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Research Article

First report of epibiont communities on narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 (Decapoda: Astacidae) from Shiyan Dam reservoir, western Iran

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Abstract

This study investigates epibiont communities, including both protozoan and metazoan species, associated with *Astacus leptodactylus* in the Shiyan Dam reservoir situated in the Kermanshah province of western Iran. A total of 220 crayfish were collected for comprehensive parasitological surveys, targeting various body parts, including the gills, exoskeleton, mouth cavity, and internal organs. Protozoan species identified included *Cothurnia sieboldii*, *Chilodonella* sp., *Vorticella similis*, *Zoothamnium* sp., and *Pyxicola annulata*. In the metazoan category, notable species consisted of *Branchiobdella kozarovi* (Annelida), *Philodina acuticornis* (Rotatoria), *Rabdochona* sp. (Nematoda), and *Mesocyclops strennus* (Copepoda). *P. acuticornis* exhibited the highest prevalence among gill epibionts at 18.2%, while *Chilodonella* sp. showed the lowest prevalence at 0.9%. The study also revealed seasonal variations, with the highest infestation occurring in spring (82%), and the lowest in winter (24%). This research presents the first documented evidence of epibionts on *A. leptodactylus* in western Iran.

Keywords: Biodiversity, Parasitological surveys, Protozoan species, Metazoan diversity, Kermanshah

Introduction

In recent years, several studies have explored epibiosis among Crustaceae (e.g., Fernandez-Leborans, 2001, 2003, 2010; Fernandez-Leborans & Fernandez-Fernandez, 2002; Fernandez-Leborans & von Rintelen, 2007; De Smet and Verolet, 2016; Tanabe et al., 2017; Lu et al., 2020; Spiridonov et al., 2020; Dvoretsky & Dvoretsky, 2022). Epibiosis involves two organisms: the epibiont and the basibiont (Fernandez-Leborans, 2010; Martin Wahl, 1989). The term epibiont refers to organisms that attach to the surface of a living substrate during their sessile phase, while basibionts act as hosts, providing support to the epibionts (Threlkeld et al., 1993; Wahl et al., 1997). The presence of epibionts on crustaceans, also known as commensals, symbionts, epizoic, and so on, has long been recorded, but this association has only recently been investigated from a new perspective (Fernandez-Leborans & von Rintelen 2007). This has not only resulted in the discovery of numerous newly described species but has also underscored the significance of epibiotic interactions across various biological disciplines, encompassing physiology, ecology, and evolution, as well as studies of biodiversity and conservation (Fernandez-Leborans et al., 2009; Fernandez-Leborans & Gabilondo, 2006; Fernandez-Leborans & von Rintelen, 2007; Utz, 2004; Williams & McDermott, 2004).

Several protozoan ciliate species have been identified as crustacean epibionts, and epibiosis can explain the ecology and biology of their basibiont crustacean groups (Abelló et al., 1990; Pere Abelló & Macpherson, 1992; Smit et al., 2019). Cladocerans, cirripeds, copepods, isopods, decapods, and amphipods are crustacean groups that have been discovered to be basibionts for invertebrate and protozoan epibionts (Ross, 1983). Ascidiacea, Bivalvia, Bryozoa, Cirripedia, Cnidaria, Decapoda, Gastropoda, Nematoda, Nemertea, Phoronida, Platyhelminthes, Polychaeta, Porifera, and Rotifera, among others, are examples of invertebrate epibionts. Protozoan epibionts are classified as apostomatids, chonotrichids, heterotrichs, peritrichs, and suctorians (Corliss, 1979; Lynn & Small 2000). The study of epibiont communities on basibionts in various lake systems could provide comparable data from different lakes, increase our understanding of evolutionary patterns, and provide additional details about the biodiversity and conservation of these unique environments. Even though the lake systems are unrelated, their diverse faunas benefit from similar ecological conditions (Fernandez-Leborans & von Rintelen, 2007).

The narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 (Decapoda: Astacidae), which is native to the regions between western Asia and eastern Europe, has been intentionally introduced to several countries, including Poland, Germany, England, Spain, Italy, and France (Harlioglu, 2004). These introduction efforts aimed to establish populations of *A. leptodactylus* in new environments, often for economic and ecological reasons. In some cases, it has become a valuable resource for aquaculture, fisheries, and even culinary purposes (Ackefors, 2000; Holdich et al., 2004; Souty-Grosset et al., 2006). However, such introductions can also have ecological implications, as non-native species can sometimes disrupt local ecosystems (Harlioğlu & Harlioğlu, 2006; Westman, 2002). The ability of *A. leptodactylus* to thrive in diverse geographical locations demonstrates its resilience and adaptability, making it a species of interest not only for economic purposes but also for scientific study and conservation efforts (Harlioglu, 2004).

Almost 90 years ago, Iranian fishermen from the local community initiated the capture of native crayfish from the Anzali Lagoon, a practice that involved selling these crustaceans to foreign nationals and diplomats (Karimpour et al., 2011). Subsequently, in 1985, a deliberate introduction of *A. leptodactylus* into suitable freshwater environments, with a particular focus on the Aras reservoir, marked a significant development. Over the years, this reservoir has evolved into a pivotal source of crayfish (Karimpour et al., 2011). Fast forward to 2005, and the Shiyan Dam reservoir, strategically situated in the western region of Iran within Kermanshah, was constructed with its primary function being the storage of water sourced from the Shiyan springs. Notably, in 2007, a deliberate release of *A. leptodactylus* was carried out in the reservoir as part of proactive environmental management initiatives. This strategic move aimed to enhance the ecological balance and sustainability of the reservoir's aquatic ecosystem (Heshmatzad et al., 2013).

This study represents a comprehensive examination of the epibiont communities associated with *A. leptodactylus* in the Shiyan Dam reservoir. Our primary objective was to provide valuable insights into the analysis and description of distribution patterns and relationships among the protozoan and metazoan epibiont communities that inhabit this endemic crayfish species, predominantly found within the Iranian Dam reservoir. Concurrently, it's important to emphasize the crucial role of pathological studies in our research. These studies are of great significance as they provide essential insights into the health status of introduced crayfish. Additionally, they allow for a comprehensive evaluation of the success of crayfish farming, with a particular emphasis on disease prevention. In addition to examining epibiont communities, we broaden our investigation to include the overall health of the freshwater crayfish community in western Iran. This holistic effort involves a thorough analysis of the epibiont communities associated with *A. leptodactylus*. Such an integrated approach provides us with a deeper understanding of the ecological and health dynamics within this aquatic ecosystem.

Materials and methods

Study area

From September 11, 2019, to August 6, 2020, this study investigated the epibiont communities of *A. leptodactylus* crayfish in the Shiyan Dam reservoir (34° 4'54.42"N; 46°41'38.86"E) in the Kermanshah province of western Iran (Fig. 1). The Dam sources its water supply from the Shiyan Springs, which have an average annual discharge of approximately 658 liters per second. Furthermore, the Shiyan Dam has a regulated output capacity of around 13.5 million cubic meters of water per year. Under normal conditions, the surface area of the mentioned reservoir is approximately 114 hectares, with a total reservoir volume of about 9 million cubic meters. The useful volume of the reservoir is approximately 8.78 million cubic meters. The Dam's crest has an elevation of about 1005 meters with a width of approximately 8 meters (Heshmatzad et al., 2013).

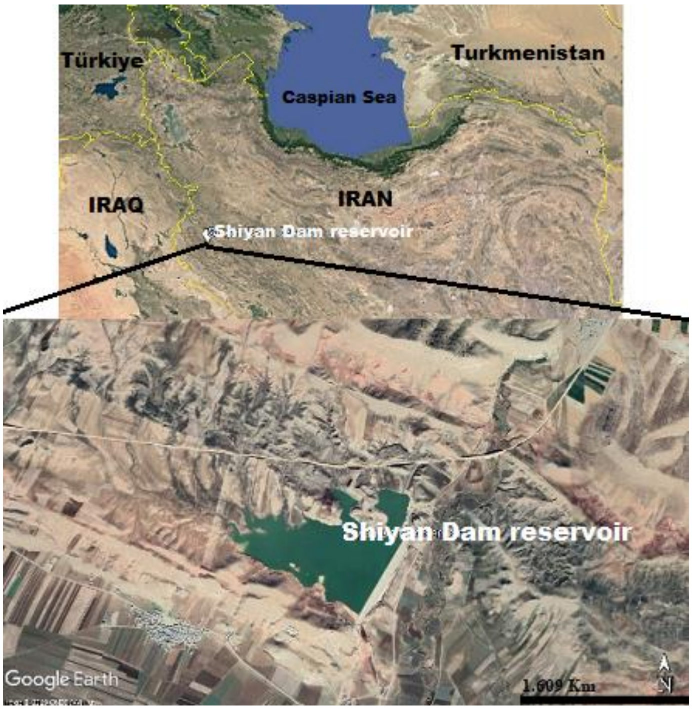

Figure 1. Study area. Shiyan Dam reservoir is situated in the Kermanshah province of western Iran.

Sampling

We collected monthly samples from various areas of the lake, particularly at the crest of the Dam, using a 15-mm mesh net (Fig. 2). We prepared an adequate amount of bait (dead fish) at the lake location and placed it in the nets before releasing 15 pairs of these nets at appropriate intervals onto the lake floor (Fig. 2). Trapping took place in the afternoon, and the collection occurred the following day before sunset. Using an icebox and sponge layers, we transported the live crayfish from the nets to aquarium containers in the laboratory. These containers were aerated by an air pump and maintained at a temperature of 22±3°C. We also recorded the physicochemical characteristics of the water, including pH, dissolved oxygen, water temperature, and air temperature, during the study. We used the Palintest photometer 7100 and the Pro1020 dissolved oxygen and pH meter for these measurements. After transferring the crayfish to the laboratory, we identified their gender and subsequently biometrically measured and weighed (total weight) them using a 0.1 mm digital caliper and a digital scale with an accuracy of 0.1 g. The carapace length was defined as the measurement from the tip of the rostrum to the posterior median edge of the cephalothorax (Sint et al., 2005).

In this study, gender determination in freshwater crayfish (*A. leptodactylus*) relied on external morphological features. Key characteristics for distinguishing between male and female crayfish include: (1) Complexity of first pereiopods: male crayfish displayed more intricate first pereiopods (primary walking legs) compared to females, primarily due to testes and sperm reservoirs. Structural variations in these pereiopods were a crucial distinguishing feature for males. (2) Size and shape of male gonopods: male crayfish had larger and more robust gonopods, specialized appendages located ventrally in the abdomen, used for sperm transfer to females. Male gonopods' size and shape beneath the abdomen were prominent for gender identification. (3) Shape and size of copulatory swimmerets: female crayfish had smaller and less prominent copulatory swimmerets, located in the abdominal region, used for carrying and transferring eggs to pleopods. Differences in swimmeret shape and size aided gender determination. Additional characteristics, such as rostrum shape and overall body size (sexual dimorphism), were examined to confirm gender. A combination of these features allowed for accurate gender identification (Malekzadeh-Viayeh & Tafi, 2017).

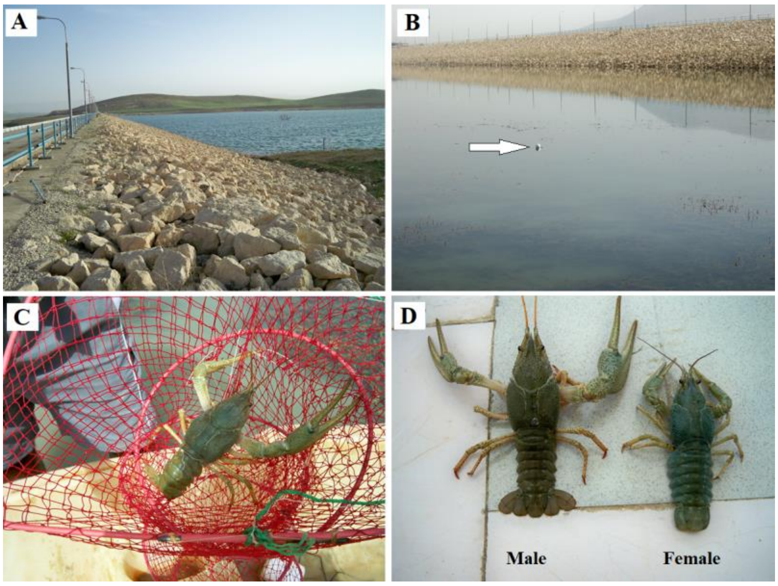

Figure 2. Shiyan Dam reservoir (A, B) with a white arrow (B) indicating the float of the mesh net used for trapping crayfish (*Astacus leptodactylus*) (C). Both male and female crayfish are shown (D).

Parasitological investigations

The hosts were dissected into antennules, antennae, chela, scales, mouthparts, carapace, pleopods, pereiopods, abdomen segments, uropods, telson, and gills. Live symbionts were detected under a light microscope using both phase contrast and light field tools. Small pieces of the exoskeleton, including epibionts, were then fixed in 5% formalin and stained following the methods described by Foissner, (1979), Lee et al., (1985), and Mayén-Estrada and Aladro-Lubel, (2001). Next, the carapace was opened to investigate the internal organs. All parts were examined with a magnification lens, and the digestive system (intestine) and hepatopancreas were placed in separate dishes containing physiological serum. They were then inspected with a loupe before being prepared as wet slides for examination under a light microscope at magnifications of 4×, 10×, 40×, and 100×.

After observing the parasites, the protozoa were exposed to a fixative for at least 15 minutes. Subsequently, they were washed in alcohol for a few minutes with an additional drop of iodine solution. Following the procedure outlined by Fernando (1972), both wet and dry smears were mounted in generally dehydrated Canada balsam. Gills require particular attention due to their sensitivity to epibionts and parasites, as well as their significant role in osmoregulation. A small section of this organ was excised for microscope evaluation at 10-40× magnification. Metazoan parasites were separated using the same methods employed for isolating external epibionts. The keys provided by Hoffman (1967), Matthes and Guhl (1973), Kudoo (1977), Alderman and Polglase (1988), and Hall (2001) were used for identifying epibionts and parasites.

Branchiobdellidans were extracted from each specimen, preserved in 70% ethanol, counted, and clarified using lactophenol. Staining of the specimens was achieved using borax carmine, and they were then affixed with glycerin jelly or Hoyer's fluid. An optical microscope was used to examine all of the branchiobdellidans, and specimens were identified based on their jaw, spermatheca, and spermathecal duct morphology (Gelder et al., 1994; Moszyński, 1938; Pop, 1965).

Results

Table 1 provides the physicochemical characteristics of water in the Shiyan Dam reservoir, including pH, dissolved oxygen, and water and air temperatures. The air temperature data show seasonal variation, with the highest values in the summer (June, July, and August) when temperatures reached 32°C, 42°C, and 42°C, respectively. In contrast, the lowest air temperature was recorded in February (3°C), which is in the winter season. Water temperature also displays seasonal trends, with warmer temperatures observed in the summer (June, July, and August), reaching their highest point at 25°C in August. The lowest water temperature was recorded in February (2°C), indicating colder conditions during the winter. Dissolved oxygen levels remained relatively stable over the sampling period, with values mostly ranging between 6.4 mg/L and 8.2 mg/L. The highest dissolved oxygen level (8.2 mg/L) was recorded in March. The pH values ranged from 7.2 to 8.5 over the sampling period, indicating variations in water chemistry. Notably, the pH was 7.2 in March and 8.5 in August (Table 1).

Table 1. Data on sampling dates and times, as well as physicochemical characteristics of water between September 2019 and August 2020.

Date	Time	Air temperature (°C)	Water temperature (°C)	Dissolved oxygen (mg/L)	pH
11 Sep	12	34	20	8	7.5
10 Oct	13:10	22	16.5	7.9	7.7
7 Nov	12:45	19	15	7.8	8.1
5 Dec	13:30	7	9	7.3	8.2
9 Jan	11:00	6	4	7.1	7.8
9 Feb	12:00	3	2	7	7.6
8 Mar	12:00	11	8	7.5	7.2
29 Mar	11:40	14	12.5	8.2	7.4
3 May	12:15	19	15	8.1	7.5
10 Jun	12:30	32	22	8	7.9
8 Jul	11:00	42	24	6.4	8.5
6 Aug	12:00	42	25	6.4	8.2

The mean carapace length and weight of *Astacus leptodactylus* (N = 220) were 87.92±30.77 mm and 143.60±21.52 g. The crayfish population was 69% male (N = 152) and 31% female (N = 68). Table 2 shows the number and percentage of crayfish and epibionts. The parasites and epibiont species found on *A. leptodactylus* are summarized in Table 3. *Cothurnai sieboldii*, *Chilodonella* sp., *Vorticella similis*, *Zoothaminum* sp., and *Pyxicolla* sp. were discovered as peritrichous ciliates (Fig. 3, Table 3). *Cothurnai sieboldii* was the most frequently observed organism (Table 3). The gill was the most common location for the establishment of these peritrichous ciliates. Except for ciliates, some species of epibiont and ectosymbiont were also identified, including *Branchiobdella kozarovi* (Annelida), *Philodina acuticornis* (Rotatoria), *Rabdochona* sp. (Nematoda), and *Mesocyclops strennus* (Copepoda) (Fig. 4; Table 3). *B. kozarovi* was discovered on the exoskeleton (N = 2) and, in particular, on the gill (N = 5). *Rabdochona* sp. and *Mesocyclops* collect in the gills. There was no parasite infestation observed in the intestines or hepatopancreas.

Table 2. The number of captured *Astacus leptodactylus* and the percentage of their parasite infestation in the Shiyan Dam reservoir in Kermanshah province, western Iran, from September to August.

	No. crayfish	No. parasite	Infestation percent	Ratio of infestation /220
Autumn	76	30	39.5	13.63
Winter	25	6	24	2.73
Spring	58	48	82	21.82
Summer	61	34	55.7	15.45

Table 3. Results of parasitological examination in the Shiyan Dam reservoir in Kermanshah province, western Iran, from September to August.

Species	Autumn	Winter	Spring	Summer	Frequency	Ratio of infestation /118	Ratio of infestation /220
Cothurnia sieboldii	5	1	7	5	18	15.25	8.18
Vorticella similis	1	0	3	2	6	5.08	2.7
Zoothamnium sp.	1	0	4	2	7	5.9	3.18
Chilodonella sp.	0	2	0	0	2	1.7	0.9
Pyxicola annulata	2	0	2	2	6	5.08	2.7
Protozoan frequency	9	3	16	11	39	33	17.73
Branchiobdella kozarovi	1	0	4	2	7	5.9	3.18
Philodina acuticornis	10	2	15	13	40	33.9	18.2
***Rabdochona* sp.**	8	1	10	7	26	22	11.8
Mesocyclops strennus	2	0	3	1	6	5.08	2.7
Metazoan frequency	21	3	32	23	79	67	35.9
Total	30	6	48	34	118	100	

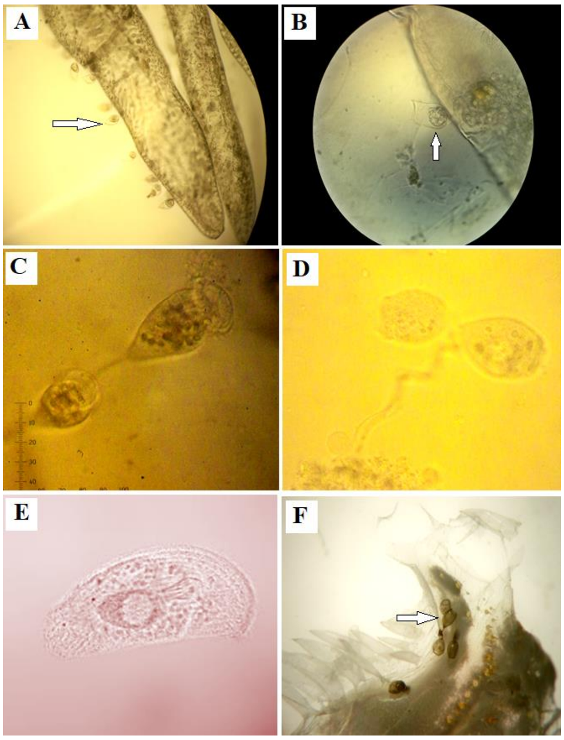

Figure 3. Protozoan species isolated from the gills of the freshwater crayfish *Astacus leptodactylus* in the Shiyan Dam reservoir in Kermanshah province, western Iran. *Cothurnia sieboldii* (A, 10×; B, 40×), *Vorticella similis* (C, 40×), *Zoothamnium* sp. (D, 40×), *Chilodonella* sp. (E, 100×), *Pyxicola annulata* (F, 40×). The white arrow indicates the identified epibionts on the gills.

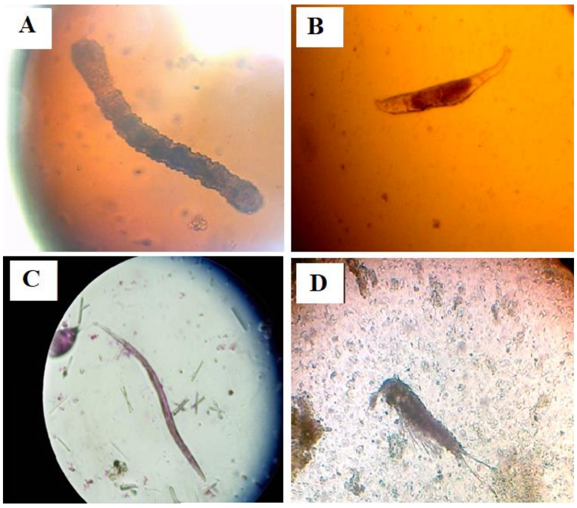

Figure 4. Metazoan species isolated from the gills of the freshwater crayfish *Astacus leptodactylus* in the Shiyan Dam reservoir in Kermanshah province, western Iran. *Branchiobdella kozarovi* (A, 10×), *Philodina acuticornis* (B, 40×), *Rabdochona* sp. (C, 10×), *Mesocyclops strennus* (D, 10×).

Discussion

Ciliates have significant ecological importance in free-living habitats, particularly in benthic regions where they exhibit high growth rates and trophic diversity (Fernandez-Leborans & Fernandez-Fernandez 2002). Epibionts and peritrichous protozoans on *A. leptodactylus* are common in freshwater crayfish fauna. The majority of them attach to crayfish exoskeletons and gills, primarily feeding on bacterial cells associated with eutrophic resources that increase in the summer and decrease in the winter (Nekuie Fard et al., 2015). Water quality significantly impacts infestation levels, and, in general, mortality occurs in aquaculture conditions characterized by poor water quality, dense host populations, and high temperatures (Morado & Small 1995).

In this study, a total of 220 specimens of *A. leptodactylus* were analyzed, of which 152 were male and 56 were female. The five protozoa (*C. sieboldii*, *V. similis*, *Zoothamnium* sp., *Chilodonella* sp., and *P. annulata*) and four metazoans (*B. kozarovi*, *P. acuticornis*, *M. strennus*, and a *Rabdochona* sp.) were the epibiont species discovered for this species in the Shiyan Dam reservoir in western Iran. These ciliate species belong to the order Sessilida and are associated with the genera *Cothurnia*, *Vorticella*, *Zoothamnum*, *Chilodonella*, and *Pyxicola*. They have previously been found as epibionts on *A. leptodactylus* in the Aras reservoir in northwest Iran (Nekuie Fard et al., 2011; Nekuie Fard et al., 2015; Asgharnia, 2017). Additionally, *Zoothamnium*, *Vorticella*, and *Cothurnia* have been identified as epibiont protozoans on crustaceans (Morado & Small, 1995; Fernandez-Leborans, 2001, 2003). Rotifers are well known as epibionts in close association with other organisms, but little is known about the types of relationships involved (Fernandez-Leborans & von Rintelen 2007). The rotifer species found in this study, *P. acuticornis*, has previously been documented on *A. leptodactylus* in the Aras reservoir (Nekuie Fard et al., 2011).

Quaglio et al., (2004) found an increased prevalence of *Epistylis* spp. in red swamp crayfish (*Procambarus clarkii*). In our study, *C. sieboldii* (8.18%) was the most frequently isolated epibiont protozoan, while *Chilodonella* sp. (0.9%) was the least frequently isolated. *C. sieboldii* had the highest frequency in spring and the lowest in winter. *Cothurnia* is covered by a lorica that surrounds the body and has a notably short stalk, allowing the ciliate to cling to the basibiont's surface. In Quaglio, (2006) study on the parasitic and fungal pathogens of freshwater crayfish, *Astropotmobius pallipses*, in northern Italian rivers, 82.9% of the samples collected were affected by *C. sieboldii*. In the investigation conducted by Nekuie Fard et al., (2015) on the parasitic and fungal diseases of *A. leptodactylus* in the Aras reservoir, 68.5% of the samples were infected with this parasite. Mortalities associated with *Cothurnia* in Italian crayfish (*Astacus fluviatilis*) were also reported by Ninni (1865).

Branchiobdellidans, commonly known as crayfish worms, are possibly the most well-known crayfish epibionts (Duris et al., 2006). Most branchiobdellidans species are epicommensals, but some are parasites (Grabda & Wierzbicka 1969). Some induce gill damage and melanization of gill filaments (Vogt, 1999). The gills of our sample contained 3.18% Annelida *B. kozarovi*. Duris et al. (2006) collected four Branchiobdella species in three crayfish specimens: *B. pentodonta* (52%), *B. balcanica* (24%), *B. parasita* (18%), and *B. kozarovi* (6%). In the Nekuie Fard et al. (2015) research on *A. leptodactylus*, the most common protozoan species was *Zoothamnium* sp. However, in our study, this species ranked second (3.18%) in terms of frequency. A comparison of our findings with the data of Fernandez-Leborans and von Rintelen (2007) on the shrimp *Caridina ensifera* reveals that three peritrich species, *Zoothamnium*, *Vorticella*, and *Cothurnia*, are similar. The percentage of freshwater nematode (*Rabdochona* sp.) infestation in the Shiyan reservoir was 11.8%, which was lower than the 38.8% reported by Nekuie Fard (2010) in the parasitic investigation into this species. Crayfish act as an intermediary host for nematode larvae of the genus *Rhabdochona*, which do not have parasitic properties and only have a commensal state or free-living form (Asgharnia, 2017). They are observed in the gills, and crayfish act as a vector for them.

P. acuticornis was found in 18.2% of the crayfish in the Shiyan reservoir, but in Nekuie Fard (2010), the proportion of infestation was 74.6%. *P. annulata* infected 2.7% of the samples in the Shiyan reservoir, but in Nekuie Fard et al. (2011 and 2015), this parasite affected 66% of the samples. In this study, 2.7% of crayfish showed symbiosis with *M. strennus* of copepods, which was mentioned in Nekuie Fard et al. (2011) studies. The abundance and diversity of epibiont communities in this study were significantly lower than in the previous survey by Nekuie Fard et al. (2015). This finding could be attributed to the reduced abundance of *A. leptodactylus* in the Shiyan Dam reservoir compared to the Aras reservoir, as well as the shorter period required for culturing in the Shiyan reservoir.

Crayfish mortality can be used to show a serious chemo-physical concern in a stream or lake. The effect of hazardous and toxic substances (e.g., pesticides, fertilizers) as well as industrial and farming contamination on narrow-clawed crayfish has not been adequately assessed and requires more study (Nekuie Fard et al., 2015). On the other hand, peritrichic ciliates may be dependent on nutrients derived from crustacean activity (Fernandez-Leborans & von Rintelen, 2007). Protozoa from lake ecosystems are important players in energy transfer and cycling in freshwater ecosystems and are regarded as a significant component in the limnic food web (Porter et al., 1985). The distribution and abundance of lower and higher species, as well as other components of the aquatic food web, may be greatly impacted by changes in the community structure of protozoa (Currick et al., 1992; Cairns & McCormick 1993).

Conclusion

This study investigates the ecological dynamics of epibiont and parasite communities associated with *A. leptodactylus* crayfish in the Shiyan Dam reservoir, Kermanshah province, Iran. Through a comprehensive analysis of the host's interactions with various microorganisms, including protozoa and metazoans, this research sheds light on the intricate ecological relationships in aquatic ecosystems. The study reveals variations in the prevalence and diversity of epibionts compared to previous research in other reservoirs, likely influenced by factors such as host population density and cultivation duration. Findings underscore the importance of considering environmental factors in crayfish health and ecosystem conservation. Recommendations for future research include assessing the impact of pollutants and contaminants on crayfish mortality and exploring the role of microorganisms in aquatic food webs. This study contributes valuable insights into the ecological dynamics of aquatic environments and highlights the need for robust environmental management practices in the region.

Acknowledgments

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