Commensalism and parasitic infestation in crayfish (*Astacus leptodactylus* Eschscholtz, 1823) of Aras Dam Reservoir, Iran

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Abstract

The freshwater crayfish of the Aras Reservoir is an important economic fisheries resource of West Azarbaijan, Iran. This study was conducted to evaluate the prevalence of parasitic infestation of Crayfish seasonally in this area in 2010. Among 390 different sizes of *Astacus leptodactylus* which were examined, a range of ectocommensals or ectosymbionts from a number of different phyla including 9 orders and 11 classes infested the different anatomic units of the surface and appendages such as gills, head, thorax, abdomen, walking legs, uropod, telson, antennae and antennulae of freshwater crayfish. Common groups such as peritrich ciliates, suctorian ciliates, free living nematodes, branchiobdellids, and algae, copepods, rotifers and oligochaetes have also been observed in association with freshwater crayfish.

Keywords: *Astacus leptodactylus*, Parasitic and commensal infestation, Aras Dam, West Azarbaijan, Iran.

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Introduction

Astacus leptodactylus of Aras Reservoir is considered as one of the important economic aquatic animal resources of Iran. It provides a luxurious and delicious but expensive meal in most countries. Regarding the increase of resource management and culture of freshwater crayfish in the USA and European countries, its economic values, health and disease status has become of great important recently. Though it has received worldwide attention, the health condition and pathogen agents of this valuable species especially from parasitic infestation have not been studied completely. The complexity, variety and abundance of opportunistic and pathogen factors in natural and culture environments of aquatic animals and their impact on survival, growth, reproduction of aquatic animals and human being health hazards are among restricting and hazardous factors including freshwater crayfish. Parasitic infestations of freshwater crayfish are varied all over the world regarding the geographical distribution, natural habitats and parasitic sites. They can cause infestations and even epidemic diseases depending on bioenvironmental conditions. Crayfish has an important role as a co existent among most invertebrates and may be a host for a variety of parasites (Lodge and Hill, 1994; Edgerton *et al*., 2002; Evans and Edgerton, 2002). Parasitic infestation and diseases of fresh water crayfish are divided into protozoa, metazoans and epicommensal organisms. This study was performed to determine and identify the parasitic organisms in *A. leptodactylus* of Aras Dam.

Materials and methods

390 samples of *A.leptodactylus* of various lengths and weights were captured randomly with conical traps in different seasons of 2010. All samples were transferred live to the laboratory of Iranian Artemia Research Center, Urmia in polyurethane boxes containing ice. In the laboratory, the samples were weighted, measured and examined. In order to determine protozoan and metazoan infestations, external parts of all samples were examined and wet mounts from carapace, branch, eye, intestine and hepatopancreas were prepared and examined by light and stereomicroscope. All collected parasitic agents were isolated, fixed, stained and classified using Moszynsky, 1938; Bykhovskyaya-Pavlovskaya *et al*., 1964; Alderman and Polglase, 1988; Hall, 2001. Then some external parts such as branch, telson and carapace were placed separately in petri dishes, NaCl solution was added to them and they were examined for external metazoan and parasites of internal organs using a stereomicroscope.

Results

Some biometrical characters of sampled *A. leptodactylus* are shown in Table 1.

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Table 1: Some biometrical characters of sampled *Astacus leptodactylus.*

A. leptodactylus		Weight (g)			Length (mm)		
Sex	**Number**	**Maximum**	**Minimum**	**Mean**	**Maximum**	**Minimum**	**Mean**
M	232	118	4	31.39	155	60	82.9
F	157	78	7	28.97	150	75	36.10

In this study 9 phyla and 11 classes consisting 40 species of various opportunistic and epicommensal protozoa and metazoans were isolated from some parts of crayfish body. These included various ciliate (Prêtrich, Loricate), free living Nematodes, Annelids, Copepoda, Rotatoria, etc. the list of isolated parasites and commensal organisms' species were summarized and illustrated in Tables 2-4. In this study, parasitic infestation of internal organs was not observed.

Most of these organisms live as epibiont on cephalothoraxes, branch, pereiopods, antennules, rostrum and eyes of *A. leptodactylus*. Ciliophora and Arthropoda, Prorodontidae, Adenophorea, Naviculaceae, Gastrotricha, Tubulinea had the highest and lowest abundance, respectively.

Table2: Isolated parasites and species of commensal organisms from the Aras Dam crayfish.

Kingdom	Phylum	Class	Family	Genus/ Species
Animalia	*Rotifera*	*Monogonta*	*lepadelilidae*	*Lepadella patella*
"	"	*Monogonta*	*Trichocercidae*	*Trichocerca cylindrical*
"	"	*Monogonta*	*Notommatidae*	*Monommata grandis*
"	"	*Monogonta*	*Brachionidae*	*Brachionus plicatilis*
"	"	*Bdelloidea*	*Philodinidae*	*Philodina roseola*
"	"	*Monogonta*	*Epiphanidae*	*Epiphanes senta*
"	*Annelida*	*Clitellata*	*Branchiobdellidae*	*Branchiobdella sp.*
"	"	*Oligochaeta*	*Naididae*	*Nais sp.*
"	*Gastrotricha*	*Gastrotriches*	*Chaetonotidae*	*Chaetonotus sp.*
"	*Arthropoda*	*Maxillopoda*	*Cyclopidae*	*Mesocyclops sp*
"	*Nematoda*	*Chromadorea*	-	*Nematod sp.*
protozoa	*Ciliophora*	*Ciliatea*	*Parameciidae*	*Paramecium sp.*
"	"	*Ciliatea*	*Vaginicolidae*	*Pyxicola sp.*
"	"	*Ciliatea*	*Halteriidae*	*Halteria sp.*
"	"	*Ciliatea*	*Cyclidiidae*	*Cyclidium sp.*
"	"	*Ciliatea*	*Urostylidae*	*Urostyla sp.*
"	"	*Ciliatea*	*Amphileptidae*	*Amphileptus sp.*
"	"	*Ciliatea*	*Dendrosomatidae*	*Tokophrya sp.*
"	"	*Ciliatea*	*Chilododontidae*	*Chilodonella sp.*
"	"	*Ciliatea*	*Vorticellidae*	*Vorticella sp*

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Table 2 (continued):

Kingdom	Phylum	Class	Family	Genus/ Species
"	"	*Ciliatea*	*Vorticellidae*	*Zoothamnium sp..*
"	"	*Ciliatea*	*Strombidiidae*	*Strombidium sp.*
"	"	*Ciliatea*	*Tetrahymenidae*	*Tetrahymena sp.*
"	"	*Ciliatea*	*Acinetidae*	*Acineta sp.*
"	"	*Ciliatea*	*Colepidae*	*Coleps sp.*
"	"	*Ciliatea*	*Operculariidae*	*Opercularia sp.*
"	"	*Ciliatea*	*Didiniidae*	*Didinium sp.*
"	"	*Ciliatea*	*Oxytrichidae*	*Stylonychia sp.*
"	"	*Ciliatea*	*Tracheliidae*	*Trachelius sp.*
"	"	*Ciliatea*	*Tracheliidae*	*Dileptus sp.*
"	"	*Ciliatea*	*Euplotidae*	*Euplotes sp.*
"	"	*ciliatea*	*Podophryidae*	*Paracineta sp.*
"	"	*Ciliatea*	*Podophryidae*	*Podophrya sp.*
"	"	*Cciliata*	*Prorodontidae*	*Prorodon sp*
"	"	*Ciliatea*	*Uronematidae*	*Uronema sp*
"	*Protozoa*	*Heliozoa*	*Actinophyridae*	*Actinophrys sp.*
"	"	*Lobosa*	*Amoebidae*	*Amoebae sp.*
Chromista	*Heterokonta*	*Bacillariophyceae*	*Naviculaceae*	*Navicula sp.*
Chromista	"	*Bacillariophyceae*	*Cymbellaceae*	*Cymbella sp.*
plantae	*Chlorophyta*	*Chlorophyceae*	*Chlamydomonadaceae*	*Chlamydomonas sp.*

Table 3: Distribution of parasitic and commensal organism infestation on different parts of *Astacus leptodactylus* captured from Aras Dam.

Parasitic agents	Skin	Abdomen	Branch and cephalothorax
Lepadella patella	+	+	+
Trichocerca cylindrica	-	+	+
Monommata grandis	-	+	+
Philodina roseola	-	+	+
Brachionus plicatilis	-	+	+
Epiphanes	-	+	+
Paramecium	+	+	+
Pyxicola	-	+	+
Halteria	-	-	+
Cyclidium	-	+	+
Urostyla	-	+	+
Amphileptus	-	+	+
Tokophrya	-	+	+
Chiliodenella	-	+	+
Vorticella	-	+	+
Strombidium	-	+	+
Tetrahymena	-	+	+
Acineta	-	-	+
Coleps	-	+	+
Didinium	-	+	+
Stylonchia	+	+	+

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Table 3 (continued) :

Parasitic agents	Skin	Abdomen	Branch and cephalothorax
Trachelius	-	+	+
Dileptus	-	+	+
Euplotes	-	+	+
Chlamydomonas	+	-	+
Paracineta	-	-	+
Podophrya	-	-	+
Uronema sp	-	+	+
Zoothamnium	-	+	+
Navicula	-	+	+
Cymbella	-	-	+
Nais	-	+	+
Branchiobdella	+	-	+
Chaetonotus	-	-	+
Amoebae	-	-	+
Prorodo sp	-	+	+
Opercolaria	-	+	+
Mesocyclops	-	-	+
Actinophrys	-	-	+

Table 4: The presence of parasitic agents on *Astacus leptodactylus* during different seasons.

Parasitic factors	Winter	Autumn	Summer	Spring
Lepadella patella	*	*	*	*
Trichocerca cylindrica	*	-	-	*
Monommata grandis	*	-	-	-
Philodina roseola	*	*	-	*
Coleps	*	*	*	*
Paramecium caudatum	*	*	*	*
Pyxicola	*	*	*	*
Cyclidiidium	*	*	*	*
Urostyla	*	*	-	*
Amphileptus	*	*	*	*
Tokophrya	*	*	*	*
Ciliodenella	*	*	*	*
Vorticella	*	*	*	*
Stombidium	*	-	-	-
Opercularia sp	*	*	-	*
Tetrahymena Pyriformis	*	*	*	*
Mesocyclops	-	-	-	*
Prorodon sp	*	*	-	*
chlamydomoras	*	-	-	-
Uronema sp	*	*	*	*
Navicula	-	*	*	*
cymbella	-	*	*	*
Acineta	*	*	-	*
Amoebae Protcus	*	*	*	*
Didinium	*	*	*	*
Branchiobdella	-	-	-	*
Nais	*	*	-	-

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Table 4 (continued):

Parasitic factors	Winter	Autumn	Summer	Spring
branchiobdella	-	*	*	-
Chaetonotus	-	*	*	*
mesocyclops	-	*	*	*
Actinophrys	-	*	*	*
Stylonchia	-	*	*	*
Brachionus plicatilis	-	*	*	-
Epiphanes	-	*	*	-
Halteria	-	*	*	*
Trachelius	-	*	*	-
Dileptus	-	*	*	*
Euplotes	*	*	*	*
Paracineta	-	*	*	*
Zoothamnium	*	*	*	*

Discussion

Most of the epibionts were distributed on maxillipods, mandible, maxilla, branch, pereiopods, Antennules, rostrum, eyes, uropods and pleopods and telson.

Different species of crustaceans can occur as symbionts, commensal and pathogenic parasites. Invading of opportunistic and commensal parasites such as ciliates, free living nematodes, *Branchiobdla, Rotifer, Copepods* have been reported on different crustaceans (Leborans and Tato-Porto , 2000a, b; Mayen - Estrada and Aladro - Lubel , 2001a; Edgerton *et al*., 2002; Duris *et al*., 2006; Quaglio *et al*., 2006a; Fernandez –Leborans, 2009; Abedian Amiri et al.,2008) . Different parasitic agents isolated from various parts of *A. leptodactylus* including sessile and suctorian ciliates, free living nematodes, annelids, *Branchiodenella*, copepod and rotatoria. The distribution of isolated species varied depending on parasite type so that some of them were restricted to branch carapace or distributed all over the body. Also, their variety and abundance in larger animals and on bucal area and branch was higher than smaller ones and upon uropods and telson which might be distributed on larger body surfaces for organism attachment and lower molting compared to younger ones (Fernandez–Leborans, 2001, 2009). 15 species of peritrich ciliates have been isolated from different parts of *Capmburcnus patzcuarensis* of the Michigan Lake (Mayen-Estrada *et al*., 2001b).

Distribution and abundance of parasites is observed more frequently in proximal parts rather than distal parts due to nutritional behavior and morphology of appendices of both crayfish and organisms. The infestation outbreaks of ciliates in fresh water crayfish were varied probably due to water quality, environmental change, molting and various species of crayfish (Edgerton *et al.*, 2002). Although histopathologically, a great deal of ciliates isolated from branch and external surfaces of freshwater crayfish in this study were opportunistic and epicommensal similar to observation of

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Brown *et al.* (1993); Morado and Small (1995); Harlioglu (1999); Edgerton *et al*. (2002); Huseyin and Selcuk, (2005); Quaglio *et al.* (2006b); Fernandez–Leborans (2009), some of them may have a negative impact on the host and if they invade branch they can cause respiratory problems, hypoxia and susceptibility to microorganisms and mortality due to environmental factors especially in culture conditions with poor water quality, increased water temperature and overcrowding. Mortality has been reported in penaeid shrimps under poor culture conditions due to severe invading of peritrish ciliates (Shields and Overstreet, 2003) and *Cherax tenuimanus* (Villarreal and Hutchings, 1986; Brown *et al*., 1993). Also, systemic infection of redclaw crayfish by *Tetrahymna peryformis* was reported from north Queensland and Australia (Edgerton *et al*., 1996). Ciliated protozoa are the most common and abundant parasites in different crustaceans. Most of them considered as epibionts in fresh water crayfish the heavy infestation of which can affect growth, molting, larvae survival, other organ functions such as that of the eyes, branch, and appendices, and restrict locomotion and cause occlusion of female genital pores, decrease fecundity, feed intake competition and mortality specially in culture environments (Mayen-Estrada and Aladro- Lubel, 2001a; Fernandez–Leborans, 2004; Fernandez– Leborans *et al*., 2006; Fernandez–Leborans, 2009). Suctorian ciliates such as *Acineta, Tokophrya, Opercolaria, Coterins*, isolated from the Aras Dam *A. leptodactylus* had no contractile stalk, sucker organ **(**antennules) or pseudo chitin cover and were observed on branch, and the external surface of body as epicommensal organisms. Their transmission is via the aquatic environment and they attach to the body surface of motile larvae and grow up to adult stage and feed on free living ciliated protozoa. In sever infestations they can cause hypoxia on branch (Edgerton *et al.*, 2002). In this study heavy infestation of branch from *Cothurina* spp. was observed. The metazoan parasites including free living nematode, *Branchiobdella* Annelids, and Oligocheta that were isolated in this study were reported as epicommensal or cymbiont which did not cause important diseases in freshwater crayfish (Alderman and Polglase, 1988; Edgerton *et al*., 2002). Isolated nematodes belonging to free living nematodes were observed on external body surfaces, the bucal area and branch of *A. leptodactylus* samples. There may be a close relationship between the nematodes and the host as they need the crayfish to complete their life cycle (Edgerton *et al*., 2002). Isolated Branchiobodella species were leech like small nematodes from *Analidae* with a segmented body and ventral and dorsal sucker to attach to

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the host. These worms were mentioned as Ectocommensal and Ectosymbiont that settled on branch and body surfaces especially carapace, and bucal area but some of them were external facultative parasites in freshwater crayfish and crustaceans (Alderman and Polglase, 1988; Gelder *et al*., 1999). *Branchiobodella* were the most common organisms existing on the freshwater crayfish that included several genera. It was estimated that up to now, nearly 150 species were identified from 21 genera the highest numbers of which belonged to *Cambricola* and *Branchiobodella*. The distribution of infestation to these organisms in freshwater crayfish in the north hemisphere has been reported from the north and central parts of America, Europe, Asia (Alderman and Polglase, 1988), cultured crayfish of Iran (Asgharnia, 2008) whereas no infestation has been reported from the southern hemisphere (Edgerton *et al.*, 2002). Also, in this study higher infestation from *Branchiobodella* species was observed on larger *A. leptodactylus* from the Aras Dam than smaller sized ones , especially on the maxillopods and head which could be related to larger body size to parasite colonization, nutritional richness, and lower molting (Cenni *et al.*, 2002; Mori *et al.*, 2002). Also, characterization of their colonization area could be seasonal and related to the presence of *Branchiobodella* species (Edgerton *et al.*, 2002). The life cycle of *Branchiobodella* is not exactly known. They grew up and developed by laying eggs in cocoons attached to the external surface of the host. It is believed that transmission was through direct contact (Thune, 1994; Edgerton *et al*., 2002). Also, *Branchiobodella* species have been observed independent and hostless but it is believed that they cannot continue to live without a host and reproduce only when attached to a live crayfish host. However they have been reported on the body surface of isopods and crabs (Brown *et al*., 2002; Edgerton *et al*., 2002; Evans and Edgerton, 2002).

There is evidence of branch injury due to accumulation of *B. hexodenta* and *B. actasi* and feeding from tissue specially in sever infestations (Alderman and Polglase, 1988; Vogt, 1999). On the other hand, there are some reports on their positive symbiotic effects on cleaning and eradication of organisms existing on body surface of crayfish, increasing growth and decreasing mortality (Keller, 1992; Brown *et al*., 2002; Lee *et al*., 2009).

The Aras Dam is the only natural and economic harvesting resource for *A. leptodactylus* in Iran which enjoys a good stocking capacity. The flowing of domestic, industrial and agricultural sewages into this reservoir can affect the water quality and infection of aquatic animals.Therefore, preservation, conservation, monitoring and management of this vulnerable and economic resource are very important. It seems that interaction among opportunistic or commensally parasitic

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agents with their host (crayfish) depends on environmental conditions, immune defense of the host and invasive rate of agents (interaction among host, parasite and environment). Existence of parasitic agents and effects of anthropogenic factors such as overfishing, industrial, domestic and agricultural pollution on aquatic ecosystems and euthrophic condition of the Aras Dam (Mohsenpour Azari, 2010) could change this interaction and due to adverse environmental conditions, weakening of crayfish immunity acts as a predisposing factor for viral, bacterial and fungal diseases of crayfish and other aquatic resources of the Aras Dam Reservoir. Therefore, due to economic and bio-ecological importance of *A. leptodactylus* and less information about its health status, continuous monitoring and good management of the Aras Dam is needed.

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