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Research Article

New record of digenean species in two mugilid fish (*Chelon auratus* and *Chelon saliens*, Risso, 1810) in southern Caspian Sea, Iran

Alizadeh-Noudeh M.[1]; Pazooki J.[1*]

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Abstract

The present study was carried out on digenean infection in two species of mugilid fish (*Chelon auratus* and *Chelon saliens*, Risso, 1810) in southern coast of Caspian Sea. One hundred and ninty eight fishes were totally studied. We identified the digenetic trematodes of three families, Haploporidae (adult Digenea), Heterophyidae (Metacercariae), and Diplostomidae (Metacercariae). Eyes, gills, stomach, intestine and pyloric caeca of fishes were infected with Diplostomidae (*Diplostomum spathaceum*), Heterophyidae (*Ascocotyle (Phagicola) longa* and *Ascocotyle* sp.), Haploporidae (*Saccocoelium obesum and S. tensum*), respectively. The results revealed that the stomach was the most infected organ (P=57%), and there was a significant difference between mean intensity of infection and sampling stations in Haploporidae (X^2= 11.6, df= 3, p= 0.009) and Heterophyidae (X^2= 10.2, df= 2, p=0.006). There was no significant difference between mean intensity of infection in three years and fish genders. *Ascocotyle (Phagicola) longa* and *Saccocoelium tensum* are reported for the first time in Iran. Another noticeable point is that the digenean parasites, which are prevalent in mullets, can cause disease in fishes. They are zoonotic (*Ascocotyle (Phagicola) longa*) and are important in point of view of ecology, economy and public health.

Keywords: Mullet, Metacercariae, Digenea, Zoonosis, Caspian Sea, Iran

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1-Department of Biology, Aquatic and Marine Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.
*Corresponding author´s Email: pazooki2001@yahoo.com

Introduction

Caspian Sea is the largest lake on earth containing a vast range of fish species (Aladin and Plotnikov, 2004). It has very important role in ecology and economy of the region. Mullet is one of the main fish species in Caspian Sea. This fish is rated as the second substantial target in the fishing industry (Fazli *et al.*, 2014; Coad, 2017). As a matter of fact, mullets are cosmopolitan, found in a wide temperature range. They have been regarded as an ecological link for trematodes of fresh water and marine fishes (Overstreet, 1971). Digeneans are heteroxenous parasites and need an intermediate host in their life cycle; this is the main limiting factor in their distribution (Paperna and Dzikowski, 2006). The digenean infestations often cause intense economic loss in mullets (Al-Bassel *et al.,* 1999; Kotb *et al.*, 2014). On the other hand, some of digeneans are considered potential zoonotic agents (Nguyen *et al*., 2021). So far, many articles are published about digenetic infection of mullets all over the world (Overstreet, 1971; Blasco-Costa *et al.*, 2009; Kotb *et al*., 2014; Sarabeev, 2015; Bottari *et al.*, 2020; Nguyen *et.al.* 2021). Mikailov (1958), Mokhayer (1980) and Mirnategh *et al.,* (2017) investigated parasites of Caspian Sea mullet. There are few studies of mugilids digenetic parasites on Iranian coasts of Caspian Sea; the only paper in this subject is Taghavi *et al.* (2013). They reported *Saccocoelium obesum* from intestines of mullet. According to the importance of mullets and scarcity of studies on their digeneans, purpose of this research was to identify the digenean parasites, and their distribution, prevalence and intensity in mullets on the Iranian coasts of Caspian Sea.

Materials and methods

Present study was performed for a period of three years from 2017 till 2019. A total number of 64 *Chelon auratus* and 134 *Chelon saliens* were captured from four locations including Kiashahr, Anzali, Chalus and Mahmoudabad on southern coasts of Caspian Sea (Fig. 1). After biometrical measurements the internal and external organs of fish were examined. Digenean parasites were prepared based on standard parasitological methods and identification process was also performed considering valid keys. The isolated parasites were fixed using a 10% formalin and mounted on a microscopic slide so as to be identified. Parasite samples were photographed using a Nikon microscope equipped with a SSC-DC378P Sony video camera. For electron microscopy on properties of some parasites, the samples were washed in distilled water for 24 hours. The water was changed every 5 hours The samples were dehydrated in varying degrees of ethanol; 30, 40, 50, 70, 90 and 99 percent, every 2 hours. Finally, the parasites were placed in acetone solution for 2 hours. The parasites were mounted on metallic stub and coated with gold by a sputtering chamber (SCDOOS, BalTec, Switzerland).

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Parasites were photographed at 15 kV by scanning electron microscope (JSM-6380 (JEOL, Tokyo, Japan)).

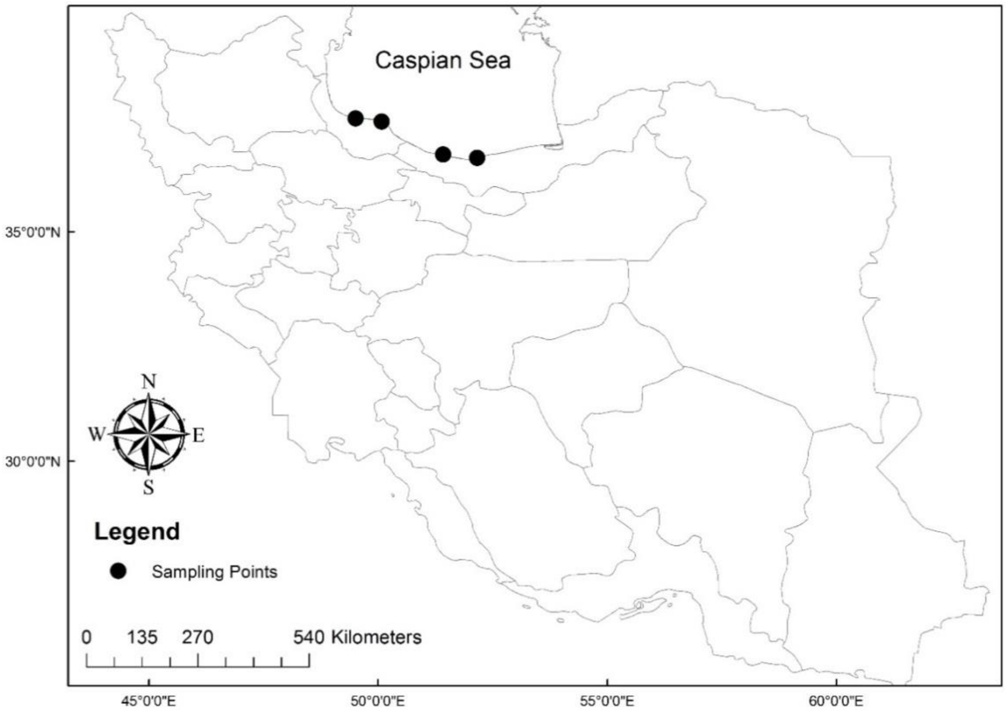

Figure 1: Study area and the sampling locations.

Parasitological measures, including prevalence (P), mean intensity (MI) and mean abundance (MA) of each parasite species were calculated according to Bush *et al.* (1997). Kruskal-Wallis analysis (nonparametric ANOVA) was used to evaluate mean intensity of infections among fish species, sampling years and locations. The differences in mean intensity of the parasite infestation between male and female fish were tested by Mann-Whitney U test. SPSS Version 25.0 statistics software was used at a significant level of 5% for statistical significance.

Results

A total number of 198 mullet fish specimens of *Chelon auratus* (N= 64) and *Chelon saliens* (N=134) were surveyed indicating that they were infected with 42 (65.62%) and 73 (54.47%) digenetic trematodes, respectively (Table 1).

Prevalence (P %), mean intensity (MI±SD) and mean abundance (MA±SD) in four locations were calculated (Tables 2 and 3). The mean intensity of Haploporidae infection (X^2=11.6, df =3, p=0.009) and Heterophyidae (X^2=10.2, df=2, p= 0.006) were significantly different in various locations (Table 4).

Digenetic trematodes, including *Saccocoelium obesum and Saccocoelium tensum* (Haploporidae) were found mainly in the intestine and partially in the stomach and Pyloric

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caeca. They were very prevalent on the surface of intestine. The mean intensity of Haploporidae in *Chelon auratus* and *Chelon saliens* were 42.13±47.21and 38.24±74.52. There was no significant difference (X^2=1.06, df = 1, p=0.30) between the two mullet species (Table 4).

Table 1: Incidence of digenetic trematodes and some biometric parameters in *Chelon auratus* and *Chelon saliens* (mean ± standard deviation).

		Weight of fish (g)	Total length of fish (cm)	No. of examined fish	No. of Infected fish
Anzali	*C. saliens*	205.66±94.40	31.5±4.48	12	10
		227.75±52.58	30.9±2.3	12	6
		128.17±17.91	26.26±0.86	18	3
	C. auratus	245.10±10.84	33.06±0.99	10	6
		217.06±47.5	30.9±3.5	8	8
		348.44±16.66	34.55±1.22	8	8
Kiashahr	*C. saliens*	157.18±35.05	29.06±1.90	26	16
		282.65±90.4	33.36±4.05	10	10
		95.25±20.85	23.76±1.56	26	24
	C. auratus	185.89±35.37	29.47±1.2	8	2
		327.99±92.39	35.5±3.09	6	6
		134.81±32.84	25±1.9	9	9
Chalus	*C. saliens*	116.68±30.85	25.35±3.1	12	0
	C. auratus	156.46±6.1	27.25±0.93	6	3
Mahmoudabad	*C. saliens*	146.26±40.45	26.58±1.9	18	4
	C. auratus	175.25±8.54	28.43±0.27	9	0

Table 2: Site and rate of digenean infection (Haploporidae, Heterophyidae and Diplostomidae) in *Chelon auratus* and *Chelon saliens*. P: prevalence, MA: mean abundance, MI: mean intensity, SD: standard deviation.

		Host	Site of infection	No. of examined fish	No. of Infected fish	P (%)	MA ±SD	MI ±SD
Haploporidae	*Saccocoelium obesum* and *S. tensum*	*Chelon auratus*	Stomach/ Intestine/ Pyloric Caeca	64	37	57.81	27.21±42.67	42.13±47.21
		Chelon saliens	Stomach/ Intestine/ Pyloric Caeca	134	73	54.47	20.83±58.06	38.24±74.52
Heterophyidae	*Ascocotyle* (*Phagicola*) *longa* and *Ascocotyle* sp.	*Chelon auratus*	Stomach/ Intestine/ Gill	64	11	17.18	15.48±80.56	90.09±183.02
		Chelon saliens	Stomach/ Intestine/ Gill	134	19	14.17	1.35±4.85	9.73±9.40
Diplostomidae	*Diplostomum spathaceum*	*Chelon auratus*	Eye	64	10	15.62	0.25±0.64	1.6±0.69
		Chelon saliens	Eye	134	6	4.47	0.07±0.39	1.6±1.03

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Table 3: Rate of digenean infection (Haploporidae, Heterophyidae and Diplostomidae) in different years and locations of *Chelon auratus* and *Chelon saliens*) in Southern coasts of Caspian Sea. P: prevalence, MA: mean abundance, MI: mean intensity, SD: standard deviation.

		Sampling years			locations			
		2017	**2018**	**2019**	**Anzali**	**Kiashahr**	**Chalus**	**Mahmoudabad**
	N	**74**	**63**	**61**	**68**	**85**	**18**	**27**
Haploporidae	MI±SD	19.54± 19.76	56.17± 101.79	44.05± 51.16	73.75± 101.01	24.43± 29.90	11± 0.01	6.5± 6.35
	MA±SD	9.7± 17.01	30.31± 79.44	31.16± 46.92	41.73± 82.01	19.25± 28.34	1.83± 4.21	0.96± 3.19
	P (%)	50	53.96	63.93	52.94	78.82	16.66	14.81
Heterophyidae	MI±SD	6.29± 6.32	95.36± 180.58	10± 0.01	69.37± 152.78	3.83± 3.27	0	10± 0.01
	MA±SD	1.44± 3.98	16.65± 81.18	0.32± 1.79	16.32± 78.13	0.54± 1.78	0	0.74± 2.66
	P (%)	22.97	17.46	3.27	23.52	14.11	0	7.40
Diplostomidae	MI±SD	2± 0.01	2± 0.01	1.5± 0.9	1.66± 0.81	1.60± 0.84	0	0
	MA±SD	0.54± 0.32	0.06± 0.35	0.29± 0.71	0.14± 0.52	0.18± 0.58	0	0
	P (%)	2.70	3.17	19.67	8.82	11.76	0	0

Table 4: Results of Kruskal-Wallis test showing differences between digenean parasites in mullet species, sampling years and locations. P: prevalence, MA: mean abundance, MI: mean intensity, SD: standard deviation.

	Haploporidae	**Heterophyidae**	**Diplostomidae**	**Haploporidae**	**Heterophyidae**	**Diplostomidae**	**Haploporidae**	**Heterophyidae**	**Diplostomidae**
Chi-square	1.061	.136	.015	4.076	5.947	2.637	11.606	10.283	.059
df	1	1	1	2	2	2	3	2	1
Asymp. Sig.	.303	.712	.904	.130	.051	.267	.009	.006	.809

Encysted metacercariae of *Ascocotyle* sp. were found in fish gill, this specimen had two rows of spines around its oral sucker (Fig. 3B). The adult parasite had similar characters with their metacercariae, except in definitive host and site of infection. On the other hand, *Ascocotyle (Phagicola) longa* specimens were found as single or multiple cysts in fish stomach and intestine. This species had one row of spines around oral sucker (Fig. 3A). *A. (Phagicola) longa* metacercariae were found mainly in mugilids and the mature form infected mammals and fish-eating birds. Differences between adult and immature Phagicola is mainly

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in definitive host, site of infection, and size (adults are larger).

Ascocotyle infections in *Chelon auratus* and *Chelon saliens* were 90.09±183.02 and 9.73±9.40, respectively. No significant difference (X^2=0.13, df=1, p=0.71) was seen between infection of the two mullet species.

Diplostomum spathaceum metacercariae infected the fish eyes (Fig. 2E); the infection rate of *D. spathaceum* is presented in Tables 2 and 3. Results of Kruskal-Wallis test showed that *D. spathaceum* was not significantly different among the two fish species, sampling years and locations (Table 4).

Mann-Whitney test results indicated that there was no relationship between parasitic infections and fish sexes (Table 5).

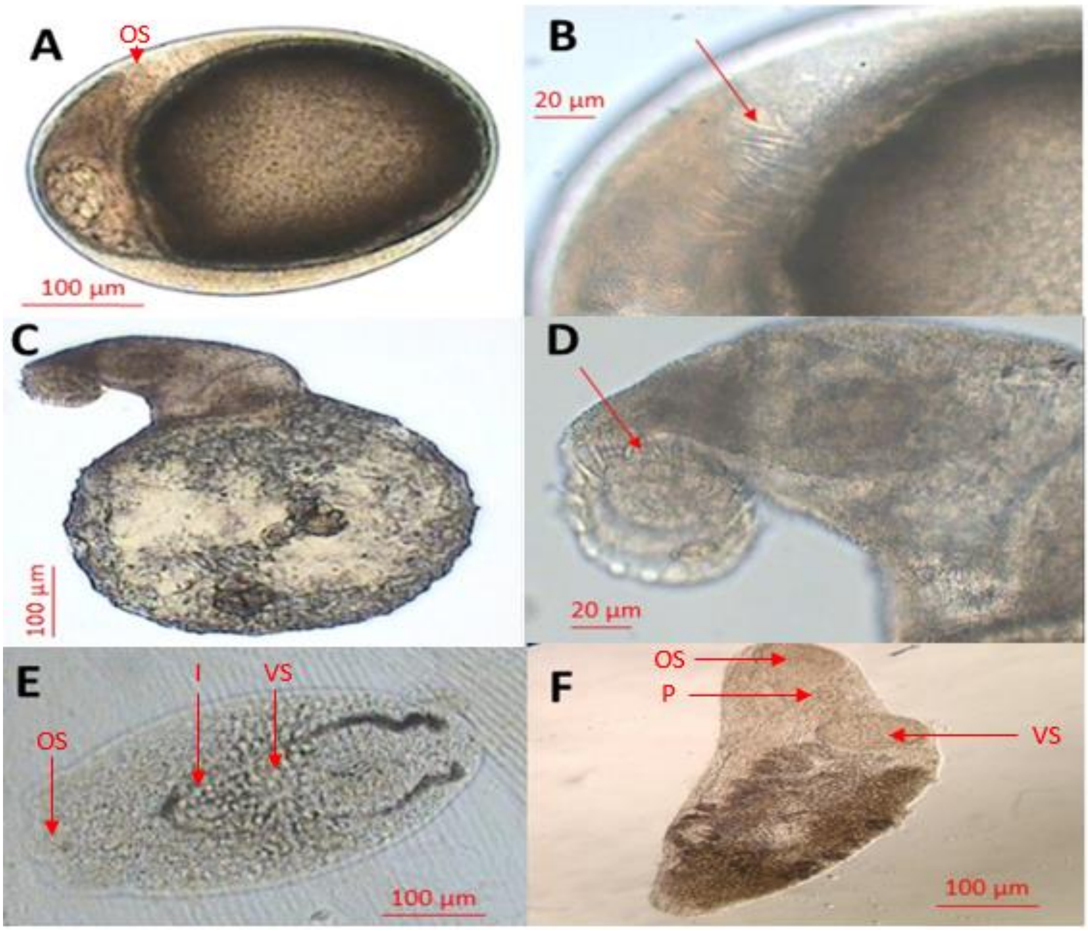

Figure 2: Digenetic trematodes isolated from (*Chelon auratus* and *Chelon saliens*). A and B: encysted metacercariae of *Ascocotyle sp*., oral sucker (OS) with two rows of spines (red arrow). C: excysted metacercariae of *Ascocotyle sp*. D: Two rows of spines (red arrow). E: *Diplostomum spathaceum* metacercariae; OS: oral sucker, I: intestine, VS: ventral sucker. F: wet mount of *Saccocoelium tensum* specimen; OS: oral sucker, P: pharynx.

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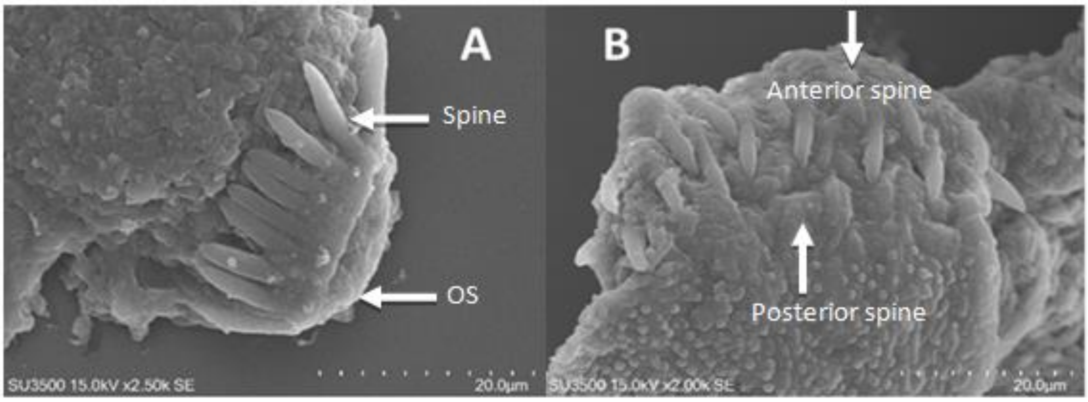

Figure 3: SEM micrograph of, A: Metacercariae of *Ascocotyle (Phagicola) longa*; oral sucker (OS) with a single row of spine (Spine) B: Metacercariae of *Ascocotyle sp.* with two rows of spines around oral sucker (anterior and posterior spines).

Table 5: Results of Mann-Whitney test showing significant differences between digenean parasites and fish gender.

	Fish gender		
	Haploporidae	**Heterophyidae**	**Diplostomidae**
Z	-1.82	-1.19	-0.58
df	1	1	1
Asymp. Sig.	0.68	0.23	0.55

Discussion

Few studies have identified and introduced digenean trematodes of mullets in southern coasts of Caspian Sea. The isolation and identification of the digenetic trematodes of the two mullets were made in four locations of Caspian Sea. Metacercariae of *Diplostomum spathaceum*, *Ascocotyle (Phagicola) longa* and *Ascocotyle sp.* were found from mullets for the first time, and also new information about intensity and prevalence of these parasites were provided.

Diplostomum spathaceum had a high prevalence in different fish species, but in this study it was reported from *Chelon auratus* and *Chelon saliens* of southern coasts of Caspian Sea for the first time. Moghainemi *et al.* (1995) reported the presence of *D. spathaceum* infestation in *Planiliza abu* of Hoor-AL-Azim Lagoon. *Diplostomum spathaceum* is reported in 40 fresh and brackish water fish species (Barzegar *et al.,* 2008). In other parts of the world, *Diplostomum spathaceum* showed a high distribution, Höglund (1991) described the metacercariae of this species in 125 fish species. *Diplostomum sp.* infestation was found in two mullets of *Mugil cephalus* and *Planiliza haematocheilus* in Ukraine (Sarabeev, 2015).

Metacercariae of *Ascocotyle (Phagicola) longa* and *Ascocotyle sp.* metacercariae infected stomach, intestine and gill of examined fish specimens, respectively. *Ascocotyle coleostoma* is reported from *Chelon*

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ramada and *Chelon saliens* of Caspian Sea (Mikailov, 1958). Also, these species are reported from Azerbaijan and Turkmenistan (Ibragimov, 1988; Seidli, 1990; Mamedova and Veliyeva, 2017). *Ascocotyle tenuicollis* metacercariae were isolated from Iran's imported ornamental fish, *Xiphophorus maculatus* (Shoaibi Omrani *et al.*, 2010). *Ascocotyle (Phagicola) longa* infestation is reported in *Mugil incilis* (Galván-Borja *et al.*, 2010), *M. liza* (Simões *et al.*, 2010; Martorelli *et al.*, 2012; Santos *et al.*, 2013), *M. cephalus* (Scholz, 1999; Özer and Kırca, 2015; Masala *et al.*, 2016), *Chelon labrosus*, *C. ramada*, *C. saliens*, *C. auratus* (Masala *et al.*, 2016), *M. curema*, *M. trichodon* (Scholz, 1999), *Mugil liza* (Oliveira *et al.*, 2007). Heterophyid trematodes were also reported from fish-eating birds (Farahnak *et al.*, 2004), human and carnivorous animals (golden jackal, red fox, stray dog and cat) from Khuzestan (Massoud *et al.*, 1981).

Heterophyid parasites are zoonotic and important in point of view of public health and economic loss (Simões *et al.*, 2010; Martorelli *et al.*, 2012). To our knowledge, *Ascocotyle (Phagicola) longa* as a zoonotic fishborne trematode has not been reported from Iranian coasts of Caspian Sea. It is transmitted to humans through the consumption of raw and undercooked fish meat. However, it is found in snails and fish (mullets) as intermediate hosts and fish-eating birds, dogs, cats, pigs as reservoir hosts (Dung *et al.*, 2007). The hosts are common in this country, so there is a need for more attention and research to control *Ascocotyle (Phagicola) longa*.

Other digenetic trematodes species found in this study were *Saccocoelium obesum* and *Saccocoelium tensum* which belong to the family Haploporidae. There are numerous reports of this family in the Caspian Sea. *Saccocoelium obesum* has been reported from mullet fish species, especially *Chelon auratus* and *Chelon saliens* (Mikailov, 1958). *Saccocoelium obesum* has been reported from the intestine of the mullet fish in Iranian waters (Mokhayer 1980; Moghainemi *et al.*, 1995; Taghavi *et al.*, 2013).

Infection prevalence, mean intensity and mean abundance of aforementioned digenean parasites were determined in *Chelon auratus* and *Chelon saliens*, as it is shown in Table 2. As mentioned before most of the introduced parasites, except *Saccocoelium obesum*, were the first to be reported from mullets in this area, therefore there was no comparable data about the infection rate that have occurred. In the present study, infection prevalence of Haploporidae (*S. obesum* and *S. tensum*) in *Chelon auratus* and *Chelon saliens* determined to be 57.8% and 54.4%, respectively. Taghavi *et al.* (2013) reported a prevalence of 10% for *Saccocoelium obesum* in *Chelon auratus*.

Due to some biological differences between fish sexes, there may be parasitic differences between them, but in this study no parasitic infection difference was observed between fish sexes. It is reported that *Saccocoelium*

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obesum showed a higher prevalence in female than male fish (Aydoğdu *et al.*, 2015). Balling and Pfeiffer (1997), Özer *et al.* (2004) and Karvonen and Lindström (2018) observed infection parasites differences between male and female fish. Fish species and locations are other factors that were examined in this study and it was found that fish species do not affect their parasitic infections, in contrast sampling in different locations showed significant infection differences among fish. Sasal *et al.* (1996) described the difference in parasitic infection levels of fish between two protected and unprotected areas. Also, Karvonen and Lindström (2018) showed that the abundance of parasites of sand gobies and common gobies are different in sampling locations.

Chelon auratus and *Chelon saliens* were heavily infected with the digenetic parasites (Heterophyidae, Haploporidae, and Diplostomidae). The prevalence of some trematodes varied spatially between different regions, but, rate of infections was not affected by season and fish sex. On the other hand, expansion of unhygienic fish farming, anthropogenic activity and inappropriate food processing in most parts of the world may increase the distribution of trematode parasites. *Ascocotyl* parasites were reported for the first time from mullets in Iranian coasts of Caspian Sea. Also, these parasites are important in terms of public health and economic issues. Therefore, with an increase in their prevalence, they may cause severe health, economic, and ecological damage and need to be given more attention.

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