Sever occurrence of *Branchiobdella kozarovi* Subchev, 1978 in cultured freshwater crayfish

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Abstract

Freshwater crayfish of Aras dam, is one of the most important economic aquatic animals of Iran. Three specimens of *Astacus leptodactylus* maintained in a static culture condition in a concrete pool were examined for the occurrence of *Branchiobdella* sp. We reported for the first time the severe intensity of *Branchiobdella kozarovi* (mean=1359.3±43.6) isolated from the gills and exoskeleton of *A. leptodactylus* maintained in static culturen. All heavily infected specimens died. A culture system with non-circulating water is not suitable for freshwater crayfish maintenance.

Keywords: *Astacus leptodactylus*, *Branchiobdella kozarovi*, non-circulating water, culture system.

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Introduction

Astacus leptodactylus (Eschscholtz, 1829) is one of the most economic and luxury species in the world that captured from Aras Dam, Iran and exported to Europe and neighboring countries for many years as an expensive and delicious meal. Regarding the increase of its culture in American and European countries, its health status and diseases is very important. But different aspects of parasitic and opportunistic infestation of this species have scarely studied. Due to complexity of opportunistic and pathogens in natural and cultural environments and their interaction with survival, growth and reproduction of aquatic animals and human health hazards, freshwater crayfish is under threat of various infestations and diseases. Therefore, identification of harmful organisms is of special importance (Yahyazadeh, 2016).

Three families of freshwater crayfish have been recognized in the world including Cambaridae from east America, Carraieb, East Asia and Japan, Astacidae from Europe, West United States and Parastacidae from South hemisphere, Australia, News land, Chili , South equator (Holdich, 2002). European freshwater crayfish have been divided into 2 genera of *Astacus* (consisting of *A. pachypus*, *A.leptodactylus* and *A. astacus)* and *Austropotamobius* (consisting of *A. pallipes* and *A. torrentium*) (Reynolds and Souty-Grosset, 2003). Iranian freshwater crayfish (*A. leptodactylus*) is a burrowing, dark and shade loving animal. Habitat conditions severely affect its growth, occurrence and mortality. Habitats with constant current and high oxygen levels provides a lower mortality and higher mean total length of captured crayfish. Freshwater crayfish lives in fresh and brackish water and the best water salinity for this species is 4-14 g/L. younger populations are more susceptible to salinities less than 4 g/l, but salinity fluctuations does not affect the mortality and growth of adult population (Mohsenpour Azary, 2016).

Nearly 90 years ago, Iranian fishermen started the capture of crayfish from Anzali Lagoon and sold them to foreign countries. Subsequent introduction of *A. leptodactylus* into favorable environments in 1985, with special focus on the Aras reservoir, caused this reservoir as the main source of crayfish (Karimpour *et al*., 2011; Heshmatzad and Vaissi, 2024). Therefore, the main economic distribution of freshwater crayfish in Iran is Aras dam lake and Anzali lagoon. But the only source of its catch and export is the Aras Reservoir Dam, which has been caught and exported to European countries since 1997. Freshwater crayfish of Aras dam reservoir has been introduced to some water resources in 13 provinces of Iran include East and West Azarbaijan, Ardabil, Zanjan, Lorestan, Fars, Kohkiloye and BoyerAhmad, Markazi, Esfahan, Elam, Khorasan, Golestan and Kerman (Matinfar, 2016; Mohsenpour Azary, 2016).

Damages caused by diseases can affect freshwater crayfish health status. Freshwater crayfish can be the host of a

wide ranges of different commensals, parasites and living pathogens include viruses, fungi, bacteria, Rickettsia-like organisms, branchiobdellids, protozoa and metazoa. Also, the most virulent pathogen is *Aphanomyces astasi*. Parasitic infestations in crayfish are diverse due to their geographical distribution and habitat and can cause infections and in some cases epidemic diseases. Freshwater crayfish as a symbiont can host many invertebrate parasites (Evans and Edgerton, 2002; Yahyazadeh *et al*., 2017). Anthropogenic displacement of freshwater crayfish with the purposes of aquaculture or aquarium trade has led to displacement of disease outbreaks and reduction in native crayfish populations (Longshaw, 2016).

Branchiobdella sp. are ectocommensals or ectosymbiont cilitellate small leech like annelid worms inhibit either gill chambers or external surface of crayfish distributed in East Asia, all parts of Europe (especially Norway, Ireland, Spain, Lithuania, Italy, Bulgaria and Slovakia, Croatia, and parts of Bosnia and Herzegovina), North and Central America (Nekuie Fard and Gilder, 2011; Subchev, 2014; Szenejko *et al*., 2023; Subchev *et al*., 2024). However, *B. kozarovi* reaches Asian Turkey and Iran in South-East and Georgia and Kazakhstan in North-East (Subchev, 2014). Their life cycle is little known and spend most of their life on the surface of crayfish body. They show less host specificity and for this reason they are able to live on the surface of freshwater crayfish species. Astacoidae are their common host and their pathogenicity is not very important in crayfish. They are epicommensal and sessile to body surface. Also, They are hermaphroditic and lay coccons on surface and carapace of crayfish host. Although by being in large numbers in the gills, caused damage due to feeding or movement and provides the ground for the accumulation of microbial and fungal agents. There were no confirmed reports of mortality due to invasion or parasite infection in freshwater crayfish (Yahyazadeh, 2016). In general, branchiobdellids may be pathogenic or may busy with cleaning symbiosis of their hosts to improve growth rates (Alderman and Polglase 1988; Lee *et al*., 2009; Longshaw, 2016). Gill injuries have been observed in crayfish infested by *B. astaci* but such damages cannot be a reason for these species pathogenicity as they could be due to bacterial or fungal infections (Quaglio *et al*., 2006; Rosewarne *et al*., 2012; Subchev *et al*., 2020). This study was conducted to report high density occurrence of *B. kozarovi* resulting mortality in *A. leptodactylus* in static culture condition.

Materials and methods

A.leptodactylus from Aras Dam Reservoir, Iran were captured in winter with funnel traps and transferred to a concrete pool (1 m height) and aerated with air pump in a cascade manner and maintained for 2 months in static water condition feeding with *Abramis brama* pieces kept in the refrigerator.

Specimens of *A.leptodactylus* examined for *B. kozarovi*. *Branchiobdella* species were isolated from gills, carapace and buccal part of crayfish by washing with physiological serum on a Petri dish using tweezers and counted, separately.

Results

Accidental laboratory studies of parasites from cultured freshwater crayfish in concrete pool, reveals sever occurrence of *Branchiobdella kozarovi* (Table 1 and Fig. 1).

Table1-Biometrical characteristics and the number of *B. kozarovi* Isolated from freshwater crayfish samples.

Gender	Weight (g)	Total Length (mm)	Number of isolated *B.kozarovi*
Female	19.17	87.43	1368
Male	25.15	98.77	1312
Female	20.41	91.83	1398

The Mean(±SD) number of isolated *B.kozarovi* Was 1359.3±43.6 individuals.

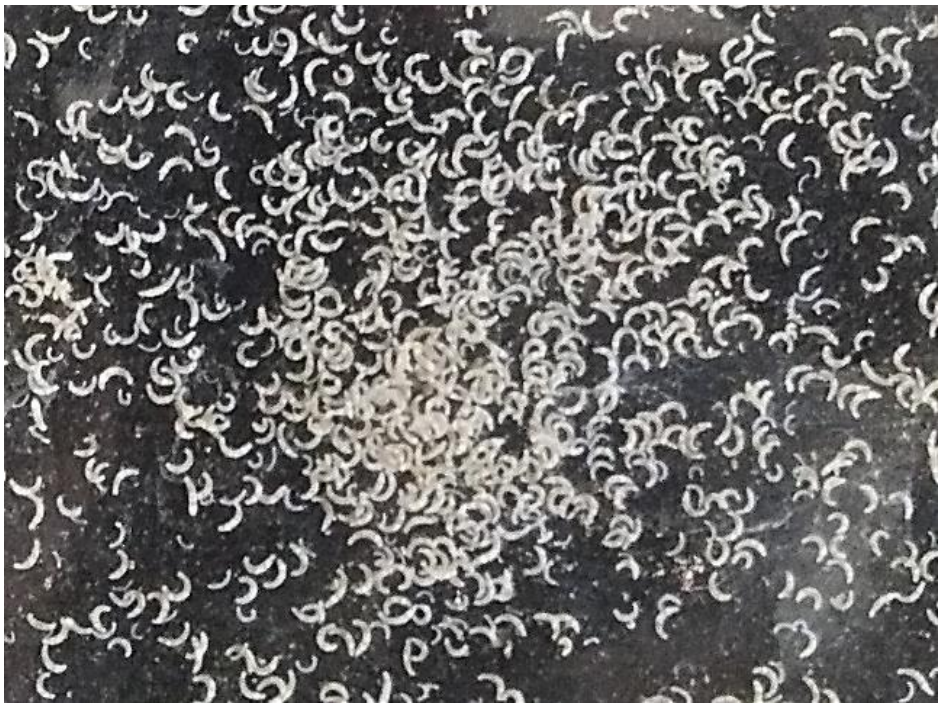

Figure 1: High occurance of *Branchiobdella kozarovi* isolated from the gills and exoskeleton (present study).

We reported for the first time the sever intensity of *B. kozarovi* (mean=1359 individuals) isolated from the gills and exoskeleton of *A. leptodactylus* maintained in static culture condition. All heavily infected specimens died.

Discussion

Mohsenpour Azari *et al*.(2015) revealed that the number of eggs of Aras dam freshwater crayfish were changing from 200 to 400 eggs depending on spawner size, environmental condition and available food. Besides, crayfish with higher lengths and weights had increased fecundity. They suggested a ban on fishing during the reproduction season, use of appropriate nets and proper management to preserve the stocks of this species. Mohsenpour Azari *et al*. (2014) revealed that among captured crayfish from Aras dam, only 13.67% and 19.9% exceeded than the standard commercial size of 120mm and 50 g, respectively. Also, male crayfish slightly dominated. They concluded that *A. leptodactylus* by decreasing catch value and size had a critical condition in Aras dam and their conservation and improving stocking density is crucial. Crustaceans can host parasitic agents including pathogenic, opportunistic, commensal and symbiotics. Invasion by opportunistic and epicommensal parasites including *Branchibodella* sp. has been reported from freshwater crayfish (Nekuiefard *et al*., 2011; Yahyazadeh *et al*., 2017).

In this study the intense prevalence of *B. kozarovi* (average=1359, n=3) as a parasitic agent isolated from gills, body surface and carapace of Aras dam freshwater crayfish maintained in static water condition was reported that caused mortality. It has been found that the establishment of the diversity and number of opportunistic parasitic agents is more in the anterior region of the body than in the posterior region and in larger crayfish more than in smaller ones (Yahyazadeh *et al*., 2017). Nekouiefard *et al*. (2011) and Nekouiefard (2010) reported maximum intensity of contamination of freshwater crayfish harvested from Aras dam with *B. kozarovi* with mean total length of 1.35mm and mean width of 0.32 in spring and summer as 100 percent (Fig. 2). They were isolated on the exoskeleton, walking leg, antenna, antennules, base of eyes and gills of Aras dam crayfish. Previously, *Branchiobdella* species were considered as crayfish external parasites but nowadays they are considered as crayfish ectobionts. Habitat characteristics, mobility and host crayfish species can influence diversification rate of *Branchiobdella* species (Szenejko *et al*., 2023).

The Aras dam reservoir is a main source of capture and release of *A.leptodactylus* to other water resources. protozoans attached to the exoskeleton and gills of fresh water crayfish are common whereas, water quality has a key role on infestation levels and disease outbreak of crayfish (Nekoiefard *et al*., 2015). Heshmatzad and Vaissi (2024) studied protozoan and metazoan epibiont communities of *A. leptodactylus* in the Shiyan Dam

reservoir of Kermanshah province, Iran with the highest (82%) and the lowest (24%) infestation in spring and winter, respectively. They noted that the epibiont prevalence and diversity can be affected by multiple factors such as host population density, environmental conditions and cultivation duration. Also, environmental factors can influence crayfish health and ecosystem conservation state (Heshmatzad and Vaissi, 2024). Epibionts and peritrich protozoans are common in freshwater crayfish fauna.

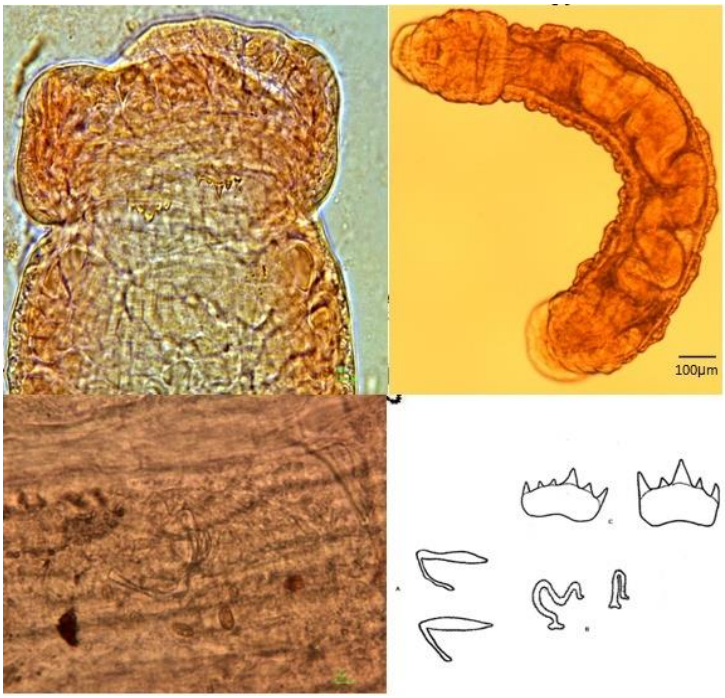

Figure 2: *Branchiobdella kozarovi* isolated from the gills and exoskeleton of freshwater crayfish in Aras dam reservoir (Nekouiefard, 2010)

Most of them attach to crayfish exoskeletons and gills, feeding on bacterial cells associated with eutrophic resources that dominate in the summer and decline in the winter (Nekuie Fard *et al*., 2015).

B. astaci is pathogenic by sucking blood from gills and causing abscesses on attaching places and destroy gills by mass occurrence. Gill damages were observed in crayfish infested by *B. astaci* with mortality when were kept in artificial condition or during transportation with sever lesions in the gills of dead crayfish (Mažylis and Grigelis, 1979; Quaglio *et al*., 2006). Also, higher melanization of gill filaments was observed in crayfish infested by *B. astaci* (Rosewarne *et al*., 2012). Subchev *et al*. (2020) considered 3 phases of gill filaments damages by *B. astaci* including sporadic attacks causing melanized spots on injured gill filaments, several worm punches causing plugging up the filament at a certain place and decaying and drops off its lateral part. They concluded that *B. astaci* do feed on gill filaments and is

pathogenic to its host and other multiple factors which initiate/stimulate or oppress the feeding of this species on host crayfish gills could also be involved (Subchev *et al*., 2020).

There is evidence of damage and melanization of the gills due to the establishment of *Branchiobdella* species in the gills and feeding on the tissues, especially in cases of severe contamination. On the other hand, there are observations that they are symbiotic about cleaning and destroying sediment organisms on the body surface of crayfish and reducing mortality. However, the effect of *Branchiobdella* in crayfish has not been definitively stated. The interactions between opportunistic or epicommensal parasites with the host seem to depend on environmental conditions, host immune defenses, and the extent of invasion. Considering the presence of parasitic agents in aquatic environments and the body surface of crayfish, the impact of man-made factors can cause this interaction to be disturbed and due to the unfavorable environmental conditions and the weakening of crayfish, cause diseases and casualties (Alderman and Polglase,1988**;** Vogt, 1999; Brown *et al*., 2002; Lee *et al*., 2009; Yahyazadeh, 2016).

We can conclude that closed condition with static culture condition is not suitable for freshwater crayfish maintenance as most of parasites can not be washed and removed from the host skin with water flow. Therefore, the culture medium water should have a gentle flow. Because freshwater crayfish do not move much therefore, the parasites that exist in the environment around them stick to the surface of their bodies and get implanted. The number of *B. kozarovi* isolated was more in females than in males. Also, male crayfish weighed more than females. The presence of more parasites in females can be related to egg-laying, more sensitivity to stress and less movement of females than males.

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