Unravelling the environmental correlates influencing the seasonal biodiversity of aquatic Heteropteran assemblages in northern Africa

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Abstract

Heteropteran communities form a key component of aquatic ecosystems but have not been widely studied compared to other freshwater faunal groups. This research examined the environmental parameters influencing the diversity, seasonal distribution and structure of aquatic Heteroptera assemblages in the Mediterranean region of Tunisia, northern Africa. Heteropterans were most abundant during spring and summer, coinciding with the emergence of several species and the most favorable environmental conditions for benthic aquatic fauna. Three-way multivariate analyses (combining community composition data from all sites and seasons) highlighted the longitudinal spatial organization of Heteropteran communities. Headwater regions were dominated by halophobic sensitive taxa, and lowland sites were characterized by high salinity resistant taxa (halophilic taxa). The longitudinal organization was driven by gradients of mineralization (salinity and electrical conductivity) and oxygen (DO, COD and BOD) concentrations. Taxonomic composition differed between river catchments, with significantly higher diversity (taxa richness) in the streams with adjacent riparian forest cover. These sites were characterized by the presence of endemic species, such as *Velia africana* and *Velia eckerleini*, and rare species, *Notonecta meridionalis*, and *Aquarius najas*. Results recorded highlight the importance of aquatic vegetation and water quality in driving the seasonal and spatial variability of Heteropterans, and provide important information to inform the management and conservation of freshwater biodiversity in Northern Africa.

Key words. freshwater ecosystems, water quality, macroinvertebrate, aquatic ecology, Mediterranean.

Introduction

Freshwater ecosystems in the Mediterranean region of North Africa are increasingly recognized as global biodiversity hotspots (Beauchard et al., 2003). However, there remains a fundamental lack of research considering the structure and functioning of north African Mediterranean freshwater ecosystems and in the insect communities they support (Slimani et al., 2019). This limited ecological knowledge currently hinders our scientific understanding of how aquatic ecosystems and their biodiversity will respond to anthropogenic pressures across North Africa in the future (Schilling et al., 2012). The primary threats to the freshwater biodiversity of north Africa, and globally, include excessive freshwater water abstraction (for domestic, agricultural and industrial use) and the construction of engineered infrastructure (e.g. dams, weirs and channelization), which have driven the rapid loss of natural freshwater habitats (Reid et al., 2019). Anthropogenic flow regime modifications and projected climatic change threatens freshwater environments (Döll and Schmied, 2012; Colin et al., 2016), their high biodiversity and the endemic freshwater fauna (including the freshwater insects) they support (García et al., 2010) across North Africa.

Heteropterans represent a subgroup of the Hemiptera, an insect order forming an integral part of freshwater ecosystems globally. There are over 4500 Heteropteran species thought to inhabit aquatic environments globally, making a significant contribution to the biodiversity of freshwater ecosystems (Polhemus and Polhemus, 2008). They have been recorded in virtually all aquatic habitats, including surface and subsurface environments associated with lotic (e.g. headwater streams, lowland rivers) and lentic (e.g. pond, lakes, coastal rock pools) waterbodies (Slimani et al., 2015, 2016, 2017). Heteropterans are important for the functioning of aquatic ecosystems as they help regulate fluxes of energy and matter across aquatic food webs, through facilitating nutrient cycling and providing a source of food for higher trophic levels (Brown and McLachlan, 2010; Amaral et al., 2016). Aquatic Heteropteran taxa vary considerably in their ecological requirements (Aukema et al., 2013; De Figueroa et al., 2013; Damgaard and Zettel, 2014; Turić et al., 2015), and many species display specific habitat preferences (Bloechl et al., 2010; Carbonell et al., 2011; Annani et al., 2012; Dudgeon, 2012). As such, Heteropteran communities are widely used as indicators of aquatic ecosystem health and to characterize ecological responses to anthropogenic pressures (e.g., Hershey et al., 2010; Lock et al., 2013; Slimani et al., 2017). In addition, many Heteropteran species are recognized as reliable surrogates for the wider biodiversity of aquatic ecosystems and have been used to support the conservation and restoration of freshwater habitats (Whiteman and Sites, 2008; Cunha and Juen, 2017).

Globally, the majority of aquatic Heteropterans belong to one of two major groups: Nepomorpha (water bugs) which largely live below the water surface, and Gerromorpha (water striders) comprising species that inhabit the water surface film (Polhemus and Polhemus, 2008). Both Nepomorpha and Gerromorpha are particularly common within Mediterranean freshwater ecosystems (De Figueroa et al., 2013), including those of northern Africa (Aukema et al., 2013; Slimani et al., 2015, 2016). The distribution and diversity of both subgroups may be influenced by a range of environmental factors, including physicochemical conditions (particularly dissolved solutes and oxygen concentrations), flow conditions (lentic or lotic habitats) and water temperature (Tully et al., 1991; Hufnagel et al., 1999; Barahona et al., 2005; Karaouzas and Gritzalis, 2006; Skern et al., 2010). The size and shape of the water body has been reported to strongly influence their distribution; with clear distinctions between small shallow waterbodies and larger deeper lakes (Macan, 1954; Savage, 1994).

Heteropterans display a range of traits which allow them to survive extreme hydroclimatic conditions, from torrential floods during winter to the severe summer droughts which occur consistently in the Mediterranean region of North Africa (Annani et al., 2012). For example, species from the genus *Notonecta* spp. (Notonectidae) have a prolonged period of development over multiple months and are typically be found in freshwaters at higher altitude during the drier summer months (Annani et al., 2012). While a limited number of studies have explored the taxonomic composition and diversity of aquatic Heteropteran communities in northern Africa (e.g. L'mohdi et al., 2008 -Morocco; Annani et al., 2012 -Algeria; Slimani et al., 2015, 2016 - Tunisia), to our knowledge none have tested their sensitivity to varying environmental conditions beyond individual river basins or seasons.

In order to better understand the key drivers underpinning the spatiotemporal distributions of aquatic Heteropteran assemblages, we examined seasonal variations in community structure and environmental controls across 45 sites within 4 catchments in northern Tunisian. A total of 21 environmental variables, identified as drivers of aquatic Heteroptera assemblages were measured: including salinity, conductivity, dissolved oxygen, water depth, and surface area of open water. The main objectives of the study were to: (i) examine differences in Heteropteran community composition and diversity between the four river catchments examined; and (ii) quantify the environmental correlates driving spatiotemporal variability of Heteropteran communities across the catchments.

Materials and Methods

Study site

This research was undertaken in northern Tunisia (Fig. 1 and Table S.1 in Supplementary Material), a Mediterranean region located in the Maghreb (North Africa) and included 45 sites within four catchments (Medjerda; Kroumirie-Mogods; Ichkeul; and Meliane-Cap Bon).

All sites maintained perennial surface water and encompassed the entire diversity of freshwater habitats preferred by the target group, including streams, springs, ponds and lakes with both lotic/lentic water conditions; comprising 34 lotic and 11 lentic waterbodies (Fig. 1, Table S.1 in Supplementary Material). Substrates within the freshwater habitats were characterized by cobbles, gravel, sand, silt and clay; with some sites appearing muddy and having a loamy character. Sampling sites were established on each catchment longitudinally from upstream to downstream (St 1 to St 45). Site 1 was in the headwaters (upstream) of the Wadi Medjerda in North-West Tunisia, and site 45 was located the furthest downstream on the Wadi Lebna in the North-Eastern extremity of Tunisia in the Cap Bon region (A summary of sites is presented Table 1). The northern area of Tunisia is characterized by a typical Mediterranean climate, ranging from humid to sub-humid and supports a rich floral diversity including *Typha angustifolia* L., *Quercus canariensis* Willd., and *Quercus suber* L. This northern region occupies 17% of the land mass but includes 60% of the national freshwater resource.

Biological data

Aquatic Heteroptera samples were collected on 4 separate occasions: February (spring), May (summer), September (autumn), and December (winter) 2013 from the 45 sites (Fig. 1, Table S.1 in Supplementary Material). The samples were obtained using a combination of both Surber samples (300 μm mesh net with an opening 0.20 m wide and 0.10 m deep) and a kick / sweep net (filet Troubleau). A total of 25 minutes of sampling and searching was undertaken at each site ensuring that the entire habitat heterogeneity was considered at each site. Samples were preserved in 70% ethanol and returned to the laboratory for processing and species level identification. Laboratory identification of fauna was undertaken using available identification keys (Poisson, 1957; Tamanini, 1979; Jansson, 1986; Andersen, 1990; Zimmermann and Scholl, 1993), a binocular microscope, and the Heteroptera collections deposited at the National Museum of Natural History in Paris and the Naturalis Biodiversity Centre in Leiden to verify the identifications with the assistance of three experts.

Environmental parameters

The following environmental parameters were measured in situ at each site at the time of sampling aquatic Heteroptera comprising pH, salinity (SAL), electrical conductivity (COND), total dissolved salt (TDS) and dissolved oxygen (OXY) with portable probes (WTW, PP350). Air and water temperature (ATEMP, WTEMP) were measured simultaneously in the field using a mercury glass thermometer graduated to 0.1°C. Altitude (ALT) was calculated using a GPS (Garmin etrex 10). Flow velocity (FV) was measured using the time (seconds) taken by a float (cork stopper) to cover a minimum distance of one meter. Visual estimates of percentage substrate composition in shallow water and riparian cover were made at each site (Table S.1 in Supplementary Material). Water samples were collected at each site using 4 L polyethylene bottles and transported on ice back to the "Centre International des Technologies de l'Environnement de Tunis" CITET laboratory for further analyses. Turbidity was measured in the laboratory using a turbidity meter (Hach model 2100A). Major cation and anion concentrations: Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). Chloride (Cl^-), and sulphates (SO_4^{2-}) were measured by liquid chromatography (DX-120 Ion Chromatograph). The concentration of nutrients: nitrates (NO_3^-), nitrite (NO_2^-), ammoniac (NH_4^+), and Orthophosphate (PO_4^{3-}), were measured by spectrophotometry (JASCO V-530 UV/VIS) at 630 nm. Chemical oxygen demand (COD) was measured using the amount of potassium dichromate ($K_2Cr_2O_7$) consumed by the dissolved solids in suspension. Biological Oxygen Demand (BOD_5) was measured by incubation of the water sample in the presence of a solution of phosphate and allyl thiourea in darkness and at 20°C over 5-days. Other parameters, including channel width (WIDT) and water depth (DEPT) were measured in the field at each site. The concentration of NO_2^- was consistently below the device's detection limit (0.4 mg L^{-1}), and therefore were not presented in the results below.

Data analysis

Statistical analyses were undertaken in the R environment (R Development Core Team, 2018). The STATICO multivariate analysis method (Thioulouse et al., 2004; Thioulouse, 2011) was used to investigate the relationships between aquatic Heteroptera communities and environmental variables (including morpho-dynamic parameters) over the four seasons. The STATICO method is based on the use of two analyses: Co-inertia Analysis (Dolédec and Chessel, 1994; Dray et al., 2003) and Partial Triadic Analysis (Thioulouse and Chessel, 1987; Thioulouse, 2011). This method allows the simultaneous analyses of three dimensions of the dataset: faunal matrix × environmental parameters matrix × four seasons.

Co-inertia analysis was initially performed by building a crossed table between the two datasets: one comprising the 21 environmental parameters and the other for the 36 Heteroptera species. Each of these contained 180 rows, corresponding to the 45 sampling sites over four seasons (spring, summer, autumn and winter). Computations were undertaken on these tables using the *statico* function in the *ade4* package (Slimani et al., 2017; Thioulouse et al., 2018). Co-inertia analysis computes axes that maximize the covariance between the scores of the rows (sampling sites) of the environmental parameters table, as well as the scores of the rows of the species table.

Partial Triadic Analysis is a K-tables data analytical method. The STATICO analysis consists of computing the four (one for each season) crossed tables (environmental parameters x species) for the four corresponding co-inertia analyses. A Partial Triadic Analysis is then undertaken on the series based on these four crossed tables. The interstructure step computes a weighted mean of the four seasons (the compromise). The compromise step includes a PCA using this table, providing environmental parameter scores and species scores. The intrastructure step computes the scores of the rows (environmental parameters) and columns (species) of the four crossed tables (one for each season) and of the rows of the initial tables (sampling sites of the environmental parameters table and of the species table). The scores computed in the intrastructure step of the STATICO analysis describe the temporal (within and between seasons) and spatial (within and between catchments) variability of the associations between aquatic Heteropteran communities and environmental parameters.

When using the STATICO method, the variable scaling and the row weights must be chosen with great care, as they imply different ecological considerations (Dray et al., 2003). In this study, the separate analysis of each table was performed using a PCA because the species responses to the environmental parameters are approximately linear (verified graphically in preliminary analysis). The table of environmental variables was submitted to a "partial Bouroche standardization", which means that it was first standardized (i.e. centered and normalized) for the whole dataset (globally), and then standardized within each season. The PCA of species abundance data was performed using log10 (x+1) transformed data to reduce the influence of highly abundant species and also submitted to a partial Bouroche standardization. Sampling site weights were considered uniform because the sampling method was homogeneous throughout the time-series and the taxonomic identification was performed consistently by the same individual.

Results

Environmental characterization

Environmental factors displayed spatial and temporal differences between sampling sites and seasons respectively (Table 1). One-Way Analysis of Variance (ANOVA) indicated significant seasonal differences for the following physicochemical parameters between seasons: pH (DF = 3, F = 25.94, = $P < 0.001$, R^2 =0.30), dissolved oxygen (DF = 3, F = 13.13, = $P < 0.001$, R^2 =0.18), turbidity (DF = 3, F = 9.69, = $P < 0.001$, R^2 =0.14), water temperature (DF = 3, F = 258.35, = $P < 0.001$, R^2 =0.81), air temperature (DF = 3, F = 180.96, = $P < 0.001$, R^2 =0.75), flow velocity (DF = 3, F = 15.32, = $P < 0.001$, R^2 =0.20), nitrate - NO_3^- L^{-1} (DF = 3, F =

9.09, = $P < 0.001$, $R^2 = 0.13$), and ammonia - $NH_4^+ L^{-1}$ (DF = 3, F = 4.57, = $P < 0.001$, $R^2 = 0.07$). The PCA of physicochemical data indicated that the first and second axes explained 22.91% and 12.46% of the variance in the dataset, respectively (Fig. 2). PCA-axis 1 primarily reflected a gradient of altitude and mineralization, with high altitude low mineralization samples located on the positive end and low altitude high mineralization (COND, SAL, TDS) samples on the negative side of the axes. PCA-axis 2 reflected a gradient of dissolved oxygen concentrations and pH on the positive side and temperature (air and water) on the negative side.

Differences in Heteropteran communities between catchments

A total of 36 aquatic Heteroptera species representing 19 genera and 13 families were recorded; 23 species belonging to the Nepomorpha subgroup and 13 species representing Gerromorpha. The densities (individuals m^2) and percentage (%) contribution to abundance during the study period for all taxa recorded are presented in Table 2.

Nepomorpha contributed 66.54% of the total relative abundance, with *Micronecta scholtzi* (Fieber) representing the most abundant species (22.43%). The Gerromorpha species contributed 33.46% of the total abundance, with *Aquarius cinereus* (Puton) being the most common (17.18%) (Table 2). Species richness and the distribution of Heteroptera varied between catchments, reflecting the variable contribution of different species throughout the study period (Fig. 3). The total density of Heteroptera was greatest at Meliane-Cap Bon (2382 ind/m^2) and the highest species richness was recorded at Kroumirie -Mogods (33 species).

The aquatic Heteroptera community compositions varied considerably between catchments (Fig. 3). The Medjerda and Kroumirie-Mogods catchments were dominated by two species *Micronecta scholtzi*, and *Aquarius cinereus*. The Ichkeul catchment was characterized by higher densities of *Micronecta scholtzi*, *Nepa cinerea* L., *Naucoris maculatus conspersus* (Stål). *Nepa cinerea*, *Naucoris maculatus conspersus*, and *Hydrometra stagnorum* L.. *Parasigara favieri* (Poisson), *Sigara scripta* (Rambur), and *Notonecta maculata* (Fabricius) were more abundant at Meliane-Cap Bon.. *M. scholtzi* and *A. cinereus* occurred at all sites at varying abundances. A number of species were relatively rare in the study region: *Ranatra linearis* L., *Ochterus marginatus marginatus* (Latreille), *Corixa panzeri* (Fieber), *Hesperocorixa linnaei* (Fieber), *Hesperocorixa moesta* (Fieber), *Hesperocorixa furtiva* (Horvath), *Sigara stagnalis stagnalis* (Leach), *Sigara selecta* (Fieber), *Sigara nigrolineata nigrolineata* (Fieber), *Notonecta meridionalis* (Poisson), *Hebrus montanus* (Kolenati) and two endemic taxa to Maghreb (Algeria, Tunisia) - *Velia eckerleini* (Tamanini), and *Velia Africana* (Tamanini).

Seasonal community responses to environment variables

Weights obtained from the interstructure process of STATICO were 0.49, 0.50, 0.50, and 0.51 for Winter, Spring, Summer and Autumn, respectively (Fig. 4A). This indicated that the four seasons had equal importance and that the relationships between aquatic Heteropteran communities and environmental parameters could be directly compared between seasons (Fig.4B).

The compromise step of the STATICO method identified the main environmental structures persisting across the four seasons within the PCA. The first PCA-axis reflected the longitudinal gradient from headwater sites (high altitude, high flow velocity, high dissolved oxygen and pH - higher axis values) to lowland sites (high salinity and conductivity – lower axis values). The second PCA-axis reflected a pollution gradient; with sites with high concentrations of nitrates, phosphates, sulfates, and higher values of BOD_5 and COD in the upper/positive sector of the graph, and deeper sites with high dissolved oxygen levels on the lower/negative part of the graph (Fig.4C). Heteroptera species were ordered along these two gradients reflecting their preferences in relation to the environmental parameters (Fig.4D).

Inter-catchment differences in community responses to different environmental parameters

Within the intrastructure step of the analysis, the environmental parameters and Heteroptera communities were averaged across all four seasons (Fig. 5). The first axis reflected a mineralization gradient with higher values of salinity (SAL), electric conductivity (COND), and total dissolved salts (TDS) on the left, and high altitude (ALT), flow velocity (FS), and pH on the right of the factorial plan. The second axis reflected a nitrate (NO) gradient from the top of the factorial plan and dissolved oxygen (OXY), and depth (Dept) at the bottom of the factorial plan. The structure of the species reflected the environmental gradient on the first axis (Fig. 4), with species displaying saline intolerance (*Aquarius cinereus* (Acin), *Hydrometra stagnorum* (Hysta), and *Notonecta glauca glauca* (Ngla)) located on the low mineralization side of the factorial plan (right) and halophilic species (*Sigara scripta* (Sscr), and *Anisops debilis perplexus* (Aper)) on the high salinity side (left). Nepomorpha and Gerromorpha species were distributed according to their individual salinity affinities. The distribution of the species along the second axis indicated that *Parasigara favieri* (Pfav) *Naucoris maculatus conspersus* (Ncon) and *Notonecta maculata* (Nmac) were associated with high dissolved oxygen concentrations and greater water depth. As a result, the species–environment relationships primarily consisted of a salinity gradient linked to a freshwater - brackish water faunal gradient. *Gerris brasili, Gerris maculatus, Hydrometra stagnorum, Microvelia pygmaea, Velia africana, Velia eckerleini, Hesperocorixa linnaei, Hesperocorixa furtiva and Notonecta meridionalis* were associated with low mineralization and relatively high altitude and higher flow velocities. In contrast, *Sigara stagnalis, Sigara lateralis, Sigara selecta, Anisops debilis perplexus, and Anisops sardeus sardeus* were associated with higher mineralization levels and relatively low altitude and reduced flow velocities. *Parasigara favieri* were associated with high dissolved oxygen concentrations.

Inter- and intra-basin variations in communities in relation to different environment controls

The Intrastructure between the two sets of descriptors (environmental parameters - red labels- and aquatic Heteroptera - blue labels) on the PCA of the compromise output for each catchment is presented in Figure 6. For the Medjerda catchment, sites are roughly ordered along an upstream-downstream gradient from right to left along the first axis. This reflects the mineralization and salinity gradient. In all seasons, site 5 appears at the top of axis 2 due to increased pollution, higher water and air temperature, an absence of precipitation and reduced flow velocities. The low values of dissolved oxygen (OXY) at these sites (0.3 mg/l) are consistent with this interpretation (Fig. 6A). However, aquatic Heteroptera displayed clear seasonal variability mainly because of the differences in the density of species between seasons (Fig. 6B).

For the Kroumirie-Mogods catchment, the environmental parameters clustered on the positive side of axis I, associated with mild mineralizion, linked to lithology; the catchment is dominated by clay-sandstone soils and dense vegetation cover (Fig. 6A). The distribution of species from the sampling sites indicated a strong structure, which indicates a good fit between the aquatic Heteroptera (corresponding to a majority to freshwater affinities) and environmental parameters (the arrows were mostly short). In spring and summer, large differences were observed for stations 1 and 9 when higher Heteroptera species richness and/or abundances were recorded (Fig. 6B).

For the Ichkeul catchment, during winter and autumn, environmental trajectories for most sites clustered at the center of the axes. However, during spring and summer sites 11, 14 and 15 were located on the negative side of axis I, reflecting an upstream-downstream gradient and seasonal variation of temperature and salinity (Fig. 6A). Most species were located centrally and / or on the negative part of axis I (Fig. 6B). Environmental and aquatic Heteroptera trajectories followed similar patterns for most seasons.

For the Meliane-Cap Bon catchment, the upstream-downstream gradient was less clear; site 9 was clearly distinguished from other sites due to pollution and higher COD and BOD_5 (Fig. 6A). A poor association between the fauna and the environment parameters was observed (Fig. 6B).

Discussion

Our results provide an important contribution to the study of freshwater communities from the north African Mediterranean region including North Tunisia, which has been identified as a biodiversity hotspot (Marignani et al., 2017; Abdou et al., 2018). This is consistent with previous Mediterranean ecosystem assessment studies, and results that identified macroinvertebrate diversity as contributing disproportionately to ecological knowledge for the North African region (Beauchard et al., 2003; Ball et al., 2013; De Figueroa et al., 2013; Slimani et al., 2019). Benthic macroinvertebrates depend on a wide range of abiotic factors, including climate, water physiochemistry, anthropogenic pressures and riparian habitat availability (White et al., 2019; Ceron et al., 2020) and this study confirms that this is also true for aquatic insects such as Heteroptera. This study also supports the finding of research linking the distribution and abundance of some Aquatic Heteroptera to water physicochemistry from other regions (Savage, 1994; Hufnagel et al., 1999). However, there were marked differences in the Heteropteran assemblage in the semiarid Mediterranean study region (Northern Tunisia). The Heteropteran fauna displayed significant differences in species richness and abundance. Richness of Heteroptera declined from 33 species in Kroumirie-Mogods (Northern western) to 19 species in the Meliane-Cap Bon catchment (Northern western), with a similar reduction in the relative density of individuals. This reflects changes in with water quality, with the least impacted sites displaying a positive correlation between good water quality and Heteroptera density for eight of the environmental parameters (out of 21). This indicates a strong link between the faunal functional composition and local in-stream habitats conditions (micro-habitats) rather than the larger-scale environmental conditions (Townsend and Hildrew, 1994).

The aquatic Heteroptera densities recorded in northern Tunisia displayed clear spatial variation reflecting the preference and tolerances of individual species. *Micronecta scholtzi*, *Naucoris maculatus conspersus*, and *Aquarius cinereus* occurred in high numbers highlighting their generalist characteristics in relation to most abiotic, allowing them to thrive in extreme environmental conditions (Carbonell et al., 2011). The reduction in macroinvertebrate densities in the Kroumirie-Mogods catchment may reflect interspecific competition at sites with high species richness (Boumaiza, 1994). However, no species were recorded at all of the sampling sites. This reflects the heterogenous abiotic conditions of the habitats studied as well as the preferences and adaptations (ecological valence) of the individual Heteroptera species.

The results of STATICO analyses indicate that environmental and spatio-temporal gradients were particularly important determinants of community structure in northern Tunisia (providing evidence to support our hypothesis). The distribution of fauna reflected several physicochemical parameters which varied among the four river catchments. The analysis highlights that the distribution of Heteroptera most strongly reflect a mineralization gradient which has been shown to primary factor structuring communities (Verneaux and Tuffery, 1967). Other factors, such as altitude and flow velocity were also strongly correlated with mineralization. This study provides further evidence that mineralization represents one of the principal forces driving heteroptera community composition and distribution a global scale (Tully et al., 1991; Karaouzas and Gritzalis, 2006; Bloechl et al., 2010; Carbonell et al., 2011; Scheibler et al., 2016). Previous studies on inland aquatic systems have shown that richness and diversity decline with increasing mineralization (Sánchez et al., 2006; Kefford et al., 2011; Millán et al., 2011; Slimani et al., 2019; Gutiérrez-Cánovas et al., 2019). In addition, mineralization was reported to be the most important factor influencing the macroinvertebrate community richness of wetland aquatic systems (Kefford et al., 2011; Millán et al., 2011; Cañedo-Argüelles et al., 2012; Velasco et al., 2019).

Our analysis demonstrated aquatic heteroptera diversity decreased downstream with increasing mineralization (Fig.5). The salinity (SAL), electric conductivity (COND), and total dissolved salts (TDS) of stream waters were identified as the primary factor controlling Heteroptera diversity. Many oligo- and mesotrophic taxa, such as Veliidae and Notonectidae, were abundant in streams with low mineralization and higher DO concentrations, but some Corixidae displayed an association with brackish water systems; comparable to results reported in other studies (Slimani et al., 2015, 2016).

The specific richness of Heteroptera displayed a general reduction with increasing salinity from headwaters to downstream (ST1 to ST44), and were absent from sites subject to high pollution stress (ST5 and ST45 - the site with the highest BOD_5 and COD concentrations). This pattern was reflected in the Heteroptera species recorded, with some species more common in freshwater, for example *N. meridionalis*, *V. eckerleini*, *V. africana* and *A. najas* were only recorded in the Kroumirie-Mogods (SAL ≤ 0.23 PSU), where clayey-sandstone soils dominated and riparian woodland occurred (oak - *Quercus canariensis* and cork tree - *Quercus suber*) (Boumaiza, 1994). In the other catchments (SAL ≥1 PSU; EC ≥ 1900 µs/cm at 20°C), Heteroptera communities were characterised by *A. cinereus*, *M. scholtzi*, *N. glauca* in the headwaters and *S. scripta*, *S. stagnalis*, *S. lateralis* and *N. conspersus* in the more mineralized sites downstream. Such distributional patterns have also been reported observed in other saline Mediterranean streams (Millán et al., 2011). In particular, some Corixidae appear to be well adapted to osmotic stress and some, such as are *Sigara scripta* are considered halophilic taxa and indicative of hypersaline streams (Savage, 1982, 1990; Millán et al., 2011; Slimani et al., 2015). There is great variation among species regarding salinity tolerance with species-based ecophysiological and metabolically mechanisms enabling them to tolerate such stress (Oren, 2000; Velasco et al., 2006; Elevi Bardavid and Oren, 2008). Although Heteopterans are sensitive to anthropogenic pollution of the aquatic ecosystems, their respiratory physiology (air breathing) and mating behavior enable them to persist even when exposed to harsh environmental conditions (Lytle, 2015). However, in the current study Heteroptera were totally absent from the most severely polluted sites at Kasseb and Lebna. These results are consistent with results reported by Zhang et al. (2018) in response to chronic pollution and habitat modification. The ability of Heteroptera to withstand relatively extreme environmental conditions may partially explain the negative correlations between water temperatures, dissolved oxygen, depth and turbidity in this study and resulted in the high species richness recoded during Spring and Summer. These observations support the findings of Slimani et al. (2017) and previous studies in the study area highlighting correlations between aquatic macrophytes (Casagranda &

Boudouresque, 2007), phytoplankton (Casagranda and Boudouresque, 2010) and Coleoptera - water beetles (Touaylia et al., 2013) with temperature and salinity.

The results reported suggest Heteroptera assemblages could be more widely used as indicators of freshwater biodiversity in biomonitoring programs and the conservation of freshwater biodiversity in Mediterranean freshwater ecosystems (Calapez et al., 2018). They have considerable utility for monitoring changes in environmental parameters, as bioindicators of either deteriorating or improving habitat quality that may be related to changing patterns of land use or other anthropogenic stressors (Buchwalter et al., 2003). The data from this study could be used to inform the development of an index of biological richness and aquatic ecosystem health when combined with wider data from the macroinvertebrate community from the region. These measures could be used to provide measures of both biodiversity and wider environmental quality which could be compared to those developed in adjacent regions in Europe and beyond. In addition, Heteroptera may have utility as agents of biological control. With the exception of the omnivorous Corixidae, aquatic Heteroptera are insect predators and have been recorded to help in the regulation of larval populations of the larvae of mosquito vectors of disease (Aditya et al., 2004; Banerjee et al., 2010). Their use in the biocontrol of mosquitoes could be used alongside the targeted application of insecticides to avoid or minimize the effects of chemical contamination on wetland ecosystems.

Future research is needed to combine the results of abiotic factors within a geographical information system (GIS) to track species current and potential future distributions under climate change scenarios and anthropogenic impacts. Many species of aquatic Heteroptera have been reported to be responding to climate change by shifting their distributional ranges, changes in recorded abundances, phenology, voltinism, physiology, behaviour and community structure, thereby serving as particularly good bioindications of climate change (Musolin, 2007). Rabitsch (2008) referred to the process as Mediterranization, given that five new species including *Micronecta scholtzi* (Corixidae), *Microvelia buenoi* (Veliidae), *Microvelia pygmaea* (Veliidae), *Notonecta meridionalis* (Notonectidae), *Velia currens* (Veliidae) were recorded in Central Europe as their geographical range expanded from their native Mediterranean region. Similarly, Klementová and Svitok (2014) demonstrated that *Anisops sardeus* (Notonectidae), native to the Sahelo-Sindian area, was actively expanding its geographical range from the Mediterranean and is now expanding its distribution in Central Europe.

Conclusion

This study examined Heteropteran communities inhabiting the understudied Mediterranean region of north Africa. The results presented highlight how different environmental parameters structure Heteropteran communities, principally altitude, mineralization and dissolved oxygen levels. Strong seasonal differences in environmental conditions in the study area resulted in the temporal variability of Heteropteran assemblages. Moreover, intra- and inter-basin variations in environmental conditions associated with both natural and anthropogenic resulted in a strong spatial structuring of Heteropterans. This study represents the first step towards understanding the relationship between ecological factors and biodiversity of true water bugs, knowing that freshwater management will become increasingly pivotal as increasing human populations and projected climatic change threaten freshwater ecosystems. Findings from this study highlighted that some Heteropteran taxa, including *N. conspersus*, *N maculata*, *A. najas*, *Aquarius cinereus*, and *Micronecta scholtzi*, were indicative of more pristine semi-natural environments and could provide the basis for characterising reference conditions for other sites in the north African Mediterranean region. Our results also provide clear evidence of how aquatic Heteroptera can be used as indicators of different environmental stressors, including water quality issues, and could be used more widely in biomonitoring assessments across northern Africa.

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Figure 1. Map indicting the location of the study sites within the four catchments (Medjerda, Kroumirie-Mogods, Ichkeul and Meliane-Cap Bon) in northern Tunisia (Site codes and names are presented in Supplementary Table S1) .

Figure 2. PCA biplot of indicating the physicochemical variables with the greatest loading on the first two axes –see Table 1 for definition of environmental codes.

Figure 3. (a) Plot of the spatial changes in aquatic Heteroptera Global density (ind/m^2) and Species richness during the study period (b) Plot of the spatial changes in mean density for each of the 36 species in the four catchments (Medjerda, Kroumirie-Mogods, Ichkeul and Meliane-Cap Bon) in northern Tunisia (codes for species names are defined on Table 2).

Figure 4. General plot of the STATICO method: Interstructure factor map (A) and the table weight and Cos^2 are shown in (B). Compromise factor map for environmental variables (C) and for taxa (D) using STATICO analysis on the 45 sites data in the four catchments of northern Tunisia from January to December 2013. See Tables 1 and 2 for definitions of environmental and taxonomic codes respectively.

Figure 5. Intrastructure plot of the STATICO analysis for environmental parameters (red, left) and water bugs (blue, right) at each season. See Tables 1 and 2 for definitions of environmental and taxonomic codes respectively

Figure 6A. Intrastructure plot of the STATICO analysis detailing the environmental parameters for the sampling sites by catchment and season. See Tables S1 for site names and further details.

Figure 6B. Intrastructure plot of the STATICO analysis of the water bugs for the sampling sites by catchment and season. See Tables S1 for site names and further details.

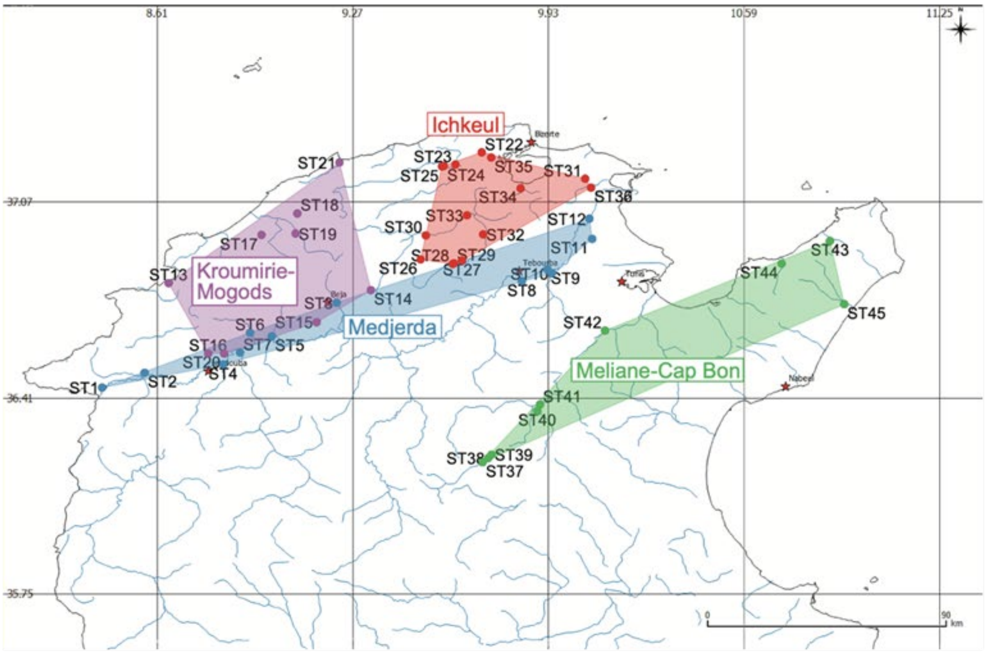
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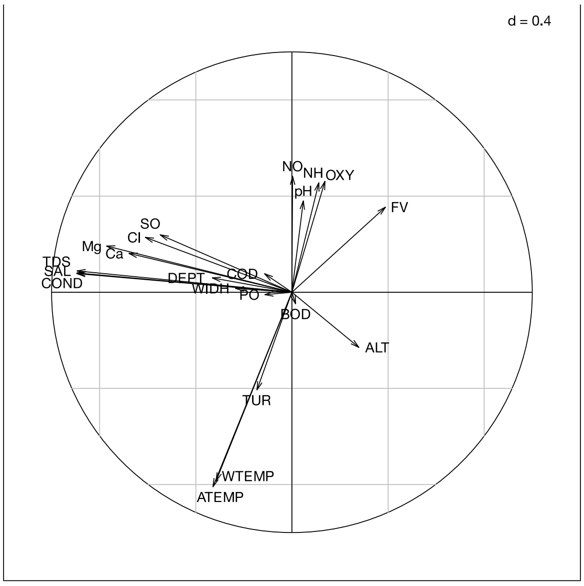
Figure 1.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WTEMP
ATEMP

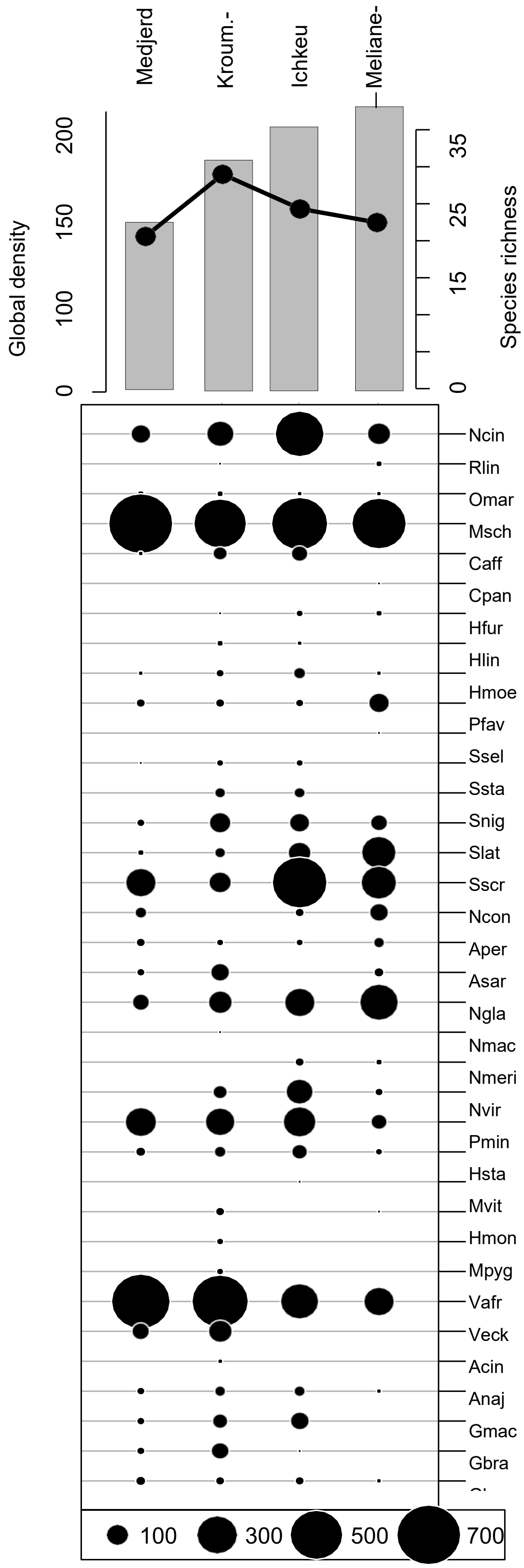

Medjerd
Kroum.-
Ichkeu
Meliane-
Global density
0
100
150
200
Species richness
0
15
25
35
Ncin
Rlin
Omar
Msch
Caff
Cpan
Hfur
Hlin
Hmoe
Pfav
Ssel
Ssta
Snig
Slat
Sscr
Ncon
Aper
Asar
Ngla
Nmac
Nmeri
Nvir
Pmin
Hsta
Mvit
Hmon
Mpyg
Vafr
Veck
Acin
Anaj
Gmac
Gbra
100
300
500
700

Figure 3.

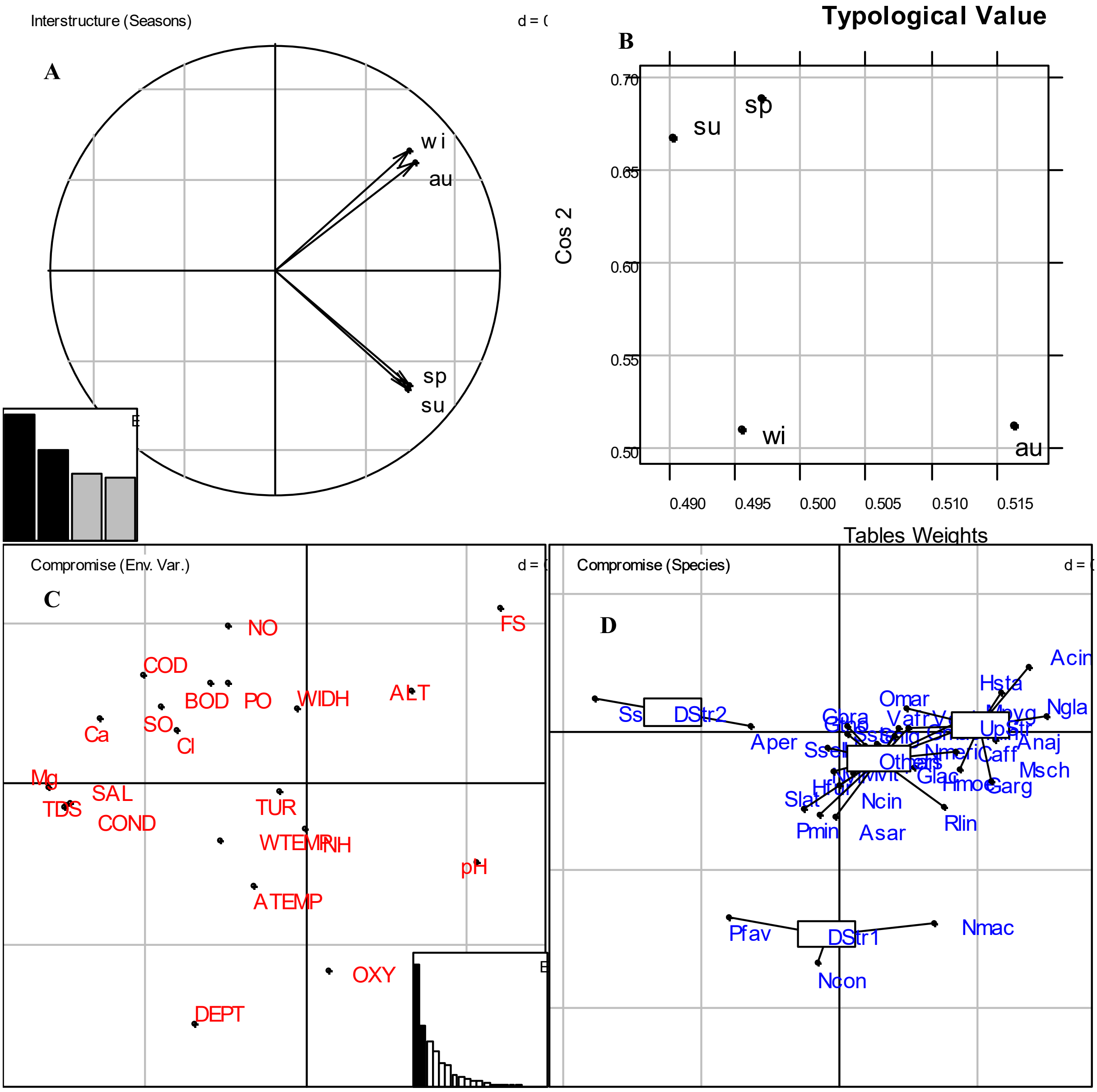
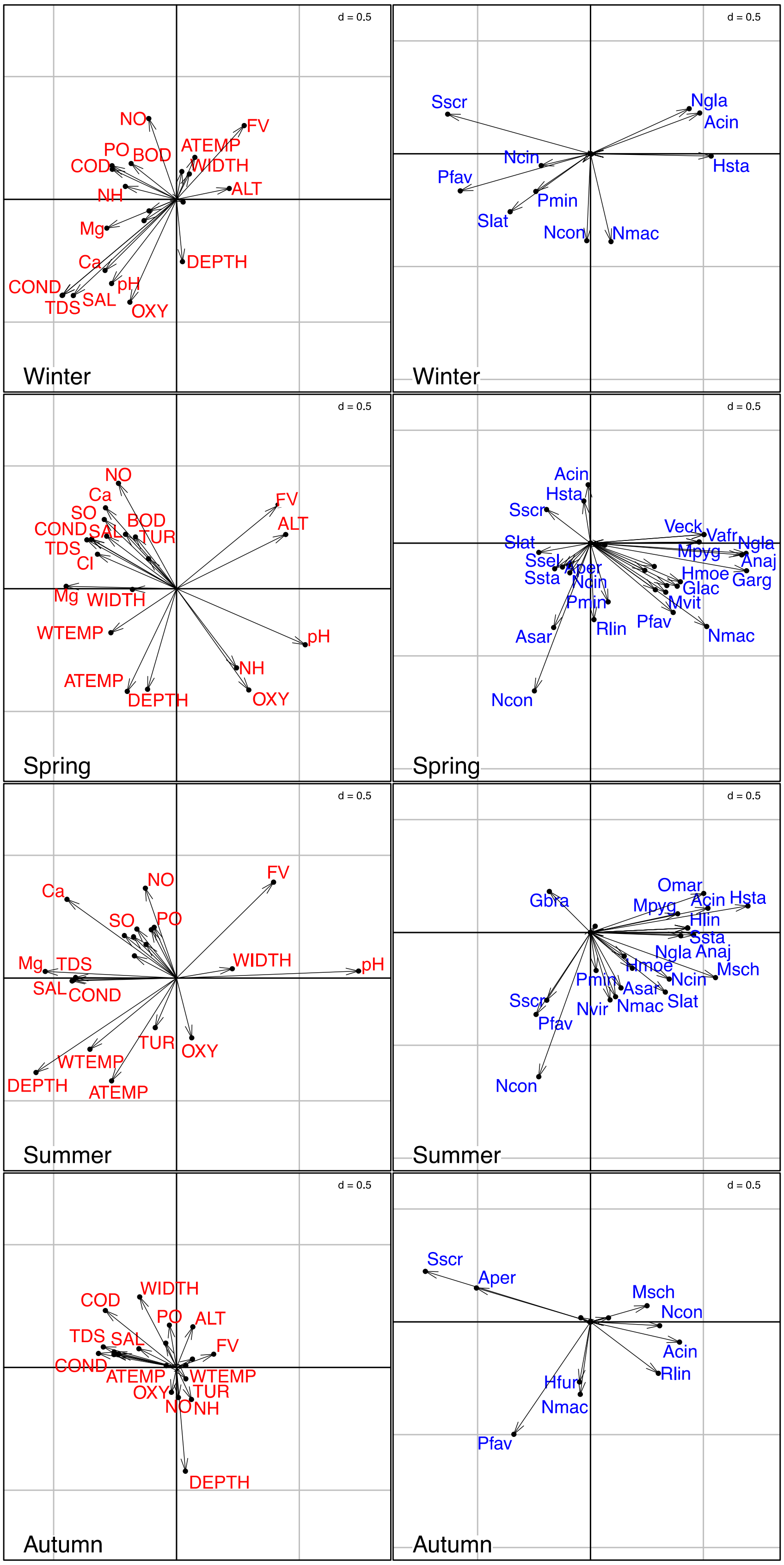

Interstructure (Seasons)
d = (
A
wi
au
sp
su
E
B
Typological Value
Cos 2
0.70
0.65
0.60
0.55
0.50
su
sp
wi
au
0.490
0.495
0.500
0.505
0.510
0.515
Tables Weights
Compromise (Env. Var.)
d = (
C
NO
FS
COD
BOD
PO
WIDH
ALT
Ca
SO
Cl
Mg
SAL
TDS
COND
TUR
WTEMP
NH
pH
ATEMP
OXY
DEPT
E
Compromise (Species)
d = (
D
Acin
Hsta
Omar
Ngla
Ss
DStr2
Aper
Vafr
UpStr
Anaj
Ssel
Others
Nmeri
Caff
Msch
Glac
Hmoe
Garg
Slat
Hfur
Ncin
Rlin
Pmin
Asar
Pfav
DStr1
Nmac
Ncon

Figure 4.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NO
FV
PO
BOD
ATEMP
COD
WIDTH
NH
ALT
Mg
DEPTH
Ca
COND
pH
SAL
TDS
OXY
Winter
d = 0.5
Sscr
Ngla
Acin
Ncin
Hsta
Pfav
Pmin
Slat
Ncon
Nmac
Winter
d = 0.5
NO
Ca
SO
BOD
FV
ALT
COND
SAL
TUR
TDS
Cl
Mg
WIDTH
WTEMP
pH
NH
ATEMP
DEPTH
OXY
Spring
d = 0.5
Acin
Hsta
Sscr
Slat
Veck
Vafr
Ngla
Mpyg
Anaj
Ssel
Aper
Ssta
Ncin
Hmoe
Garg
Glac
Mvit
Pmin
Rlin
Pfav
Asar
Nmac
Ncon
Spring
d = 0.5
FV
NO
Ca
SO
PO
Mg
TDS
WIDTH
pH
SAL
COND
TUR
OXY
WTEMP
DEPTH
ATEMP
Summer
d = 0.5
Omar
Gbra
Mpyg
Acin
Hsta
Hlin
Ssta
Ngla
Anaj
Hmoe
Msch
Pmin
Ncin
Asar
Sscr
Nvir
Nmac
Slat
Pfav
Ncon
Summer
d = 0.5
WIDTH
COD
PO
ALT
TDS
SAL
FV
COND
ATEMP
WTEMP
OXY
TUR
NO
NH
DEPTH
Autumn
d = 0.5
Sscr
Aper
Msch
Ncon
Acin
Rlin
Hfur
Nmac
Pfav
Autumn

Figure 5.

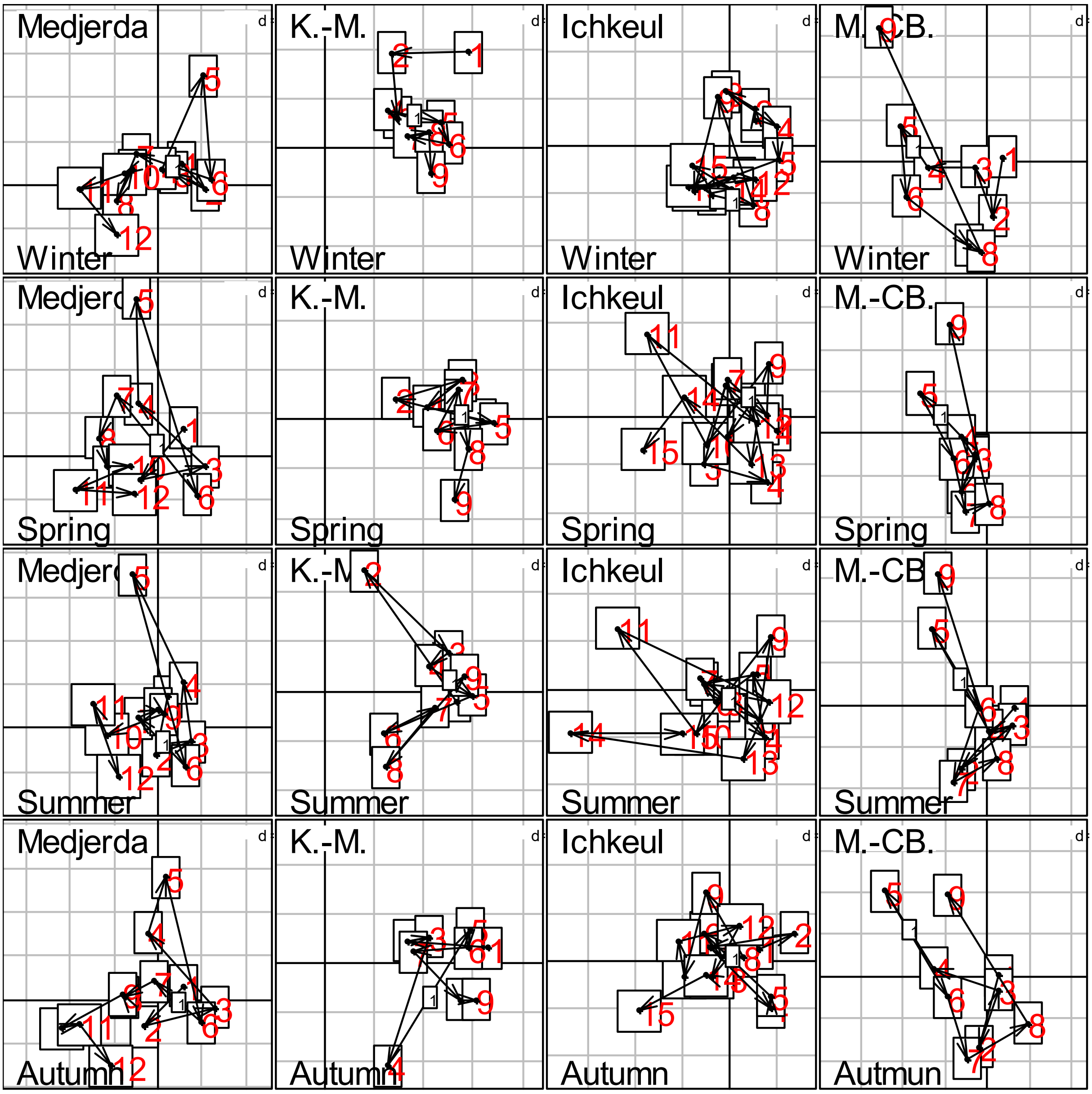
Medjerda
K.-M.
Ichkeul
M.-CB.
Winter
Spring
Summer
Autumn
Autmun
d

Figure 6A.

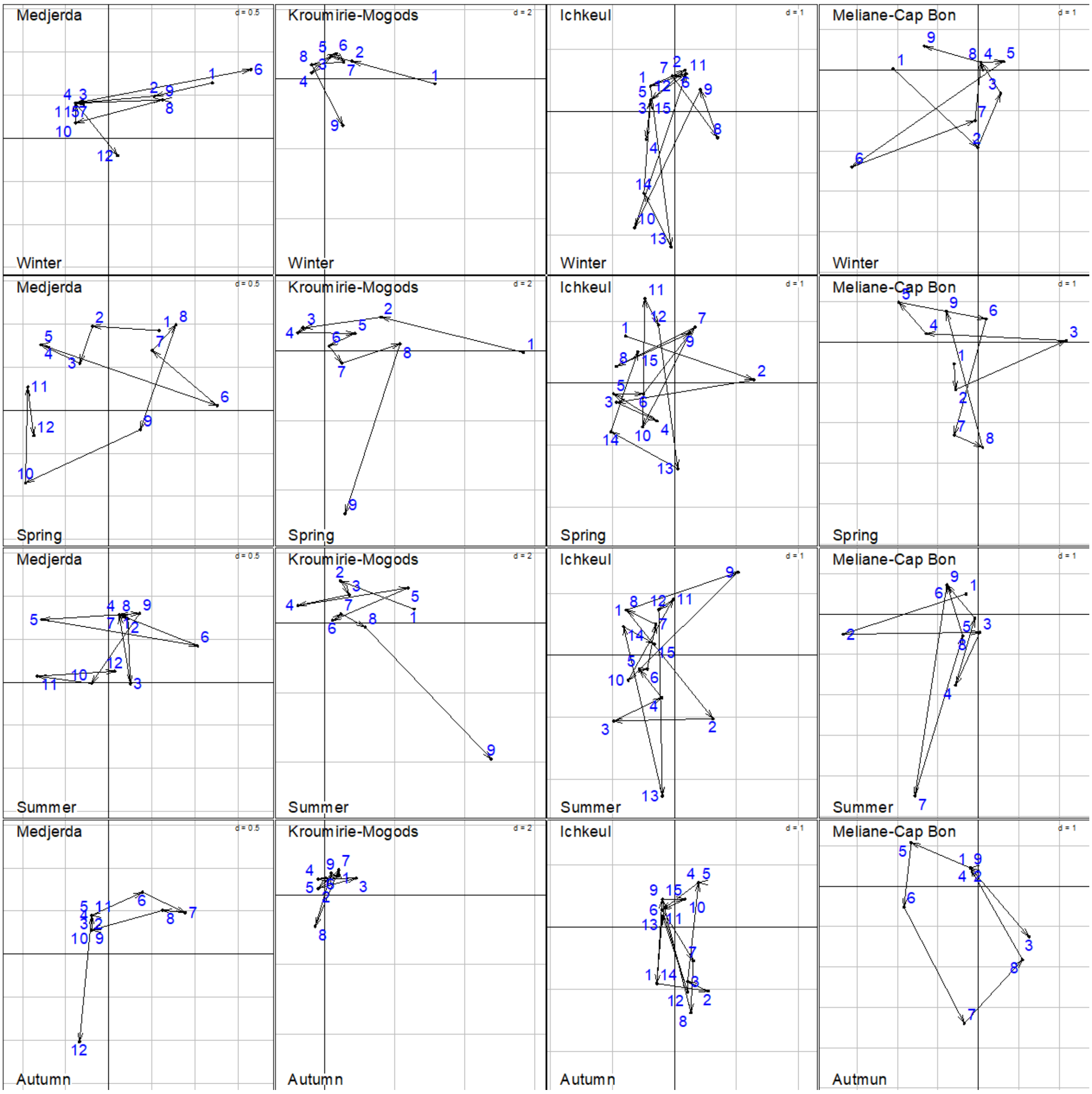
Medjerda
d = 0.5
Winter
Kroumirie-Mogods
d = 2
Winter
Ichkeul
d = 1
Winter
Meliane-Cap Bon
d = 1
Winter
Medjerda
d = 0.5
Spring
Kroumirie-Mogods
d = 2
Spring
Ichkeul
d = 1
Spring
Meliane-Cap Bon
d = 1
Spring
Medjerda
d = 0.5
Summer
Kroumirie-Mogods
d = 2
Summer
Ichkeul
d = 1
Summer
Meliane-Cap Bon
d = 1
Summer
Medjerda
d = 0.5
Autumn
Kroumirie-Mogods
d = 2
Autumn
Ichkeul
d = 1
Autumn
Meliane-Cap Bon
d = 1
Autmun

Figure 6B.

Table 1. Summary of environmental parameters for the 45 sites from the four catchments (Medjerda; Kroumirie-Mogods; Ichkeul; and Meliane-Cap Bon) in northern Tunisia during the study period. Definition of environmental parameter codes:channel width (WIDT) and water depth (DEPT)Altitude (ALT), salinity (SAL), electrical conductivity (COND), total dissolved salt (TDS) dissolved oxygen (OXY) Water temperature, (WTEMP) Air temperature (ATEMP), Flow velocity (FV), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), sulphates (SO_4^{2-}) Chloride (Cl-), nitrates (NO_3^-), nitrite (NO_2^-), ammoniac (NH_4^+), Orthophosphate (PO_4^{3-}) Chemical oxygen demand (COD), Biological Oxygen Demand (BOD5).

Environmental	Global			Winter			Spring			Summer			Autumn			F values (df)
parameters	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	
ALT (m)	1	398	105.4±117	1	398	105.4±117	1	398	105.4±117	1	398	105.4±117	1	398	105.4±117	0
Width (m)	2	40	12.01±10.3	2	40	11.8± 10	2	40	11.8±10	2	40	11.8± 10	2	40	11.8± 10	0.03
Depth (cm)	5	50	19.66±14.15	5	50	19.6±14.2	5	50	19.6±14.2	5	50	19.6±14.2	5	50	19.6±14.2	0
pH	6.8	8.69	7.8±0.33	7.2	8.45	7.82±0.34	7.4	8.7	8±0.24	6.8	8.06	7.5±0.26	7.3	8.42	7.84±0.28	25.94* **
SAL (psu)	0.1	7.6	1.2±1.15	0.1	3.8	1.06±0.97	0.1	3.8	1.4±1.1	0.1	7.6	1.5±1.4	0.1	3.5	1.16±1	1.52
COND (µS cm^{-1})	212	13100	2515±2071	212	7400	2058± 1736	382	7520	2806±2036	388	13100	2883±2495	245	6510	2314±1895	1.65
TDS (mg L^{-1})	154	10558	1864±1570	154	5508	1523± 1302	259	5597	2080±1542	263	10558	2141±1930	179	4845	1710±1420	1.61
OXY (mg L^{-1})	1	11.8	6.38±2.11	1	11.8	6.59±2.8	1.4	9.25	5.83±1.83	1.2	6.68	5.36±1	1.1	9.77	7.74±1.53	13.13* **
Turbidity (NTU)	0	71	4.44±13.22	0.1	16	3±3.9	0.0	60	7.45±12.84	0.3	64	16.5±16.6	0.1	71	6.77±12.41	9.69* **
Water Temp (°C)	2	30	16.66±6.42	2	20	10.5±3.6	16	24	19.92±2.42	18	30	24.4±2.9	8	18.5	11.7±1.95	258.3* **
Air Temp (°C)	5	42	19.08±8.93	5	17	13.2±3.25	12	35	22.64±6.43	23	42	30±5	7	14	10.5±1.63	180.96* **
FV (cm s^{-1})	2.5	159	36.4± 31.95	3.3	159	42.07±30	2.6	63	21 ±17.7	2.6	58.82	21.8±15.9	4.5	153	54.85±39	16.24* **
Ca^{2+} (L^{-1})	14	540.8	156.1± 90.9	14	341.3	136.9± 78.6	30	540.8	167.8±103	33	540.8	166±96	30	389	153±82.2	1.12
Mg^{2+} (L^{-1})	3.5	277.9	59.77± 52.9	3.5	201.7	51.94±47.64	5.9	277.9	67.57±45.7	5.9	277.9	61±54.6	7.7	207	58.2±49.8	0.68

SO_4^{2-} (L^{-1})	13.5	3100	239.3±568.1	13	872.3	176.72±185	21	3100	273.6±488	33	3100	304±492	13	872	202±197	1.16
Cl^- (L^{-1})	0.1	2600	268.9± 457	0.1	1491	194.96±268	37	2600	324±589.7	37	2600	347±584	0.1	1491	209±267	1.3
NO_3^- (L^{-1})	0.26	581.9	44.47±90.57	0.5	114	27.5±31	0.5	103.7	26.4±29.7	0.5	92	22.24±24.7	0.2	581	101±162	9.09* **
NH_4^+ (L^{-1})	0.02	81.2	2.3±14.47	0.06	12.3	1.7±2	0.02	2.34	0.64±0.54	0.1	2.42	0.7±0.49	0.03	81.2	6.3±16.7	4.57* **
PO_4^{3-} (L^{-1})	0.1	8.73	0.31±0.77	0.1	8.73	0.38±1.28	0.15	2.6	0.28±0.43	0.15	4	0.31±0.68	0.1	2.47	0.28±0.4	0.15
COD (mg L^{-1})	15	1025	39.89±84.77	16	1025	57.87±155.2	16	181	31.1±26.8	16	33	33±34	15	323	37.5±52	0.94
BOD5 (mg L^{-1})	0.3	102	2.98±10.5	0.5	31	2.39±2.39	0.3	56	3.1±10.43	0.3	8.72	2.88±8.7	0.5	102	3.5±15	0.08

F values: between groups mean square/within-groups mean square.

Significant difference between sampled seasons: (***$p < 0.001$).

Table2. List of species and their codes used in STATICO analysis.
Average (range) species density (ind/m^2) and abundance (%) of aquatic Heteroptera during the study period.
Species in bold represent the most abundant species.

	Family	Species	Codes	Global density (ind/m^2)	Abundane (%)
Nepomorpha	Nepidae	*Nepa cinerea* L.	Ncin	508.33	7.82
	Ranatridae	*Ranatra linearis* L.	Rlin	25	0.38
	Ochteridae	*Ochterus marginatus* L.	Omar	16.66	0.26
	Micronectidae	***Micronecta scholtzi*** **F.**	Msch	**1458.33**	**22.43**
	Corixidae	*Corixa affinis* L.	Caff	58.33	0.89
		Corixa panzeri F.	Cpan	8.33	0.13
		Hesperocorixa furtiva H.	Hfur	25	0.26
		Hesperocorixa linnaei F.	Hlin	16.66	0.26
		Hesperocorixa moesta F.	Hmoe	58.33	0.89
		Parasigara favieri P.	Pfav	100	1.54
		Sigara selecta F.	Ssel	8.33	0.13
		Sigara stagnalis stagnalis F.	Sstag	50	0.77
		Sigara nigrolineata nigrolineata F.	Snig	83.33	1.28
		Sigara lateralis L.	Slat	175	2.69
		Sigara scripta R.	Sscr	250	3.84
	Naucoridae	***Naucoris maculatus conspersus*** S.	Ncon	**658.33**	**10.13**
	Notonectidae	*Anisops debilis perplexus* P.	Aper	91.66	1.41
		Anisops sardeus sardeus H.	Asar	50	0.77
		Notonecta glauca L.	Ngla	66.66	1.02
		Notonecta maculata F.	Nmac	433.33	6.66
		Notonecta meridionalis P.	Nmer	41.66	0.13
		Notonecta viridis D.	Nvir	16.66	0.26
	Pleidae	*Plea minutissima* L.	Pmin	166.66	2.56
Gerromorpha	Hydrometridae	*Hydrometra stagnorum* L.	Hsta	450	6.92
	Mesoveliidae	*Mesovelia vittigera* H.	Mvit	125	1.92
	Hebridae	*Hebrus montanus* K.	Hmon	8.33	0.13
	Veliidae	*Microvelia pygmaea* D.	Mpyg	33.33	0.51
		Velia eckerleini T.	Veck	33.33	0.51
		Velia africana T.	Vafr	33.33	0.5
	Gerridae	***Aquarius cinereus*** P.	**Acin**	**1116.66**	**17.18**
		Aquarius najas De G.	Anaj	41.66	0.64

		Gerris maculatus T.	Gmac	50	0.26
		Gerris brasili P.	Gbra	58.33	0.89
		Gerris lacusrtis L.	Glac	116.66	1.79
		Gerris argentatus S.	Garg	91.66	1.41
		Gerris thoracicus S.	Gtho	50	0.77

Table S1. List of localities sampled, indicating the code, location and habitat based variables.

Code	Watershed	Location	**Altitude (m)**	**GPS**	**Riparian vegetation**	**Type of substrate**
ST1	Medjerda	Ghardimaou	197	36°27'01.87"N 08°26'01.56"E	Very abundant	Rocks, stones and sands
ST2	Medjerda	Chemtou	173	36°30'00.38"N 08°34'33.23"E	Little abundant	Sands
ST3	Medjerda	Béja	147	36°44'11.04"N 09°13'25.15"E	Very abundant	Stones and sands
ST4	Medjerda	Mellègue	136	36°31'42.18"N 08°50'28.93"E	Abundant	Clay
ST5	Medjerda	Kasseb	131	36°37'22.90"N 09°00'17.52"E	Very abundant	Muddy
ST6	Medjerda	Bouhertma	130	36°38'05.44"N 08°55'53.35"E	Very abundant	Stones and sands
ST7	Medjerda	Tessa	127	36°34'05.91"N 08°53'51.98"E	Abundant	Muddy
ST8	Medjerda	Batan	24	36°48'29.99"N 09°50'53.43"E	Abundant	Rocks, stones and sands
ST9	Medjerda	Djedeida	23	36°50'52.00"N 09°56'05.03"E	Abundant	Rocks, stones and sands
ST10	Medjerda	Chafrou	18	36°4'54.67"N 09°56'54.62"E	Abundant	Muddy
ST11	Medjerda	Khlaidia	5	36°57'02.71"N 10°05'06.72"E	Abundant	Muddy-Silty
ST12	Medjerda	Kalaat El Andalous	6	37°01'07.45"N 10°04'33.27"E	Very abundant	Muddy
ST13	Kroumirie - Mogods	Ennour	398	36°48'06.64"N 08°39'25.37"E	Very abundant	Rocks, stones and sands
ST14	Kroumirie - Mogods	Ksar Mezouar	224	36°46'42.02"N 09°20'20.37"E	Little abundant	Rocks, stones and sands
ST15	Kroumirie - Mogods	Hammam Sayala	210	36°40'12.56"N 09°09'21.43"E	Abundant	Muddy-Silty
ST16	Kroumirie - Mogods	Aïn Gnaâa	142	36°33'53.33"N 08°47'25.26"E	Little abundant	Muddy
ST17	Kroumirie - Mogods	Titria	66	36°57'50.31"N 08°58'12.34"E	Very abundant	Rocks, stones and sands
ST18	Kroumirie - Mogods	Belif	37	37°02'10,67"N 09°05'28,22"E	Abundant	Rocks, stones and sands
ST19	Kroumirie - Mogods	Maâden	34	36°58'07.79"N 09°05'05.67"E	Very abundant	Rocks, stones and sands
ST20	Kroumirie - Mogods	Bouterfes	16	36°57'12.05"N 08°54'45.52"E	Abundant	Rocks, stones and sands
ST21	Kroumirie - Mogods	Ziatine	2	37°12'27.93"N 09°13'58.89"E	Little abundant	Sands
ST22	Ichkeul	Kraâa	12	37°14'30,53"N 09°42'45,87"E	Abundante	Muddy
ST23	Ichkeul	Douymiss	7	37°12'04.26"N 09°37'26.91"E	Little abundant	Rocks, stones and sands
ST24	Ichkeul	Kloufi	4	37°11'46,77"N 09°35'06,56"E	Little abundant	Clay and Muddy

ST25	Ichkeul	Sejnane	4	37°11'39,61"N 09°34'44,36"E	Very abundant	Rocks, stones and sands
ST26	Ichkeul	Tout	388	36°52'53.96"N 09°30'25.02"E	Very abundant	Muddy
ST27	Ichkeul	Zammit	127	36°52'39.76"N 09°38'48.72"E	abondante	Muddy-Silty
ST28	Ichkeul	Tine I	123	36°51'59.18"N 09°36'54.77"E	Very abundant	Rocks, stones and sands
ST29	Ichkeul	El Malaha	122	36°52'06.91"N 09°37'10.17"E	Little abundant	Muddy
ST30	Ichkeul	Joumine I	102	37°57'44.95"N 09°31'27.88"E	Very abundant	Rocks, stones and sands
ST31	Ichkeul	Henna	45	37°09'10.77"N 10°03'42.43"E	Little abundant	Muddy-Silty
ST32	Ichkeul	Tine II	36	36°57'56.87"N 09°43'03.37"E	Abundant	Clay and muddy
ST33	Ichkeul	Joumine II	16	37°01'48.48"N 09°39'47.47"E	Abundant	Rocks, stones and sands
ST34	Ichkeul	Sidi Hissin	15	37°07'14.64"N 09°50'38.01"E	Little abundant	Clay and muddy
ST35	Ichkeul	Hima	2	37°13'27.76"N 09°44'41.50"E	Little abundant	Clay and muddy
ST36	Ichkeul	El Melah	1	37°07'23.09'N 10°04'53.63"E	Little abundant	Muddy
ST37	Meliane-Cap Bon	El Kebir I	380	36°11'57.53"N 09°42'56.16"E	Abundant	Clay and muddy
ST38	Meliane-Cap Bon	El Kebir II	368	36°12'47.49"N 09°44'04.65"E	Abundant	Clay and muddy
ST39	Meliane-Cap Bon	El Kebir III	353	36°13'28.40"N 09°44'45.10"E	Abundant	Sands
ST40	Meliane-Cap Bon	Boudhebène	175	36°22'16.99"N 09°54'01.44"E	Abundant	Muddy
ST41	Meliane-Cap Bon	Miliane I	162	36°23'36.35"N 09°54'33.11"E	Abundant	Muddy-Silty
ST42	Meliane-Cap Bon	Miliane II	38	36°38'31.85"N 10°07'43.44"E	Abundant	Clay
ST43	Meliane-Cap Bon	M'Gaiez	18	36°56'38.08"N 10°53'14.92"E	Abundant	Muddy
ST44	Meliane-Cap Bon	Abid	4	36°52'02.94"N 10°43'27.62"E	Abundant	Sands
ST45	Meliane-Cap Bon	Lebna	4	36°43'51.91"N 10°56'07.79"E	Abundant	Muddy