year and tegument respiration, which were positively correlated with SISs in the RLQ model and also positively correlated with depth and chlorine, were identified as vulnerable traits. Crawling locomotion and full water swimming were identified as tolerant traits. The distribution patterns of the OCH taxa revealed a robust correlation between the taxa and the sampling sites. Notably, taxa such as Nepidae, Naucoridae, and Corixidae exhibited widespread distribution and a strong association with the SISs. On the other hand, traits related to living macroinvertebrate food sources and reproduction in vegetation, specifically clutches, exhibited a negative correlation with total dissolved solids. Incorporating OCH functional traits into biomonitoring programs allows for a more comprehensive assessment of river ecosystems. This approach provides a nuanced understanding of how different stressors impact the community composition and overall ecological health.

Keywords: Odonata, Coleoptera, and Heteroptera traits; site impact categorization; RLQ; fourth-corner test; larva aquatic stage; clutches in vegetation reproduction; living macroinvertebrates and spiracles (aerial); trait co-occurrence; Rif region



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1. Introduction

The substantial and multifaceted impact of various human activities on aquatic ecosystems cannot be overstated. Challenges like inappropriate land use for cattle and agriculture, widespread deforestation, diverse forms of industrial and domestic pollution, the deterioration of natural habitats, the construction of hydropower dams, and the introduction

of invasive species have evolved into notable issues over time [1–3]. The loss of species threatens the delicate balance of ecosystems, jeopardizing their resilience and ability to withstand environmental changes. Aside from the loss of substantial integrity and the decline in species diversity, human activities also endanger ecosystems' functionality and the services they provide, for example, organic matter decomposition, water filtration, and water cycle regulation [4,5]. These challenges are quite pronounced in the freshwater ecosystems in the Mediterranean region of the world.

Freshwater ecosystems in the Mediterranean region go through a dynamic process impacted by sequential, unpredictable, and seasonal flooding as well as drying episodes that occur throughout the year [6]. Aside from these intrinsic stressors, they are confronted with major human-induced pressures such as flow management, intensive agricultural practices, and other numerous sources of pollution [7–9]. These anthropogenic stressors have a significant impact on both the aquatic ecosystems and their constituent biota [10,11].

In particular, the Rif region is unique due to its complicated biogeographical history, paleogeographic origins, and proximity to the Iberian Peninsula. Bennis et al. [12]. and Blondel et al. [13] identified the Mediterranean refugial area as an ideal location for faunistic and biogeographical investigations due to its high endemism of aquatic fauna, including the OCH group. Additionally, tracking changes in the population of these insect group over time might help identify potential threats and improve conservation efforts targeted at preserving these critical aquatic habitats.

As emphasized, this environmental shifting poses significant challenges to the structural and functional composition of freshwater ecosystems and their biota [14]. This transformation reflects the climate's critical role in shaping the distribution, behavior, and survival of numerous aquatic taxa. Nevertheless, these aquatic organisms have evolved specific sets of biological traits and ecological preferences. According to [15–18], these adaptive characteristics, such as body shape, locomotion, habitat preferences, and feeding habits, enable their survival, reproduction, and growth under increasingly constraining conditions. The "functional approach", also known as the study of biological and ecological traits, entails an extensive examination of the characteristics, behaviors and preferences displayed by organisms (e.g., macroinvertebrates) in an ecosystem.

Furthermore, the assessment of benthic macroinvertebrates is crucial in the monitoring of freshwater ecosystems [19] because of their high sensitivity to various pollutants originating from agricultural and urban sources [20,21], as well as habitat degradation and fragmentation [22,23]. Moreover, when assessing the effects of anthropogenic impacts on benthic macroinvertebrates, trait-based approaches offer distinct advantages [24]. Substantial and extensive documentation of functional responses to environmental changes within freshwater ecosystems in the Rif region have been reported across a variety of taxonomic groups (e.g., the EPT group), as discussed in our previous work [25]. To the best of our knowledge, little or nothing has been done with regard to the OCH (Odonata, Coleoptera, and Heteroptera) taxonomic group's responses to environmental changes in this study's area of interest.

Three primary considerations guided our choice of the OCH taxonomic group. Firstly, they are representative of the benthic community in a variety of aquatic environments ranging from stagnant to flowing water. Second, their importance in the ecosystem is emphasized by their pivotal roles in the macroinvertebrate food web [26]. Third, this taxonomic group responds differently to environmental factors such as pollution and changes in land use within watersheds [27–29]. Therefore, these advantages that come with using multiple traits and the fact that this approach is still relatively new when it comes to determining how the studied OCH taxonomic group reacts to human-induced changes is an eye opener in the science of functional-based monitoring. This interesting group provides important information about the biological health, ecological state, and habitat diversity of the areas they live in. Their practicability in this context made them one of the leading indicators for ecological monitoring and assessment, as has been emphasized by [30]. By using the taxonomic and trait-based assemblage of indicator insects like Odonata,

Coleoptera, and Heteroptera, we can evaluate the conservation status of intermittent rivers in the Mediterranean [31].

Using trait-based approaches can be helpful in identifying the particular trait combinations that allow some taxa to withstand the pressures imposed by anthropogenic activities. This method explores a range of characteristics, including morphological, physiological, and phenological characteristics [32–35]. Hence, in this study, we attempted to systematically categorize traits and ecological preferences that may serve as indicators of sensitivity or tolerance to an impact gradient. Among these traits are the larval aquatic stage and the reproduction trait of clutches in vegetation that were classified as vulnerable traits in previous studies [18–34]. In the context of this study, the selected traits refer to distinctive characteristics exhibited by macroinvertebrate taxa that render them either resilient or vulnerable to declining water quality. Europe is currently making the most progress in the use of trait-based biomonitoring systems [31–42]. There have also been studies on trait-based biomonitoring approaches in Australia [43] and New Zealand [44]. Trait-based biomonitoring approaches have also been successfully explored and developed in freshwater systems in North America [45,46] and Latin America [47], and to lesser degrees in Africa [25,48–54].

The present study hypothesized that human-induced pressures have a significant impact on the functional ecology of freshwater systems in Morocco's northwestern region. The study's specific goal was to determine how these pressures affect the functional composition of OCH in streams in the northwest Rif region of Morocco. This research is notable as the first attempt in Africa and most likely globally to investigate the functional structure of the OCH group for freshwater assessments using the functional trait approach, and to describe their responses to prevailing environmental gradients. According to this hypothesis, human activities will shape the functional composition of the OCH group by acting as selective drivers, influencing specific trait combinations or features.

It is noteworthy that most of the chosen sites display a Mediterranean hydrological regime, which is typified by a high degree of irregularity. This hydrological pattern is characterized by extreme summer droughts and powerful winter floods that have a major influence on the flow regime [55,56]. This study is important because it sheds light on the intricate dynamics of the freshwater systems under study in Morocco while taking place in the distinctive ecological framework of the northwest Rif region.

Studying the functional traits of indicator taxa like OCH provides insights into how these organisms respond to environmental stressors, including pollution. Traits like feeding habits, reproductive strategies, and habitat preferences act as indicators of ecosystem health, aiding in the assessment of pollutant impacts on aquatic communities.

2. Materials and Methods

2.1. Study area Description

The study area is located in Morocco's northwest Rif region, which is an important area both geologically and geographically. This location is in the northwestern region of the nation; it falls between 35°23' and 35°25' north latitude and 5°25' and 5°30' west longitude. The Rif region is distinguished by its elevated (sloppy) landscape, which stretches along Morocco's northern coast and provides stunning views of the Mediterranean. Geographically, it is located inside the Alborán Sea or Gibraltar Arc, which is a geological formation that is a part of the larger Alpine orogenic belt [57].

For this study, we selected 36 sites, which were all identified by a unique code: S1 (Afeska), S2 (Maggo I), S3 (Maggo II), S4 (Ouara I), S5 (Ouara II), S6 (Kalaa I), S7 (Kalaa II), S8 (Kalaa III), S9 (Ferda I), S10 (Ferda II), S11 (Tassikeste), S12 (Zaouiat), S13 (Talambote), S14 (Afertane), S15 (Tizgharine), S16 (Madissouka), S17 (Beni M'hamed), S18 (Kannar I), S19 (Kannar II), S20 (Igouraine), S21 (Amazithen), S22 (Sidi Yahya Aarab), S23 (Taghassa), S24 (Aarkoub), S25 (Jnane Niche), S26 (Asemilil I), S27 (Asemilil II), S28 (M'tahen), S29 (Tquaraa), S30 (Tisgriss), S31 (Taïda), S32 (El Hamma), S33 (Mlilah), S34 (Loukkos), S35

(Mansoura), and S36 (Qoub). As shown in Figure 1, these sites were selected from 11 distinct hydrographic networks and catchment areas.

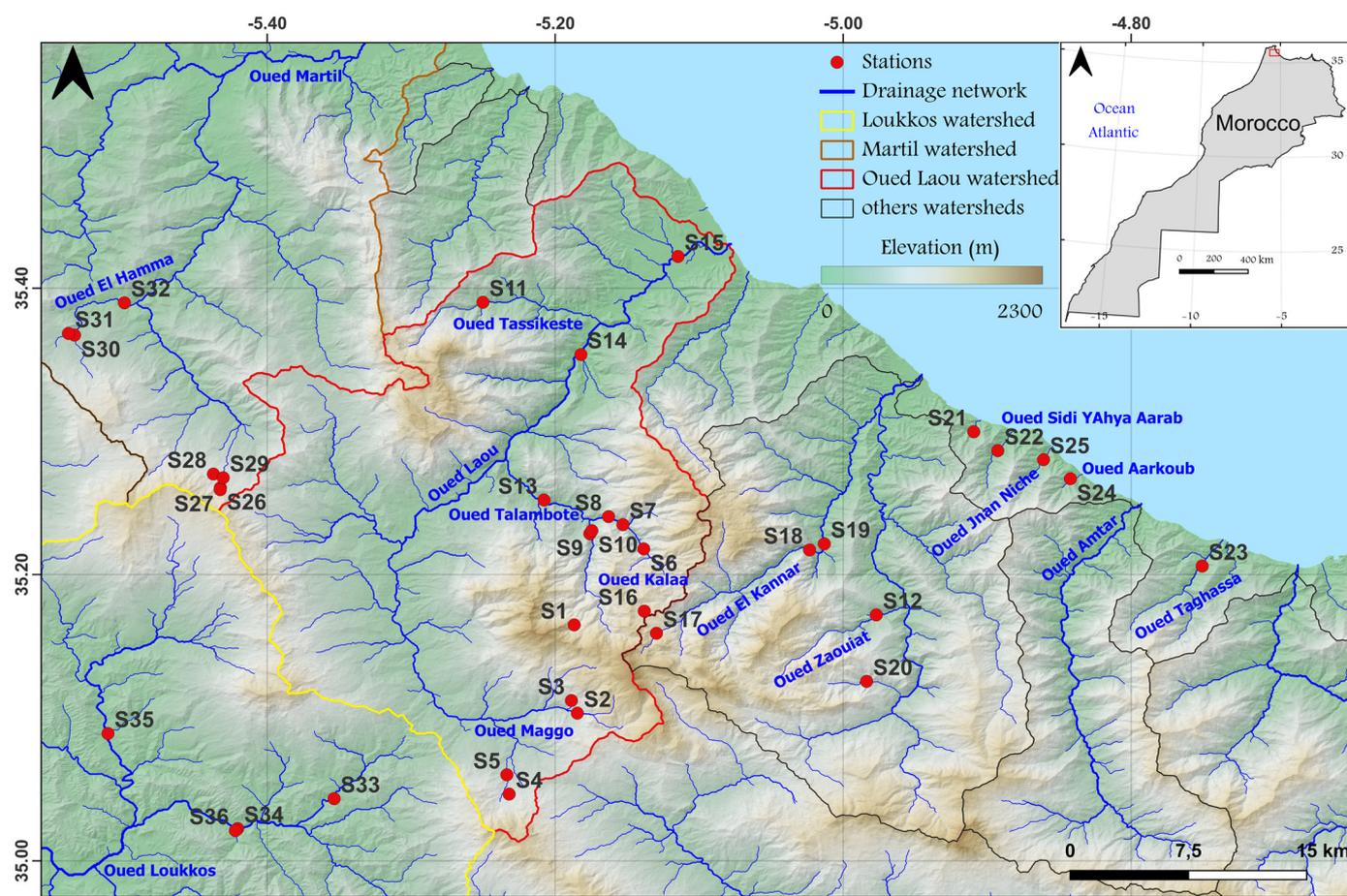


Figure 1. Distribution map of investigated sites.

2.2. Evaluation of OCH Sampling and Analyses of Physico-Chemical and Hydraulic Parameters

2.2.1. Odonata, Coleoptera, and Heteroptera Group Sampling and Identification

A Surber sampler with a mesh size of 500 μm and dimensions of 20 \times 20 cm was used to capture aquatic insects at each sampling site during each sampling event. Sampling took place at each site during all seasons (spring, summer, autumn, and winter) over two consecutive years, from 2021 to 2023. The process included a three-minute sampling duration, covering all available microhabitats such as sand, silt, mud, stone, and vegetation at each sampling location. These locations were distinguished by various depths, substrates, and water velocities.

Following collection, the fauna were thoroughly cleaned to remove rocks, debris, and leaves. The collected specimens were preserved in 96% ethanol to allow for subsequent laboratory analyses. Sorting, identification, and abundance counts were carried out using Tachet et al. [58]'s identification key, which allowed for the identification of all OCH (Odonata, Coleoptera, and Heteroptera) specimens at the family level. Supplementary Table S1 contains a comprehensive list of the OCH families collected in the course of the study.

2.2.2. Physico-Chemical and Hydraulic Parameter Measurements

In order to comprehend the ecological dynamics of the freshwater systems under study, a consistent evaluation of a number of physico-chemical and hydraulic parameters was required. The water temperature ($^{\circ}\text{C}$), pH, electrical conductivity (μ/cm^2), dissolved

oxygen (mg/L), total dissolved solids (ppm), and salinity (psu) were measured *in situ*, using a waterproof portable multiparameter meter (CyberScan Series 600). These parameters were regularly and seasonally measured at each site (Supplementary Materials Table S2). Hydraulic parameters, which included depth, width, and current velocity, were also measured on-site with a tape measure (flexile ruler or strip for measurement) in three replicates for increased accuracy and reliability. Samples of water were collected in 1 L plastic bottles and stored at 4 °C for later analysis. Samples were collected and sent within 24 h to the Loukkos Hydraulic Basin Agency Laboratory (ABHL), Tetouan, Morocco, for in-depth analyses.

Numerous parameters were examined in the analysis, including and five-day biochemical oxygen demand (mgO₂/L), total suspended solids (mg/L), and chemical oxygen demand (mgO₂/L). The portable Pastel UV analyzer was used to perform the examination. The stringent analysis procedure followed Rodier's recommendations [56–59], guaranteeing accuracy and adherence to the recommended methods for monitoring water quality. Furthermore, the nutrient content of the water samples was analyzed, including the levels of nitrites (NO⁻² mg/L), nitrates (NO⁻³ mg/L), chlorine inputs (Cl⁻ mg/L), and complexometric calcium (Ca²⁺ mg/L). However, ammonium, phosphorus, and sulfate characteristics were limited to specific sites and were thus excluded from further statistical analyses.

2.3. Odonata, Coleoptera, and Heteroptera Trait Selection Fuzzy Coding System

We described the OCH taxonomic group's functional assemblage by selecting 67 modalities from 11 trait classes (Supplementary Materials Table S3). Among the selected trait classes were behavioral (e.g., locomotion and substrate relation), life cycle (e.g., life cycle duration, aquatic stages, potential number of reproductive cycles per year), trophic (e.g., food and feeding habits) and morphological (e.g., maximal potential size). Traits and ecological preferences were selected based on their mechanistic relationship with anthropogenic stressors such as land use types, sedimentation, and nutrient influx from nearby catchments [37,39]. Furthermore, information on each trait and ecological preferences at the family level was primarily obtained from Bis and Usseglio-Polatera [60] and confirmed by Dr. Dr. Augustine Ovie Edegbene, a trait-based expert at the Department of Biological Sciences, Federal University of Health Sciences, Otukpo, Nigeria. Each registered taxon in the database that was confirmed by the expert was classified using a fuzzy coding approach based on its association with each trait category [61].

As detailed in Supplementary Materials Table S3, each taxon was assessed and assigned an affinity score for each trait category. According to the framework established by Usseglio-Polatera et al. [42] and Tachet et al. [58], a score of 0 denoted a lack of affinity, while a score of 3 denoted a robust or strong affinity for a given trait category. These affinity scores not only highlighted the frequency of trait categories used by the taxa, but also accounted for the inherent uncertainty, drawing on a foundation of published data and expert insights. These scores were further processed to establish a relative frequency distribution to allow for a more comprehensive interpretation. This was accomplished by dividing the taxon affinity scores for the fuzzy-coded trait categories and normalizing them.

2.4. Data Analysis

2.4.1. Sampling Delineation into Impact Categories

To determine the relationships between the environmental dataset and the 36 sampling locations, we used Principal Component Analysis (PCA). Prior to analysis, log₁₀(x + 1) transformations were applied to all environmental parameters and OCH group abundances to approximate a normal distribution. As a result, a total of 15 environmental factors were considered for a thorough analysis of the survey data. This method enabled us to efficiently explore the dataset's interrelationships and variations.

The study classified sampling sites into distinct impact categories using the results of the PCA and the analytical framework outlined by [50]: slightly impacted sites (SISs), moderately impacted sites (MISs), and heavily impacted sites (HISs). This categorization, initially established by the PCA, forms the basis for subsequent comparisons of varying levels of anthropogenic stressors in this study. The classification of the 36 sites was performed by following the thinking in [62]. This was accomplished by extracting the coordinates of each of the sites from the PCA. The first component of the PCA was used for this categorization as it explained over 24% of the total variance which is the highest among the first three PCA components. In categorizing the sites, we first calculated the inter-site distances by subtracting the lowest scoring site from the highest scoring site, and the scores of the other sites from the highest scoring site. We converted the inter-site distances to percent distances and thereafter percentile distributions of 100–90th, <90–50th, and <50th were used to classify the sites into slightly impacted sites (SISs), moderately impacted sites (MISs), and heavily impacted sites (HISs), respectively. The PCA ordination was performed using the Vegan package in R version 4.0.5 (R Development Core Team 2021).

2.4.2. RLQ and Other Associated Tests—Identifying and Classifying Vulnerable and Tolerant OCH Traits

To find a substantial relationship between the environment and biological features, we employed a three-table ordination strategy that combines RLQ ordination with fourth-corner analysis. In contrast to traditional ecological approaches, this establishes a mechanistic link between organisms and their environment, allowing for the development of predictive models. If functional traits are linked to specific environmental conditions, families with those traits should be affected when those conditions change. An RLQ analysis was performed to determine the relationships among physico-chemical parameters, aquatic insect (OCH) taxonomic abundance, and OCH functional traits. An RLQ analysis is a three-table co-inertia analysis that involves independent ordinations of the R (environmental variable and in this case, physico-chemical and hydraulic parameters), L (taxonomic composition: OCH abundance data), and Q (functional traits: OCH traits and ecological preferences) tables [63]. Then, we conducted a Monte Carlo permutation with 49,999 replicates, employing models 2 and 4 of the RLQ analysis. Subsequently, a fourth-corner analysis was carried out on both the physico-chemical and OCH datasets. This analysis explored the relationship between physico-chemical characteristics, traits, and ecological preferences using a multivariate method. To further confirm the level of significance of the OCH traits and physico-chemical parameters, we calculated the global level of significance based on the total inertia of the RLQ analysis. Further, we represented the level of significance on the first two axes of the RLQ on a factorial map. The RLQ model, fourth-corner test, and all additional statistical analyses and graphical outputs were processed in R version 4.0.5 (R Development Core Team 2021) using the R-scripts provided in [64].

2.4.3. Inter-Trait Co-Occurrence Based on Cluster Analysis

To visualize the associations among inter-trait attributes across the 36 sites, we employed cluster analysis, using Bray–Curtis similarity. This method allowed us to explore the patterns of similarity and dissimilarity among trait attributes, providing insights into the grouping or clustering of sites based on their trait profiles. Therefore, the cluster analysis using Bray–Curtis similarity is very important in exploring the functional-based (trait-based) interaction of taxa in an ecosystem in the face of prevailing environmental deterioration. This analysis demonstrates how traits from various categories or classes work together to help species survive in different environmental conditions. While this approach is relatively new, it has revealed that certain traits, which may not typically survive in disturbed environments, can thrive because the organism possesses multiple traits that suit various conditions [53–65].

3. Results

3.1. Site Delineation in the Study Area Catchments along an Impact Gradient in the Study Area

We correlated physico-chemical parameters with the sampling sites using the correlation matrix from the Principal Component Analysis. From the PCA biplot, Axes 1 and 2 explain 24.75% and 17.20% of the total variance, respectively (Figure 1).

The eigenvalues of axes 1 and 2 are 85.61 and 12.50, respectively. Based on the results of the PCA, physico-chemical parameters such as DO, BOD₅, nitrate, and current were negatively associated with S2, S3, S12, S17, and S20 on axis 1 (Figure 2). Chlorine and TA were positively associated with S35 in axis 1 (Figure 2). pH was negatively associated with S5, S7, S31, and S32 on axis 2 (Figure 2).

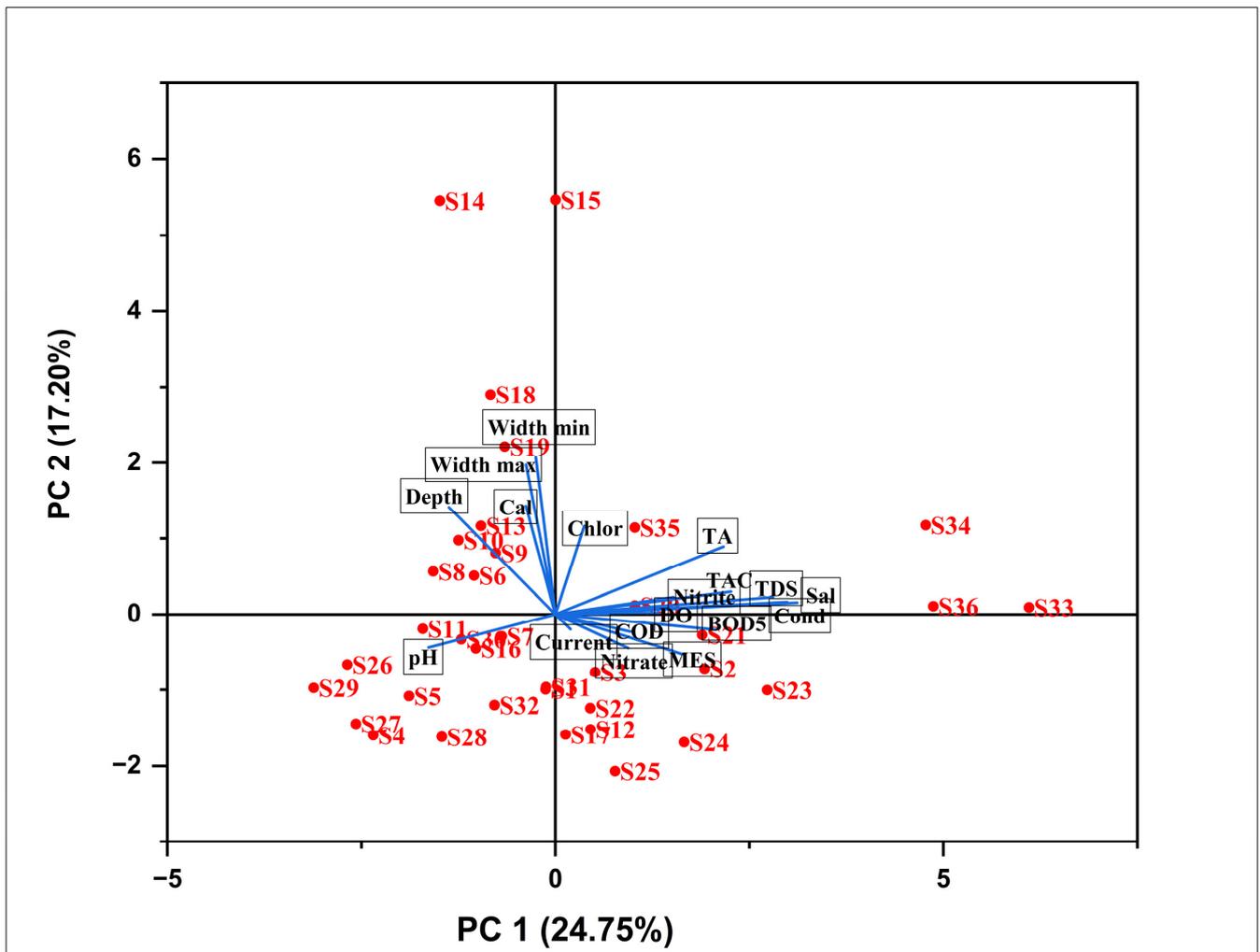


Figure 2. Principal component analysis showing the relationship between physico-chemical parameters and sampling sites in the study area.

Next, we delineated the sites along the gradient of impacts in the study area catchments; we categorized seven sites as slightly impacted sites (SISs), 19 sites as moderately impacted sites (MISs), and 10 as heavily impacted sites (HISs) (Table 1).

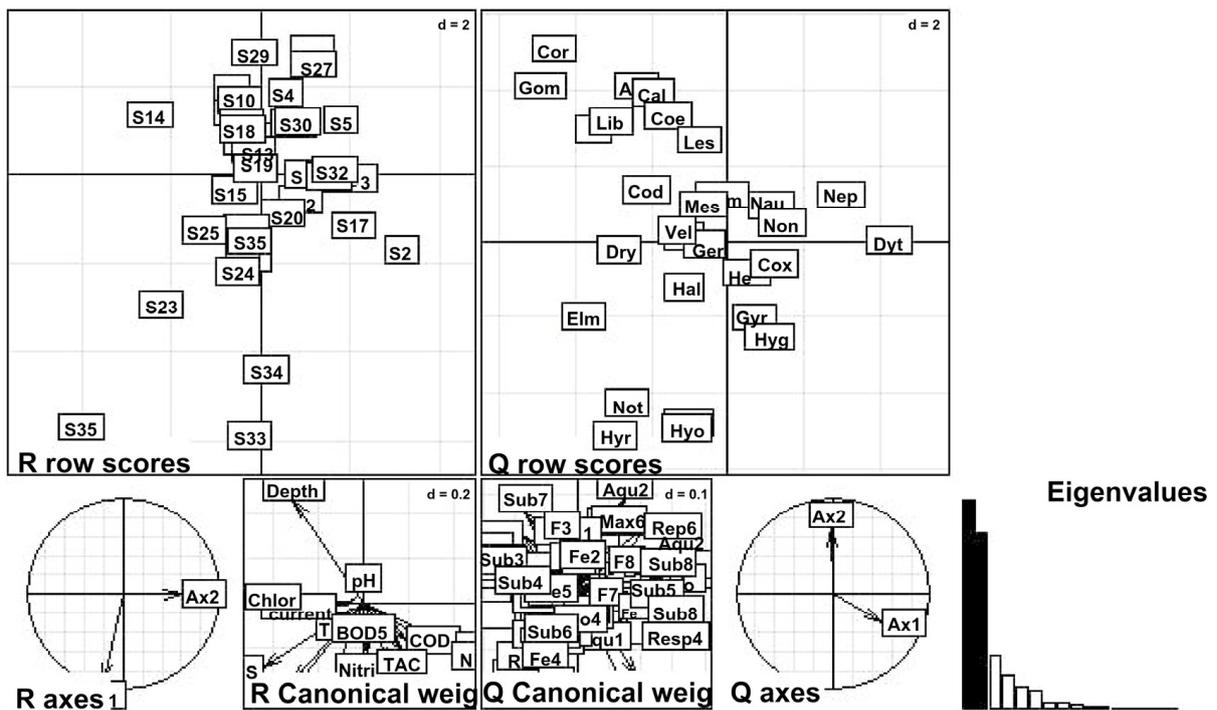
Table 1. Summary of the PCA coordinates for axis 1, inter-site distances, percentage inter-site distances, and categorization of sampling sites. Note: SIS = slightly impacted site, MIS = moderately impacted site, HIS = heavily impacted site.

Site	Axis 1	Inter-Site Distance	Percentage Inter-Site Distance	Impact Categorization
S1	0.1141	0.2274	50.1759	MIS
S2	0.1192	0.2223	65.09517	MIS
S3	0.1085	0.233	68.2284	MIS
S4	0.07525	0.26625	77.96486	SIS
S5	0.07832	0.26318	77.06589	SIS
S6	0.1184	0.2231	65.32943	MIS
S7	0.1357	0.2058	60.26354	MIS
S8	0.1258	0.2157	63.16252	MIS
S9	0.1074	0.2341	68.55051	MIS
S10	0.1321	0.2094	61.31772	MIS
S11	0.1318	0.2097	61.40556	MIS
S12	0.1458	0.1957	57.306	MIS
S13	0.1358	0.2057	60.23426	MIS
S14	0.2067	0.1348	39.47291	HIS
S15	0.1689	0.1726	50.54173	MIS
S16	0.1318	0.2097	61.40556	MIS
S17	0.1406	0.2009	58.8287	MIS
S18	0.1443	0.1972	57.74524	MIS
S19	0.1489	0.1926	56.39824	MIS
S20	0.1777	0.1638	47.96486	HIS
S21	0.2261	0.1154	33.79209	HIS
S22	0.2108	0.1307	38.27233	HIS
S23	0.3415	0	0	HIS
S24	0.237	0.1045	30.60029	HIS
S25	0.2008	0.1407	41.20059	HIS
S26	0.03733	0.30417	89.06881	SIS
S27	0.03674	0.30476	89.24158	SIS
S28	0.07779	0.26371	77.22108	SIS
S29	0.05678	0.28472	83.37335	SIS
S30	0.09439	0.24711	72.36018	SIS
S31	0.1147	0.2268	66.41288	MIS
S32	0.1031	0.2384	69.80966	MIS
S33	0.3218	0.0197	5.768668	HIS
S34	0.2033	0.1382	40.46852	HIS
S35	0.1653	0.1762	51.5959	MIS
S36	0.326	0.0155	4.538799	HIS

3.2. Odonata, Coleoptera, and Heteroptera (OCH) Taxa and Trait Distribution Patterns along Impact Gradient in the Study Area—Selection of Vulnerable and Tolerant OCH Traits

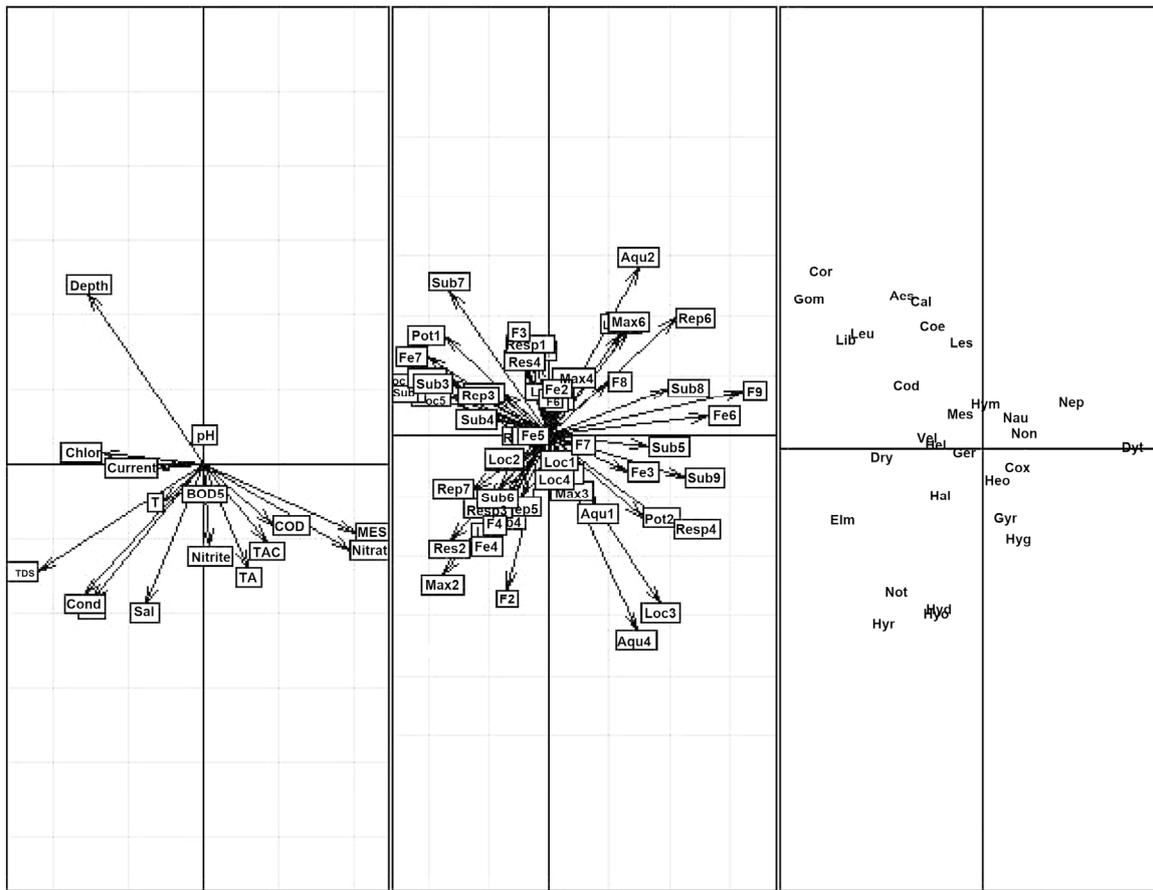
3.2.1. Relationships between Sampling Sites and OCH Taxa

The visualization of the relationship between the sampling sites and Odonata, Coleoptera, and Heteroptera taxa, with a specific representation of the R and Q axes for sampling sites, is showed in Figure 3a. The eigenvalues of the first and second RLQ are 1.013 and 0.85, respectively, with a projected inertia for axes 1 and 2 of 38.94% and 32.71%. The cumulative projected inertia for the first RLQ axis is lower (38.94%) compared to that of axis 2 (71.65%). The adjustment methods for multiple comparisons using a Monte Carlo permutation with 49,999 permutation arguments for models 2 and 4 of the RLQ showed no significant difference ($p > 0.05$; model 2 p -value = 0.315; model 4 p -value = 0.416). The distribution patterns of the OCH taxa showed a strong correlation with the sampling sites (Figure 3a). For instance, taxa such as Nepidae, Naucoridae, and Corixidae were widely distributed and strongly associated with slightly impacted sites (SIS; S4, S5, S27 and S30; Figure 3a). Taxa such as Cordulegasteridae, Gomphidae, Calopterygidae, Lestidae, Corduliidae, and Mesoveliidae were widely distributed and strongly associated with S10, S12, and S18, the moderately impacted sites (MISs), while Corixidae, Gyrinidae, Hygrobiidae, Elmidae, Notonectidae, Hydrochidae, and Hydrophilidae were widely distributed and strongly associated with highly impacted sites (HISs; S15, S23, S24, S25, S35, and S36; Figure 3a).



(a)

Figure 3. Cont.



(b)

Figure 3. (a) RLQ model visualizing the relationship between sampling sites and Odonata, Coleoptera, and Heteroptera taxa, with specific representation of R and Q axes for sampling sites and taxa and eigenvalues. Site abbreviations: S1=, S2=, S3=, S4=, S5=, S6=, S7=, S8=, S9=, S10=, S11=, S12=, S13=, S14=, S15=, S16=, S17=, S18=, S19=, S20=, S21=, S22=, S23=, S24=, S25=, S26=, S27=, S28=, S29=, S30=, S31=, S32=, S33=, S34=, S35=, S36=. Odonata, Coleoptera, Heteroptera (OCH) taxa abbreviations: Aes = Aeshnidae, Cal = Calopterygidae, Coe = Coenagrionidae, Cor = Cordulegasteridae, Cod = Corduliidae, Gom = Gomphidae, Les = Lestidae, Lib = Libellulidae, Dry = Dryopidae, Dyt = Dytiscidae, Elm = Elmidae, Gyr = Gyrinidae, Hal = Haliplidae, Hel = Helodidae, Heo = Helophoridae, Hyd = Hydraenidae, Hyr = Hydrochidae, Hyo = Hydrophilidae, Hyg = Hygrobiidae, Not = Noteridae, Cox = Corixidae, Ger = Gerridae, Hym = Hydrometridae, Mes = Mesoveliidae, Vel = Veliidae, Nau = Naucoridae, Nep = Nepidae, Non = Notonectidae. (b) RLQ model visualizing the relationship among physico-chemical parameters and Odonata, Coleoptera, and Heteroptera traits and taxa. **Physico-chemical parameter abbreviations:** T = temperature, Sal = salinity, DO = dissolved oxygen, TDS = total dissolved solids, Chlor = chlorine, Cond = conductivity, DO = dissolved oxygen, BOD₅ = five days biochemical oxygen demand, COD = chemical oxygen demand, MES = suspended matter, TA = total alkalinity, TAC = total alkalinity complete. **Trait abbreviations—maximal potential size:** Max1 ≤ 0.25 cm, Max2 ≥ 0.25–0.5 cm, Max3 ≥ 0.5–1.0 cm, Max4 ≥ 1.0–2.0 cm, Max5 ≥ 2.0–4.0 cm, Max6 ≥ 4.0–8.0 cm, Max7 ≥ 8.0 cm. **Life cycle duration:** Lif1 ≤ 1 year, Lif2 ≥ 1 year. **Potential number of reproductive cycles per year:** Pot1 ≤ 1 year, Pot2 = 1 year, Pot3 ≥ 1 years. **Aquatic stage—**Aqu1 = eggs, Aqu2 = larva, Aqu3 = pupa, Aqu4 = adult. **Reproduction:** Rep1 = ovoviviparity; Rep2 = isolated eggs, free; Rep3 = isolated eggs, cemented; Rep4 = clutches, cemented or fixed; Rep5 = clutches, free; Rep6 = clutches in vegetation; Rep7 = clutches, terrestrial; Rep8 = asexual reproduction. **Resistance forms:** Res1 = eggs, statoblasts, Res2 = cocoons, Res3 = housing against desiccation, Res4 = diapause or dormancy, Res5 = none.

Respiration: Resp1 = tegument, Resp2 = gill, Resp3 = plastron, Resp4 = spiracle (aerial), Resp5 = hydrostatic vesicle (aerial). **Locomotion and substrate relation:** Loc1 = flier, Loc2 = surface swimmer, Loc3 = full water swimmer, Loc4 = crawler, Loc5 = burrower (epibenthic), Loc6 = interstitial (endobenthic), Loc7 = temporary attached, Loc8 = permanently attached. **Food:** F1 = microorganisms, F2 = detritus ≤ 1 mm, F3 = plant detritus ≥ 1 mm, F4 = living macrophytes, F5 = living microphytes, F6 = dead animal > 1 mm, F7 = living macroinvertebrates, F8 = living macroinvertebrates, F9 = vertebrates. **Feeding habits:** Fe1 = absorber, Fe2 = deposit feeder, Fe3 = shredder, Fe4 = scraper, Fe5 = filter feeder, Fe6 = piercer (plants or animals), Fe7 = predator (carver/engulfer/swallower). **Substrate preference:** Sub1 = flags/boulders/cobbles/pebbles, Sub2 = gravel, Sub3 = sand, Sub4 = silt, Sub5 = macrophytes, Sub6 = microphytes, Sub7 = twigs/roots, Sub8 = organic detritus/litter, Sub9 = mud.

3.2.2. Relationships between Physico-Chemical Parameters and OCH Traits in the Site Impact Categories

Figure 3a,b shows the relationship between the physico-chemical parameters and OCH traits in relation to the OCH taxa in the sampling site impact categories. Based on the results of the RLQ model, OCH traits such as larval aquatic stage, maximal potential body sizes (>1.0 – 2.0 cm and >4.0 – 8.0 cm), living macroinvertebrate and vertebrate food sources, piercer (plants or animals) feeding habit preference, clutches in vegetation reproduction trait, and organic detritus/litter substrate preference were strongly correlated with pH in the slightly impacted sites (SISs; Figure 3a,b). OCH traits such as sand, silt, and twig/root substrate preferences, plant detritus food source, predatory feeding habit, tegument respiration trait, isolated egg reproduction trait, and diapause or dormancy resistance form were strongly correlated with depth and chlorine in the moderately impacted sites (MISs; Figure 3b). In the heavily impacted sites, the following OCH traits were strongly correlated with COD, MES, nitrate, nitrate, TAC, and BOD₅: flying, full water swimming and crawling locomotion and substrate relation preferences, and egg and adult aquatic stages (Figure 3b). Other OCH traits associated with these physico-chemical parameters in HISs included 1-year life cycle duration, >0.5 – 1.0 cm maximal potential body size, living macroinvertebrate food source, shredding feeding habit, spiracle (aerial) respiration trait, and macrophyte and mud substrate preferences (Figure 3b).

3.3. Relationships between Physico-Chemical Parameters and OCH Traits in the Site Impact Categories Based on Fourth Corner Test—Identifying Vulnerable and Tolerant Traits

Following the results of the RLQ tests in Figure 3a,b, we further explored the further performed a fourth-corner test to confirm the relationship between the physico-chemical parameters and OCH traits. Full water swimming preference, adult aquatic stage, and detritus ≤ 1 mm food sources were negatively correlated with depth (Figure 4). Pupal aquatic stage and cocoon resistance form were positively correlated with DO while pupal aquatic stage and mud substrate preference were positively correlated with nitrate (Figure 4). Surface swimming preference and spiracle (aerial) respiration traits were positively correlated with suspended matter; pupal aquatic stage and hydrostatic vesicle (aerial) respiration traits were positively correlated with total alkalinity (Figure 4). Living macroinvertebrate food source and clutches in vegetation reproduction traits were negatively correlated with total dissolved solids, while larval aquatic stage and clutches in vegetation reproduction traits were negatively correlated with conductivity (Figure 4). Absorption feeding habit and twig/root substrate preference were negatively correlated with suspended matter, and >2.0 – 4.0 cm maximal potential body size was negatively correlated with salinity (Figure 4). Egg resistance form and vertebrate food source were positively correlated with chlorine, while >1 year life duration cycle, silt substrate preference, and twig/root substrate preference were positively correlated with conductivity, temperature, and depth, respectively (Figure 4). Larval aquatic stage was negatively correlated with salinity and DO, and >0.25 – 0.5 cm maximal potential body size was positively correlated with TDS and conductivity (Figure 4).

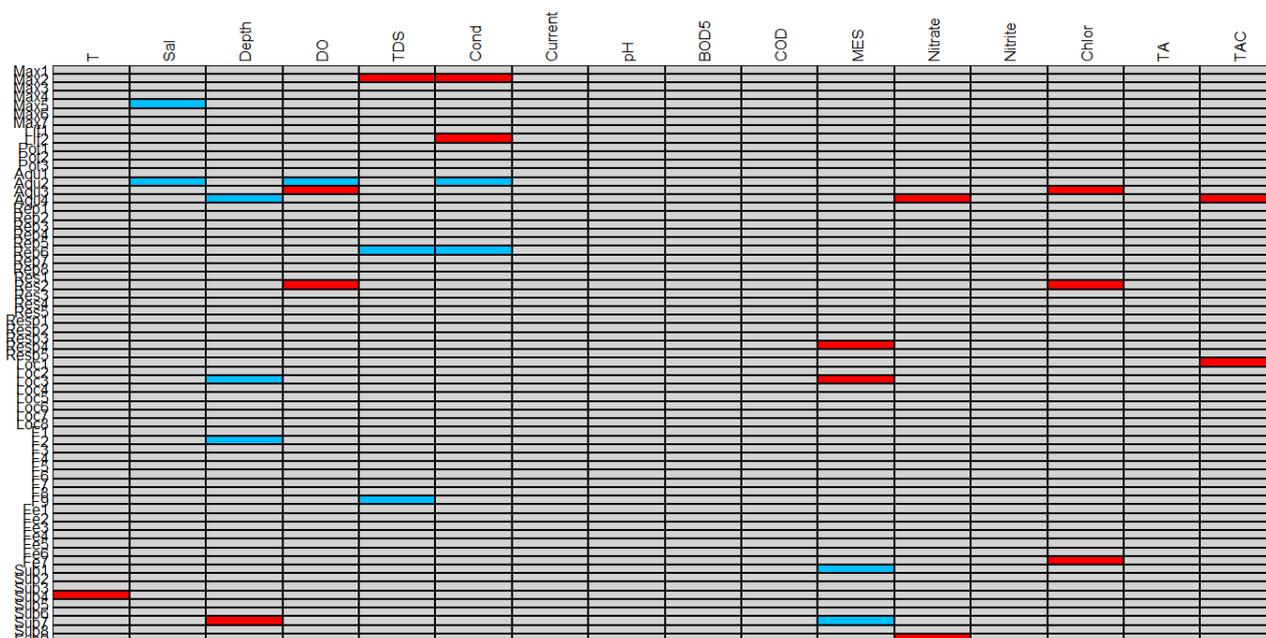


Figure 4. Fourth-corner model confirming the relationship between physico-chemical parameters and OCH traits. **Note:** blue shading indicates negative relationship between physico-chemical parameters and given trait and red shading indicates positive relationship between physico-chemical parameters and given trait. The grey shading indicates no relationship between physico-chemical variables and any trait.

The results of the RLQ models in Figure 3a,b showed the relationships between the OCH traits and physico-chemical parameters in the three sampling site impact categories (SISs, MISs, and HISs), and through further confirmation of the traits' relationships with the physico-chemical parameters using the fourth-corner test, vulnerable and tolerant traits were identified and classified. Hence, larval aquatic stage, clutches in vegetation reproduction trait, and living macroinvertebrates as source of food were identified and classified as vulnerable traits based on their association with SISs in the RLQ model and either a positive correlation with a physico-chemical indicator (DO) or negative correlation with any of the pollution-related physico-chemical indicators (e.g., conductivity, MES, TDS, BOD₅, and nutrients) on the fourth-corner map. Due to the spiracle (aerial) respiration trait's strong association with HISs and positive correlation with MES on the fourth-corner map, we identified and classified the trait as being tolerant to pollution.

3.4. Testing the Strength of Fourth-Corner Test to Detect the Level Significance Difference between Physico-Chemical Parameters and OCH Traits

To test the ability of the fourth-corner test to detect the level of significance between the physico-chemical parameters and OCH traits, we used a Monte-Carlo permutation test with 49,999 permutation arguments. The result showed that there was a large significant difference between the physico-chemical parameters and OCH traits ($p = 0.001$; $p < 0.05$). To further confirm this level of significance, we evaluated the global significance between the physico-chemical parameters and OCH traits using the total inertia of the RLQ analysis. The result showed that there was no significance difference between the physico-chemical parameters and OCH traits based on models 2 and 4 (Figure 5). Further, we constructed a factorial map to show the RLQ axes' level of significance between the physico-chemical parameters and OCH traits and the results also revealed no significant correlation between the physico-chemical parameters and OCH traits (Figure 6).

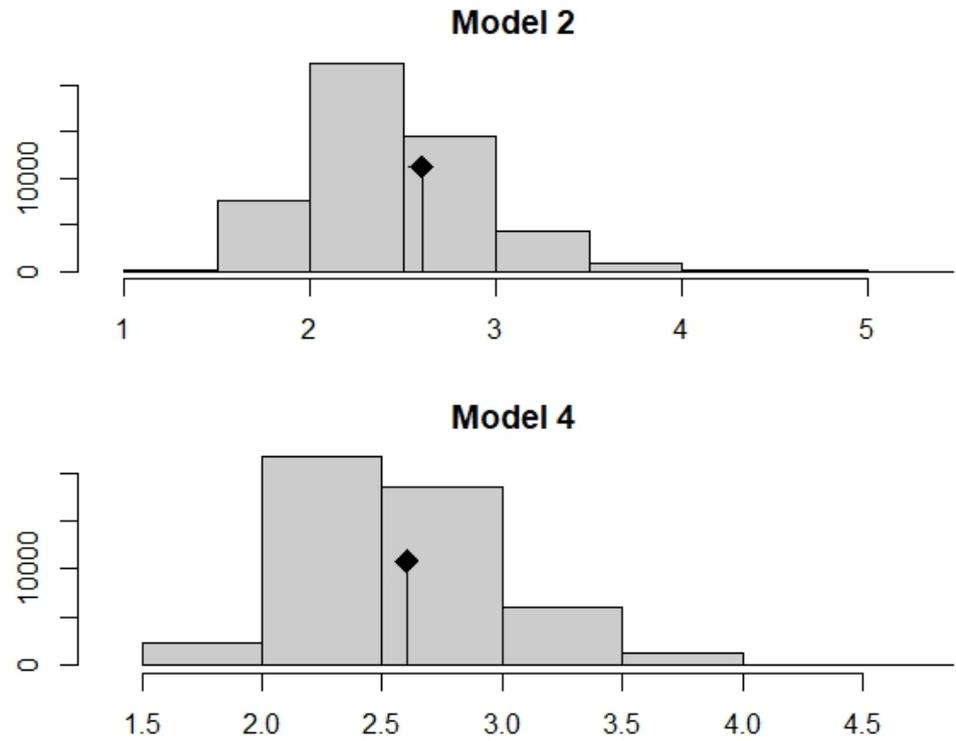


Figure 5. Total RLQ inertia showing the global significance level between physico-chemical parameters and traits.

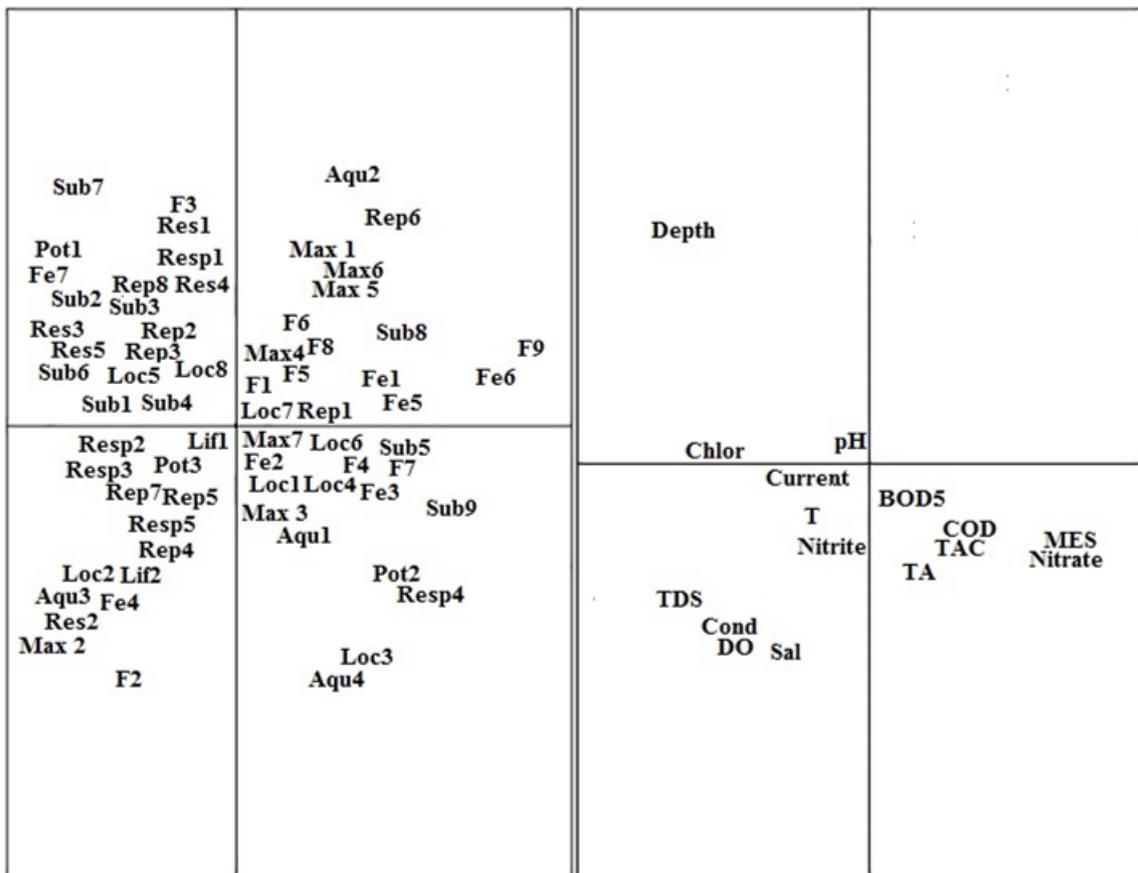


Figure 6. Factorial map showing the significant association between RLQ axes for OCH traits (Q axes) and physico-chemical parameters (R axes).

3.5. Visualization of Odonata, Coleoptera, and Heteroptera (OCH) Inter-Trait Classes and Attribute Relationships in the Sampled Sites

We visualized the inter-traits attribute association over the 36 sites using cluster analysis (Bray–Curtis similarity) which returned a similarity of 86% (0.86), with 14 distinct clusters of inter-trait attribute co-occurrence (Figure 7 and Table 2). Of the 67 trait attributes selected, 17 trait attributes did not form clusters: microorganisms (F1), living macroinvertebrates (F8), and vertebrates as sources of food (F9); full water swimming (Loc3), temporarily attached (Loc7), and permanently attached (Loc8) as modes of locomotion and substrate relations; twig/root substrate preference (Sub7); ≤ 0.25 cm (Max1) and > 8 cm (Max7) maximal potential size; cocoons (Res2) and housing against desiccation (Res3) resistance forms; absorber (Fe1) and filter feeder (Fe5) feeding habits; clutches, free (Rep5) and asexual reproduction (Rep8) modes of reproduction; and hydrostatic vesicle (aerial; Resp5) mode of respiration.

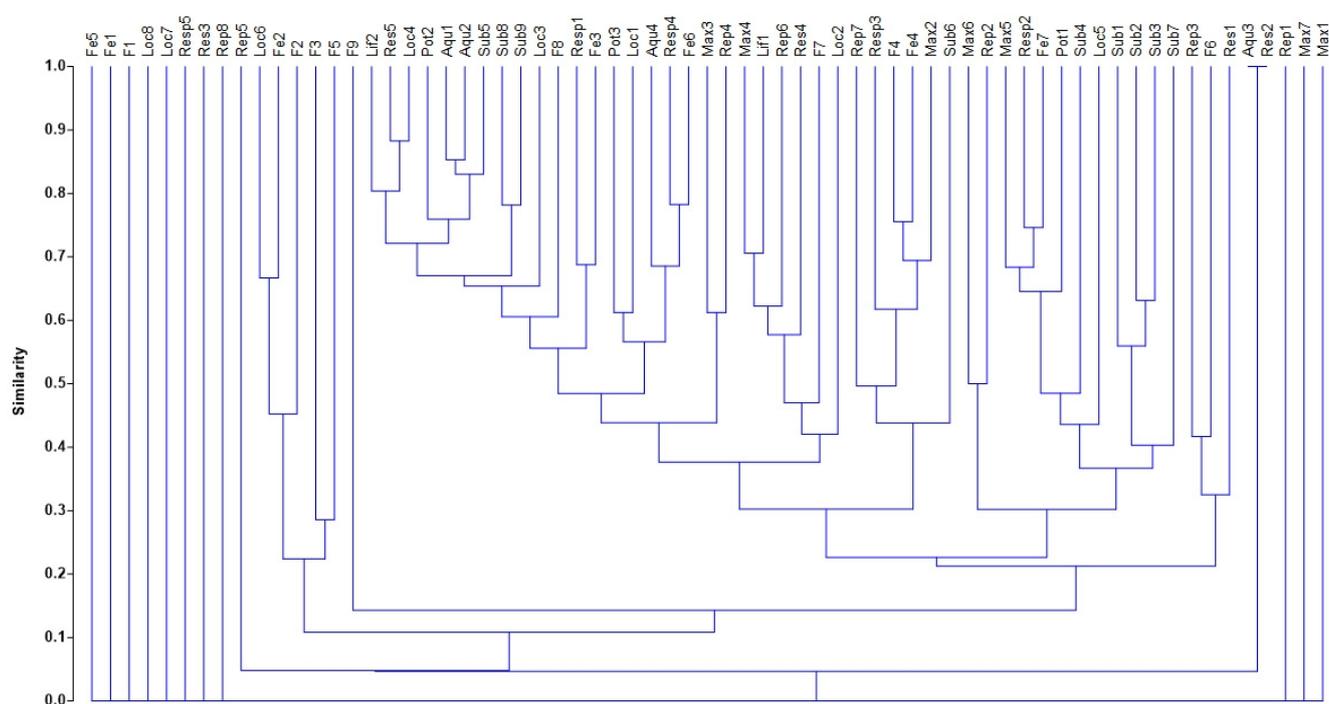


Figure 7. Cluster analysis (Bray–Curtis similarity) showing the co-occurrence of traits in the study area.

The remaining 50 trait attributes formed clusters and were well represented in all the 11 trait classes selected for this study (Table 2). Three trait classes (locomotion and substrate relation, feeding habits, and food) and five trait attributes (interstitial (endobenthic), deposit feeder, detritus ≤ 1 mm, plant detritus ≥ 1 mm, and living microphytes) were represented in cluster 1 (Table 2).

Cluster 2 explained three trait classes (life cycle duration, respiration, and locomotion and substrate relation) represented by one trait attribute each (Table 2); cluster 3 was also represented by three trait classes (potential number of reproductive cycles per year, aquatic stage, and substrate preference) and four trait attributes (1-year reproductive cycle, egg and larval aquatic stages, and silt substrate preference; Table 2). Only one trait class (substrate preference) and two trait attributes (organic detritus/litter and mud substrate preferences) were represented in cluster 4 (Table 2). Clusters 5 (respiration and feeding habits), 6 (potential number of reproductive cycles per year and locomotion and substrate relation), 8 (maximal potential size and reproduction), and 11 (maximal potential size and reproduction) were represented by two trait classes and attributes each (Table 2). Clusters 7 (aquatic stage, reproduction, and feeding habits) and 14 (reproduction, food, and resistance

form; Table 2). Cluster 13 was represented by one trait class (substrate preference) and three trait attributes (flags/boulders/cobbles/pebbles, gravel, and sand substrates preferences; Table 2).

Table 2. The 14 distinct clusters of macroinvertebrate traits co-occurrence clustering at >0.86 (86%) Bray–Curtis similarity in selected rivers in the study area based on Figure 6.

Cluster	Clustered Trait Attributes
1	Loc6, Fe2, F2, F3, F5
2	Lif2, Res5, Loc4
3	Pot2, Aqu1, Aqu2, Sub5
4	Sub8, Sub9
5	Resp1, Fe3
6	Pot3, Loc1
7	Aqu4, Rep4, Fe6
8	Max3, Rep4
9	Max4, Lif1, Rep6, Res4, F7, Loc2
10	Rep7, Resp3, F4, Fe4, Max2, Sub6
11	Max6, Rep2
12	Max5, Resp2, Fe7, Pot1, Sub4, Loc5
13	Sub1, Sub2, Sub3
14	Rep3, F6, Res1

4. Discussion

The habitat characteristics play a significant role in patterning the functional structure of aquatic communities in the specific reach. Various elements and conditions within the habitat directly influence the structure and dynamics of aquatic communities [66]. However, the life cycles, feeding behaviors, ecological requirements, and dispersal abilities of the OCH group in this study differed substantially. Despite the common perception of OCH taxa as a slightly sensitive group, which makes them valuable indicators for assessing environmental disturbance, this sensitivity can manifest in various ways, including changes in abundance, distribution, or behavior in response to alterations in environmental conditions [67,68]. Additionally, they have evolved a wide range of ecological and morphological adjustments to adapt to seasonal changes and the loss of habitat quality [69,70].

The current study adds to the ongoing research regarding the importance of trait-based development in monitoring the ecological health of freshwater ecosystems, aligning with previous investigations, especially in the Afrotropic region of the world [25–72]. This research, conducted in the African research area, represents a pioneering endeavor in the field of trait-based biomonitoring.

4.1. Odonata, Coleoptera, and Heteroptera (OCH) Taxa and Trait Distribution Patterns along Impact Gradient in the Study Area—Selection of Vulnerable and Tolerant OCH Traits

4.1.1. Relationships between Sampling Sites and OCH Taxa

The distribution patterns of the OCH taxa, as depicted in Figure 3a, reveal a noteworthy correlation between these taxa and the respective sampling sites. A distinctive pattern emerged, indicating a strong association between certain taxa and the impact level of the site. For example, taxa such as Nepidae, Naucoridae, and Corixidae exhibited a widespread distribution, showing a robust connection with slightly impacted sites (SISs), specifically S5. This site is exposed to various sources of impact that could affect its water quality, including agricultural contamination and fecal pollution from livestock grazing and watering. Conversely, taxa like Cordulegasteridae, Gomphidae, Calopterygidae, Lestidae,

Corduliidae, and Mesoveliidae demonstrated a wide distribution with a strong association with moderately impacted sites (MISs) such as S10, S12, and S18 (Figure 2). This implies that these taxa may have preferences that align with the conditions found in moderately impacted environments, for example, fast to moderately flowing water and a considerable supply of dissolved oxygen (DO). Meanwhile, reach-scale parameters such as current velocity, salinity, and altitude significantly influenced Heteroptera distribution, and the availability of microhabitats (e.g., specific vegetation patches within a river reach characterized by a high current velocity) can similarly impact the distribution of Heteropteran taxa, which is characterized by high mobility and low habitat specificity [73–75].

4.1.2. Relationship between Physico-Chemical Parameters and OCH Traits in the Site Impact Categories

Traits such as the number of reproductive cycles per year and tegument respiration, which were positively correlated with depth and chlorine, were identified as vulnerable traits. This classification was based on their association with SISs (based on RLQ analysis). Additionally, traits that exhibited positive correlations with the physico-chemical parameters, including electrical conductivity, nutrient inputs, and BOD₅, or negative correlations with increasing dissolved oxygen were recognized as pollution-tolerant traits and indicated ecological preferences for impacted sites. Remarkably, these pollution-tolerant traits were significantly more prevalent at HISs compared to SISs. In this study, crawling locomotion was identified as a tolerant trait, contradicting findings from previous studies [53–77]. These disparate findings could be explained by factors such as sample size, geographic location, and the specific metrics used to assess impact gradients. Variations in local environmental conditions, including sediment composition, flow dynamics, and pollutant types, could also account for the observed differences. On the other hand, swimming as an ecological preference aligns with the claim made by Wilkes et al. [78], who suggested that macroinvertebrates that exhibit swimming as their mode of locomotion possess the ability to escape impending pollution, rendering them insensitive to pollution.

The prevalence of pollution-tolerant traits, such as the tegument respiration trait, significantly increased in moderately impacted sites (MISs). Extending beyond the scope of this study, organisms with tegumental or cutaneous respiration can tolerate low dissolved oxygen (DO) concentrations [54–80]. In streams characterized by low levels of dissolved oxygen (DO), the physiological adaptation of organisms employing tegumental or cutaneous respiration emerges as a noteworthy trait [79,80]. The emphasis on tegumental respiration as a pollution-tolerant trait expands our understanding of how aquatic communities navigate and thrive in polluted environments. This study contributes to the existing research that underscores the importance of the trait-based development in monitoring the health of freshwater systems, aligning with previous studies that have highlighted its relevance [54,71,72,81,82].

In the slightly impacted sites (SISs), OCH traits such as larval aquatic stage, maximal potential body sizes of >1.0–2.0 cm and >4.0–8.0 cm, living macroinvertebrate and vertebrate food sources, and piercer (plants or animals) feeding habit preference demonstrated strong correlations with pH. The type of substrate has also been used to predict macroinvertebrate abundance and diversity [83]. Several taxa showed higher frequencies in specific substrates, which are related to the nature of the occupied substrate, water flow speed, and food resource type. For example, *Progomphus* (Odonata) has been reported to be associated with sandy substrates, and this genus' nymphs burrow and are adapted to prey on other animals buried in the sandy substrate [84,85], a pattern that has been reported to be linked to the substrate's characteristics and the feeding resource type it contains [86].

In heavily impacted sites (HISs), various OCH traits, including flying, full water swimming, crawling locomotion and substrate relation preferences, and egg and adult aquatic stages were strongly correlated with the physico-chemical parameters indicative of pollution such as COD, MES, nitrate, TAC, and BOD₅. The correlation between biological characteristics and physico-chemical factors aligns with how organisms adapt to the

physical thresholds within their microhabitat. Smaller invertebrates (less than 10 mm) with flexible, streamlined body structures tend to thrive in challenging conditions with high degrees of human effects [87]. These body shapes minimize drag and allow them to navigate through small spaces within sediment layers [88–91]. Conversely, large-bodied organisms are less associated with polluted streams [25,92,93], and are instead found slower-moving environments where they can navigate through the fine sediments. This adaptation to local habitat conditions reflects what is seen in stream fish, where smaller, streamlined species favor riffles [94,95].

According to [96], organisms' life cycle patterns are expected to be linked to seasonal patterns of water flow. Voltinism, for example, is a key reproductive trait that indicates how many life cycles an organism goes through in a given year. It has been established that organism with multiple life cycles per year, such as bivoltinism and multivoltinism, are more resistant to environmental changes than organisms with only one life cycle per year (univoltinism) [44,53,54,80,93]. This hypothesis proposes that the frequency of life cycles influences an organism's ability to cope with and adapt to environmental stressors, particularly pollution [54]. The reasoning behind this proposition could be rooted in the increased reproductive opportunities and adaptability associated with multiple life cycles, allowing for a more resilient species. As a result, voltinism appears to be an important factor in how organisms navigate and respond to environmental stressors, particularly pollution. The accumulation of evidence from various studies emphasizes the importance of voltinism as a fundamental component in ecological assessments, laying the groundwork for informed predictions about the effects of pollution in freshwater ecosystems.

Furthermore, the trait of maximal potential size is closely related to the same set of parameters; this suggests that large-bodied organisms may exhibit resistance to high TDS levels or stream loading. Large and extremely large body sizes would predominate in the least impacted sites [25,54]. This adaptive strategy becomes particularly significant in environments prone to frequent changes or disturbances. The quicker recovery facilitated by small individuals with shorter life cycles (1-year life cycle) is a testament to the community's ability to bounce back and continue functioning even in the face of dynamic environmental challenges [20,44,53,54,80,93]. The high predictability of flow fluctuation observed in Mediterranean-climate regions is likely to influence how organisms formulate their life-history patterns, allowing them to effectively adapt to these predictable alterations [7].

Indeed, when disturbances occur at regular intervals, life cycles tend to adapt to the long-term dynamics of the disturbances rather than responding to specific flow fluctuations [97]. These findings highlight the adaptive nature of OCH taxa in response to varying environmental stressors. For instance, dragonflies which excel at long-distance flight have the ability to venture into new territories when their surroundings become unfavorable [98]. In sites characterized by low disturbance levels, our investigation of the OCH group revealed that sensitive taxa exhibit early and noticeable responses compared to their more tolerant counterparts [99–101]. Additionally, certain aquatic Coleoptera species, particularly those in the Gyrinidae, Haliplidae, and Elmiidae families, have been identified as indicators for reliable water bioassessments [102,103]. Despite the historical presence of these families in impacted streams, their current status may be influenced by changing environmental conditions or human-induced disturbances.

4.2. Confirming the Relationship between Physico-Chemical Parameters and OCH Traits Based on Fourth Corner Test—Identifying Vulnerable and Tolerant Traits

Heteroptera families, for example, have a high dispersal capacity, as evidenced by their migration when faced with unfavorable environmental conditions [104,105]. Similar complexities arise in other insect groups, such as the Dytiscidae under Coleoptera, which frequently shares biological and ecological characteristics with the Heteroptera order [42,106,107]. Pupal aquatic stage and cocoon resistance form showed positive correlations with dissolved oxygen (DO), indicating a potential preference for oxygen-rich

conditions. Taxa that rely on gills and plastrons were significantly more prevalent in forest sites (e.g., Tisgriss, Taïda, and Stah) with higher dissolved oxygen (DO) concentrations, a lower organic matter load, and greater canopy coverage.

According to previous research [42,45,108], taxa that breathe through a bubble, plastron, or gills are sensitive to elevated sediment and organic material levels. This observation aligns with previous research that highlights low levels of dissolved oxygen as a crucial factor in altering respiration methods in highly impacted environments [44,109]. However, aerial respiration can also serve as a defense mechanism against oxygen depletion and the associated stress [25].

4.3. Visualization of Odonata, Coleoptera, and Heteroptera (OCH) Inter-Trait Classes and Attribute Relationship in the Sampled Sites

Respiratory mechanisms, for instance, are likely interlinked with the taxa's feeding habits, while reproductive strategies may be linked to locomotion and substrate preferences. Additionally, the size-related traits could play a role in influencing both reproductive strategies and locomotion, reflecting their ecological versatility. Alterations in trophic groups could be influenced by inherent environmental gradients, such as stream order, stream width, and elevation. This natural variability introduces challenges in accurately forecasting the reactions to disturbances associated with changes in land use [92,98]. Predicting these responses with precision becomes challenging due to the multifaceted and sometimes unpredictable relationships between these natural gradients and the impacts of human-induced stressors [110].

4.4. Implications of the Use of OCH Traits for Riverine Ecosystem Biomonitoring in the Mediterranean Region

Our findings, based on the functional traits of OCH (Odonata, Coleoptera, and Heteroptera), can be instrumental in river biomonitoring, particularly in the Mediterranean region. By understanding how these indicator orders respond to environmental stressors, biomonitoring efforts can effectively assess the health of river ecosystems and detect the impact of pollution. Their specific functional traits, such as feeding habits, reproductive strategies, and habitat preferences, can serve as valuable indicators of ecological integrity.

In the context of the Mediterranean rivers, where water resources face various challenges, the use of functional traits can offer a nuanced understanding of the ecological dynamics. This information can guide conservation and management strategies, helping to protect and restore the health of rivers in this region.

Overall, the hypothesis of this study suggests that human-induced pressures significantly affect the functional ecology of freshwater systems in Morocco's northwestern region. This study is noteworthy as the first attempt to explore the functional structure of the OCH group for freshwater assessments, using the functional trait approach to understand their response to environmental gradients. According to this hypothesis, human activities shaped the functional composition of the OCH group by acting as selective drivers, influencing specific trait combinations or features.

5. Conclusions

The use of trait-based approaches provides robust evidence for identifying sources of impairment and serving as a valuable complement to taxonomic-based methods. These characteristics govern how organisms interact, influence nutrient cycling, and shape the overall structure and function of freshwater ecosystems. Nevertheless, specific categories of traits demonstrated significant associations with typical local environmental stressors. However, it is crucial to note with caution that, despite our sampling sites encompassing 2nd–3rd-order streams, elements like altitude and other natural factors might overshadow the impacts of human-induced alterations, potentially diminishing the sensitivity of the bioassessment approach. These insights play a pivotal role in guiding conservation and management endeavors directed at preserving and restoring aquatic ecosystems within the studied area. Further, caution should also be employed when using our outlined vulnerable

and tolerant traits, as the trait-based approach was explored at the family level. To confirm the results of this study, the OCH traits' response to disturbances should be explored at the genera or species level to avoid undue variability at the level of genus or species traits within the same family.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ecologies5010009/s1>, Table S1. List of families captured within the taxonomic group of Odonata, Coleoptera, and Heteroptera (OCH); Table S2. Environmental parameters measured in study area sites; Table S3. Fuzzy coding of functional traits of the Odonata, Coleoptera, and Heteroptera (OCH) group.

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