Parasites and bacteria isolated from ctenophore invaders, *Mnemiopsis leidyi* and *Beroe ovata*

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Mnemiopsis leidyi (Comb Jelly) is an exotic species which has not been reported from the Caspian Sea till 1999, but it was observed and reported from the southern coasts of Caspian Sea in 1999 (Esmaili Sari, 1999). Invasion of the *Mnemiopsis leidyi* to the Caspian Sea pelagic ecosystem has been one of the main difficulties in the past decade (Parafkandeh Haghighi and Kaymaram, 2011). Mnemiopsis leidyi has been brought from the Black Sea to the Caspian Sea by ballast water of ships and it has settled in the southern Caspian Sea through the time.There is a little information regarding the microbial flora in comb jelly (Estes et al., 1997) but a large number of comb jelly carry natural flora and pathogens located in the coastal waters such as *Aeromonas* and *Vibrio*. However, it might have been entered into the Caspian Sea many years ago, but it has recently caused execessive reduction in kilka fish stocks because it is considered as a food competitors for them. The comb jelly may also carry some new microrganisms like parasites and microbial flora to the Caspian Sea. This study was carried out to investigate the parasites and bacterial flora of *B. ovata* and *M. leidyi* as probable dangers for the Caspian Sea ecosystem.

During one year investigation (2003-2004), sea water specimens and *M. leidyi* were obtained from the southern Caspian Sea. In order to detect the parasite, all of the specimens were collected randomly by the plankton net (mesh size 90- 100 µm). A total of 2160 specimens of *M. leidyi* from the Caspian Sea and 47 specimens of *B. ovata* from the Marmara and Black Sea were investigated. Parasite specimens were sedimented by centrifuge (1000-1500 rpm) and then were invesigated by light microscope.*Trichodina* species was identified using silver nitrate impregnation. For Microbial flora 36 specimens of *M. leidyi* and 10 specimens of *B. ovata* were studied. Specimens were first homogenized in sterile normal saline and, then, were inoculated on tryptic soya agar at 30°C for 72 h. The morphological, physiological and biochemical characteristics of the grown bacteria from the second passages on tryptic soya agar were studied using standard bacteriological examinations.

No parasites were isolated from *M. leidyi* in the Caspian Sea.However, 73% and 64% of *B. ovata* from Marmara and Black Seas (Table 1) were parasitized with *Trichodina ctenophorii*, respectively. Significant differences were found between infestation rates (%) in the different salinities ($P<0.02$; $X^2=9.309$)(Table 2). The intensity of infestation increased with the rise of salinity. In lower salinity (12.6- 14.9 ppt) the intensity of Infestation was reduced.

Table 1: Percentage and intensity of infection with *T. ctenophorii* in *M. leidyi* and *Beroe ovata*

Place of Sampling	Ctenophore species	Number of specimens	Infestation rate (%)	Intensity of Infestation
South Caspian Sea	*M. leidyi*	2160	0	0
Marmara Sea	*B. ovata*	22	73	420-2100

Table 2: infestation rate and intensity of *T. ctenephorii* in *Beroe ovata* in different salinities of black Sea

Salinity (ppt)	No. of specimens	No. of specimens	Infestation rate (%)	Intensity of infestation
12.6	5	2	40.0%	130
14.9	6	4	66.7%	250
19	6	4	66.7%	140-500
21.6	8	6	75.0%	260-1050

14 bacterial isolates including gram negative and gram positive bacteria were recognized (shown in Table 3). *Micrococcus* sp., *Aeromonas* sp. and *Bacillus coagulans* were isolated from both ctenophora.Some other bacteria such as *Agromobacterium* sp., *Chromobacterium* sp., *Shewanella* sp., *Vibrio harveyi* and *Bacillus linens* were only isolated from *B. ovata*.The most common pathogenic isolated bacteria were *Micrococcus* sp. and *Vibrio* sp.

Table 3: Isolated bacteria from *M.leidyi* and *Beroe ovata* in this study

Ctenophora	Isolated bacteria	
B. ovata	*Agromobacterium tumefaciens* ,*Aeromonas* sp. , *Choromobacterium violacceom*, *Bacillus linens*, *Shewanella* sp. *Micrococcus* sp. , *Bacillus coagulans*, *Vibrio harveyi*	
M.leidyi	depth of Body	*Micrococcus* sp., *Staphilococcus* sp. , *Vibrio metschinokovii*,*Burkholderia mallei*, *Aeromonas* sp.
	surfce of Body	*Bacilus circulans*, *B. sphrericoccus*, *B. coagulans*, *Micrococcus* sp. , *M. kristinae* , *Vibrio metschinokovii* ,Enterobacteriaceae, *Vibrio* sp., *Streptobacillus* sp.,*Cytophaga* sp.

Ctenophora are a host for several parasites such as Trematodes (Stunkard, 1980), amphipoda (Harbison et al., 1977), and unicellulars (Crowell, 1976). The most variety of unicellular and multicellular parasites have been reported in *M. mccradyi* (Martorelli, 2001; Moss et al., 2001). It seems that there are many differences between the comb jellies of Black Sea and the Caspian Sea from ecological point of view, especially salinity of the two regions .When the comb jelly from the Black Sea with the salinity of 24 ppt enters into the Caspian Sea via Volga Canal by ships ballast water, it passes several salinity changes that caused high changes in osmotic pressure. In this case most of the parasites particularly unicellular parasites are destroyed. In fact the comb jelly was devoid of any parasite. On the other hand, comb jelly invaded the Caspian Sea some years ago (Esmaili Sari, 1999), so it could be a new host for the Caspian Sea parasitic fauna if they adapt themselves to the physiological and anatomical condition of comb jelly tissues.

Nowadays, the final salinity in the Caspian Sea is estimated less than 14.9 ppt resulting in high reduction in *Trichodina* population while in salinity of 12.6 ppt they are going to be destroyed. It means that if even the *B. ovata* adapts itself to the Caspian Sea water its parasitic fauna due to the high reduction of salinity would be eliminated.So it is considered as a good biological control agents for *M. leidyi* which finally would lead to reduction in *m. leidyi* stocks. The most common pathogenic bacteria which were isolated including *Micrococcus* sp. and *Vibrio* sp. Many bacteria are common in *B. ovata* and *M. leidyi*, except for *Chromobacterium* sp., *Agromobacterium* sp., *Shewanella* sp. and *Bucillus linens*. Five genus of gram negative bacilli and one genus of gram positive coccus plus a gram positive bacillus have been isolated from *B. ovata*. In a comparison between microbial flora of *B. ovata* and *M. leiydi*, *V. harveyi*, *B. linens*, *Agromobacterium* sp., *Chromobacterium* sp. and *Shewanella* sp. were not isolated in *M. leiydi*. But *Aeromonas* sp., *Micrococcus* sp. and *Bacillus coagolance* were observed in both of them. Of course *Agrobacterium* and *Chromobacterium* were recently reported in the Caspian Sea but none of them were known to be pathogens. All the isolated bacteria are presently called as natural flora of sea water (Austin, 1989). Bacterial flora usually can be easily transferred from a place to another by ballast water of ships, ships body and aquatic migratory birds. In this case each liter of ballast water can trasfer about 240000 bacteria. In a five-year study from 1995 to 1999 on water, sediments in the Black Sea, the following pathogenic viruses were recognized: Coxsackievirus B1 and B2, human Poliovirus type 2, Hepatitis A virus (HAV) and Rota & Reo and Adeno virus (Stepanova, 2001). If the ballast water is not changed, these groups of virus can be transferred by ships ballast water. Hence the transfer of such microrganisms from the Black Sea to the Caspian Sea, should be prevented. Since *Beroe ovata* is considered as a biological control agent, before transferring *B. ovata* from the Black Sea to the Caspian Sea using antibiotics and anti-parasitic baths are required.Virological assessment on *B.*

ovata should confidently be done for transferring the *B. ovata*.

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