Iranian Journal of Fisheries Sciences 14(2) 308-320 2015

Protozoan epibionts on *Astacus leptodactylus* (Eschscholtz, 1823) from Aras Reservoir, Northwest Iran

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Received: April 2013 Accepted: November 2014

Abstract

The *Astacus leptodactylus* specimens were collected from four sites of Aras reservoir, North-West of Iran and examined for the epibionts during 2009. Protozoan epibionts from ciliophora (one genus and seven species) and tracheophyta were isolated from the cuticular surface of different body parts of narrow-claw crayfish, *A.leptodactylus*. Seasonal prevalence of infestation was determined in 394 individuals of *A.leptodactylus*. The facultative ciliate *Tetrahymena pyriformis* was identified on the gills and gill haemocoel with 0.5% prevalence. Futhermore , epibiont fouling organisms such as *Epistylis chrysemidis* (52.3%); *Vorticella similis* (45.9%); *Cothurnia sieboldii* (68.5%); *Pyxicola annulata* (66%); *Chilodonella* spp.(0.5%); *Zoothamnium intermedium*(57.1%); *Opercularia articulate* (20.6%) and *Podophrya fixa* (8.6%) were also isolated from 13 body parts of *A.leptodactylus*. The presence of *Chilodonella* infestation is the first record of this genus on freshwater crayfish species. The comparison of biometrical data of the epibionts showed no significant differences in prevalence of seasonal infestation between sampling sites. The current work represents the first documentation for the presence of protozoan epibionts on *A.leptodactylus* in Aras Reservoir, Iran.

Keywords: Protozoan, *Astacus leptodactylus*, Epibiont, Aras Reservoir, Iran

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Introduction

The epibiosis (facultative association of two organisms: the epibiont and the basibiont) is a frequent phenomenon on the crustaceans (Wahl, 1989). The term “epibiont” includes organisms that are attached to the surface of a living substratum, during the sessile phase of their life cycle, while the basibiont lodges and constitutes a support for the epibiont (Tânia *et al*., 2010). Both concepts describe ecological functions (Wahl, 1989). Among the epibiont organisms on crustaceans, the protozoans are common. The groups of protozoans more frequently found as epibionts, are the ciliates and, especially, the peritriches, suctorians and chonotrichids (Fernandez-Leboranz and Tato-Porto, 2000; Fernandez-Leboranz, 2001; Fernandez-Leboranz and Rintelen, 2007). Peritrich infestations of freshwater cray fish have been widely reported (Jensen, 1947; Nenninger, 1948; Hamilton, 1952; Krucinska and Simon, 1968; Sprague and Couch, 1971; Matthes and Guhl, 1973; Lahser, 1975; Johnson, 1977; Suter and Richardson, 1977; Kellicott, 1984; Mills, 1983, 1986; Scott and Thune, 1986; Herbert, 1987; Alderman and Polglase, 1988; Vogelbein and Thune, 1988; Owens and Evans, 1989; O’Donoghue *et al*., 1990; Evans *et al*., 1992; Thune, 1994; Boshko, 1995; Edgerton *et al*., 2002). Peritrichs have been described in *A. leptodactylus* (Nenninger, 1948; Krucinska and Simon, 1968; Matthes and Guhl, 1973; Boshko, 1995). Common peritrich ciliates infesting freshwater crayfish include species of the genera *Epistylis*, *Cothurnia*, *Lagenophrys* and *Zoothamnium*. Less well known are those species in the genera *Vaginicola*, *P.*, *Vorticella*, *Carchesium* and *cothurnia*. Sessile peritrichs are found on the external surfaces, including the branchial chamber. Peritrichs are generally filter-feeding bactivores (Corliss, 1979). Under eutrophic conditions, as sometimes occurring in aquaculture ponds, infestation levels increase (Scott and Thune, 1986). Some authors have suggested that if the peritrichs are localized in the gill cavity, dense populations may interfere with respiratory processes (Johnson, 1977; Villareal and Hutchings, 1986). Crayfish mortalities associated with heavy infestations of sessile peritrichs have been reported (Ninni, 1864; Kent, 1881–1882; Johnson, 1977; Villareal and Hutchings, 1986; Brown *et al*., 1993). Another possible mechanism of pathogenesis has also been investigated (Vogelbein and Thune, 1988). *T. pyriformis* may occur both as free-living organisms and as opportunistic parasites in both vertebrate and invertebrate hosts, including fish (Longshaw, 2011). The present study was performed to evaluate the mean prevalence and intensity of seasonal infestations with ciliates on the only endemic freshwater crayfish *A.leptodactylus* from Aras River (one of the largest rivers in the Caspian Sea basin), Aras Dam reservoir and explain the relationship between epibiont ciliates and water quality in this ecosystem (Nekuie Fard, 2010). The Aras dam reservoir is considered as a main resource of capture and release of *A.leptodactylus* juveniles to other water resources aiming at developing the culture of this crustacean. The goal of this study was to identify the risk and suppressive factors affecting

A.leptodactylus transmission to other water resources.

Materials and methods

During 2009, a total of 394 (255 males, 139 females) live *A.leptodactylus* were collected with 15-mm mesh net at four sites (site 1: 39.20° 91′ 14″ N 45.15° 83′ 33″ E; site 2: 39.19° 07′ 17 N 45.23° 88′ 75″ E; site 3: 39.18° 11′ 11″ N 45.31° 92′ 81″ E; site 4: 39.13° 27′ 19″ N 45.33° 96′ 42″ E) in the Aras Dam reservoir (Fig.1). Specimens were placed in separate insulated plastic bags and kept cool until they were transported to the Shahid Kazemi Fisheries Office laboratory in Poldasht town. The specimens were maintained in the laboratory for less than a week in aquaria containing unfiltered water of the same sampling site, with submerged plants, gently aerated at a temperature of 22±3°C. The hosts were dissected into 13 body parts (rostrum, antennules, antennae, scale, chela, carapace, mouthparts, pereiopods, pleopods, abdominal segments, telson, uropods, and gills). Live symbionts were observed under a light microscope using both light field and phase contrast techniques. Permanent preparations of small pieces of exoskeleton bearing epibionts were made by fixing samples in 5% Formalin, which were then stained according to Foissner (1979), Lee *et al.* (1985) and Mayén-Estrada *et al.* (2001). Computer measured the line drawing and length of the species, projected there by a video camera. Measurements of the epibionts were related to the scale of an objective micrometer, projected to the screen in the same way. The validity of the methods was checked by measuring the same organs with microscope micrometers. For the identification of epibionts the keys given by Hoffman, 1967; Matthes and Guhl, 1973; Kudo, 1977 and Alderman *et al.*, 1988 were used. Additionally, The physical and chemical factors such as: visibility, pH, O_2, conductivity and biogens (TN, PO_4, N-NH_4, N-NO_3) were recorded. Visibility, pH, O_2 and conductivity were determined in situ using the Scchi disc and electrode Jenway 3405 and remaining factors were analyzed in the laboratory, according to Hermanowicz *et al*. 1976. All statistical analyses were performed using SPSS version 17 (SPSS, Inc., Chicago, IL).

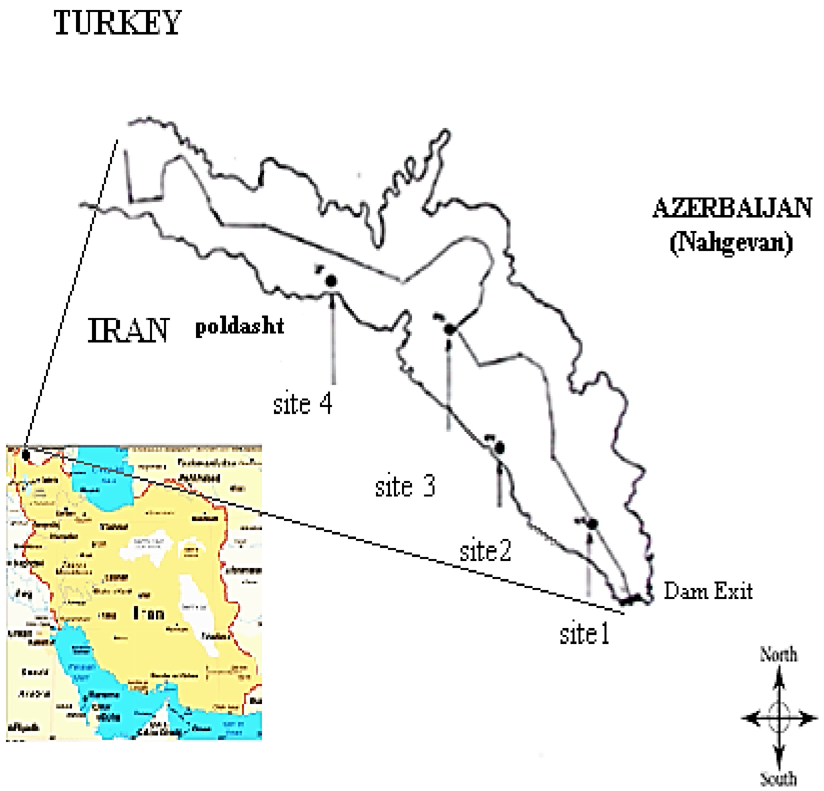

Figure 1: Location of the sampling stations on Aras reservoir, Northwest Iran.

Results

Eight different taxa (1genus, 7species) of ciliates and species of tracheophyta were observed on *A.leptodactylus*. The results of the morphometric data of detected epibionts are summarized in Table 1. Prevalence and mean intensity of these protozoan epibionts were measured (Table 2). Overall intensity (Mean±SE) of epibionts infestation were included *T. pyriformis*; *E. chrysemidis*; *V. similis*; *C. sieboldii*; *P. annulata*; *Chilodonella* spp.; *Z. intermedium*; *O. articulata* and *P. fixa* were 16.87±0.89, 0.01±0.0, 14.96±0.78, 24.40±0.96, 21.95±0.94, 0.21±0.15, 6.82± 0.73 and 1.27±0.21, respectively (Table 1). Statistical analysis of seasonal prevalence and intensity of ciliates were illustrated in Table 3. No significant seasonal infestations were observed between the sampling sites (p>0.05). The physico-chemical properties of Aras Reservoir water during the study period are presented in Table 4. These parameters confirm the eutrophic status of the reservoir and are similar to those observed in other eutrophic lakes (Carlson, 1977; Wetzel, 1983; Mohsenpour *et al*., 2010).

Table 1: Biometric features of epibionts (*measurements in µm, range, mean, standard deviation, sample sizes and body part of detected).

Species/genus	*Body length	*Body width	Detected region
E.chrysemidis	67-80 *X* 73, SD 7 n = 30	22-47.6 *X* 36 , SD 5.6 n = 30	Rostrum, gills, telson pereiopods, pleopods, uropods, abdominal segments
T.pyriformis	39-44 *X* 41, SD 3.5 n = 2	22-26 *X*23, SD 2.9 n = 2	Gills, gill haemocoel
Z.intermedium	52-79 *X* 56, SD 10 n = 30	30-43 *X* 36, SD 3.6 n = 30	Uropods, abdominal segments, telson, pereiopods, pleopods, scale carapace chela
C. sieboldii	77-92 *X* 85, SD 8 n = 30	41-55 *X* 45, SD 5.2 n = 30	Gills
P. annulata	78-82 *X* 80, SD 2 n = 30	29-32 *X* 31, SD 1.5 n = 30	Gills
***Chilodonella* spp.**	38-42 *X* 38, SD 2 n = 2	21-30 *X* 25, SD 4.5 n = 2	Gills
O. articulata	86-132 *X*109, SD 23 n = 30	35-73 *X* 39, SD 4 n = 30	Gills, antennules, antennae, scale, carapace chela, mouthparts, pereiopods, pleopods, abdominal segments, telson, uropods
P. fixa	46-53 X49,SD 3 n = 30	35-43 X39,SD 4 n = 30	Pereiopods, pleopods, abdominal segments, telson, uropods, mouthparts
V. similis	79-108 X93, SD 15 n = 30	69-80 X75, SD 5 n = 30	Gill, pereiopods, pleopods, abdominal segments, uropods

Table 2: Prevalence (%), mean intensity (±SE) and frequency of epibionts (during 2009).

	Winter		**Sprig**		**Summer**		**Fall**		**Overall**	
Epibionts	**Prevalence**	**Intensity**	**Prevalence**	**Intensity**	**Prevalence**	**Intensity**	**Prevalence**	**Intensity**	**Prevalence (Frequency)**	**Intensity**
E.chrysemidis	71.1	21.37±1.62	47.5	17.83±1.71	42.4	27.34±2.61	48.5	27.55±2.07	52.3 (206)	16.87±0.89
T.pyriformis	2.1	0.02±0.01	0	0	0	0	0	0	0.5 (2)	0.01±0.0

Table 2 continued:

Z.intermedium	55.7	10.66±1.22	62.4	20.42±1.55	58.6	31.52±1.25	51.5	24.79±1.86	57.1 (225)	14.96±0.78
C. sieboldii	100	36.87±1.06	76.2	32.36±1.13	38.4	25.22±2.94	59.8	35.36±1.41	68.5 (270)	24.40±0.96
P. annulata	89.7	24.55±1.47	76.2	31.04±1.08	38.4	29.21±3.02	59.8	37.10±1.41	66 (260)	21.95±0.94
***Chilodonella* Spp.**	2.1	0.84±0.58	0	0	0	0	0	0	0.5 (2)	0. 21±0.15
O. articulata	7.2	1.29±0.48	14.9	4.81±1.15	32.3	20.28±2.61	27.8	17.45±2.67	20.6 (81)	6.82±0.73
P. fixa	2.1	0.30±0.21	13.9	2.68±0.66	11.1	2.66±0.73	7.2	1.88±0.67	8.6 (34)	1.27±0.21
V. similis	97.9	16.36±0.62	43.6	9.99±1.14		7.41±1.62	24.2	11.05±1.82	45.9 (181)	8.72±0.54

Table 3: Seasonal statistical prevalence and incidence comparison of epibionts (during 2009).

	*** Prevalence%**							**Incidence**						
Epibiont	**Win-Spr**	**Win-Sum**	**Win-Fal**	**Spr-Sum**	**Spr- Fal**	**Sum- Fal**	**0verall**	****Win-Spr**	****Win-Sum**	****Win- Fal**	****Spr-Sum**	****Spr- Fal**	****Sum- Fal**	*****0verall**
E.chrysemidis	S	S	S	S	S	NS	S	S	S	S	NS	NS	NS	S
T.pyriformis	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Z.intermedium.	NS	NS	NS	NS	NS	NS	NS	S	S	NS	NS	NS	NS	S
C. sieboldii	S	S	S	S	S	S	S	S	S	S	S	NS	S	S
P. annulata	S	S	S	S	S	S	S	S	S	NS	NS	NS	NS	S
***Chilodonella* spp.**	NS	NS	NS	NS	NS	NS	NS	S	NS	S	S	NS	NS	NS
O. articulata	S	S	S	S	S	NS	S	NS	S	NS	NS	S	NS	S
P. fixa	S	S	NS	NS	NS	NS	S	NS	S	S	S	NS	NS	S
V. similis	S	S	S	S	S	NS	S	NS	S	NS	S	NS	NS	S

Win= Winter, Spr =Spring, Sum=Summer, Fal =Fall. S= Significant difference ($p<0.05$); *NS* = Non significant difference ($p>0.05$).* Chi-2 test. ** Kruskal Wallis Test. *** Mann-Whitney U test.

Table 4: Physico -Chemical properties of water on Aras Reservoir during the study.

Seasons	O_2 [mg.l^{-1}]	T [°C]	pH	BOD [mg.l^{-1}]	NO^{2-} [mg.l^{-1}]	NO^{3-} [mg.l^{-1}]	Cond. [µs/cm]	NH_3 [mg.l^{-1}]	T Phos [mg.l^{-1}]	T Nit [mg.l^{-1}]	Mg^{2+} [mg.l^{-1}]	Ca^{2+} [mg.l^{-1}]	Chl-a [µgl^{-1}]
Winter	14.2	6	8.8	7.2	0.21	21.2	633	0.08	0.07	4.8	62.6	54.5	24.2
Spring	9.4	20	7.5	3.5	0.13	7.8	395	0.06	0.1	1.8	55.9	25.3	26.2
Summer	9.8	26	8.5	4	0.06	8.7	233	0.23	0.07	1.8	54.1	30.9	17
Fall	10.7	14	8.5	5.8	0.21	13.9	262	0.31	0.09	3.4	70.7	54.3	19.8

T Phos=Total Phosphate; T Nit= Total Nitrogen; Chl-a=Chlorophyll a

Discussion

Although ciliates are commonly associated with crayfish, they are normally not considered a problem in the wild. Generally, mortalities occur under aquaculture conditions where poor water quality, elevated temperature and high host densities increase the risk of problems (Morado and Small, 1995). Most ciliates are found on the external surfaces of crayfish, including pleopods, pereiopods, telson, gills and carapace. Host-parasite checklists of ciliates on crustaceans are provided by Sprague and Couch (1971) and Morado and Small (1995).

The other major ciliates affecting crayfish are in the order Sessilina, whose defining characteristics are that they are attached permanently to the host (Morado and Small, 1995). Genera occurring on crayfish were included *Epistylis, Carchesium, Lagenophrys, Paralagenophrys, Zoothamnium, Opercularia, Vorticella* and *Cothurnia*. Most reports related to the peritrichous ciliates *Epistylis* spp. suggest that they are innocuous, acting as commensals (Vogelbein and Thune, 1988; Brown *et al*., 1993; Harlioglu, 1999; Hüseyin and Selcuk, 2005; Quaglio *et al*., 2006). However, mortalities have been associated with *Epistylis* sp., usually reported under culture conditions (Brown *et al*., 1993). Also, mortalities associated with Cothurnia in Italian crayfish were reported by Ninni (1864).

The protozoan epibiont genera determined in our study (*T. pyriformis*; *E. chrysemidis*; *V. similis*; *C. sieboldii; P. annulata; Chilodonella* spp.; *Z. intermedium*; *O. articulata* and *P.fixa*) have not been already recorded as epibionts on *A.leptodactylus*, although species of the genera *Epistylis, Cothurnia, Zoothamnium, Pyxicola* and *Vorticella* had previously been observed as epibionts on freshwater crayfishes (Matthes and Guhl 1973, Lahser 1975, O'Donoghue *et al*., 1990 and Evans *et al*., 1992). *A.leptodactylus* peritrichs have been studied mainly by Nenninger 1948; Krucinska and Simon, 1968; Matthes and Guhl, 1973; Boshko, 1995. Suctorian ciliates are from numerous genera, the most common being *Acineta* and less common genera including *Tokophrya, Podophrya* and *Opercularia*. Suctorian ciliates have been described in *A.leptodactylus* by Krucinska and Simon, 1968; Matthes and Guhl, 1973.

Many peritrich ciliates exhibit a highly specific host–commensal relationship. An investigation of life stages of *C. variabilis* found in the gill chamber of *Pacifastacus gambeli* showed a synchrony between metamorphosis of the ectocommensals and the moult stage of the crayfish host (D'Eliscu, 1975). It is likely that similar synchrony occurs with other sessile peritrich ciliates and their respective hosts. The close interaction between the symbionts and its host may have implications for the likelihood of exotic peritrichs successfully colonizing related host organisms elsewhere. However, little research has been conducted in this area, most reports being restricted to documenting the occurrence of the organisms in a given host crayfish population rather than the experimental infestation of different crayfish species with a given symbionts (Longshaw, 2011).

Protozoa of the lake environments are considered as a major link in the limnic food web and they have key functions in energy flow and cycling in freshwater ecosystems. Protozoa are very important components in the energy transfer to the higher trophic levels and they are a common nutrient for crustaceans and fish larvae (Porter *et al*., 1985). The changes in the community structure of protozoa may significantly affect other components of the aquatic food web, and thus may influence the distribution and abundance of both lower and higher organisms (Beaver and Crisman, 1989; Carrick and Fahnenstiel, 1992; Cairns and McCormick, 1993). Ciliates have important ecological significance in free environments, especially in benthic areas, where they show high growth rates and an important trophic diversity (Patterson *et al*., 1989; Fenchel, 1990; Fernandez-Leboranz and Fernandez-Fernandez, 2002). In general, the epibionts and peritrichous protozoans of *A.leptodactylus* are common fauna of fresh water crayfish. Most of them attach to the exoskeleton and gills of crayfish, and feed primarily on bacterial cells associated with eurotropic reservoir which generally increase in summer and reduce in winter. Water quality has a significant effect on infestation levels and turbidity is reported to be an excellent water quality indicator of potential peritrichs infestation in commercial crayfish ponds (Scott and Thune, 1986). Infestation levels in farmed and wildstock crayfish of the same species have been shown to vary (O'Donoghue *et al*., 1990; Evans *et al*., 1992), probably as a result of variation in the aquatic environmental conditions. Therefore, we can come to conclusion that parallel to increasing of eutrophication of Aras reservoir (Mohsenpour *et al*., 2010), prevalence and intensity of epicommensals like *Epistylis* spp. on crayfish population will be significantly increased and may have an adverse effect on health status which may lead to disease outbreak and mortality. Crayfish mortality can be graphic evidence of a serious chemo-physical problem in lakes or streams. The impact of toxic and harmful substances (fertilizers, herbicides) and of industrial and agricultural pollution on narrow-clawed crayfish has not been sufficiently evaluated and needs further study.

Acknowledgements

We would like to thank Iranian Fisheries Research Organization, Iranian Artemia Research Center and Fisheries Department of West Azarbaijan for supporting this study.

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