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Environmental drivers and seasonal distribution of endemic macroinvertebrates in the Upper Loukkos Watershed (Northwestern Morocco)

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Abstract

Freshwater ecosystems of the Mediterranean basin are increasingly exposed to hydroclimatic fluctuations, often interacting with sustained anthropogenic pressures. However, in North African rivers, the ecological patterns of endemic aquatic invertebrates remain poorly documented. In this context, the Upper Loukkos Watershed (northern Morocco), a mountainous system within the Intercontinental Mediterranean Biosphere Reserve, provides a relevant setting to examine how environmental gradients and seasonal dynamics influence these assemblages. Endemic macroinvertebrates were sampled across 18 sites during four sampling campaigns (spring and autumn 2014-2015), capturing contrasting hydro-seasonal conditions from drought-induced low flows to post-flood periods. A total of 667 individuals belonging to 24 endemic species were recorded, including Moroccan, Maghrebian, and Ibero-Maghrebian taxa. Diversity metrics varied across sites and seasons, with a clear peak in spring, coinciding with increased flow permanence and partial recovery of hydrological connectivity. The mayfly *Choroterpes atlas* was the most abundant taxon, whereas the genus *Hydraena* showed the highest diversity. Multivariate analyses (NMDS, CCA) revealed that environmental gradients strongly structured species distribution. Sites characterized by elevated organic load and suspended solids were associated with disturbance-tolerant taxa (mainly Coleoptera and Hemiptera), whereas well-oxygenated lotic habitats supported rheophilic Ephemeroptera and Plecoptera. Spatial and seasonal patterns revealed a marked turnover in endemic assemblages based on their temporal stability, dominated by intermittent taxa (41.7%); only *Hydraena allomorpha* was classified as permanent, being recorded throughout all sampling periods. The Jaccard similarity index revealed consistently low overlap in community composition among sampling campaigns, indicating pronounced temporal turnover in the macroinvertebrate assemblage. The predominance of Ibero-Maghrebian taxa underscores the biogeographical significance of the Betico-Rifan region as a link between Europe and Africa. Overall, these findings identify Rif headwaters as key habitats for range-restricted freshwater taxa and highlight their potential role as climatic refugia under increasing hydroclimatic variability. This underscores the need for sustained, long-term monitoring and for explicitly incorporating intermittent and headwater streams into conservation frameworks, which remain underrepresented despite their ecological importance in North African river networks.

Key words: endemic macroinvertebrates; Mediterranean rivers; intermittent streams; hydroclimatic fluctuations; Loukkos Watershed; Morocco.

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Introduction

Freshwater ecosystems cover barely 2.3% of the Earth's surface, yet they sustain a disproportionate share of biodiversity and ecosystem services (Reid *et al*., 2019; Tickner *et al*., 2020). Their structure and functioning are increasingly shaped by climate change, hydroclimatic variability, and growing human control of the water cycle (Kundzewicz and Gerten, 2015; Huggins *et al*., 2022). Land-use changes, river regulation, nutrient enrichment, and water abstraction have altered flow regimes and biogeochemical functioning, accelerating eutrophication, habitat degradation, and biodiversity loss, especially in Mediterranean rivers with high endemic richness (Bonada and Resh, 2013; Poikane *et al*., 2019; Dorado-Guerra *et al*., 2023).

The Mediterranean Basin combines exceptional biological diversity with pronounced hydroclimatic instability. Cool, wet winters are followed by hot, dry summers, while floods alternate with droughts, constraining aquatic ecosystems (Figueroa *et al*., 2013). Projections indicate lower precipitation, higher temperatures, and more frequent droughts (Estrela-Segrelles *et al*., 2021). River discharge should decline, connectivity shift, and pollutant concentrations rise, increasing thermal and water stress in catch-

ments long transformed by human use (Dewson *et al*., 2007; Espinar-Herranz *et al*., 2025).

Benthic macroinvertebrates integrate environmental conditions across spatial and temporal scales and differ markedly between perennial and intermittent reaches, highlighting both seasonal and interannual variability (Bonada *et al*., 2007; Alcaraz-Hernández *et al*., 2024; García-García *et al*., 2024).

Persistence in temporary systems depends on resistance or resilience traits, whereas many endemic taxa remain tied to well-oxygenated headwaters. Warming and longer droughts favour tolerant and invasive species, promote homogenisation and contract specialised ranges, increasing the risk of functional and regional loss (Stewart *et al*., 2013; Strachan *et al*., 2015; Carey *et al*., 2023).

Situated at the intersection of the Atlantic, Mediterranean, and Saharan regions, Morocco occupies a key biogeographical position within the western Mediterranean Basin. Its complex topography and paleogeographic history, particularly in the Rif Mountains, support remarkable freshwater diversity and endemicity. However, increasing drought, water abstraction, agricultural pressure, and the limited consideration of invertebrates in conservation planning pose growing threats to these systems (Bennas *et al*., 2009; Benamar *et al*., 2021a).

Despite the recognised importance of Mediterranean-climate rivers, knowledge of endemic freshwater biodiversity in the North African sector remains fragmentary and uneven. Much of the existing Moroccan work, moreover, has been taxonomically narrow, usually centred on single orders and often framed around conservation status assessments rather than broader ecological processes (Bennas *et al*., 2009; Errochdi *et al*., 2014; El Alami *et al*., 2022). Most aquatic insect species are absent both from the IUCN Red List and from Appendix IV of Moroccan Law 29-05 (Ennabili *et al*., 2025), highlighting a critical disconnect between biodiversity value and formal protection (Sánchez-Fernández *et al*., 2008; Darwall *et al*., 2014; Guellaf *et al*., 2025).

Many sensitive species from intermittent or disturbed systems remain poorly documented, and bioassessment may underestimate degradation when flow intermittence removes specialist taxa (Sánchez-Fernández *et al*., 2008; Troudet *et al*., 2017; Belhaj *et al*., 2023a). Furthermore, limited knowledge of life-history traits, and conservation frameworks focused mainly on vertebrates continue to limit effective protection (Nogueira *et al*., 2021; Rodríguez-Lozano *et al*., 2023).

Studies from Morocco (e.g., Bennas *et al*., 2009; Benamar *et al*., 2011; Errochdi *et al*., 2014; El Haissoufi *et al*., 2015; Slimani *et al*., 2016; El Bazi *et al*., 2017; Benamar *et al*., 2021a, 2021b; Guellaf *et al*., 2021a; Benamar *et al*., 2022; El Alami *et al*., 2022; Belhaj *et al*., 2023a, 2023b; Belhaj *et al*., 2024; El Yaagoubi *et al*., 2024; Guellaf *et al*., 2025) indicate that endemic macroinvertebrates are largely confined to high-altitude sectors, often within protected areas, where hydrological integrity and habitat heterogeneity persist. Yet most of these studies are faunistic, relying on static distributional records rather than repeated monitoring. These approaches document where endemics occur far more than how environmental gradients and hydro-seasonal dynamics sustain them within these headwater systems.

These gaps remain unresolved in the Moroccan Rif. Whether richness, abundance and composition shift between post-flood spring and post-drought autumn assemblages has not been formally compared.

The respective roles of natural hydrological variability and anthropogenic pressures in shaping this structure have not yet been disentangled in the region, making it difficult to clearly separate climate-driven effects from those linked to land use. Likewise, it remains largely unknown whether individual taxa persist across contrasting seasons, although this information is essential for anticipating their vulnerability to increasing hydrological intermittence.

Conservation strategies, therefore, need to integrate freshwater taxa into spatial prioritisation frameworks, including priority areas for inland waters and expansion of protected areas (Leverington *et al*., 2010; Nogueira *et al*., 2021; Belhaj *et al*., 2024). At the basin scale, distribution patterns and ecological requirements can help identify climatic refugia, connectivity corridors, and high-integrity headwaters, thereby strengthening adaptive conservation planning under future hydroclimatic scenarios (Trizzino *et al*., 2015). In this context, identifying refugial systems capable of sustaining endemic assemblages under increasing intermittence becomes a central ecological and conservation challenge. The Upper Loukkos Watershed, within the Intercontinental Biosphere Reserve of the Mediterranean (IBRM) and partly encompassed by Bouhachem Natural Park, lies in the Betico-Rifan region, further enhancing its role as a potential biogeographical bridge and refugium. It provides a system for examining how hydroclimatic variability and anthropogenic pressures interact to shape endemic macroinvertebrate assemblages.

However, seasonal variation in endemic species has not been investigated. This gap is now pressing, particularly in light of a large dam planned within the watershed, expected to modify flow regimes, along with other ongoing socio-economic development projects (MEE, 2024). Identifying where endemic taxa concentrate, and how their assemblages shift between post-flood and post-drought periods, therefore provides a pre-impact baseline of direct conservation value.

This study aims to: i) characterise the taxonomic composition and chorological structure of endemic macroinvertebrates within the Upper Loukkos Watershed; ii) analyse their spatial and seasonal distribution patterns to assess changes following drought and flood periods; and iii) quantify the influence of hydro-seasonal variability and anthropogenic pressures on endemic assemblages.

METHODS

Study area

The study was conducted in the Upper Loukkos Watershed (ULW), located in northern Morocco within the Loukkos Watershed, one of the main Atlantic-draining systems of the country. The ULW covers approximately 1,370 km^2 and extends between 35°24.661'-34°49.92' N and 5°36.27'-5°32.6' W, forming a transition zone between the Rif mountainous chain and the downstream plains. This upstream sector is characterized by rugged topography, narrow valleys, and a dense hydrographic network.

The climate is Mediterranean, influenced by both Atlantic air masses and local topography. Rainfall is irregular, ranging from around 400 mm in the lower parts of the basin to nearly 1,000 mm in the higher elevations. Precipitation mainly occurs between October and March. Winters are generally mild (mean ~11°C) and summers relatively warm (mean ~25.3°C) (Salhi *et al*., 2019). Episodes of intense rainfall, combined with clay-rich geological formations and steep slopes, generate rapid runoff responses. As a consequence, streamflow shows pronounced seasonal variability, with high winter discharges contrasting with severe summer low flows and, in some secondary tributaries, temporary drying (Agharroud *et al*., 2023).

Land use differs along the altitudinal gradient. While the lower Loukkos Watershed is largely devoted to irrigated agriculture, the upper watershed remains dominated by matorral shrublands and cork oak (*Quercus suber*) formations, locally altered by grazing. These natural formations are further impacted by grazing, land clearing, and reforestation programmes involving species such as Eucalyptus, pine, and Acacia (Ballouche, 2024). Olive cultivation is widespread throughout the basin. Small-scale oil extraction units operate in rural areas, and liquid residues are sometimes discharged directly into nearby streams. Domestic effluents from scattered settlements represent an additional source of organic pressure (Eddaoukhi *et al*., 2024).

A network of 18 sampling stations was established along the principal tributaries of the Upper Loukkos Watershed in order to represent the diversity of hydromorphological conditions and disturbance levels (Supplementary Tab. S1). Sites were surveyed seasonally during 2014 and 2015, with sampling conducted in spring and autumn to characterize community structures following flood and drought periods (Fig. 1).

Macroinvertebrate sampling and identification

Macroinvertebrate communities were investigated using a combination of quantitative and semi-quantitative approaches adapted to the diversity of local substrates. Riffle habitat samples were collected using a Surber sampler (20×20 cm). Complementary sampling was carried out with a hand net (25×25 cm; 250-500 µm mesh) to explore marginal zones, fine deposits and structurally pool habitats. Successive passes were performed until no additional taxa were detected, ensuring a representative inventory of the benthic community.

All material was preserved in 96% ethanol and transported to the laboratory. Organisms were sorted under a stereomicroscope and identified primarily to the family level following Tachet *et al.* (2002). The identification of endemic taxa was performed in collaboration with taxonomic specialists from our research group at the Laboratory of Ecology, Systematics and Biodiversity Conservation, Abdelmalek Essaadi University, Tetouan, Morocco.

Physicochemical analysis

Water quality was characterized through a set of field and laboratory measurements. In situ parameters included water temperature (°C), electrical conductivity (µS cm^{-1}), salinity (ppm), pH, turbidity (NTU) and dissolved oxygen (mg L^{-1}). These variables were measured at each station using a calibrated multiparameter probe (EUTECH CyberScan PCD 650). Measurements were performed directly within the main flow to ensure that the recorded values reflected representative conditions.

Water samples were collected and transported to the Loukkos

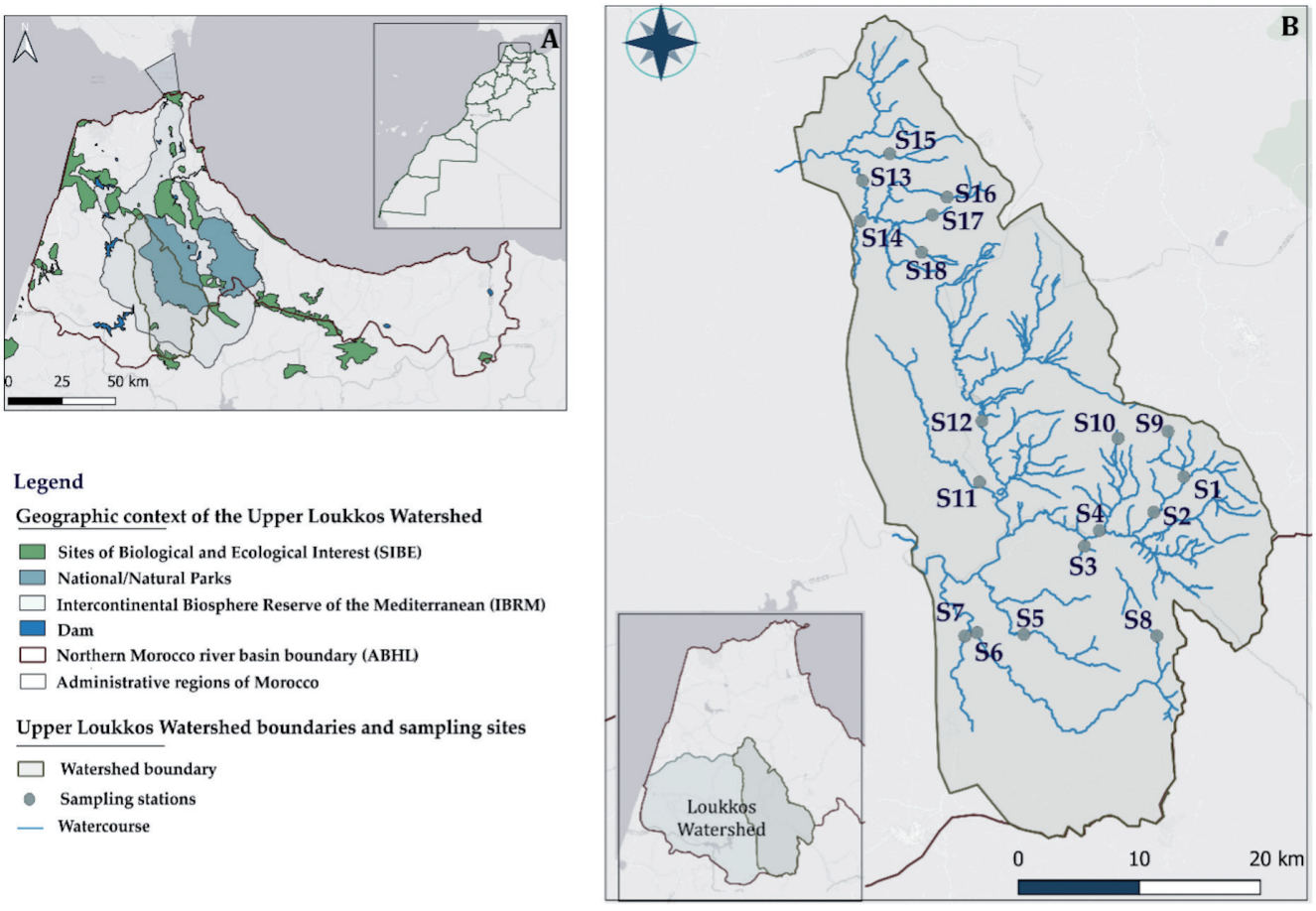

Fig. 1. Location of the Upper Loukkos Watershed showing geographic context (A) and distribution of the 18 sampling sites (B).

Hydraulic Basin Agency laboratory (ABHL, Tetouan) for further analysis. Laboratory determinations included chloride (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}), sulphate (mg L^{-1}), suspended solids (mg L^{-1}), biochemical oxygen demand (BOD_5, mg L^{-1}), and chemical oxygen demand (COD, mg L^{-1}). All analyses were carried out following standard procedures described by Rodier *et al.* (2009).

Data analysis

Temporal persistence of species was assessed based on their frequency of occurrence across the four sampling campaigns. For each taxon, occurrence frequency was calculated as the proportion of surveys in which the species was recorded relative to the total number of campaigns (n=4). According to this frequency, species were assigned to four persistence categories: permanent (0.76-1.00), frequent (0.51-0.75), intermittent (0.26-0.50), and rare (0-0.25).

Similarity in species composition between sampling campaigns was assessed using the Jaccard similarity index derived from presence-absence data. Multivariate patterns in community composition were further explored using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities. Seasonal differences in assemblage structure were tested using permutational multivariate analysis of variance (PERMANOVA).

To identify the taxa contributing most to seasonal differences in community composition, a similarity percentage analysis (SIMPER) was performed. Relationships between macroinvertebrate assemblages and environmental variables were examined using canonical correspondence analysis (CCA). Species abundance data were Hellinger-transformed prior to analysis to reduce the potential influence of dominant taxa, and environmental variables were standardised (z-scores) to ensure comparability across different units. Spatial and seasonal differences in endemic species richness and abundance were assessed using the Kruskal-Wallis test, given the non-normal distribution of count data. All statistical analyses were conducted in the R statistical environment (R Core Team, version 4.5.2).

RESULTS

Composition and distribution patterns of endemic macroinvertebrates

A total of 667 individuals were collected during four sampling campaigns carried out in spring and autumn 2014 and 2015 across the 18 investigated sites in the Upper Loukkos Watershed. These individuals belonged to 24 endemic species restricted to Morocco, the Maghreb, or the broader Ibero-Maghrebian region (Tab. 1).

Although spatial variation in endemic species richness was apparent across sampling stations (Fig. 2), Kruskal-Wallis tests revealed no statistically significant differences among sites in richness (H_{17}=12.37, p=0.777) or abundance (H_{17}=7.80, p=0.971). Oued Maleh (station S6) showed the highest abundance, reaching 107 individuals.

Tab. 1. Abundances and chorological categories of endemic species recorded in the Upper Loukkos Watershed during the survey period.

Species	Code	Chorotype	Spring 2014	Autumn 2014	Spring 2015	Autumn 2015
Ephemeroptera						
Acentrella almohades Alba-Tercedor & El Alami, 1999	Aca	Ib-Mag				3
Baetis maurus Kimmins, 1938	Bma	Ib-Mag	5		21	
Cheleocloeon dimorphicum (Soldán & Thomas, 1985).	Cdi	Mag			3	
Ecdyonurus ifranensis Vitte & Thomas, 1988	Eif	Mor	1	12		
Ecdyonurus rothschildi Navás, 1929	Ero	Ib-Mag	6		25	
Choroterpes atlas Soldán & Thomas, 1983	Cat	Ib-Mag	6	3	229	
Choroterpes volubilis Thomas & Vitte, 1988	Cvo	Mor	3	1		
Plecoptera						
Hemimelaena flaviventris (Pictet, 1841)	Hfl	Ib-Mag	1		9	
Isoperla cf. *kir* Fochetti & Vinçon, 1993	Iki	Mor	6	15	27	
Siphonoperla lepineyi (Navás, 1935)	Sle	Mor	3	2	1	
Trichoptera						
Rhyacophila fonticola Giudicelli & Dakki, 1984	Rfo	Ib-Mag			1	
Cheumatopsyche atlantis Navás, 1930	Chat	Mag			1	
Hydropsyche maroccana Navás, 1936	Hma	Mag	1		1	
Heteroptera						
Velia ioannis Tamanini, 1971	Vio	Mag	8		5	1
Parasigara transversa (Fieber, 1848)	Ptr	Ib-Mag		42	5	5
Coleoptera						
Graptodytes aequalis (Zimmermann, 1918)	Gae	Ib-Mag			1	
Helophorus algiricus Motschulsky, 1860	Heal	Mag			3	15
Hydrochus aljibensis Castro & Delgado, 1999	Hyal	Ib-Mag		2		2
Hydraena allomorpha Lagar & Fresneda, 1990	Hal	Ib-Mag	11	1	42	6
Hydraena bisulcata Rey, 1884	Hbi	Ib-Mag			9	2
Hydraena capta Orchymont, 1936	Hca	Ib-Mag				28
Hydraena rigua Orchymont, 1931	Hri	Mag		1	36	35
Limnebius fretalis Peyerimhoff, 1913	Lfr	Ib-Mag			12	6
Ochthebius anxifer Balfour-Browne, 1979	Oan	Ib-Mag			3	

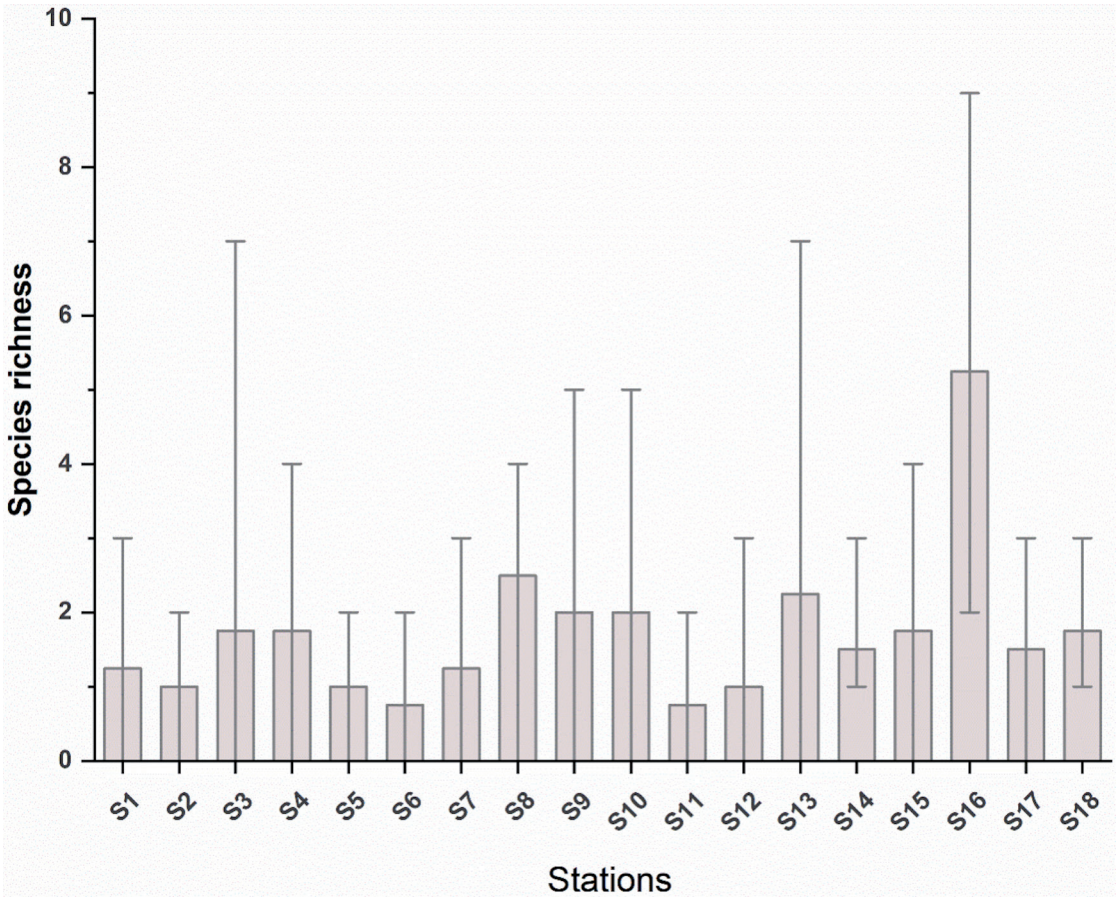

Fig. 2. Spatial distribution of endemic macroinvertebrate species richness across the sampling stations in the Upper Loukkos Watershed.

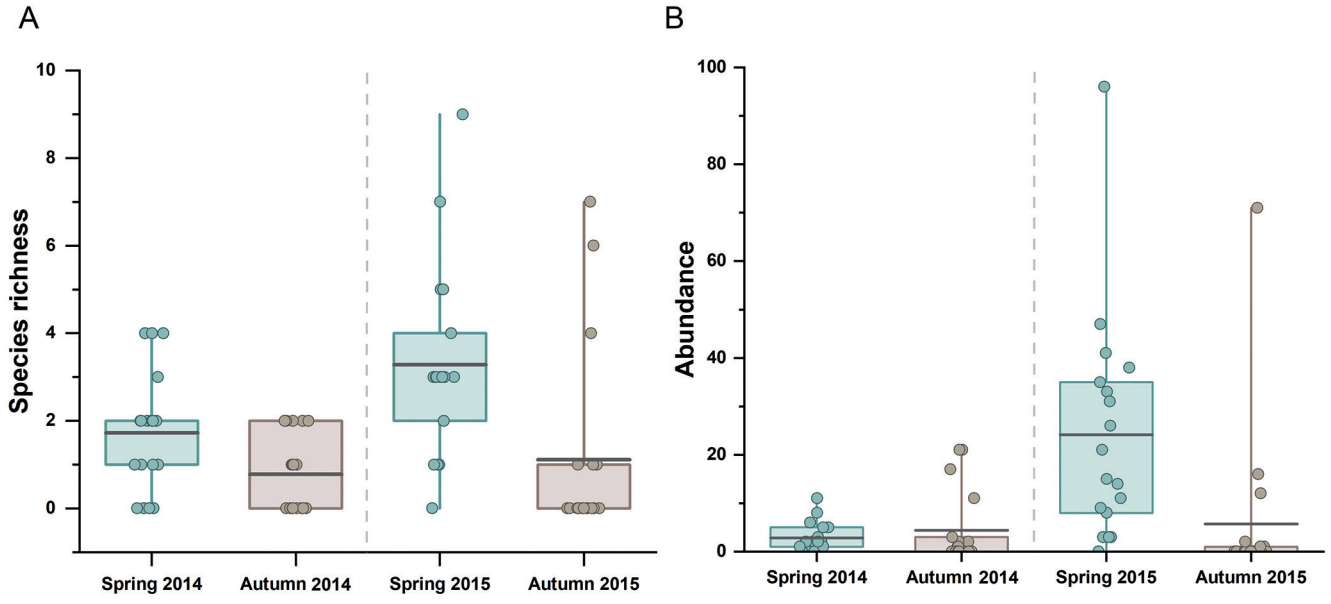

Fig. 3. Seasonal variation in species richness (A) and abundance of endemic macroinvertebrates (B) during the four sampling campaigns.

Among the recorded taxa, the genus *Hydraena* (Coleoptera) showed the highest diversity of endemic representatives, with four species. In terms of relative dominance, *Choroterpes atlas* was clearly the most abundant taxon, accounting for 36% of all collected individuals. This species was also the most widespread across the study area, being recorded at 12 sampling sites. Other abundant taxa included *Hydraena rigua* (11%) and *Hydraena allomorpha* (9%).

Kruskal-Wallis tests confirmed significant seasonal differences in both species richness (H_3=21.50, p<0.001) and abundance (H_3=25.58, p<0.001). The highest richness was recorded in spring 2015, when 20 species were detected. Lower values were found during the other sampling campaigns, with 11 species in spring 2014, 10 in autumn 2015, and 9 in autumn 2014. Abundance followed a similar seasonal pattern, with a marked peak in spring 2015, when a total of 434 individuals were collected.

Taxonomic richness showed noticeable variation among stations and sampling periods (Fig. 3). Values ranged from 0.78 taxa in autumn 2014 to 3.28 taxa in spring 2015, with the highest richness recorded at station S16 (Oued Stah) during the spring 2015 survey. A comparable seasonal pattern was also observed for abundance. Mean abundance increased substantially from 2.83 individuals in autumn 2014 to 24.1 individuals in spring 2015. The highest abundance was recorded at Oued Maleh during the spring 2015 sampling campaign, with a total of 96 individuals.

Analysis of the chorological composition of the assemblage revealed a strong predominance of Ibero-Maghrebian elements.

Tab. 2. Seasonal variation of physicochemical parameters (mean ±SD) measured in the Upper Loukkos Watershed during the four sampling campaigns (2014-2015).

Parameter	Unit	Spring 2014	Autumn 2014	Spring 2015	Autumn 2015
Temperature	°C	21.5±4.36	14.8±1.85	23.1±4.29	15.8±3.44
pH	pH	7.78±0.54	7.74±0.41	7.80±0.54	7.48±0.40
Conductivity	µS cm^{-1}	1033±1456	656±510	638±414	646±549
Chloride	mg L^{-1}	241±489	125±169	187±153	267±175
Nitrate	mg L^{-1}	0.79±0.52	4.12±3.36	0.77±0.09	1.01±0.26
Sulphate	mg L^{-1}	135±74.4	93.4±65.1	80.5±47.6	70.0±35.3
Oxygen	mg L^{-1}	3.02±1.24	6.84±1.68	3.65±1.03	1.35±0.84
BOD5	mg L^{-1}	21.3±3.92	13.3±2.30	25.4±4.58	16.1±2.98
COD	mg L^{-1}	43.2±7.83	27.5±3.79	54.0±9.79	34.5±6.38
TSS	mg L^{-1}	13.6±2.52	62.0±16.1	104±18.7	66.7±12.0

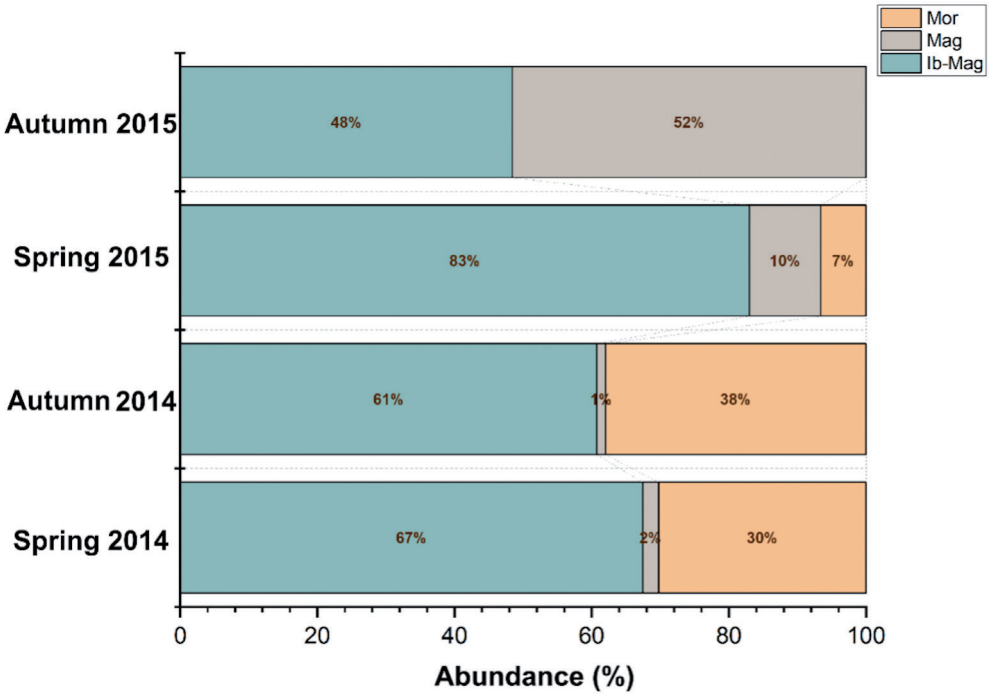

Fig. 4. Seasonal variation in the chorological composition of endemic macroinvertebrates in the Upper Loukkos Watershed.

Fourteen species belonged to this biogeographical category, representing approximately 58% of the total abundance. Maghrebian taxa formed the second most represented group, with six species accounting for 25% of individuals. Finally, four species were strictly endemic to Morocco, representing about 17% of the total abundance. Seasonal changes in species occurrences by chorotype are illustrated in Fig. 4.

Physicochemical conditions and their influence on macroinvertebrate assemblages

Physicochemical conditions varied substantially throughout the sampling period (2014-2015), reflecting both seasonal fluctuations and spatial heterogeneity among the investigated sites (Tab. 2). Water temperature showed a wide range of variation, from 11.2°C recorded at station S10 during autumn 2015 to a maximum of 29.2°C measured at station S6 in spring 2015.

Across the study area, pH values indicated generally neutral to moderately alkaline conditions. The lowest value (6.62) was observed at station S10, whereas the highest value (8.77) was recorded at station S6 during the spring 2014 survey.

Electrical conductivity displayed strong spatial variability, highlighting marked differences in ionic content among sites. The lowest value (43.9 µS cm^{-1}) was measured at station S17 in spring 2014, while the highest conductivity reached 6488 µS cm^{-1} at station S2 during the same sampling season. A comparable spatial pattern was observed for chloride concentrations, which ranged from 10.6 mg L^{-1} at station S17 in autumn 2014 to 2127 mg L^{-1} at station S2 in spring 2014.

Nutrient concentrations also differed among stations and sampling periods. Nitrate reached its highest value at station S10 in autumn 2014 (13.73 mg L^{-1}), whereas sulphate concentrations peaked at station S2 in spring 2014 (362.6 mg L^{-1}).

Dissolved oxygen levels showed noticeable spatial variation, with the highest concentration (10.11 mg L^{-1}) recorded at station S14 during autumn 2014. Indicators of organic enrichment exhibited a similar pattern. Biochemical oxygen demand (BOD) ranged from 10 mg L^{-1} at station S2 in autumn 2014 to 33 mg L^{-1} at station S18 in spring 2015, while chemical oxygen demand (COD) increased from 21 mg L^{-1} at station S2 to 70.5 mg L^{-1} at station S18 during the same period.

Total suspended solids (TSS) also showed considerable variability across the basin, with concentrations ranging from 2 mg L^{-1} at station S2 in spring 2014 to 135 mg L^{-1} at station S18 during spring 2015.

Canonical correspondence analysis (CCA) was conducted to examine the relationships between endemic macroinvertebrate species and the measured environmental variables (Fig. 5). Species abundance data were Hellinger-transformed prior to CCA to reduce the potential influence of dominant taxa. The first two canonical axes explained 42.3% of the constrained inertia, with CCA1 accounting for 25.6% of the constrained variation and CCA2 contributing an additional 16.7%. A permutation test indicated that the overall CCA model was not statistically significant ($F_{10,7}$=1.11, p=0.302), consistent with the exploratory nature of the observed associations.

The ordination diagram revealed a clear ecological gradient structuring the distribution of both sampling stations and taxa. The positive side of the first canonical axis (CCA1) was primarily associated with increasing values of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), sulphate (SO_4^{2-}), and chloride (Cl^-), reflecting sites characterized by relatively high organic and mineral loads. Stations located along this gradient were associated with several lentic or disturbance-tolerant taxa, particularly among Coleoptera, Hemiptera, and Odonata assemblages (OCH). In addition, some lotic species, such as *B. maurus* and *I. kir*, were also positioned along this gradient, suggesting a certain tolerance to altered or nutrient-enriched environmental conditions.

In contrast, the negative side of the first canonical axis (CCA1) was mainly structured by a gradient related to electrical conductivity, nitrate (NO_3^-) concentrations and dissolved oxygen levels. Stations located in this sector of the ordination were associated with taxa typically inhabiting well-oxygenated and flowing environments. Several lotic Ephemeroptera species were clearly aligned with this gradient, including *C. atlantis*, *E. rothschildi*, *C. atlas*, *C. volubilis*, *C. dimorphicum* and *E. ifranensis*.

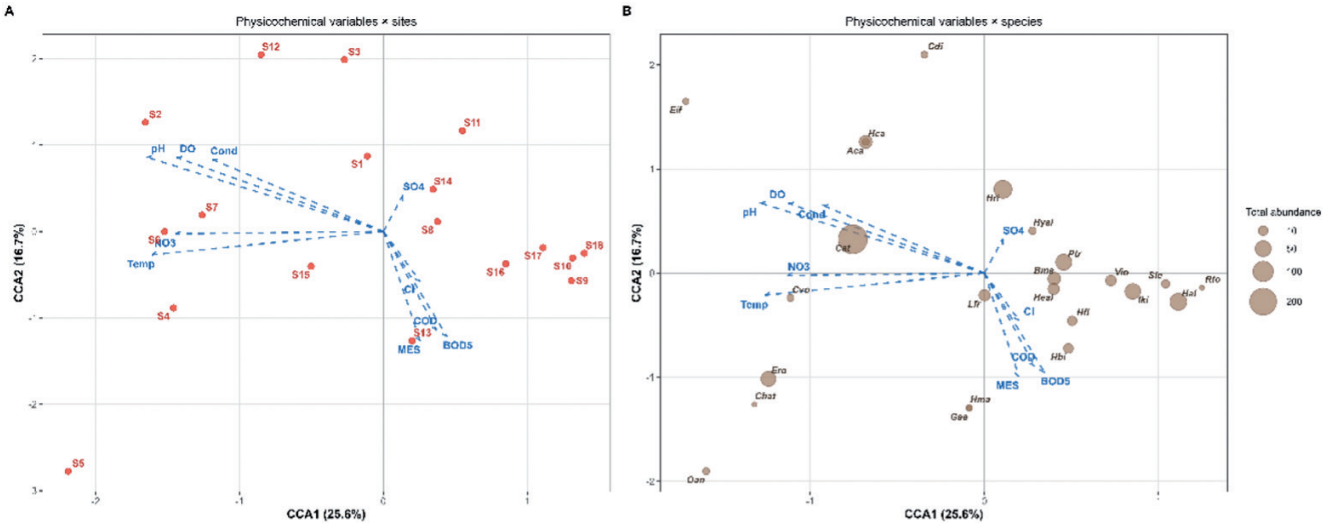

Fig. 5. Canonical correspondence analysis (CCA) ordination showing relationships between environmental variables and sampling stations (A) and endemic macroinvertebrate species (B).

Spatial and seasonal patterns of endemic macroinvertebrate assemblages

Based on species occurrence across the four sampling campaigns, taxa were classified into four persistence categories reflecting their temporal stability within the assemblage: permanent (0.76-1.00), frequent (0.51-0.75), intermittent (0.26-0.50), and rare (0-0.25). Only one taxon, *H. allomorpha*, was classified as permanent and was recorded during all sampling periods. Six taxa (*C. atlas*, *I. kir*, *S. lepineyi*, *V. ioannis*, *P. transversa*, and *H. rigua*) were classified as frequent, occurring in three of the four campaigns. Ten species were classified as intermittent (*B. maurus*, *E. ifranensis*, *E. rothschildi*, *C. volubilis*, *H. flaviventris*, *H. maroccana*, *H. algiricus*, *H. aljibensis*, *H. bisulcata*, and *L. fretalis*). Intermittent species represented the largest proportion of the assemblage (41.7% of the total species pool). In contrast, seven species were considered rare, as they were recorded only once during the study period (*A. almohades*, *C. dimorphicum*, *R. fonticola*, *C. atlantis*, *G. aequalis*, *H. capta*, and *O. anxifer*) (Fig. 6).

Pairwise comparisons of sampling periods using the Jaccard similarity index revealed relatively low similarity in community composition across campaigns, with values ranging from 0.105 to 0.429. The lowest similarity was observed between Spring 2014 and Autumn 2015 (0.105), indicating a marked shift in species composition between these two sampling events. Moderate similarity values were recorded between Spring 2014 and Autumn 2014 as well as between Spring 2014 and Spring 2015 (0.429). Comparisons between autumn and spring sampling periods generally yielded lower similarity values (0.273-0.318), highlighting a pronounced temporal restructuring of the macroinvertebrate assemblage (Tab. 3).

A PERMANOVA based on Bray-Curtis dissimilarities indicated that season accounted for 31.4% of the variation in macroinvertebrate community composition (R^2=0.314; F=0.92). However, this effect was not statistically significant (p=0.67), likely reflecting the limited statistical power of a design with only four temporal replicates rather than an absence of seasonal structuring. This result is consistent with the distribution of Bray-Curtis dissimilarities, as mean dissimilarity values were comparable within seasons (0.864±0.043) and between seasons (0.844±0.064). The substantial overlap between these values indicates that although seasonal shifts were detectable, community variability within sampling periods remained high.

The NMDS ordination (Bray-Curtis distance, stress=0.158) revealed a seasonal structure in the macroinvertebrate assemblage. Species were distributed along the ordination according to their temporal occurrence patterns (Fig. 7). Frequent and permanent taxa were generally located near the centre of the ordination, indicating their consistent contribution to community composition across sampling periods. In contrast, rare species were positioned at the periphery of the ordination space, reflecting their sporadic occurrence. Intermittent taxa occupied intermediate positions and were associated with sampling seasons.

SIMPER analysis decomposed the Bray-Curtis dissimilarity between spring and autumn assemblages, revealing that a small number of taxa accounted for most of the observed compositional differences (Tab. 4). As this approach identifies the taxa driving dissimilarity without testing the statistical significance of group separation, the following patterns should be interpreted as descriptive rather than confirmatory. The frequent species *C. atlas* contributed the most to seasonal dissimilarity (23.2%) and was markedly more abundant in spring. Similarly, the permanent species *H. allomorpha* showed higher mean abundances during spring. In contrast, *P. transversa* and *H. capta* were more abundant in autumn and contributed substantially to the compositional differences characterizing autumn assemblages. Several intermittent taxa, including *E. rothschildi* and *B. maurus*, were almost exclusively associated with spring samples, indicating a seasonal occurrence pattern.

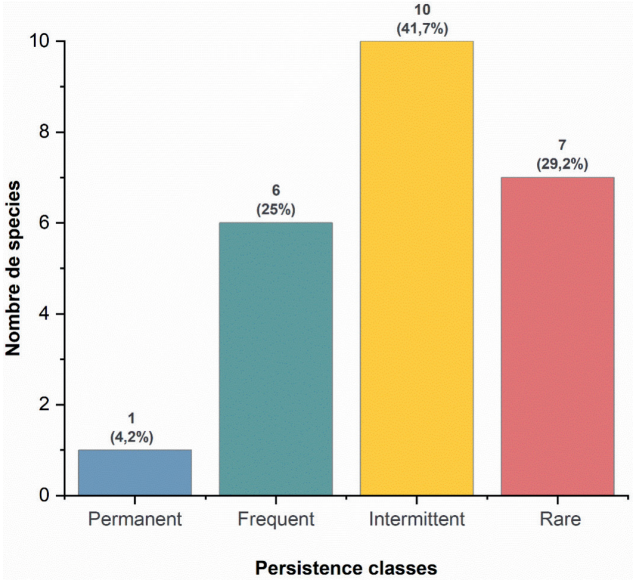

Fig. 6. Temporal occurrence of endemic macroinvertebrate species across the four sampling campaigns.

Tab. 3. Jaccard similarity and distance values between the four sampling campaigns.

Compared periods	Jaccard distance	Jaccard similarity
Spring 2014 – Autumn 2014	0.571	0.429
Spring 2014 – Spring 2015	0.571	0.429
Spring 2014 – Autumn 2015	0.895	0.105
Autumn 2014 – Spring 2015	0.727	0.273
Autumn 2014 – Autumn 2015	0.733	0.267
Spring 2015 – Autumn 2015	0.682	0.318

Tab. 4. Species contributing most to the dissimilarity between spring and autumn macroinvertebrate assemblages based on SIMPER analysis (Bray-Curtis dissimilarity).

Species	Persistence class	Mean Spring	Mean Autumn	Contribution (%)	Cum. Contribution (%)	Ratio
Cat	Frequent	117.5	1.5	23.23	23.23	1.00
Ptr	Frequent	2.5	23.5	10.69	33.92	0.73
Hri	Frequent	18.0	18.0	7.63	41.55	0.73
Hal	Permanent	26.5	3.5	6.41	47.96	2.94
Hca	Rare	0.0	14.0	5.85	53.81	0.68
Iki	Frequent	16.5	7.5	4.55	58.36	2.36
Ero	Intermittent	15.5	0.0	4.51	62.87	10.62
Bma	Intermittent	13.0	0.0	3.77	66.64	10.29
Heal	Intermittent	1.5	7.5	3.14	69.78	0.70
Vio	Frequent	6.5	0.5	3.10	72.88	1.16

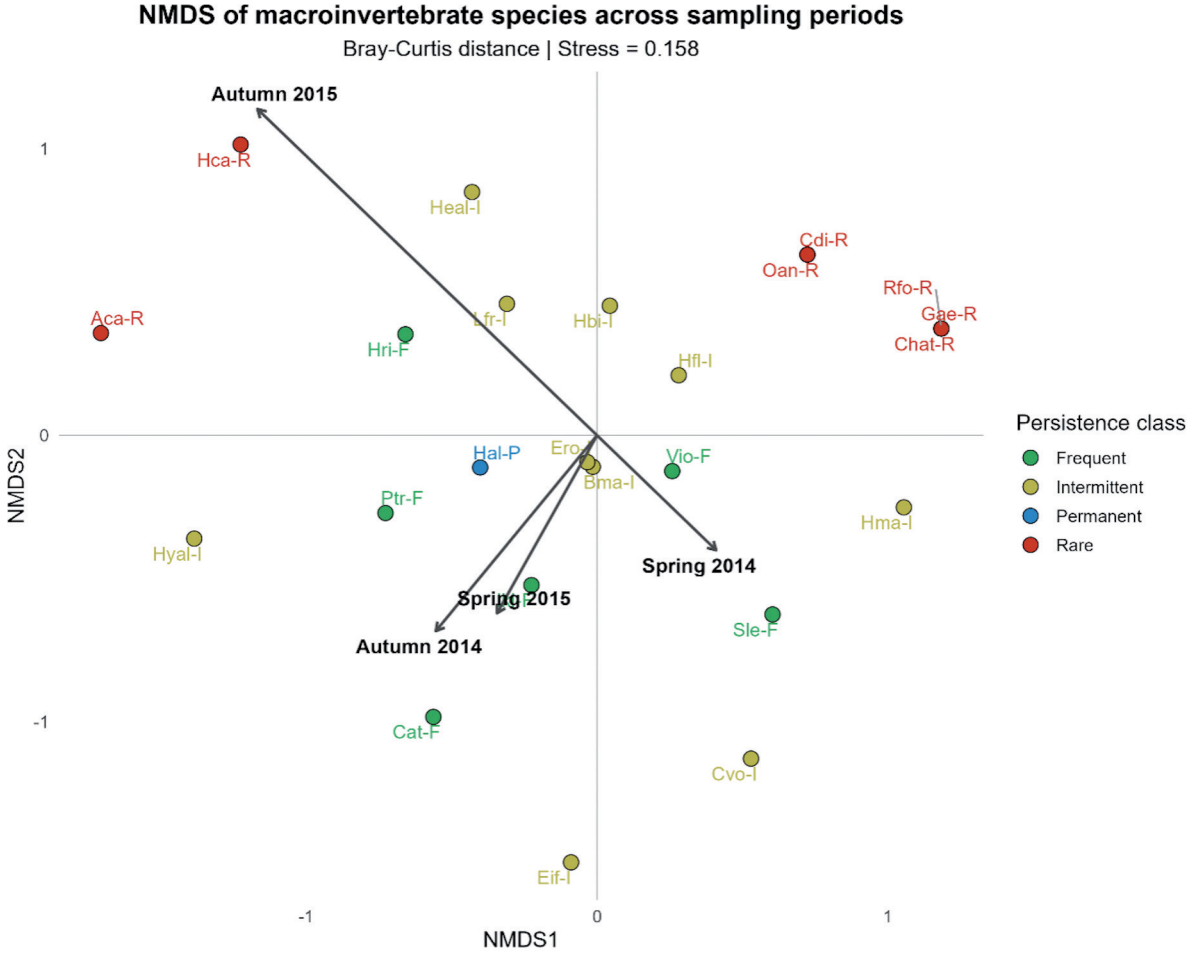

Fig. 7. NMDS ordination (Bray-Curtis distance) showing seasonal patterns in macroinvertebrate assemblage composition.

DISCUSSION

Mediterranean rivers exhibit strong hydro-seasonal differences that strongly structure macroinvertebrate communities through recurrent shifts between high-flow and low-flow phases (Gasith and Resh, 1999; Brown *et al*., 2009). In the Upper Loukkos Watershed, this was expressed as an apparent seasonal shift, with spring assemblages associated with flowing habitats and autumn assemblages persisting under reduced discharge.

During spring, when flow permanence increased and habitat connectivity was re-established, assemblages were largely dominated by rheophilic taxa typical of well-oxygenated riffles, especially Ephemeroptera and Plecoptera. Species such as *C. atlas*, *E. rothschildi*, *E. ifranensis*, *B. maurus*, *I. kir*, and *H. flaviventris* were mainly recorded under these conditions. In contrast, during periods of reduced discharge, assemblages shifted towards taxa associated with slower currents or pool habitats, including Odonata, Coleoptera, and Hemiptera (Brown *et al*., 2009; Banegas-Medina *et al*., 2021). Species such as *V. ioannis, P. transversa,* and several Hydraenidae *(H. allomorpha, H. rigua, H. capta*) became more common as discharge declined. These seasonal changes illustrate the gradual shift from lotic habitats to lentic or semi-isolated environments that typically accompanies flow contraction in temporary rivers (Bonada *et al*., 2007; Sangiorgio *et al*., 2007; Bogan *et al*., 2019; Bonada *et al*., 2020; Banegas-Medina *et al*., 2021).

Flow intermittence also influences the persistence of communities by determining the availability of refugial habitats during low-flow periods. In Mediterranean streams, isolated pools and residual wetted patches often provide short-term refuges during drought, allowing local populations to persist and later recolonize the channel when hydrological connectivity is restored (Stubbington *et al*., 2017; Banegas-Medina *et al*., 2021). However, the ecological role of these refuges varies among taxa: species with strong dispersal abilities or tolerance to lentic conditions often dominate them, whereas endemic or narrowly distributed taxa may be more vulnerable to prolonged drying (Stubbington *et al*., 2017; Bogan *et al*., 2019). As hydrological variability intensifies, these dynamics may increasingly favour opportunistic taxa while limiting the persistence of more specialized taxa (Datry *et al*., 2017; Taybi *et al*., 2020; Carey *et al*., 2023).

The CCA analysis revealed associations between physicochemical gradients and the spatial distribution of endemic macroinvertebrates within the basin. Stations characterized by higher BOD_5, COD, suspended solids and mineral concentrations were associated with taxa frequently reported as disturbance-tolerant in Mediterranean streams, mainly Coleoptera and Hemiptera (Alba-Tercedor *et al*., 2002; Touaylia, 2017), suggesting a certain tolerance to moderate organic enrichment or local disturbance. In contrast, sites with higher dissolved oxygen levels and lower organic loads supported assemblages typical of well-oxygenated flowing habitats.

This distinction is particularly relevant for endemic EPT taxa, which tend to be associated with well-oxygenated, flowing conditions, a habitat affinity broadly documented in functional trait classifications and generally linked to greater sensitivity to hydrological disturbance (Tachet *et al*., 2002; Carey *et al*., 2023). Under fluctuating conditions, physicochemical gradients were associated with assemblage composition. We therefore interpret these results as correlative associations rather than causal evidence of ecological filtering. In Mediterranean rivers, seasonal flow, water abstraction and pollution have all been shown to reduce suitable habitat and to increase the vulnerability of specialized taxa (Carey *et al*., 2023; Espinar-Herranz *et al*., 2025).

Some groups, however, have been reported to persist across a range of hydrological conditions. Hydraenidae, for instance, can occur in both running and slow-flowing waters and may recolonize channels rapidly after rewetting (Benamar *et al*., 2022), which could explain their relatively high occurrence in the study area and is consistent with observations from other Mediterranean streams (Stubbington and Datry, 2013).

More generally, taxa belonging to Odonata, Coleoptera, and Hemiptera are frequently reported as tolerant to hydrological variability and environmental disturbance (Touaylia, 2017; Sripanya *et al*., 2023; Benamar *et al*., 2024). Nevertheless, persistence was not limited to disturbance-tolerant taxa. Some rheophilic species, including *B. maurus, C. volubilis, S. lepineyi* and *I. kir*, have also been reported from northern Moroccan streams as capable of tolerating moderate environmental variation (Errochdi, 2015; Guellaf *et al*., 2021a; El Alami *et al*., 2022; El Yaagoubi *et al*., 2024). This suggests that community composition is shaped not only by tolerance, but also by a combination of environmental filtering and local habitat conditions.

The chorological composition of the assemblage, with its predominance of Ibero-Maghrebian species, underlines the biogeographical importance of the Rif region within the western Mediterranean basin (Médail and Quézel, 1997; Bonada *et al*., 2008). The Betico-Rifan massif has long been recognized as a center of biodiversity linking southern Iberia and northern Morocco. Its complex geological history, together with past land connections between Europe and Africa, likely promoted faunal exchange while also allowing endemic lineages to persist (Bennas *et al*., 1992; Yasri-Cheboubi *et al*., 2016; Touaylia, 2017; Guellaf *et al*., 2021b). In the Upper Loukkos Watershed, located within the Intercontinental Biosphere Reserve of the Mediterranean (IBRM), the strong representation of Ibero-Maghrebian taxa reflects these long-standing biogeographical connections and the shared evolutionary history of Iberian and North African freshwater faunas.

The conservation status of endemic aquatic invertebrates remains difficult to assess due to the scarcity of long-term population data. While Red List evaluations often guide conservation priorities, the application of IUCN criteria to aquatic insects remains challenging because such frameworks require robust information on population trends and distribution dynamics (Benamar *et al*., 2021a; Guellaf *et al*., 2025). As a result, most of the 24 endemic taxa documented here remain formally unassessed despite increasing environmental pressure. Our findings thus support a broader inclusion of macroinvertebrates in biodiversity assessments as a means of improving conservation planning and obtaining a more realistic picture of freshwater ecosystem condition.

Belhaj *et al.* (2023a)) identified the Loukkos watershed as a potential climatic refugium for endemic aquatic Coleoptera, highlighting its ecological importance for freshwater conservation due to the presence of protected areas within this Atlantic basin. However, endemic species richness remained relatively limited, with only 24 species recorded compared with other Mediterranean basins such as the Laou watershed (Errochdi, 2015) and the Martil watershed (Guellaf *et al*., 2025). This pattern may be related to the strong hydrological variability of the basin, characterised by recurrent floods and drought periods, together with pronounced flow intermittence.

The endemic assemblages documented here nonetheless underscore the ecological value of these headwater systems. Strengthening conservation strategies will require integrating freshwater biodiversity into protected-area planning, expanding surveys

in poorly studied basins, and preserving hydrological refugia capable of sustaining endemic populations under increasing climatic variability (Sánchez-Fernández *et al*., 2004; Belhaj *et al*., 2023a).

Several limitations of the present study warrant acknowledgement. Sampling covered four seasonal campaigns across two years, yielding limited power for formal multivariate inference. The non-significant PERMANOVA result should therefore be interpreted as exploratory rather than as evidence of weak seasonal structuring. It should also be noted that the first two CCA axes explained only 42.3% of the constrained inertia, indicating substantial unexplained variation. Our conclusions regarding seasonal compositional turnover should accordingly be treated as preliminary, and long-term higher-frequency monitoring will be needed to validate both the seasonal and distributional trends reported here. Furthermore, our findings are restricted to a single watershed (Upper Loukkos); extending surveys to other biogeographical units within the Rif Mountains and across northern Morocco would be necessary to assess regional representativeness.

CONCLUSIONS

This study provides new insights into the distribution of endemic macroinvertebrates in the Upper Loukkos watershed, a mountainous Mediterranean system located within the Intercontinental Biosphere Reserve of the Mediterranean. The communities observed across the basin reflect the combined influence of seasonal hydrological variability and local anthropogenic pressures shaping river habitats in the region. Although overall species richness remained moderate, the occurrence of several Ibero-Maghrebian taxa highlights the biogeographical importance of the Rif Mountains and the key role of headwater streams in sustaining regionally restricted freshwater fauna.

Our results also show that knowledge of freshwater invertebrates in North African rivers is still limited. Many endemic species remain poorly documented and are rarely considered in conservation strategies. Additional field surveys, together with improved taxonomic resolution, are therefore necessary to better characterize these communities. Long-term monitoring of hydrological and environmental conditions would further help to clarify species respond to ongoing environmental change.

Strengthening biodiversity assessments could facilitate the integration of freshwater macroinvertebrates into conservation frameworks, including the IUCN Red List and Moroccan Law 29-05, and contribute to the identification of priority areas for freshwater conservation in Mediterranean basins.

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Online supplementary material:

Tab. S1. List of localities and stream characteristics of the sampled sites.

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