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Notes on the aquatic macroinvertebrates of the Mujib River Basin and Azraq Wetland Reserve in Jordan for use in biomonitoring

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Abstract: Freshwater is in crisis globally. Arid countries such as Jordan are particularly challenged regarding freshwater, especially considering growing demand and increasing pollution pressure. Water resource monitoring is essential to combat the threats to freshwater and ensure effective and sustainable management of scarce freshwater resources. Biomonitoring using aquatic macroinvertebrates provides a practical, cost-effective, and holistic approach to assessing water quality and freshwater ecosystem health. We surveyed the aquatic macroinvertebrates at four sites in Jordan, three sites in the Mujib River basin and at one site in the Azraq Wetland Reserve, using biomonitoring survey methods employed and accredited in South Africa. The surveys present a first dedicated aquatic macroinvertebrate survey of the Azraq Wetland Reserve, and a first survey of the Mujib River basin using standard biomonitoring techniques, rather than exhaustive sampling. We report the taxa sampled at each site, identified in-field to family-level where possible. We recorded 20 different taxa at the Azraq Wetland Reserve, and 28 different taxa within the Mujib River basin. The diversity of taxa sampled using standard biomonitoring techniques supported previous work by Alhejoj, Bandel and Salameh (2014) demonstrating that aquatic macroinvertebrates present a useful tool for biomonitoring of water resources in Jordan. However, we suggest that follow-up research is done to build on and refine the 'Jordan Biomonitor System for Watercourses (JBSW)' developed by Alhejoj, Bandel and Salameh (2014). The JBSW should be modified to become an integrated index based on the community, identified to family-level, of aquatic macroinvertebrates present to make it easy and practical to use in-field, comparable to indices developed and validated elsewhere in the world. If this is done, biomonitoring using aquatic macroinvertebrates could become a powerful tool to help assess and manage the precious freshwater resources in Jordan as they come under ever increasing pressure from overexploitation, pollution, and growing demand.

Key words: water scarcity, water quality, Jordan, aquatic macroinvertebrates, biomonitoring

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Introduction

The Hashemite Kingdom of Jordan (hereafter Jordan) is a critically water scarce country (Jaber *et al.*, 1997; Schyns *et al.*, 2015). The importance and rarity of precious freshwater resources grows daily as unsustainable extraction practices diminish surface and groundwater reserves in an attempt to meet growing demand from rapid population growth and increasing urbanization (Hadadin *et al.*, 2010; Hellegers *et al.*, 2022). Unsustainable use and diversion of freshwater supplies have already led to degradation of freshwater systems, even resulting in local extinctions (Amr *et al.*, 2011) and ecological or social disasters (Procházka *et al.*, 2008; Mir and Hamidan, 2012; Whitman, 2019). For example, the Azraq Oasis within the Azraq Wetland Reserve is now considered an ecological disaster area after the springs which supplied it began drying in the 1980s. In 1992 the oasis was completely desiccated due to exploitation of the fresh water supply to support the cities of Azraq and Amman (Disi *et al.*, 2014). The current day oasis represents approximately 5-10% of its size prior to the 1980s, after restoration efforts by the Royal Society for the Conservation of Nature (RSCN; Whitman, 2019). Degradation, or outright loss, of freshwater ecosystems presents a major problem worldwide, but especially in water scarce regions such as Jordan. Freshwater ecosystems provide a range of goods and services that both humans and biodiversity are completely reliant on, such as water treatment, clean drinking water, disaster mitigation, fish, fibre, recreational and cultural importance, and intrinsic 'quality of life' value (Díaz *et al.*, 2018; Dudgeon, 2019; Albert *et al.*, 2021; Lynch *et al.*, 2023). Considering the critical importance of freshwater resource preservation in Jordan, it has become vital to design and implement water quality monitoring programs, and to establish which humans and biodiversity are reliant on which freshwater resources.

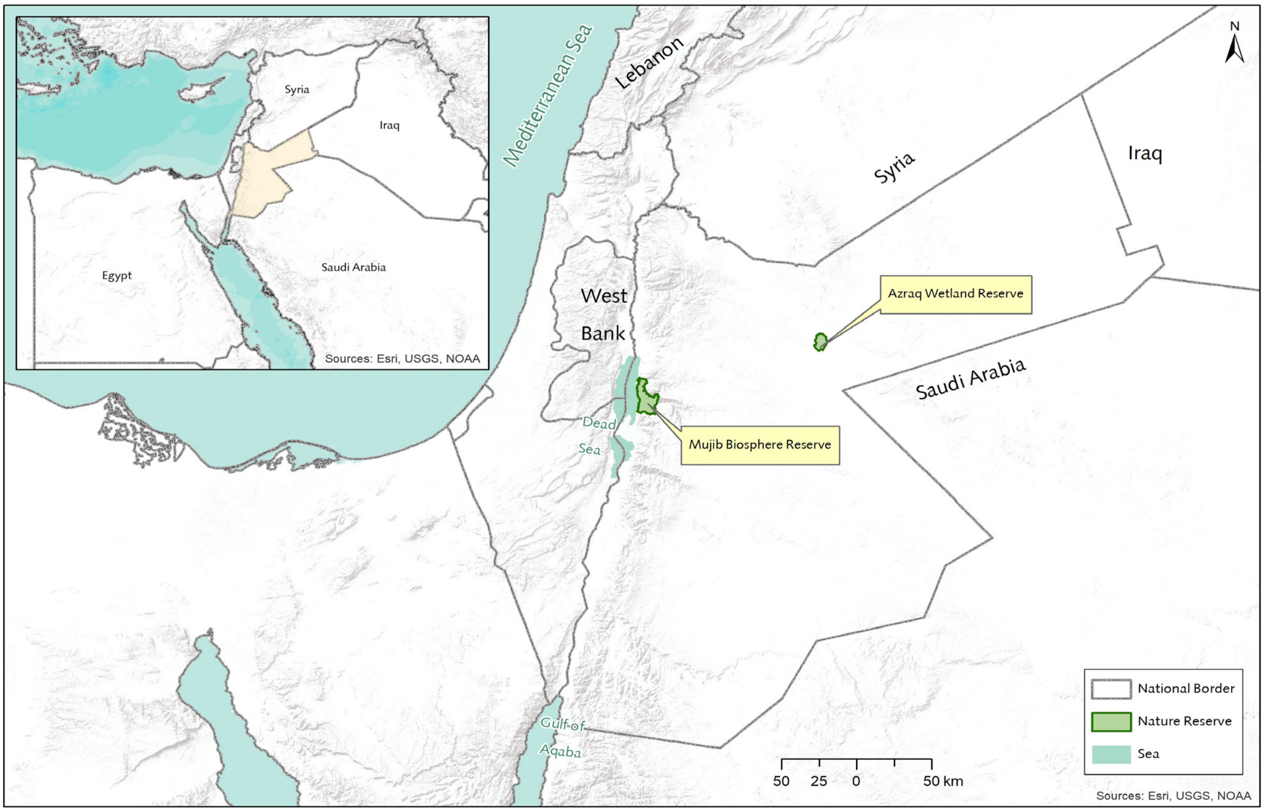
Aquatic macroinvertebrates represent a highly useful group for assessing and monitoring water quality and river or lake health. This is because different taxa have unique habitat preferences in terms of water quality parameters and physical habitat characteristics, as well as unique tolerance levels for pollution (Johnson *et al.*, 1993; Muralidharan *et al.*, 2010; Arias-Real *et al.*, 2022). Through determining what aquatic macroinvertebrates are present or absent from a system, in addition to their tolerance levels for pollution and disturbance, one can infer information about the water quality and ecological health of a system (Alhejoj *et al.*, 2014b). For example, Alhejoj et al. (2017) point out that the *Planorbis*, *Gyraulus* and *Ancylus* genera have been nearly completely excluded from Jordan freshwater systems because of increasing pollution. In this study, we sampled sites within the Mujib River basin, as well as a site within the Azraq Wetland Reserve, in Jordan to determine what macroinvertebrate taxa were present. To the best of our knowledge, this presents a first dedicated assessment of the aquatic macroinvertebrates present at the Azraq Wetland Reserve. The Mujib basin has been previously sampled, either for specific groups of invertebrates, or for all aquatic macroinvertebrates (Amr *et al.*, 2013; Haddad *et al.*, 2013; Ramadan and Katbeh-Bader, 2018). However, in this assessment, we sampled using the techniques outlined in the South African Scoring System (SASS) version 5 (Dickens and Graham, 2002) to test the efficacy and feasibility of implementing a similar aquatic macroinvertebrate biomonitoring technique at these locations. These locations were chosen because of the importance of the freshwater in the Mujib River basin and Azraq Wetland Reserve for biodiversity, as signalled by the establishment of the Mujib Biosphere Reserve in 1987 (Haddad *et al.*, 2013), and the recognition of the Azraq Wetland Reserve (Disi *et al.*, 2004) as a Ramsar site (https://rsis.ramsar.org/ris/135), an Important Bird Area (BirdLife International, 2023), and a International Union for the Conservation of Nature (IUCN) Green List site (IUCN, 2023). The freshwater supplied by the Mujib River basin is also essential for human use (e.g., abstractions for agriculture, domestic water supply, and ecotourism), especially for supplying Jordan's capital city, Amman (Hamidan, 2014). The Azraq Wetland Reserve is also a highly valued cultural, historical, and tourism site (Disi *et al.*, 2004; Maher *et al.*, 2021; Boyd *et al.*, 2022). Therefore, it is crucial to establish what biodiversity is present in the basin, and to assess the plausibility of biomonitoring for determining the water quality and health of the system to enable sustainable management. We aimed 1) to gather a first assessment of the aquatic macroinvertebrates present at the Azraq Wetland Reserve, and a first assessment of aquatic macroinvertebrates in the Mujib River basin using standard biomonitoring sampling techniques, and 2) based on these sampling efforts, to form recommendations on using macroinvertebrates for biomonitoring water quality and aquatic ecosystem health within these systems to follow up on initial work done to develop the 'Jordan Biomonitor System for Watercourses (JBSW)' developed by Alhejoj, Bandel and Salameh (2014a).

Materials and Methods

Study sites: The Mujib basin contains two major sub-catchments, the Wadi Mujib (~4500 km^2) and the Wadi Wala (or Haidan; ~2100 km^2) catchments, and ranges from an elevation of ~950 meters above sea level in the east, to ~430 meters below sea level in the west (Hamidan, 2014). Rainfall in the catchment falls largely over the Boreal Winter and Spring, falling in a gradient from ~50 mm per year in the east to ~300 mm per year in the west (El-Naqa, 1993; Al-Harahsheh and Al-Amoush, 2010). There are two large impoundments in the catchment, the Wala Dam (on the Haidan River) and the Mujib Dam (on the Mujib River). These are associated with abstraction partly for domestic and industrial use within the settlements in the catchment, and primarily for irrigation for numerous small to medium-scale rangeland practices. Perennial flow in the Mujib River is maintained by surfacing groundwater (Ijam and Al-Mahamid, 2012; Farhan and Al-Shaikh, 2017). Little water from the Mujib River actually reaches the Dead Sea, since it is diverted to a water treatment plant and siphoned off for human use just before it eventually empties (Hamidan, 2014). Approximately 40-50 of aquatic macroinvertebrate species have been recorded in the Mujib River sub-catchment, representing 27 families and five different orders (Haddad *et al.*, 2013). Approximately 75 insect species from 33 families within seven orders were recently recorded through exhaustive sampling within the Haidan sub-catchment (Ramadan and Katbeh-Bader, 2018). River flow is measured at gauging stations on the dams and at a weir located just upstream from where the water is diverted or flows into the Dead Sea. Water quality and biomonitoring data are not regularly collected in the basin.

The Azraq Wetland Reserve is within the Azraq basin, 500 meters above sea level (Disi *et al.*, 2004). The reserve falls in within the hyper arid north-eastern part of Jordan, with a mean annual rainfall of only ~70 mm per year. The wetland dried up completely in 1980 following overexploitation of the surface and surrounding groundwater. Restoration efforts by the RSCN are ongoing, with water resupplied to the reserve via groundwater extraction. The current area of the reserve is maintained at approximately 10% of its former size. However, it is still recognised as a wetland of international significance by the Ramsar Convention (https://rsis.ramsar.org/ris/135), as an Important Bird Area by Birdlife International (BirdLife International, 2023), and was added to the IUCN Green List in 2018 based on the fact that it hosts a range of fish (including the critically endangered and endemic Azraq killifish or toothcarp *Aphanius sirhani*), birds, mammals, reptiles, and macroinvertebrates (IUCN, 2023). The only assessment of macroinvertebrates in the Azraq Wetland Reserve we are aware of was a survey of the terrestrial arthropods (Amr *et al.*, 1996), we can find no published data on previous assessment of the aquatic macroinvertebrates. For more information on the Azraq Wetland Reserve biodiversity and status, see Disi *et al.* (2004).

Aquatic macroinvertebrate surveys were conducted between 22 – 24 October 2022 at three sites (Haidan Bridge, Al Banna, and Malagi) within the Mujib basin and one site within the Azraq Wetland Reserve (Figure 1, Table 1). The Haidan Bridge site was ~7.5 km upstream of the Mujib Biosphere Reserve boundary, downstream of the Wala dam. The Al Banna site was on the eastern border of the Mujib Biosphere Reserve, downstream of the Mujib dam. The Malagi site was within the Mujib Biosphere Reserve, upstream of the Mujib River waterfall within the Mujib gorge, but downstream of the confluence of the Mujib and Haidan Rivers (Figure 1).



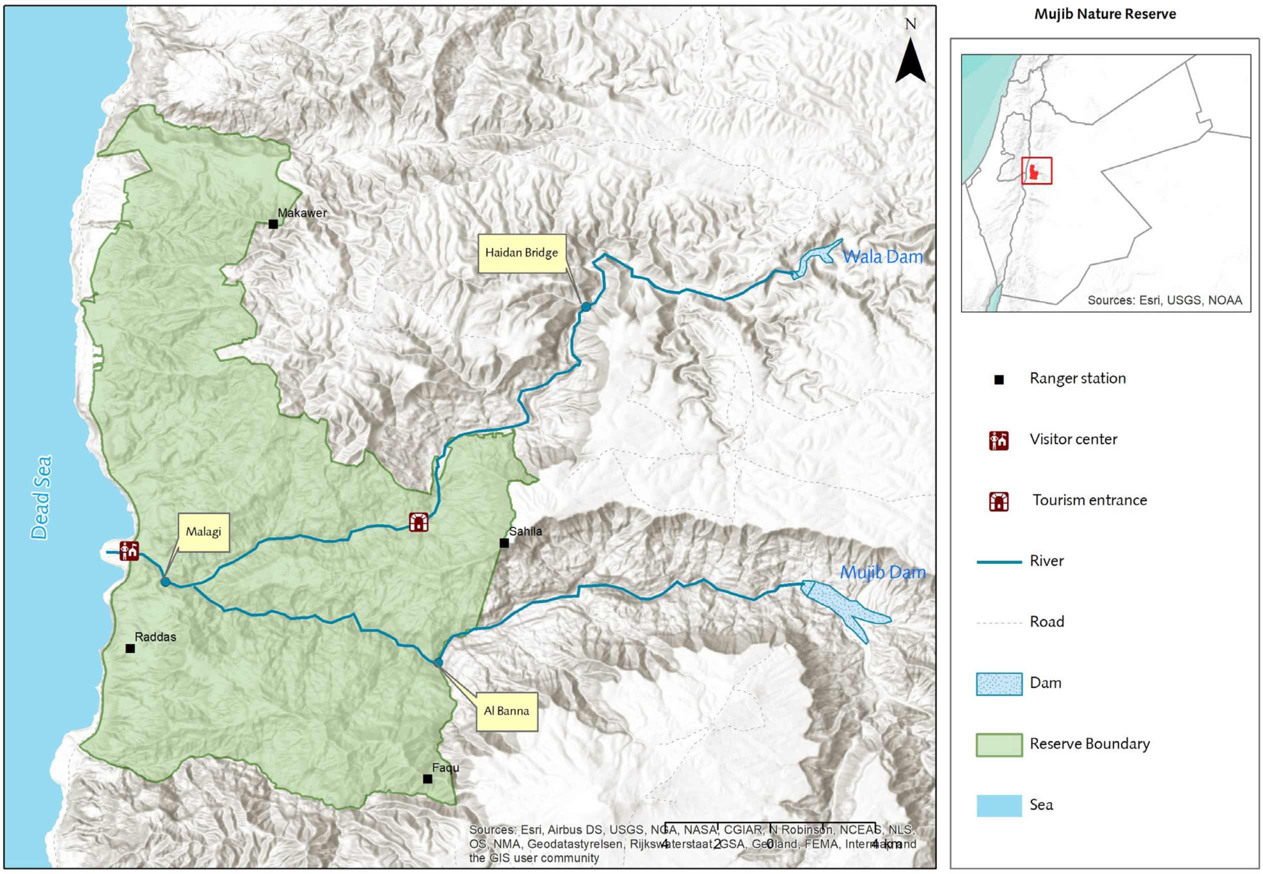


Figure 1. Map showing A) the position of the Azraq Wetland Reserve and the Mujib basin within Jordan, and B) the specific location of the aquatic macroinvertebrate survey sites within the Mujib basin, along with the location of the large Mujib and Wala impoundments, as well as the boundaries of the Mujib Biosphere Reserve. The Mujib basin drains into the Dead Sea in the west. Note the different scales.

Table 1. Details on the survey sites, showing the assigned site name, the date of sample, the river the site was on (not applicable for the Azraq Wetland Reserve), and GPS coordinates.

Site	Date sampled	River	Latitude (°N)	Longitude (°E)
Malagi	22/10/2022	Mujib	31.456317	35.585585
Al Banna	23/10/2022	Mujib	31.440868	35.692322
Hidan Bridge	23/10/2022	Haidan	31.529216	35.726725
Azraq Wetland Reserve	24/10/2022		31.501160	36.49150

Data collection: Aquatic macroinvertebrates surveys were conducted at each site using the sampling method described in the South Africa Scoring System version 5 (SASS5; for full details see Dickens and Graham, 2002). Briefly, the SASS5 method uses a standardised rapid kick-sampling protocol to thoroughly sample aquatic macroinvertebrates in streams or rivers across habitat biotopes. Sampling covers three river components; stones (in and out of current), vegetation (instream and at the river edge), and sediments (gravel, sand, and mud). Samples are collected with a 30x30 centimetre frame net with a pore size of one millimetre^2. For each component, the sample is diluted in a white tray so that the sampler can spend 15 minutes identifying each macroinvertebrate sampled. The sampler also notes taxa anecdotally that they can manually catch or see with visual observations (see Dickens and Graham, 2002). The method is accredited to ISO 17025 standards. Sampling was conducted by a South African Department of Water and Sanitation (DWS) accredited SASS5 practitioner. The method was modified in-field to effectively gather a representative sample the site at the Azraq Wetland Reserve; no stones components were available for sampling, so only the sediments and vegetation were sampled, along with anecdotal observations. Four different sites were sampled at the Azraq Wetland Reserve, one at each of the main pans, with the samples pooled for identification and reporting. The macroinvertebrates sampled were identified to family level where possible on-site. Flow velocity data were also collected at each site (with the exception of the Azraq Wetland Reserve) using a transparent velocity head rod (hereafter 'velocity plank'; Fonstad *et al.*, 2005; WRC, 2016; Graham and Taylor, 2018).

Results

Site status

The sites (Figure 2) varied in terms of the human and livestock impact, flow, and availability of instream habitat for macroinvertebrates. The Haidan Bridge site appeared popular for public visitation, leading to a build of waste pollution. Livestock were observed in the area feeding on marginal vegetation. There were a variety of pools and water depths, increasing instream flow- and depth-related habitat heterogeneity. The Al Banna site showed some evidence of use by locals and livestock, with some waste pollution present. Instream aquatic macroinvertebrate structural habitat was the most varied, though there was little variation in flow depth or velocity. The Malagi site showed minimal human or livestock impact. The instream habitat was limited by the fact that the stones were mostly embedded or armoured by mineralization. Average flow velocity at the sites were 0.036 $m^3.second^{-1}$, 0.126 $m^3.second^{-1}$, and 0.729 $m^3.second^{-1}$ at the Haidan Bridge, Al Banna, and Malagi sites, respectively.

Macroinvertebrates

The Azraq Wetland Reserve site had the highest number of taxa, with 20 different family level taxa identified from 10 orders or clades (Table 2). The Haidan Bridge site had 18 family level taxa from nine orders or clades, the Al Banna site 15 family level taxa from eight orders or clades, and the Malagi site 13 family level taxa from eight orders or clades (Table 2).

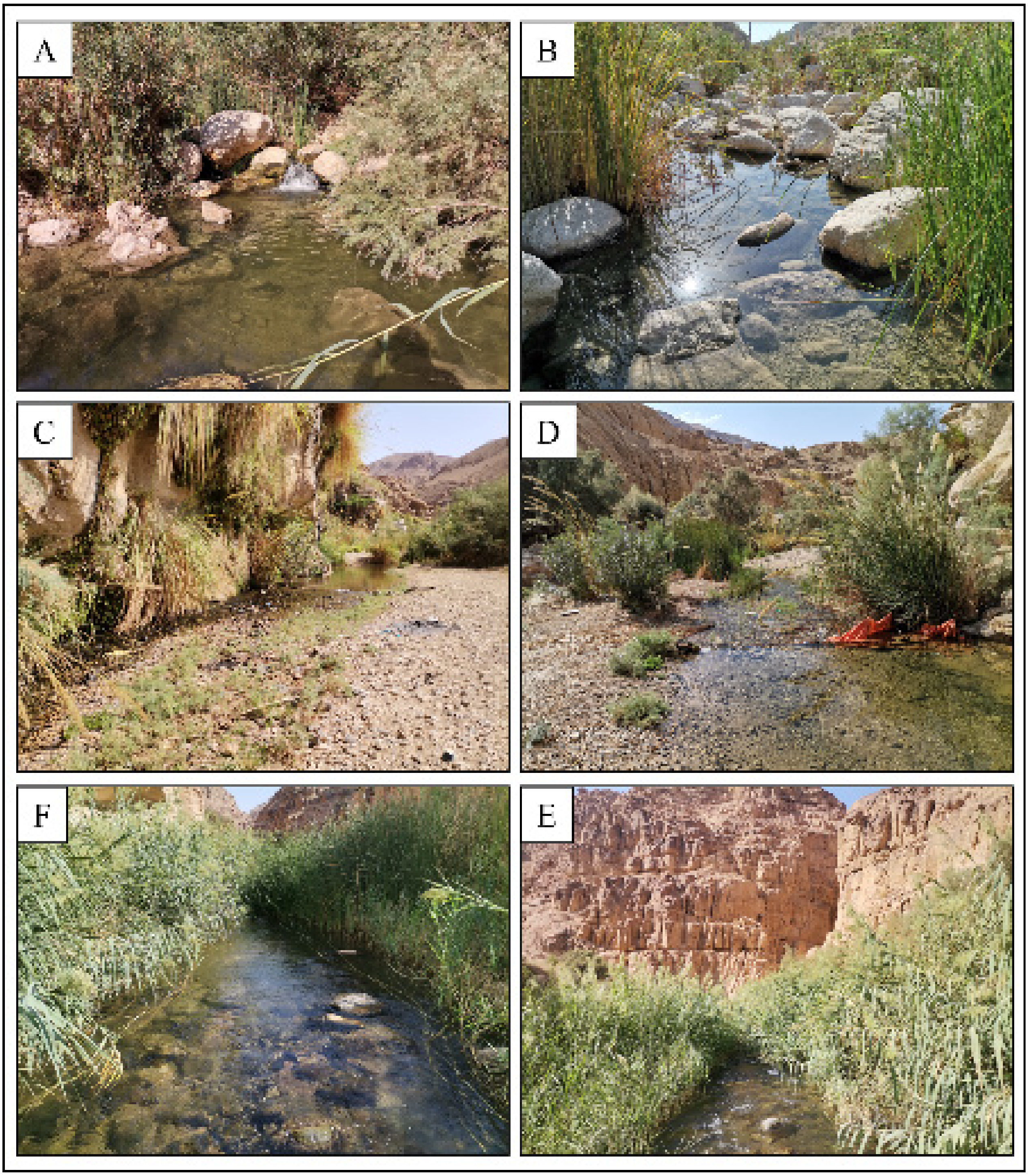


Figure 2. Upstream (left column) and downstream (right column) images of the Haidan Bridge (A and B), Al Banna (C and D), and Malagi (E and F) aquatic macroinvertebrate survey sites.

Table 2. Aquatic macroinvertebrate taxa sampled at each survey site; x denotes presence.

		Site and presence of taxon			
Order level grouping*	**Family level grouping***	Malagi	Al Banna	Haidan Bridge	Azraq Wetland Reserve
Amphipoda	Amphipoda[e]				x
Caenogastropoda[a]	Melanopsidae	x	x	x	x
	Thiaridae	x	x		x
Coleoptera	Dytiscidae				x
Cycloneritida	Neritidae		x		x
Decapoda	Potamonautidae	x	x	x	
Diptera	Chironomidae	x	x	x	x
	Culicidae			x	x
	Dolichopodidae		x		
	Simuliidae	x			
	Tabanidae		x		x
	Tipulidae	x			
Ephemeroptera	Baetidae 1 sp	x			x
	Baetidae 2 sp			x	
	Caenidae	x		x	
	Leptophlebiidae			x	
Hemiptera	Corixidae		x	x	
	Gerridae			x	x
	Naucoridae			x	
	Nepidae				x
	Notonectidae			x	x
	Pleidae				x
	Veliidae	x	x	x	x
Hirudinea[b]	Hirudinea[a]			x	
Hygrophila[c]	Lymnaeidae				x
	Physidae				x
Odonata	Aeshnidae			x	x
	Coenagrionidae	x	x	x	x
	Euphaeidae		x	x	
	Gomphidae	x	x		
	Libellulidae		x		x
Oligochaeta[d]	Oligochaeta[d]	x	x	x	
Trichoptera	Hydropsychidae	x	x		
Trombidiformes	Hydracarina[f]			x	x
	No. Order level taxa	8	8	9	10
	No. Family level taxa	13	15	18	20

Notes: * Taxa were grouped to the nearest taxonomic level as possible based on existing taxonomic classes or what is possible with in-field identification. [a] Subclass Caenogastropoda; [b] Subclass Hirudinea; [c] Superorder Hygrophila; [d] Subclass Oligochaeta; [e] Order Amphipoda; [f] Unranked grouping for many families of aquatic mites.

Discussion

Freshwater scarcity is a pressing global issue (Gleick and Cooley, 2021). Jordan faces especially severe freshwater scarcity due to its arid climate, unsustainable reliance on limited water resources, and increasing population (Jaber *et al.*, 1997; Al-Assa'd and Abdulla, 2010; Schyns *et al.*, 2015; Clemens *et al.*, 2020; Dombrowsky *et al.*, 2022; Oberhauser *et al.*, 2023). As demand surpasses supply, Jordan faces significant challenges in maintaining adequate water quantity and quality for domestic, agricultural, and industrial purposes (Whitman, 2019). As a result, it is essential to monitor the rare and precious freshwater resources in Jordan to ensure sustainable management, use, and preservation of freshwater for both humans and biodiversity alike. This study presents, as far as we are aware, a first directed survey of the aquatic macroinvertebrates in the Azraq Wetland Reserve, as well as a first survey of the aquatic macroinvertebrates using standard biomonitoring techniques in the Mujib basin, in Jordan (Disi *et al.*, 2004; Haddad *et al.*, 2013; Alhejoj *et al.*, 2014b, 2014a, 2017; Ramadan and Katbeh-Bader, 2018). A good variety of taxa across a range of suspected pollution and water quality tolerances were present in the Azraq Wetland Reserve and the Mujib River basin. As a result, following the earlier work of Alhejoj, Bandel and Salameh (2014a), we recommend that biomonitoring via surveying the aquatic macroinvertebrates in these locations could provide a powerful tool to assess the health and the quality of the water resources. However, this suggestion is subject to the provision that family-level aquatic macroinvertebrate tolerances and sensitivities are validated for Jordan to develop an integrated index for rapid, in-field assessment of water resource water quality and ecological health, as employed elsewhere in the world (e.g., Dickens and Graham, 2002; Morse *et al.*, 2007; Paisley *et al.*, 2014; Odountan *et al.*, 2019; Ndatimana *et al.*, 2023).

Notes on aquatic macroinvertebrates of the Azraq Wetland Reserve and Mujib River basin

This study provides a first dedicated survey of the aquatic macroinvertebrates at a site in the Azraq Wetland Reserve, to the best of our knowledge. We identified specimens from 20 families within 10 orders or clades. The Amphipoda (malacostracan crustaceans, Class Amphipoda), Dytiscidae (predatory diving beetles, Order Coleoptera), Lymnaeidae (pond snails, Superorder Hygrophila), Nepidae (water scorpions, Order Hemiptera), Physidae (bladder snails, Superorder Hygrophila), and Pleidae (pygmy backswimmers, Order Hemiptera) were recorded in the Wetland Reserve and not in the Mujib River basin, likely reflecting geographic and habitat variation (i.e., wetland *versus* river). More thorough sampling at regular time intervals and at more locations across the Wetland Reserve may yield additional insights into the diversity and abundance of the different taxa present. A total of 28 families of macroinvertebrate were identified in the Mujib basin, similar to the number reported in previous more exhaustive assessments (Haddad *et al.*, 2013; Ramadan and Katbeh-Bader, 2018), supporting the effectiveness of sampling using SASS5 methods for an efficient and thorough sampling of aquatic macroinvertebrates. Variations in the taxa present at each site likely reflected the variations in human impacts, flow, and habitat availability (Dickens and Graham, 2002). For example, the Haidan Bridge site had low flow rates and water in standing pools at various depths, creating heterogenous habitat for different species with different habitat-flow-depth preferences. In contrast, the Malagi site showed minimal human impact, but was more uniform in instream habitat, flow velocities, and flow depths, limiting habitat for a variety of taxa.

The potential use of aquatic macroinvertebrates for biomonitoring in Jordan

Water resource monitoring involves the regular assessment of physical, chemical, and biological parameters to evaluate the health and safety of water resources (Altenburger *et al.*, 2015; Behmel *et al.*, 2016). Monitoring helps identify patterns of use, identify potential contaminants, track pollution sources, and implement appropriate mitigation measures. Essentially, monitoring provides the core data for making informed decisions regarding water resource management, protection, and restoration (Chapman, 1996; Lovett *et al.*, 2007). Among the various monitoring methods, biomonitoring stands out as an effective, practical, and holistic approach for assessing the health of freshwater bodies (Friberg *et al.*, 2011). Aquatic macroinvertebrates present an especially useful biomonitoring tool, given they 1) are easy to sample and identify, so monitoring does not require sophisticated equipment or complex laboratory analyses, making it cost-effective method, 2) play a critical role in freshwater ecosystems, 3) are found in abundance and variety in freshwater systems around the globe, and 4) are highly responsive to changes in water quality, with taxon-specific sensitivities and tolerances (Dickens and Graham, 2002; Bonada *et al.*, 2006). Essentially, surveying aquatic macroinvertebrates provide an affordable, rapid, integrated assessment of the impacts of various stressors, including pollution, habitat degradation, and changes in water flow, providing a holistic view of water quality and ecological health of aquatic systems. Though freshwater systems are scarce in Jordan, this study supported previous work by Alhejoj, Bandel and Salameh (2014a) demonstrating that they still contain a rich biodiversity of aquatic macroinvertebrates suitable for biomonitoring purposes. However, the classifications listed in the 'Jordanian Biomonitor System for Watercourses (JBSW)' proposed by Alhejoj, Bandel and Salameh (2014a) are loosely based on the presence or absence of key taxa at various taxonomic hierarchies with known pollution tolerances, rather than an integrated index based on the ecology and sensitivity of the entire community present at a similar, easily identifiable, taxonomic hierarchy (Alhejoj *et al.*, 2014a). As a result, the JBSW largely inhibits the rapid field assessment possible using family-level taxonomic identification. For example, our results suggested that each of the sample sites could show anywhere from moderate to high water quality according to the JBSW depending on the genus or species of Neritidae (nerites, Order Cycloneritida), Melanopsidae (melanopsids, Superfamily Cerithioidea), Caenidae (small squaregill mayflies, Order Ephemeroptera), or Chironomidae (chironomids, Order Diptera) specimens at each site. Genus or species-level resolution identifications of aquatic macroinvertebrates are seldom possible without laboratory analysis by taxonomic experts. Therefore, for the JBSW to be practical in-field to develop rapid assessments, it may be necessary to refine evaluation to easily identifiable family-level taxonomic requirements. Overall, we recommend that dedicated research is done to follow up on the excellent ground work done by Alhejoj, Bandel and Salameh (2014a) to develop a reworked, thorough aquatic macroinvertebrate biomonitoring tool based on taxonomic classifications rapidly identifiable in-field, similar to those developed and employed for other nations (e.g., Dickens and Graham, 2002; Palmer and Taylor, 2004; Morse *et al.*, 2007).

Conclusions

Water quality monitoring is essential in water-scarce regions like Jordan to ensure sustainable water resource management and protect the health of ecosystems and communities. Among the various monitoring methods, biomonitoring using aquatic macroinvertebrate offers a practical and effective approach to generate a holistic view of water quality and water body health. The cost-effectiveness, ease of sampling, and standardized protocols make it particularly suitable for resource-limited regions such as Jordan. We recommend that a scoring system to calculate an aquatic macroinvertebrate biomonitoring index similar to those used elsewhere in the world (Dickens and Graham, 1998; Morse *et al.*, 2007; Paisley *et al.*, 2014; Odountan *et al.*, 2019) be developed and validated for the taxa at family level in Jordan to follow up on initial work done by Alhejoj, Bandel and Salameh (2014a). Thereafter, efficient and effective biomonitoring can be integrated into water quality monitoring programs to enhance Jordan's ability to address water scarcity challenges and promote the sustainable use of its limited water resources.

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