

Internal parasites of saposchnikovi shad, *Alosa saposchnikowii* (Grimm, 1887), from the southeastern part of the Caspian Sea, Iran

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Received: March 2015

Accepted: August 2015

Abstract

This paper presents the results of a parasitological study on internal parasitic infections in saposchnikovi shad, *Alosa saposchnikowii*, from the southern part of the Caspian Sea. In this regard 30 fish were studied for abdominal cavity parasites in February 2014. Results showed all of the specimens were infected at least with one kind of parasite and there was no significant difference in parasitic infections between males and females. According to the results three kinds of parasites including one trematode (*Pronoprymna ventricosa*) and two kinds of nematodes (*Anisakis simplex* and *Eustrongylides* sp) were isolated from the abdominal cavity of the studied fish. 43.33 % of the specimens were infected with *A. simplex*, 96.66 % were infected with *P. ventricosa* and 16.66 % of the specimens were infected with *Eustrongylides* sp. Intensity of infection to *A. simplex*, *P. ventricosa* and *Eustrongylides* sp in saposchnikovi shad was calculated as, 3.46 ± 1.76 , 131 ± 16.78 and 2 ± 0.71 , respectively. According to the results 33.33% intestine, 10% liver and 6.66% mesentery of specimens were infected with *A. simplex*, 26.66% stomach, 86.66% pyloric caeca and 66.66% intestine of studied fish were infected with *P. ventricosa* and *Eustrongylides* sp was isolated in 13.33% mesentery and 3.33% liver of the specimens. It seems this is the first record of *A. simplex* and *Eustrongylides* sp infection from saposchnikovi shad in Iran. These parasites are important because of their pathogenicity in fish and zoonotic risk in human health care.

Keywords: Trematode, Nematode, Parasites, *Alosa saposchnikowii*, Caspian Sea

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Introduction

Parasites are an important group of pathogens that cause infection and diseases in both freshwater and marine fish. Parasite infestation can be found in most fish in natural environments. This fish can not only be host to different parasites but also can be the carrier of many zoonotic parasitic diseases which can cause serious problems in many vertebrates including human. Parasitic infections in fish can lead to decrease in growth rate, weight loss and emaciation, affect yield of fish products, cause human and animal diseases, delay in sexual maturity and mortalities (Woo, 2006).

Many studies have been done on identifying parasitic infestation in some Caspian Sea fish. It can be noted that a comprehensive study on the parasites of the southern part in the Caspian Sea fishes by Mokhayer (1980) reported 29 species of fish parasites. After that parasite infections have been attributed in different fishes of the Caspian Sea by researchers. In this regard studies by Malek (1993), Satari and Faramarzi (1997), Pazooki and Masoumian (1999, 2004); Eslami *et al.* (1972), Mokhayer (1974, 1975, 1981), Eslami and Mokhayer (1977), Eslami and Kohneshahri (1978), Jalali (1998) etc. can be mentioned. Totally, at least more than 248 species of parasites were recorded from 102 fish species in Iran (Pazooki and Masoumian, 2012).

Clupeidae are pelagic fish that play a key role in many food chains. Two genera of this family including:

Clupeonella spp and *Alosa* spp can be found in the Caspian Sea. They are widely distributed in the southern part of Caspian Sea and *Alosa* spp is mainly seen in the coastal and deltaic areas. Parasite infections are expected in these fish due to their feeding habit (feeding on crustaceans and small fishes) in natural environments.

Unfortunately there are few studies on parasitic infections from clupeidae family in the Caspian Sea. There are five records of parasitic infestation from *Alosa caspia* and three species of the *Clupeonella* in the Caspian Sea (Shamsi and Dalimi, 1996; Kornijchuk and Barzegar, 2005; Varshoie *et al.*, 2010; Youssefi *et al.*, 2011; Barzegar *et al.*, 2012). There is no record of saposchnikovi shad parasites fauna from the Caspian Sea. The aim of this study is to survey prevalence and intensity of internal helminthic parasites of *A. saposchnikowii* in the southern part of Caspian Sea.

Material and methods

In the present study, parasitic fauna of the abdominal cavity of *A. saposchnikowii* in the southeast part of the Caspian Sea were studied in February 2014. Fish samples were caught by purse seine nets by fisherman in Behshahr City - Mazandaran province. Totally 30 fish specimens of saposchnikovi shad were studied. After biometry, specimens were rinsed and their abdominal cavities were opened, and the digestive tract and other organs (kidney, spleen, liver and mesentery)

were fully examined for parasites following standard parasitological methods. All isolated parasites were counted after washing, and transferred to fixative solution and sent to the Iranian National Parasitology Museum, Faculty of Veterinary Medicine of the Tehran University, for identification.

In this study isolated trematodes were washed in normal saline (0.6% NaCl), fixed in 70% ethanol, and prepared for microscopic examination by staining with iron acetocarmine (Georgiev *et al.*, 1986). Nematodes were washed in normal saline and fixed in 4 % formaldehyde, preserved in 70 % ethanol, then the parasites were cleared in glycerin and stained by lacto phenol (Moravec, 1994). Finally, all metazoan parasites were mounted in entellan (Merck Co, Germany) after staining. Drawing of parasites was carried out with the aid of a camera lucida fixed on a compound microscope. The parasite specimens were deposited in the collection of fish parasites in the museum. Identification was basically those described by Hanek and Fernando (1972) and Roberts (2001)

Prevalence of infection (%), mean intensity and abundance of infection and the limit number of infection were calculated according to the following formula:

Prevalence of parasites (%) = the number of infected fish/ the total number of examined fish \times 100

Mean intensity of infection = the number of counted parasites/ the total infected fish.

Mean abundance of infection = the total number of parasites/ the total number of surveyed fish

Data were analyzed separately using the SPSS software version no.18, and t-test was used to determine the presumptive differences between both sexes and both seasons depending on the mentioned variables. Data were presented as mean \pm SD and $p < 0.05$ was considered statistically significant.

Results

Totally three helminthes species including *Anisakis simplex*, *P. ventricosa* and *Eustrongylides* sp were found in the examined fish. According to the result, all the specimens were contaminated with at least one kind of parasite. The highest prevalence of parasitic infection belonged to *P. ventricosa* which have been isolated from pyloric caeca, intestine and stomach of 96.66 % of specimens. *Eustrongylides* sp infection showed the lowest prevalence of parasitic infection in this study which was isolated from the liver and mesentery of 16.66 % of specimens. Infection in *A. simplex* was isolated from the liver and intestine of infected fish (Tables 2 and 3). 33.33 % of intestine, 10 % of liver and 6.66 % of mesentery in studied fish were infected with *A. simplex*, as well as 26.66 % of stomach, 86.66% of pyloric caeca and 66.66% of intestine in specimens were infected with *P. ventricosa*. In relation to *Eustrongylides* sp infection, this parasite was observed in 13.33% of mesentery and 3.33% of liver in studied fish.

Table 1: Biometric characters of *Alosa saposchnikowii* specimens (Mean \pm SD).

Variables	Mean \pm SD	Minimum	Maximum
Weight (g)	378.06 \pm 310	58.00	957.00
Total length (cm)	32.40 \pm 7.82	21.00	45.00
Standard length (cm)	27.53 \pm 6.83	17.50	38.00

Table 2: Prevalence of parasite infections in *Alosa saposchnikowii*.

Kind of infestation	Prevalence (%)	Intensity (mean \pm SD)	Range	Mean abundance of infection \pm SD
Total parasite infection	100%	133.2 \pm 17.63	1 - 150	133.2 \pm 17.63
<i>A. simplex</i>	43.33%	3.46 \pm 1.76	1 - 8	1.5 \pm 1.12
<i>P. ventricosa</i>	96.66%	131 \pm 16.78	2 - 150	126.63 \pm 17.33
<i>Eustrongylides</i> sp	16.66%	2 \pm 0.71	1- 3	0.33 \pm 0.06

Table 3: prevalence, intensity, limit number and abundance of infection in *Alosa saposchnikowii*.

Infected organ	Parasites name	Prevalence (%)	Intensity \pm SD	Rang	Abundance \pm SD
Intestine	<i>A. simplex</i>	33.33	2.60 \pm 1.45	1-5	0.87 \pm 0.45
	<i>P. ventricosa</i>	66.66	34.83 \pm 11.83	2-67	33.66 \pm 13.25
Pyloric caeca	<i>P. ventricosa</i>	86.66	80.83 \pm 28.45	7- 150	78.13 \pm 28.44
Stomach	<i>P. ventricosa</i>	26.66	15.34 \pm 10.06	3 - 35	14.83 \pm 10.27
Liver	<i>Eustrongylidessp</i>	3.33	2.00 \pm 0.36	0-2	0.07 \pm 0.36
	<i>A. simplex</i>	10.00	4.00 \pm 1.54	1-8	0.40 \pm 1.54
Mesentery	<i>Eustrongylides</i> sp	13.33	2.00 \pm 0.74	1-3	0.27 \pm 0.07
	<i>A. simplex</i>	6.66	3.50 \pm 0.97	2-5	0.23 \pm 0.08

Based on data analysis, gender had no significant effects on parasite burden and parasite kinds in *A. saposchnikowii* (Tables 4 and 5). Intensity of infections from *A. simplex*, *P. ventricosa* and *Eustrongylides* sp in saposhnikovi shad

are presented in Tables 2 and 3. In this study *P. ventricosa* had the highest intensity of infection in saposhnikovi shad and was mostly isolated from pyloric caeca.

Table 4: Relationship between sex and parasite burden in *Alosa saposchnikowii*.

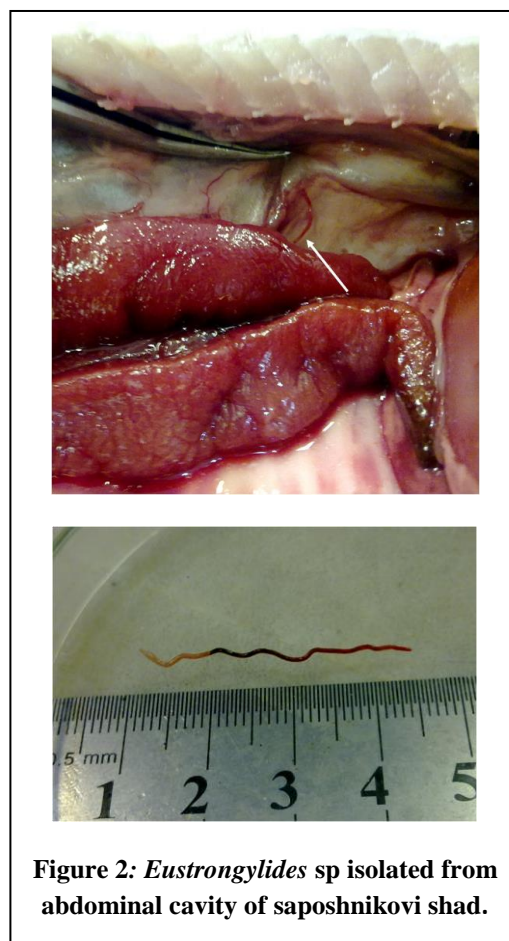
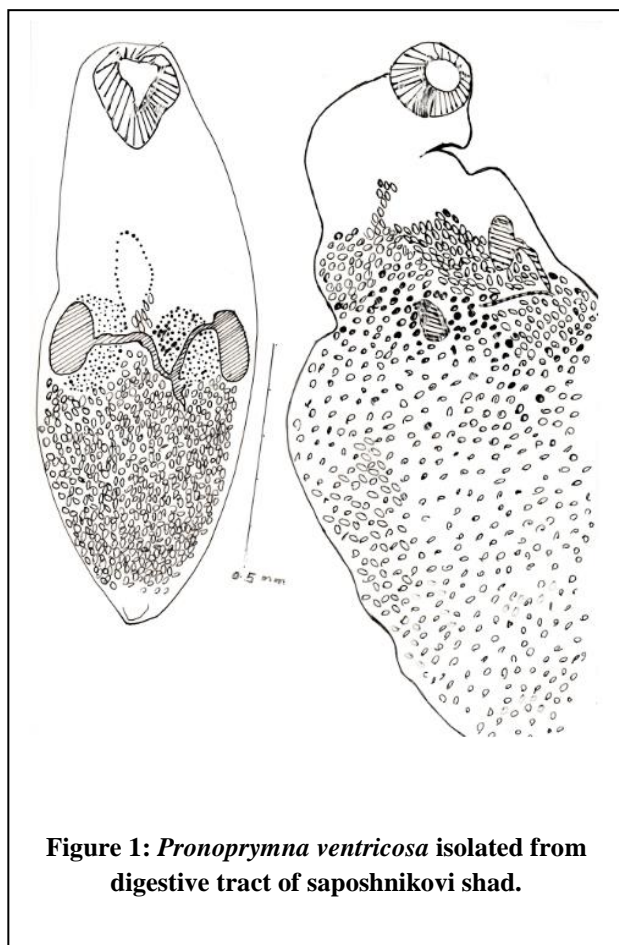
Fish Host	Sex	No. examined	No. infected	Parasitic load	Significance ($p=0.05$)
<i>A. saposchnikowii</i>	F	20	20	2643	N
	M	10	10	1211	

F: female, M: male, N: negative ($p<0.05$).

Table 5: Relationship between sex and parasite species burden in *Alosa saposchnikowii*

Parasite species	Sex	No. examined	No. infected	Total parasitic load	Mean parasitic load	Significance ($p=0.05$)
<i>A. simplex</i>	F	20	9	34	1.70	N
	M	10	4	11	1.70	
<i>P. ventricosa</i>	F	20	19	2604	8.97	N
	M	10	10	1195	1.14	
<i>Eustrongylides</i> sp	F	20	3	5	0.25	N
	M	10	2	5	0.50	

F: female, M: male, N: negative ($p<0.05$).



Discussion

Some Caspian saposchnikovi shad remain in the southern part of the Caspian Sea all year round, while others migrate long distances between the southern and northern regions of the Caspian Sea during spring and winter for spawning or nutrition (Naderi Jolodar and Abdoli, 2004), and this long distance migration may increase parasite exposure. In the present study, all specimens were infected with at least one parasite. As shown in Tables 3 and 4, there weren't any differences in parasite infection as well as in intensity of parasitic infestation between genders. This can be because of similar nutritional habits in both genders.

Despite numerous reports in the occurrences of various parasitic infections in fish of the Caspian Sea and basins, there is little information about infection to *P. ventricosa* in Caspian Sea fish. This trematode has been isolated from pyloric caeca and intestine of different marine fishes, and was largely isolated from Clupeidae too. For example, This parasitic infection has been reported in *Alosa* spp in the Northeast Atlantic Sea and Mediterranean Sea (Bray and Gibson, 2008), Azov Sea (Nizova and Syrovatka, 2000), and Black Sea (Koval, 1962; Gaevskaya *et al.*, 2003; Popjuk, 2009; Ozer *et al.*, 2013). Fish infection to *P. ventricosa* has just been reported from Clupeidae in Iran. Shamsi and Dalimi (1996) reported *Pseudopentagramma symmetrica* in three species including

C. engrauliformis, *C. cultriventris* and *C. grimmi* from the Caspian Sea. In another study, Varshoie *et al.* (2010) recorded this parasite from the three mentioned fish too. (*Pseudopentagramma*, is synonymous to *Pronoprymna*, (Chulkova, 1939). Kornijchuk and Barzegar (2005) registered *P. ventricosa* from Caspian shad (*Alosa caspia persica*) in the southern part of The Caspian Sea. Youssefi *et al.* (2011) and Barzegar *et al.* (2012) in different studies isolated this parasite from Caspian shad. In the present study *P. ventricosa* was isolated from 96.66% of sampled saposchnikovi shad. This result is in line with Barzegar *et al.* (2012) who isolated this parasite from 100 % of sampled Caspian shad. Varshoie *et al.* (2010) isolated *P. ventricosa* from 53-58% of three species of Clupeidae (*C. engrauliformis*, *C. cultriventris* and *C. grimmi*) from the Iranian coastal regions of the Caspian Sea. Due to similar migration and eating habits in the clupeide family, infection to *P. ventricosa* in other fish of this family in the Caspian Sea is expected.

As shown in Table 5, *A. simplex* and *Eustrongylides* sp were isolated from 43.33% and 16.66 % of specimens, respectively. Despite reports of these parasitic infections in different bony fish of the Caspian Sea (Pazooki and Masoumian, 2012), it seems this is the first record of *A. simplex* and *Eustrongylides* sp infection from saposchnikovi shad in Iran. These parasites have pathogenic effects on

fish and human. Dubinin (1952), Dogiel and Bykhovskiy (1939) showed the pathogenicity of *Eustrongylides* spp. in Ship sturgeon (*A. nudiventris*) causing heavy infections leading to gonad destruction.

Eiras and Rego (1988) reported similar changes in South American fishes (*Pygocentrus nattereri*) with granulomatous inflammation and exudates accumulation due to parasite penetration in somatic muscles and inner organs. In similar reports of experimental infections in rainbow trout, third-stage larvae of *A. simplex* caused considerable cellular reaction in the body cavity and in the tissue penetrated, especially in the pyloric caeca (Santamarina *et al.*, 1994; Larsen *et al.*, 2002). Some studies showed various severity destruction and necrosis of the liver parenchyma and ruptures of the wall of the blood vessels in abdominal cavity organs, in consequence of *Anisakis* spp larvae migration through infected tissues (Hauck and May, 1977; Smith, 1984).

On the other hand, *Anisakis* and *Eustrongylides* are important because of their pathogenicity in human too. These parasites can make serious problems in human (via consuming raw or uncooked infected fish). *Anisakis* spp larvae can penetrate digestive tract and migrate along intestinal wall, tongue, lung, lymphatic ganglia or pancreas in human (Rosales *et al.*, 1999) and lead to serious clinical signs such as allergic reaction, stomach pain, vomiting, nausea and gingivostomatitis (Ancillo

et al., 1997; Audicana *et al.*, 2002). This parasite has been isolated from shad in other regions (Bao *et al.*, 2015). *Eustrongylides* is another zoonotic parasite the accidental infection of which in human, by eating raw infected fish can lead to gastritis and intestinal perforation with severe pain in abdominal cavity; this parasite larva can result in serious damages to patients and sometimes surgical intervention is needed for treatment (Deardorff and Overstreet, 1991; Cole, 1999).

Acknowledgements

The authors would like to thank the staff of the Department of Parasitology - Veterinary Medicine - Tehran University for providing the necessary facilities.

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