Monthly Ecological Dynamics and Their Influence on the Prevalence and Species Richness of Dactylogyrus (Monogenea) Parasites in Fishes of the Lesser Zab River

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Abstract

The gills of fishes are considered the permanent habitat for Dactylogyrids spp., common monogenean ectoparasites. This study aims to investigate the impact of monthly aquatic ecological dynamics on the diversity of Dactylogyrus. Moreover, the prevalence and species richness were correlated as well. A total of 140 fishes, representing 12 species from the family Cyprinidae (9) and Leucisidae (3), were collected from the Lesser Zab River, located in the northeast of Altuon-Kopri town, Kurdistan Region, Iraq. The study was conducted from August 2022 to July 2023. The collected fishes included: *Acanthobrama marmid* (37), *Alburnus sellal* (9), *Capoeta* (C.) *damascina* (7), C. *umbla* (3), *Carasobarbus luteus* (10), *Carassus* (C.) *auratus* (13), C. *carassus* (3), *Chondrostoma regium* (18), *Cyprinion macrostomum* (5), *Cyprinus carpio* (26), *Luciobarbus xanthopterus* (4) and *Mesopotamichthys sharpeyi* (5). They were inspected weekly for diversity variation and richness of Dactylogyridis with different aquatic parameters such as pH, temperature, total dissolved salts (TDS) and electrical conductivity (EC). The collected data revealed the presence of various Dactylogyridis species with different prevalence and species richness. Monthly variations revealed that the highest species richness occurs lowermost the lowermost occurred in March 2023, and the lowest was observed in September 2022. According to aquatic parameters, only water temperature shows a significant negative correlation according to the species richness ($p<0.05$). In contrast, the other parameters show no significant correlation in accordance to the different months, monthly aquatic mean levels of pH, TDS and EC were not correlated with species richness and prevalence of Dactylogyridis spp.

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Introduction

As reported by Fricke *et al.* (2023), the Cyprinidae family is the most diverse among freshwater fish, with 3,023 known species (1,782 confirmed as valid) spanning 285 genera. It represents about 4.91% of the world's total fish species and is known to thrive in diverse habitats. Iraq's freshwater ecosystem is notable for its endemism, with 52 native species spanning 11 distinct fish families, including Cyprinidae. This is highlighted in the research findings of Freyhof *et al.* (2021).

This gill parasite, found in Cyprinid fish, is one of the most significant genera comprising over 900 species, as Timofeeva *et al.* (1997) noted. The wide range of Cyprinid hosts it affects, and its high level of specificity contribute to the widespread distribution of such sizeable parasitic

organisms in various aquatic habitats. For instance, many *Dactylogyrus* species demonstrate stringent host specificity by infecting only one host species or closely related host species (Simková *et al.*, 2006).

It is crucial to comprehend the ecology of monogenean fish parasites in order to create efficient management plans that will reduce their negative effects on fish populations and ecosystems. Novel insights into the molecular biology and development of these parasites are being provided by advancements in genomic and transcriptomic technology, which may result in novel strategies for limiting their effect and dissemination (Hutson *et al.*, 2018; Öktener and Bănăduc, 2023). Monogenean parasites often have direct life cycles, meaning they do not need intermediary hosts. However, factors including the parasite population's density, the host's movement and behavior, and the existence of additional potential hosts in the environment can all impact the transmission of monogeneans (Buchmann and Lindenstrøm, 2002).

Fish and other aquatic animals frequently have flatworms called monogenean parasites living on their skin and gills. These parasites can significantly harm the aquaculture industry's bottom line by slowing fish growth and efficiency and, in certain situations, even causing fish death (Buchmann and Lindenstrøm, 2002; Hoai, 2020). Their ecology is complex, influenced by biotic and abiotic elements, including host-parasite interactions and environmental conditions. The methods of parasite attachment, feeding, and reproduction, as well as the effects of these parasites on host populations and communities, have all been the subject of research on the ecology of monogenean parasites (Gehman *et al.*, 2019; Devore *et al.*, 2020; Hikmatulloh *et al.*, 2020; Gilbert and Avenant-Oldewage, 2021; Bertaglia *et al.*, 2023).

Information on the *Dactylogyrus* species that impact Cyprinid hosts in the Mediterranean region is rare. However, a handful of studies have hinted that due to their strong host specificity, the distribution of *Dactylogyrus* species aligns with the distribution of their Cyprinid host species (Lambert and El Gharbi, 1995). In the Kurdistan region of Iraq, approximately 190 confirmed species of fish parasitic fauna are found within various localities. These parasites inhabit and affect over 25 distinct species of freshwater cyprinid fish. In total, there have been reports of over 80 species of monogeneans, with roughly 50 belonging to the *Dactylogyrus* genus. Additionally, there are unidentified species categorized as *Dactylogyrus* spp., constituting the largest group among these monogeneans (Petrov *et al.*, 2016; Mhaisen and Abdullah, 2017; Koyee and Abdullah, 2019).

The primary aims of this study are to offer a comprehensive perspective on the ecological variables that impact two aspects of host-parasite relationships, namely parasite prevalence and species diversity. Specifically, we aim to investigate the monthly variations in *Dactylogyrus* spp. populations and their associations with various aquatic environmental factors [temperature, pH, salinity, and electrical conductivity (EC)]. This research focuses on cyprinid fish populations inhabiting the Lesser Zab River within the Kurdistan Region of Iraq, particularly emphasising how these environmental factors may influence the likelihood of fish gill infestations with the parasites.

Material and Methods

Ethical approval

This research was authorized by the Research Ethics Committee Office (RECO) on 25 August 2022, under protocol reference 4S/133. It was carried out by the animal ethical standards and guidelines specified in Salahaddin University-Erbil.

Description of the study area

The study site is situated along the banks of the Lesser Zab River in northeastern Iraq,

near the town of Altun-Kopri (Prde). Altun-Kopri is approximately 40 kilometres northwest of Kirkuk City and about 50 kilometres from Erbil City. The Lesser Zab River falls within the geographical coordinates of 34º to 36º north latitude and 43º to 46º east longitude. This River has its source in Iran, as documented by Abdullah and Nasraddin (2015).

Physico-chemical analysis of water

Water samples were collected weekly from the research site, specifically from a depth of 3 meters from the River. These samples were collected with the assistance of local fishermen. Various physicochemical parameters of the water, including the potential of hydrogen ion concentration (pH) level, temperature (˚C), total dissolved salts (TDS) measured in parts per million (ppm) and electrical conductivity (EC) measured in micro siemens per centimeter (µS/cm) were directly assessed at the collection where the fish were collected. This assessment was carried out using a portable meter.

Fish Collection and Taxonomic Identification

A total of 140 fish specimens were collected by local commercial fishermen using gill nets between August 2022 and July 2023. These freshly caught specimens were expeditiously transported to the Zoology Research Laboratory within the Biology Department at Salahaddin University in Erbil, Iraq. During transport, they were carefully placed in a cork box filled with local river water to ensure their well-being. Within 24 hours of collection, the fish were dissected for further examination.

The fishes were taxonomically classified into 12 species, 9 of them within the family *Cyprinidae* and 3 species within the family Leucisidae in accordance to Coad (2010), and their scientific nomenclature adhered to the designations as specified in FishBase (Froese and Pauly, 2023). In the laboratory, measurements were taken for total length (TL), standard length (SL) and body depth in centimeters, and body weight in grams, with weights ranging from 15 to 780 g. Additionally, the study distinguished between male and female individuals of each fish species.

Parasitological examination

The examination of gills involved a systematic process. The gills, the primary location for the presence of Dactylogyrids spp., were carefully isolated from the fish. They were first sorted into a dish based on their location on the sides of the head to facilitate the spreading of *Dactylogyrus* spp.; subsequently, the gills were separated. A small amount of water was introduced to the gills, then a needle was used to separate sections of the gill filaments carefully. Next, at least five live Dactylogyrids (after being extracted from the gills) were extracted from the gills using a small pipette and placed individually on a microscope slide with a minimal amount of water. To clarify the specimens, a droplet of melted glycerin-gelatin was added before covering them with a coverslip. The coverslide was gently dried using filter paper, and the specimens were carefully condensed in glycerin-gelatin (Kritsky *et al.*, 2004) as provisional preparations by the procedure outlined by Lari *et al.* (2016).

The stereo- and light microscopes were employed to ascertain the distribution and positioning of Dactylogyrids spp. on the gill arches. Measurements were conducted following the guidelines of Jarkovský *et al.* (2004), as depicted in Figure (1). Additionally, they had been recognised through observing the hard components of the haptor (anchors, ventral and dorsal connective bars, marginal hooks) and reproductive organs (vaginal armament and male copulatory organs).

The detected parasites were classified based on morphology and identified according to Pugachev *et al.* (2010).

Criteria for infection

Microscopy techniques, including stereo- and light microscopes, were employed to investigate the distribution and positioning of Dactylogyrids spp. Parasite diversity is measured as species richness. Richness is the number of species at the observed site and is measured by total species abundance (León and Choudhury, 2005). Additionally, the prevalence (%) of the parasites contributes to diversity dynamics. The prevalence (%) is the ratio of infested fish to the total number of inspected fish. Species richness refers to the number of Dactylogyrids spp.; occurring on an individual fish, as described by Margolis *et al.* (1982) and Bush *et al.* (1997).

The statistical analysis

To assess the significance of variations in prevalence values and species richness, a Chi-squared test was employed. This test was conducted in relation to temperature, pH and salinity. Pearson Correlation was implemented within a General Linear Model and correlation coefficient analysis was calculated. All nonparametric data were transformed using the natural logarithm (ln) and analyzed using the SPSS program (version 21.0) and GraphPad Prism (version 9.2). The research citations were appropriately formatted using the EndNote X7 program.

Result and Discussion

According to the fishes examined in this study, the number of infections and rate of infection with the species of Dactylogyridis are shown in Table (1). *Luciobarbus xanthopterus* had the highest infection rate (75%), while *Alburnus sellal* had the lowest (11.1%). Understanding seasonal parasite dynamics is essential for comprehending parasite biology, identifying periods fish are susceptible to diseases, and implementating control strategies (Neary *et al.*, 2012). According to traditional ecological definitions, species diversity encompasses two essential components that should be accounted for by any diversity index: species richness and evenness, the latter is the degree to which the relative abundances of the different species in the assemblage are similar to each other (Morand *et al.*, 2015).

Table 1. Scientific, number of fishes examined, infected number and the prevalence of Dactylogyridis species from Alton-kopri Lesser Zab River

Family and fish scientific name	Examined Fish No.	Infected Fish No.	Prevalence (%)
Family: Cyprinidae *Capoeta damascina* (Valenciennes, 1842)	7	3	42.9
C. umbla (Heckel, 1843)	3	2	66.7
Carassobarbus luteus (Heckel, 1843)	10	4	40
Carassus auratus (Linnaeus, 1785)	13	8	61.5
C. carassus (Linnaeus, 1785)	3	2	66.7
Cyprinion macrostomum Heckel, 1843	5	2	40
Cyprinus carpio (Linnaeus, 1758)	26	8	30.8
Luciobarbus xanthopterus (Heckel, 1843)	4	3	75
Mesopotamichthys sharpeyi (Günther, 1874)	5	1	20
Family: Leucisidae *Acanthobrama marmid (*Heckel, 1843)	37	11	29.7
Alburnus sellal (Heckel, 1843)	9	1	11.1
Condrostomum regium (Heckel, 1843)	18	6	33.3
Total	140	51	36.4

A significant aspect of parasite infections within natural host populations is the notable variation observed among members of the same host species. This variation includes both the diversity and the presence of parasites, which can differ between host population. The presence of specific habitat characteristics, whether physical or chemical in nature, can play a role in enabling the parasites to establish themselves and multiply within particular host populations. For instance, in fish hosts, factors like the size of the lake, water pH levels, or the proximity to other lakes have been linked to variations in the parasite species number found within a host population or to the average abundance of specific parasites (Bagge, 2005).

Species richness in this study ranges from 1 to 40, with the highest in March 2023 and the lowest in September 2022. The highest prevalence of Dactylogyridis spp. was observed in December 2022 and March 2023, while the lowest infection rate was reported in September 2022. A condensed condition throughout winter months may, however, increase the vulnerability of the host to immature parasite stages and result in more mean intensities (Crafford *et al.*, 2014).

Monthly average levels of potential Hydrogen ion (pH), total dissolved salts (TDS in ppm) and electrical conductivity (EC in µS/cm) were non-significantly ($p>0.05$) correlated to the species richness and the prevalence of Dactylogyridis spp. On the other hand, the annually averaged water temperature was 12.65˚C (range: 4.72˚-20˚C), which has a negatively significant ($p<0.05$) correlation to Dactylogyridis spp. richness ($r=-0.6196$), but non-significantly correlated to the prevalence of Dactylogyridis spp. (Table 2).

Table 2. Correlation analysis for some parameters of water, species richness and prevalence of isolated Dactylogyridis s.pp.

***Dactylogyrus* richness and prevalence**	**Water quality**			
	pH	**Water Temp (˚C)**	**TDS (ppm)**	**Electrical Conductivity (µS/cm)**
Species richness	-0.000981	-0.6196	-0.1302	-0.1115
p-value	0.9976	0.0317*	0.6867	0.7302
R square	9.627	0.3839	0.01696	0.01242
Prevalence (%)	-0.1884	0.08622	-0.5015	-0.4760
p-value	0.5577	0.7899	0.0967	0.1178
R square	0.03548	0.007433	0.2515	0.2265

Water temperature is considered one of the most critical factors controlling the presence and richness of monogeneans. It directly affects their reproduction and development, and indirectly impacts the host's immune response (Šimková *et al.*, 2001). Optimal temperatures for *Dactylogyrus* survival and reproduction vary depending on the species, but generally, warmer water temperatures favor their growth and reproduction. However, excessively high temperatures can negatively impact *Dactylogyrus* populations, leading to reduced survival and infectivity (Šimková *et al.*, 2001; Zhang *et al.*, 2022). However, the temperature's impact varies among parasite species. Some species, like *D. extensus*, thrive at a lower temperature around 16–17 ˚C, while others, like *D. vastator* prefer warmer water temperatures in the range of 20 to 30 ˚C (Zhang *et al.*, 2015).

The highest rate of infection (63.49%) was recorded in July 2022, when pH and TDS fall to the lowest values (5.5 and 84 ppm) respectively. Water pH is another critical factor that can influence the prevalence and species richness of *Dactylogyrus* parasites. *Dactylogyrus* species have different pH tolerances, and fluctuations outside their optimal range can lead to reduced survival, reproduction, and infectivity (Bagge, 2005; Allalgua *et al.*, 2021). Regarding to water salinity that can affect the prevalence and species richness of *Dactylogyrus* parasites. *Dactylogyrus* species are generally more prevalent in freshwater systems, but some species can tolerate low salinity conditions in estuaries and coastal areas. Salinity changes can also influence the physiology and behavior of *Dactylogyrus* parasites, leading to alterations in feeding, reproduction, and infectivity (Bagge, 2005).

The correlation analysis for different water quality parameters, species richness, and prevalence (%) of isolated Dactylogyridis spp. displayed in Figure (1) with different levels of significance regarding the correlation coefficients (Pearson's correlation coefficients) between these parameters along with their corresponding p-values (significance level) and R-squared values (coefficient of determination).

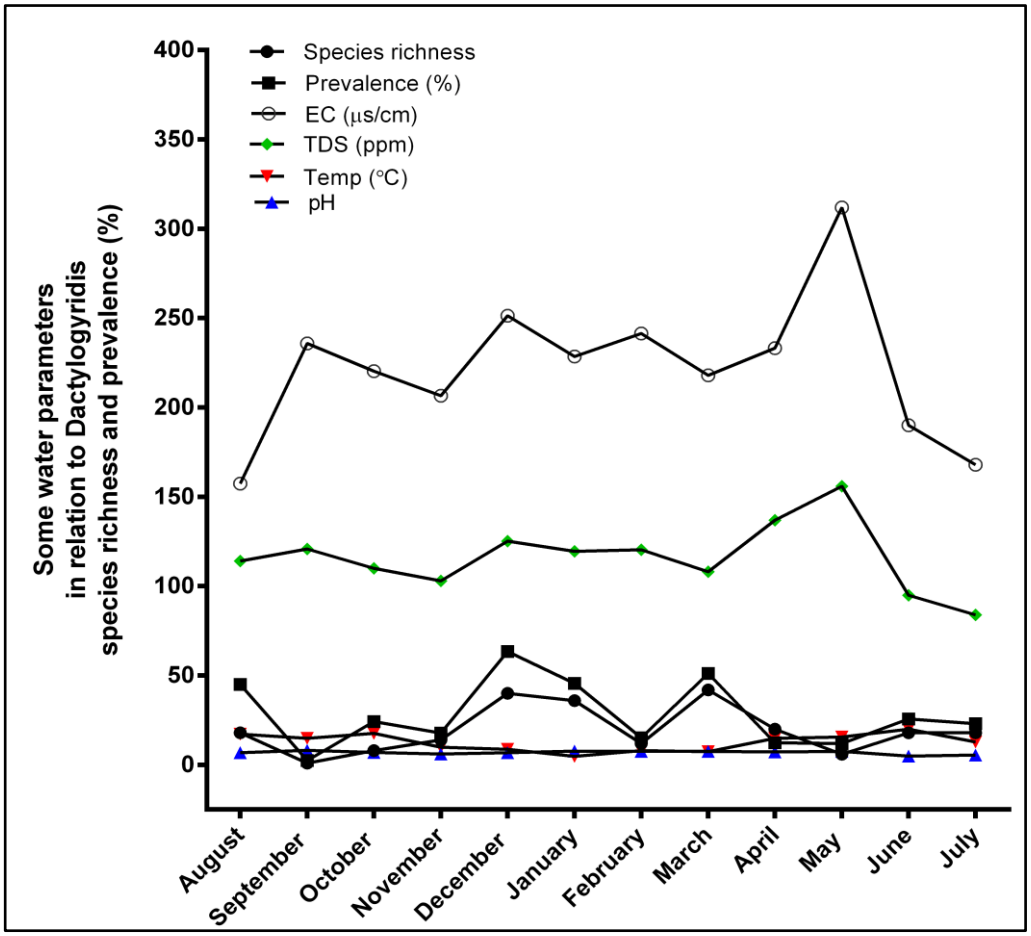

Figure 1. Water parameters degree about isolated Dactylogyridis spp. richness and prevalence (%) according to months of the study (August 2022 - July 2023)

Water salinity is also an essential factor that can affect the prevalence and species richness of *Dactylogyrus* parasites. *Dactylogyrus* species are generally more prevalent in freshwater systems, but some species can tolerate low salinity conditions in estuaries and coastal areas. Salinity changes can also influence the physiology

and behavior of *Dactylogyrus* parasites, leading to alterations in feeding, reproduction, and infectivity (Bagge, 2005). Stress conditions in fish can be attributed to a combination of ecological factors and water quality parameters (Nnadi *et al.*, 2011). Exposure to unfavorable conditions can induce stress in fish, and this stress can serve as a contributing factor that promotes an increase in parasite abundance (Kadlec *et al.*, 2003). It can be hypothesised that the species richness and abundance of helminth parasite communities in freshwater fish would be positively correlated with the nutrient status of the water bodies, as measured by pH, conductivity, and micronutrient concentrations (Thomas, 2002).

According to the species richness (Figure 2), there is an extremely weak negative correlation between species richness and pH (correlation coefficient: -0.000981). The p-value (0.9976) suggests that this correlation is not statistically significant. On the other hand, there is a moderate negative correlation regarding to species richness and water temperature (correlation coefficient: -0.6196). The p-value (0.0317) indicates that this correlation is statistically significant at a significance level 0.05 (since the p-value is less than 0.05). Moreover, a weak negative correlation exists between species richness and TDS (Total Dissolved Solids) in water (correlation coefficient: -0.1302). The p-value (0.6867) suggests that this correlation is not statistically significant. Furthermore, there is a weak negative correlation between species richness and electrical conductivity (correlation coefficient: -0.1115). The p-value (0.7302) indicates that this correlation is not statistically significant.

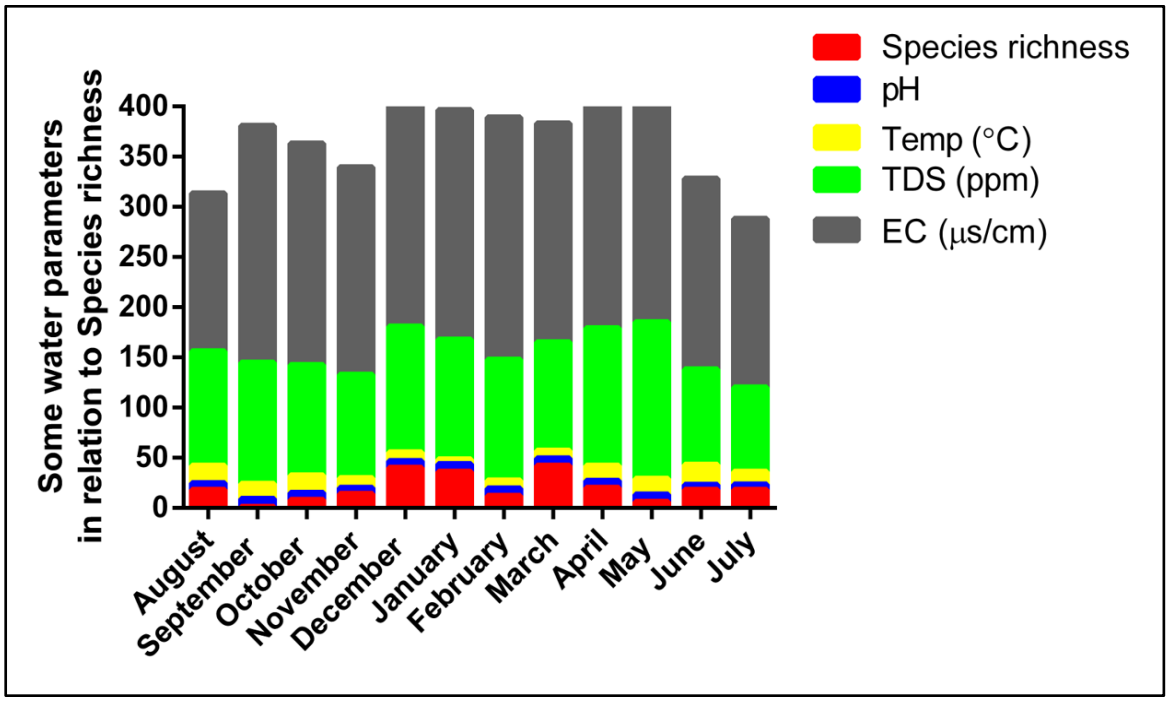

Figure 2. Aquatic ecological parameters in relation to Dactylogyridis species richness according to months of the study (August 2022 - July 2023)

Seasonal occurrence peaks during rainfall due to a decreased rate of monogeneans. Conversely, a higher rate of parasitic infections during the dry season could be attributed to the relatively heightened susceptibility of the host population. It is commonly observed that organisms experiencing stress or being in a weakened physical condition are often less resistant to parasitic infections (Zuk, 1990).

During the summer season, various conditions contribute to habitat contraction, reduced oxygen abundance, increased fish crowding, and, in some instances, decreased fish condition and elevated death rates (Chapman and Chapman, 1993a, 1993b). In the inlet stream, water availability significantly diminishes during the dry months, and soil barriers separate parts of the stream, resulting in the concentration of monogeneans in dry season refuges. If higher population densities contribute to elevated stress levels, the dry season conditions could lead to greater parasitism.

Based on the prevalence of Dactylogyridis species (Figure 3), there is a weak negative correlation between the prevalence of Dactylogyridis spp. and pH (correlation coefficient: -0.1884). The p-value (0.5577) suggests that this correlation is not statistically significant. On the other hand, there is a fragile positive association between prevalence and water temperature (correlation coefficient: 0.08622). The p-value (0.7899) indicates that this correlation is not statistically significant. Moreover, a moderate negative correlation exists between prevalence and TDS (correlation coefficient: -0.5015). The p-value (0.0967) suggests that this correlation is not statistically significant but is close to being significant at a 0.10 level. Furthermore, a moderate negative correlation exists between prevalence and electrical conductivity (correlation coefficient: -0.4760). The p-value (0.1178) indicates that this correlation is not statistically significant.

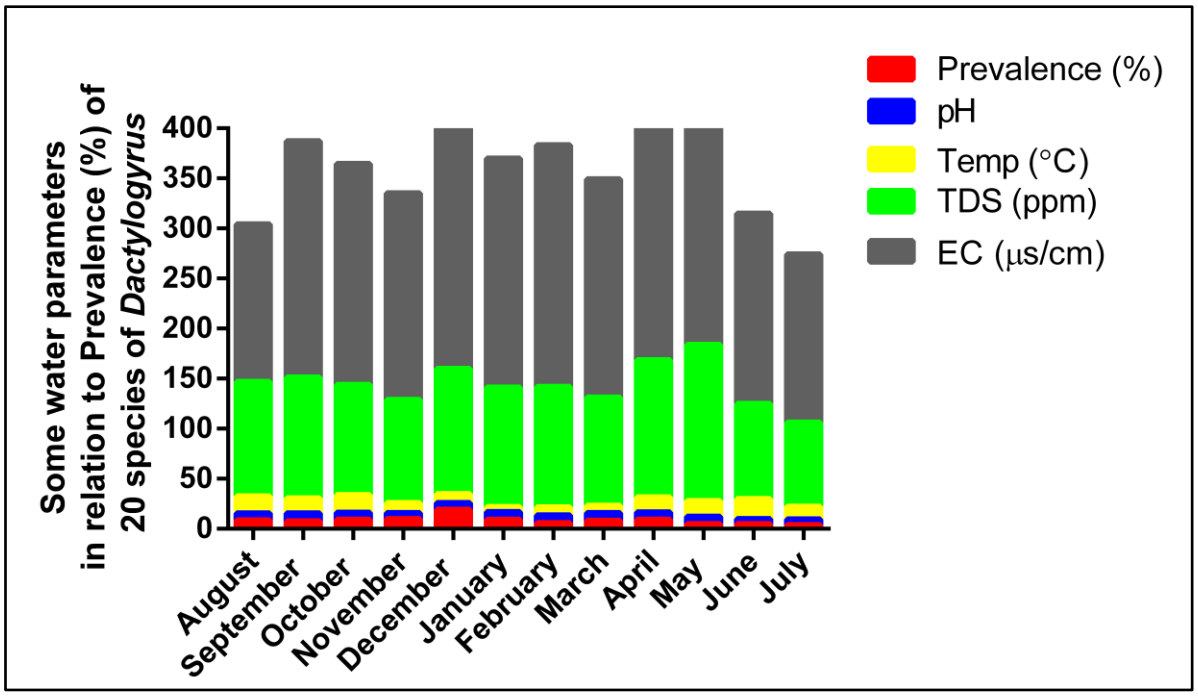

Figure 3. Aquatic ecological parameters in relation to Dactylogyridis prevalence (%) according to months of the study (August 2022 - July 2023)

Based on the correlation coefficients and significance levels of the identified species, species richness is significantly negatively correlated with water temperature, indicating that as water temperature increases, species richness tends to decrease. The prevalence of Dactylogyridis spp. is not significantly correlated with any of the water quality parameters. A study conducted in the same region by Bashe and Abdullah (2010) also observed monthly fluctuations in the infection rate with a monogenean fish parasite. These findings align with the results of the current study, which show

higher infection rates during April and May and lower rates during January and February. Another study in the same region by Abdullah and Nasraddin (2020) focused on Dactylogyrids spp. and their seasonal variations. Their findings indicated that the highest infection rate occured during the summer, while it was lowest during the winter.

These relationships only suggest statistical associations between the variables analyzed. Further research and analysis would be necessary to determine any causal relationships or additional factors influencing species richness and prevalence of Dactylogyridis spp. in relation to water quality parameters.

Conclusion

The study concerned the variety and distribution of Dactylogyridis on two fish families in the Lesser Zab River, Kurdistan Region, Iraq. The result included examining the most important native fish species in the study area. The highest level richness value was recorded in March; the lowest was recorded in September 2022. There were no significant correlated with mean level of pH, TDS and EC with species richness and prevalence of Dactylogyridis spp.; water temperature was a significant correlation with species richness.

Conflict of interests

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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