

Original Article

Occurrence and intensity of parasites in *Chelon aurata* (Risso, 1810) and *Neogobius caspius* (Eichwald, 1831) (Teleostei: Perciformes) from southern Caspian Sea

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Abstract: This study was performed to investigate ecto- and endo-parasites of golden grey mullet, *Chelon aurata* (Risso, 1810) (N=331) and Caspian goby, *Neogobius caspius* (Eichwald, 1831) (N=170) from the southern Caspian Sea. The sampling was carried out for one year in three stations, including Chamkhaleh (St1), Kiashahr (St2) and Anzali (St3) coastal areas, Guilan Province, Iran. Biometric characteristics were recorded, sexes were determined and specimens examined for ecto- and endoparasites. A total of 158 specimens (58.31%) out of 331 grey mullets, and 61 (35.88%) out of 170 Caspian gobies, were found to be infected. 1453 parasites belonging to 5 species were found consisting of *Trichodina reticulata*, unknown protozoan, nematode larvae (in golden grey mullet) and a cestode, *Eustrongylides excisus* larvae (in Caspian goby). The occurrence of unknown protozoan and nematode larvae in *C. aurata* and cestode larvae in *N. caspius* are reported for the first time in Iran. Seasonal variations, the effects of host length, weight and localities on parasite prevalence and mean intensity have been examined during present investigation.

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Introduction

The mugilids are found in freshwater, brackish and marine regions of the world (Özer and Kirka, 2013). They are known to be euryhaline, therefore are mostly coastal marine fishes and fresh water ones are less in number. Golden grey mullet, *Chelon aurata* (Risso, 1810) feeds on zooplankton, molluscs larvae, other invertebrates, debris, algae and some small aquatics (Fazli et al., 2008). There have been several studies on the parasitic fauna of *C. aurata* reporting some parasites, including *Saccocoelium obesum*, *Capillaria* sp., *Contraecaecum* sp., *Neoechinorhynchus agilis* (in Mitras Lagoon, Sardinia), *Trichodina lepsii* (at the Black Sea coast of Sinop, Turkey), *Ligophorus* spp. (in Eratino lagoon of Greece) (Merella and Garippa, 2001; Özer and Öztürk, 2004; Ragias et al., 2005). However, there are no reports on parasites of this fish species in the Caspian Sea.

Important species of the family Gobiidae in the

Caspian Sea are Monkey goby (*Neogobius fluviatilis*), Caspian goby (*Neogobius caspius*), Bighead goby (*Ponticola gorlap*) and Round goby (*Neogobius melanostomus*) (Daghigh-Roohi and Sattari, 2004). *Neogobius caspius* has wide distribution inhabiting close to the beach in shallow waters. They are benthic feeder so that feed mainly on benthic crustaceans such as Cumaceae, Gammaridae and molluscs (Daghigh-Roohi and Sattari, 2004). Parasites of some Gobiids in the southern Caspian Sea, including *Eustrongylides excisus*, *Dichelyne minutus* and *Corynosomas strumosum*, have been reported in recent works (Pazooki and Aghlmandi, 1999; Daghigh-Roohi and Sattari, 2004; Pazooki et al., 2011). As Gobiids are valuable food items for some commercially important fishes such as sturgeons, then they can transmit parasitic infections.

This study aimed to investigate the prevalence, intensity and composition of parasitic fauna in

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C. aurata and *N. caspius* in the southern Caspian Sea and to determine the influence of seasonal differences, locality, sex, weight and length of host on the prevalence, intensity and composition.

Materials and Methods

This study was carried out for a time period of one year from October 2015 to September 2016. Three sampling sites in the Guilan Province including Chamkhaleh (station 1: St1; 37°13'44.8"N 50°18'58.4"E), Kiashahr (station 2: St2; 37°28'8.6"N 49°59'34.4"E) and Anzali (station 3: St3; 37°29'16.5"N 49°28'35.2"E) were selected. Fish were caught by local fishermen using beach seine net, transported partly alive in tanks and partly as perished specimens in an ice-cooled box. All specimens were left to expire naturally before body dissection.

Fish examination and parasite collection: Total length and body weight of fishes were measured and their gender was determined after dissection. A total of 331 specimens of *C. aurata* and 170 specimens of *N. caspius* were examined for ecto- and endo-parasites using light microscope and stereoscope. The skin, fins, heart, gills, body cavity and visceral organs, including stomach, intestine, liver, swim bladder and gonads were examined. Parasites counting and also methods of fixation, preservation, slide preparation were performed according to Jalali (1999) and Palm and Caira (2008). All specimens were fixed in 10% formalin solution, stained with aqueous acetocarmine, dehydrated and mounted in premount. The worms were identified using parasites identification keys (Yamaguti, 1961; Bykhovskaya-Pavlovskaya et al., 1962; Moravec, 1994; Jalali, 1999). The parasitological parameters i.e. prevalence, mean intensity and abundance were calculated according to Bush et al. (1997). The prevalence (%) was calculated as number of hosts infected / number of hosts examined. The mean intensity was calculated as total number of individuals of a particular parasite / number of infected hosts. The mean abundance is total number of individuals of a particular parasite species in a host species / the total number of hosts examined. The dominance of a parasite species was calculated as N/N_{sum}

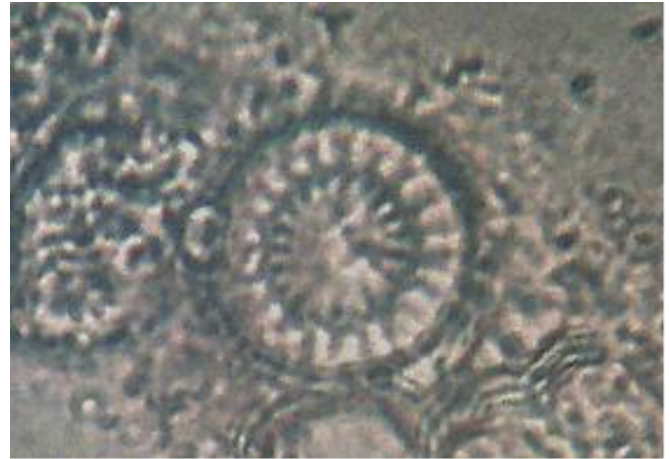


Figure 1. *Trichodina reticulata* (100X).

sum (where N =abundance of a parasite species and N_{sum} =sum of the abundance of all parasite species found) and expressed as a percentage based on Leong and Holmes (1981).

Data analysis: The dominance values were used for classification of parasites as: eudominant (>10%), dominant (5.1-10%), subdominant (2.1-5%), recedent (1.1-2%) and subrecedent (<1.0%) of given species (Niedbala and Kasparzak, 1993). Mean intensity of infection and abundances of parasite species (with prevalence >10%) among seasons and sexes were tested by Kruskal-Wallis test (KW, multiple comparisons) and Mann-Whitney U test (MW, pairwise comparisons). Z test was carried out for prevalence comparison between sexes. The results were considered significant at 95% level ($P<0.05$). Statistical analysis was carried out using SPSS 22 software.

Results

The examined golden grey mullets were 118.73 g (± 25.14 , range=38.2-310 g) in weight and 24.38 cm (± 1.11 , range=15.2-38.5 cm) in total length. The examined Caspian goby were 19.69 g (± 1.34 , range=10.22-65.21 g) in weight and 10.41 cm (± 0.26 , range=6.3-18.2 cm) in total length.

1453 individuals of 5 parasite species consisting of two ectoparasites: *Trichodina reticulata* (Fig. 1) and unknown protozoa (Fig. 2) and three endoparasites: one nematode larvae (Figs. 3, 4) in *C. aurata*, *Eustrongylides excisus* larvae (Figs. 5, 6) and one

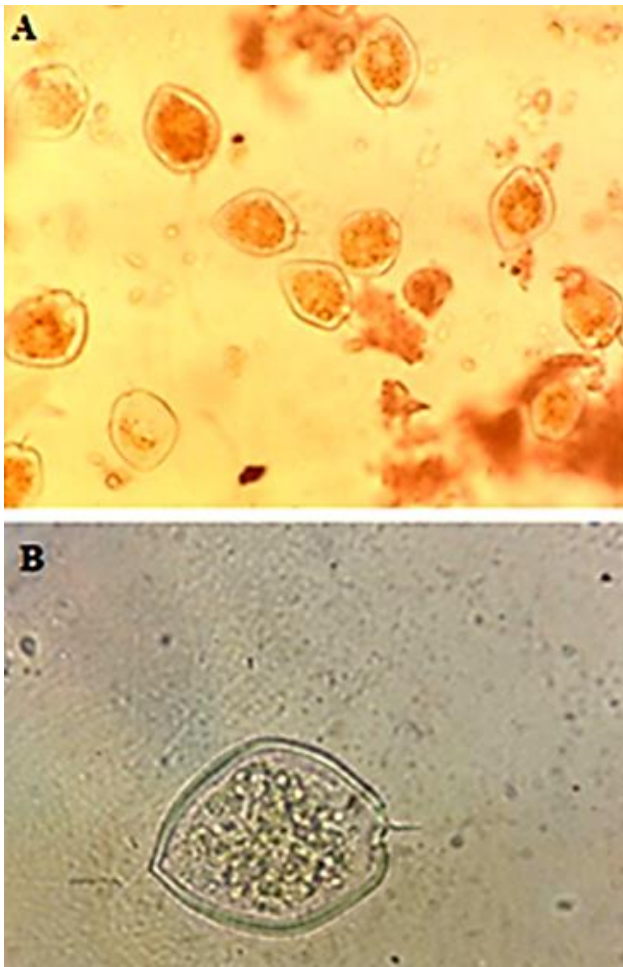


Figure 2. (A) Unknown protozoa (400X) and (B) Unknown protozoa (1000X).

Table 1. The prevalence, mean intensity, range, abundance and dominance of some parasites in *Chelon aurata* (N=331)

Parasite	Prevalence (%)	Mean±SD	Range	Abundance±SD	Dominance
<i>Trichodina reticulata</i> (N ₁ =833)	67.05	4.71±1.31	0-14	3.16±0.94	61.3
Unknown protozoa (N ₁ = 517)	3.78	51.7±26.7	9-170	1.96±26.7	38.04
Nematodes larvae (N ₁ = 8)	2	1.6±0.82	1-2	0.02±0.02	0.59

N1=number of parasite, SD=standard deviation

cestode larvae (Fig. 7) in *N. caspius* were found. The occurrence of unknown protozoans and nematodes larvae in *C. aurata* and also cestode larvae in *N. caspius* were reported for the first time. Prevalence, mean intensity and abundance of the parasites in two fish species are summarized in Tables 1-8.

Trichodina reticulata and unknown protozoa had the highest prevalence, mean intensity, abundance and dominance. The eudominant parasites of *C. aurata* (Table 1) were *T. reticulata* and unknown protozoa (D=61.3% and 38.04%, respectively). The sub-recedent parasites were nematodes larvae (D=0.59%).

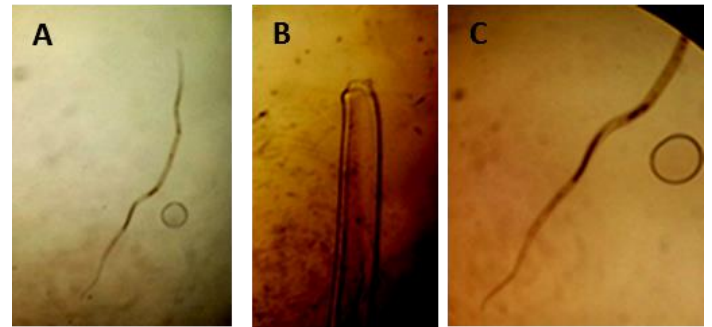


Figure 3. (A) nematode larvae (40X), (B) the anterior of nematode larvae (400X) and (C) the posterior of nematode larvae (100X).

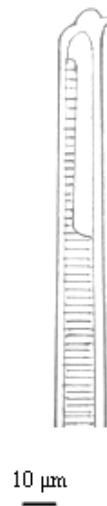


Figure 4. Line drawing of the nematode larvae showing cephalic end in *Chelon aurata*.

The maximum prevalence, mean intensity and abundance of *T. reticulata* were determined to be in autumn (72.18%, 5.42±1.42, 3.91±1.08) and spring (70.59%, 4.38±1.03, 3.09±0.84) (Table 2). The unknown protozoan parasites were observed only in autumn. There was no significance difference between seasons for parasites occurrence (KW, $P>0.05$).

The abundance of *T. reticulata* in St2 was higher than other stations, but differences were not significant (KW test, $P>0.05$), while mean intensity showed a significant relationship between St1 and the other two stations (KW, $\chi^2=8.499$, $df=2$, $P<0.05$).

Table 2. The prevalence, mean intensity, range and abundance of some parasites of *Chelon aurata* (N=331) in different seasons.

Parasite/ Season	<i>Trichodina reticulata</i>		Unknown protozoa		Nematodes larvae	
	Prevalence (%)		Prevalence (%)		Prevalence (%)	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
	Range	Range	Range	Range	Range	Range
	Abundance±SD		Abundance±SD		Abundance±SD	
Autumn N=133	72.18	5.42±1.42	7.52	51.7±26.7	0	0
		0-14		9-170		
		3.91±1.08		11.24±26.7		
Winter N=97	58.76	3.59±1.48		0	0.03	1.33±0.68
		0-10				1-2
		2.14±1.13				0.04±0.05
Spring N=51	70.59	4.38±1.03		0	0.02	2±1.03
		0-9				2
		3.09±0.84				0.04±0.07
Summer N=50		-		-	0.02	2±1.03
						2
						0.04±0.07

SD=standard deviation

Table 3. The prevalence, mean intensity, range and abundance of some parasites of *Chelon aurata* (N= 331) in different localities.

Parasite locality	<i>Trichodina reticulata</i>		Unknown protozoa		Nematode larvae	
	Prevalence (%)		Prevalence (%)		Prevalence (%)	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
	Range	Range	Range	Range	Range	Range
	Abundance±SD		Abundance±SD		Abundance±SD	
Chamkhaleh N=116	66	3.1±1.5	11	51.7±17.66	0	0
		0-10		9-170		
		2.03±1.4		5.6±17.66		
Kiashahr N=107	77	5.3± 1.3		0	0.02	1.5±0.61
		0-14				1-2
		4.1±1.4				0.03±0.1
Anzali N=108	58	5.9±1.6		0	0.03	1.67±0.67
		0-11				1-2
		3.4±1.3				0.05±0.11

SD=standard deviation

(Table 3). There was no significant difference between abundance, mean intensity of the unknown protozoa and nematode larvae among three regions (KW test, $P>0.05$).

The prevalence of *T. reticulata* in females was significantly higher than males, while the prevalence of the unknown protozoa in males was significantly higher than females (Z test, $P<0.05$) (Table 4). The differences between the prevalence of nematodes

larvae in females and males were significant (Z test, $P<0.05$). There was no significant difference between all parasites in terms of mean intensity and abundance (Mann Whitney U test, $P>0.05$).

A total of 95 individuals of 2 parasite species were found in *N. caspicus*. Of those, *E. excisus* had the highest prevalence, mean intensity, dominance and abundance (Table 5). The eudominant parasites of *N. caspicus* were *E. excisus* (D=96.88%) and the

Table 4. The prevalence, mean intensity, range and abundance of parasites of males and females *Chelon aurata*.

Parasite Sex	<i>Trichodina reticulata</i>	Unknown protozoa	Nematode larvae
	Prevalence (%)	Prevalence (%)	Prevalence (%)
	Mean±SD	Mean±SD	Mean±SD
	Range	Range	Range
	Abundance±SD	Abundance±SD	Abundance±SD
Male N=225	65.32	3.47	1.33
	4.17±0.97	6.3± -	1.33±0.68
	0-11	13-170	1-2
Female N=106	2.68±0.42	0.22±0.11	0.02±0.02
	70.33	1.1	1.89
	5.78±1.82	5± -	2±1.03
	0-14	9-101	2
	3.66±1.91	0.05±0.03	0.04±0.05

SD=standard deviation

Table 5. The prevalence, mean intensity, range, abundance and dominance of some parasites in *Neogobius caspius* (N=170).

Parasite	Prevalence (%)	Mean±SD	Range	Abundance±SD	Dominance
Cestode larvae (N ₁ =2)	1.43	2±1.03	2	0.01±0.005	1.05
<i>Eustrongylides excisus</i> (N ₁ = 93)	35.29	1.55±0.35	1-4	0.55±0.14	96.88

N₁=number of parasite, SD=standard deviation.Table 6. The prevalence, mean intensity, range and abundance of some parasites of *Neogobius caspius* (N=170) in different seasons.

Parasite/ Season	Cestode larvae	<i>Eustrongylides excisus</i>
	Prevalence (%)	Prevalence (%)
	Mean±SD	Mean±SD
	Range	Range
	Abundance±SD	Abundance±SD
Autumn N=26	0	11.54
		1.33±0.69
		1-2
		0.15±0.08
Winter N=30	0	3.3
		4±2.07
		2
		0.13±0.07
Spring N=54	1.85	18.52
	1 ± 0.52	3.6±1.86
	1	1-3
	0.02 ± 0.01	0.67±0.35
Summer N=60	0	21.67
		3.92±2.02
		1-4
		0.85±0.44

SD=standard deviation

recent parasite was cestode larvae (D=1.05%) (Table 5).

The cestode larvae was observed only in spring. The abundance of *E. excisus* in summer was significantly higher than other seasons (KW, $\chi^2=8.586$, df=3, $P<0.05$) (Table 6). The cestode larvae

was observed only in St1 (Table 7). The prevalence and abundance of *E. excisus* in St1 were higher than other stations, but differences were not significant (KW test, $P>0.05$). The prevalence of cestode larvae and *E. excisus* larvae in females were significantly higher than males (Z test, $P<0.05$). Mean intensity and

Table 7. The prevalence, mean intensity, range and abundance of some parasites of *Neogobius caspicus* (N=170) in different localities.

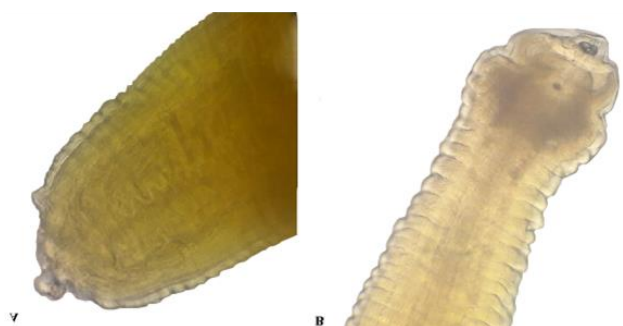
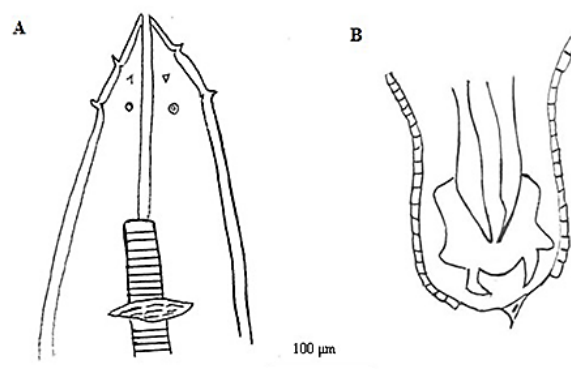
Parasite locality	Cestode larvae		<i>Eustrongylides excisus</i>	
	Prevalence (%)		Prevalence (%)	
	Mean±SD		Mean±SD	
	Range		Range	
	Abundance±SD		Abundance±SD	
Chamkhaleh N=63		2		37
		1±0.34		1.96±0.9
		1		1-4
		0.02±0.09		0.71±0.6
Kiashahr N=51		0		31
				1.44±0.6
				1-2
				0.45±0.3
Anzali N=56		0		38
				1.2±0.3
				1-2
				0.45±0.2

SD=standard deviation

Table 8. The prevalence, mean intensity, range and abundance of some parasites of *Neogobius caspicus* (N=170) in males and females.

Parasite/ Sex	Cestode larvae		<i>Eustrongylides excisus</i>	
	Prevalence (%)		Prevalence (%)	
	Mean±SD		Mean±SD	
	Range		Range	
	Abundance±SD		Abundance±SD	
Male N= 100		0		21
				1.14±0.1
				1-2
				0.24±0.07
Female N= 70		4		54
		1± -		1.79±0.6
		1		1-4
		0.05±0.03		0.97±0.28

SD=standard deviation

Figure 5. (A) The anterior of *Eustrongylides excisus* (400X) and (B) the posterior of *E. excisus* (100X).Figure 6. Line drawings of *Eustrongylides excisus* from *Neogobius caspicus*. (A) Cephalic end and (B) caudal end.

abundance of these parasites in females were higher than males, but differences were not significant (Mann Whitney U test, $P>0.05$) (Table 8).

The results showed a significant positive relation

between length and weight of *C. aurata* and the prevalence of *T. reticulata* (Fig. 8). There was also a significant positive relation between weight of

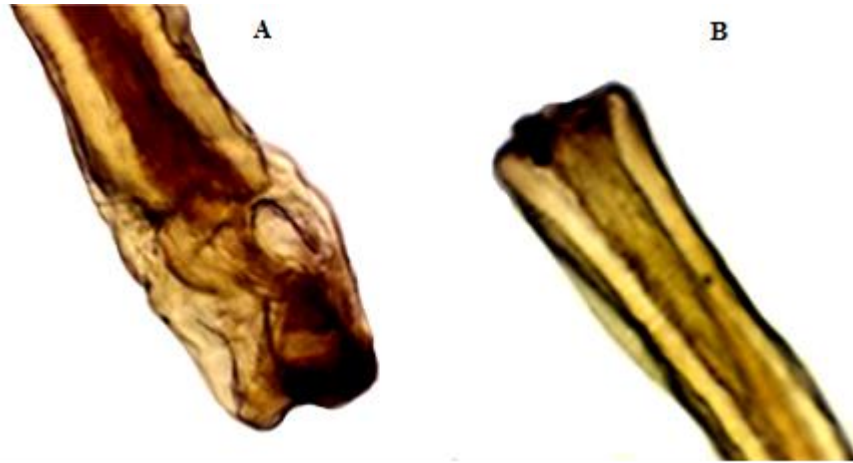


Figure 7. (A) The anterior of cestode larvae (100X) and (B) the posterior of cestode larvae (100X).

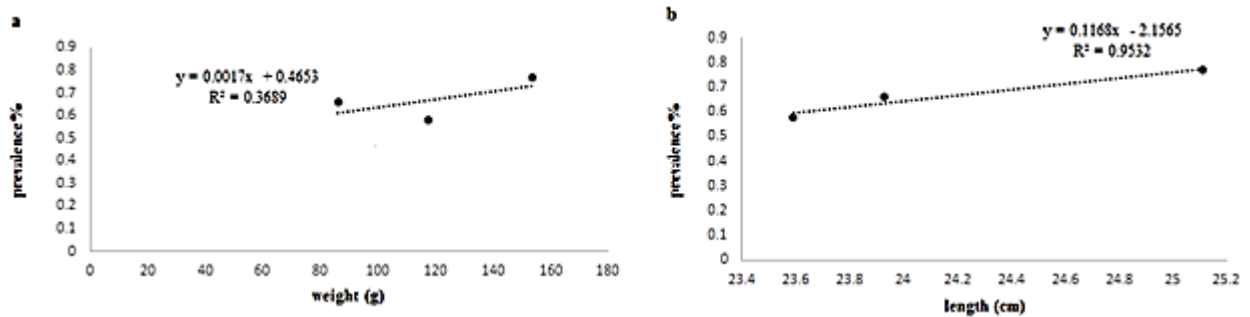


Figure 8. (a) Relationship between weight and prevalence of *Trichodina reticulata* in *Chelon aurata* and (b) relationship between length and prevalence of *Trichodina reticulata* in *Chelon aurata*.

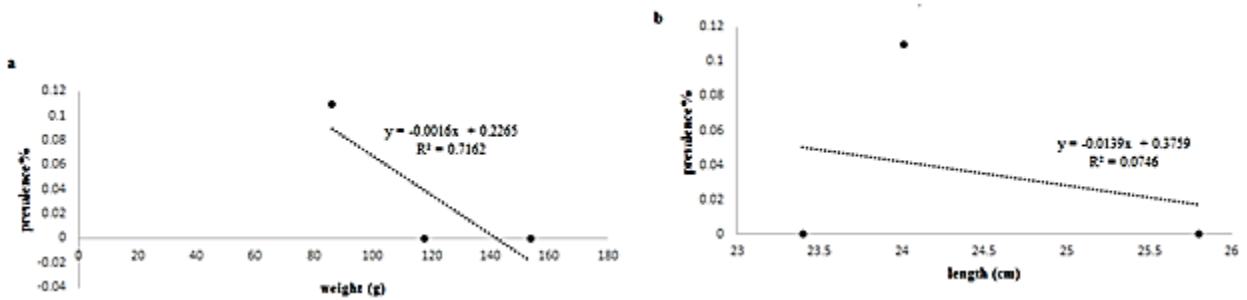


Figure 9. (a) Relationship between weight and prevalence of unknown protozoa in *Chelon aurata* and (b) relationship between length and prevalence of unknown protozoa in *Chelon aurata*.

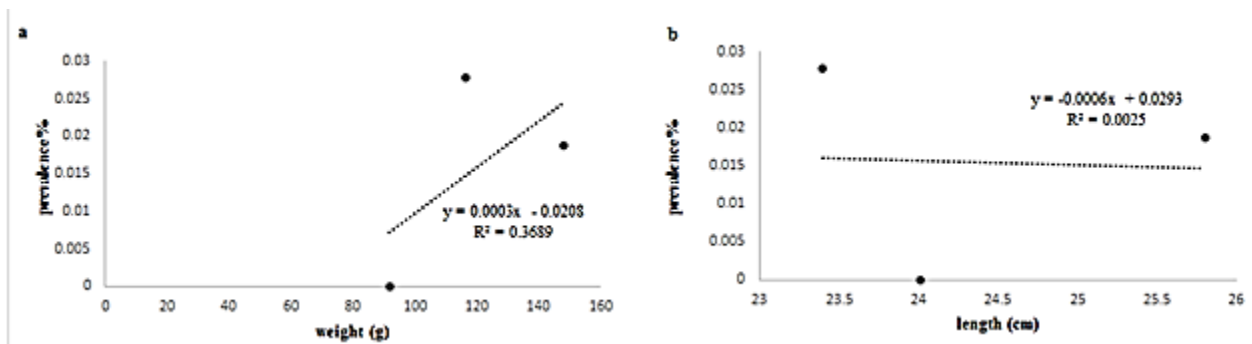


Figure 10. (a) Relationship between weight and infection of nematodes larvae in *Chelon aurata* and (b) relationship between length and infection of nematodes larvae in *Chelon aurata*.

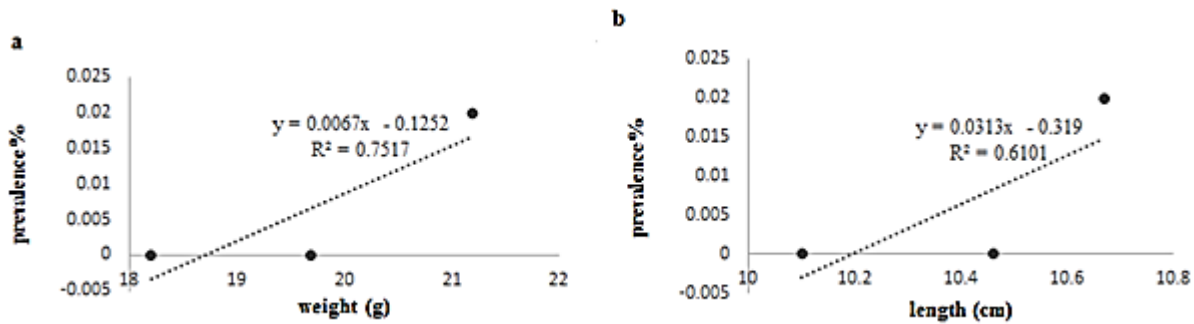


Figure 11. (a) Relationship between weight and infection of cestode larvae in *Neogobius caspius* and (b) relationship between length and infection of cestode larvae in *Neogobius caspius*.

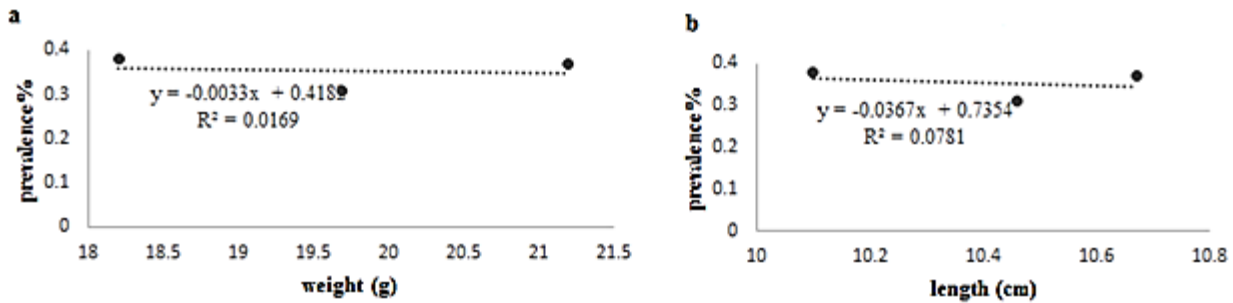


Figure 12. (a) Relationship between weight and infection of *Eustrongylides excisus* in *Neogobius caspius* and (b) relationship between length and infection of *Eustrongylides excisus* in *Neogobius caspius*.

C. aurata and the prevalence of unknown protozoa but no significant relationship was observed by length (Fig. 9). There was also significant relationship between weight of *C. aurata* and the prevalence of nematode larvae but no relation was found between length and the prevalence of these parasites (Fig. 10). The results also showed a significant relationship between length and weight of *N. caspius* and the prevalence of cestode larvae (Fig. 11). There were no relationships between length and weight of *N. caspius* and the prevalence of *E. excisus* (Fig. 12).

Discussion

In the present study, *T. reticulata*, unknown protozoa and nematode larvae were identified in *C. aurata*. There is one report concerning to infection of *C. aurata* by parasites in Iran (Taghavi et al., 2012) where four parasites, including *Ichthyobodo necator*, *Ichthyophthirius multifiliis*, *Trichodina* sp. and *S. obseum* have been reported. The occurrence of unknown protozoa and nematodes larvae in *C. aurata* is reported for the first time from Iran.

The occurrence of *Trichodina* sp. has been reported from other fish species in the southern Caspian Sea,

including *Cyprinus carpio*, *Blicca bjoerkna* and *Tinca tinca* from Anzali wetland (Sattari, 1996). There were also some reports about occurrence of *T. reticulata* in Iran (Mokhayer, 1972; Adel et al., 2015; Moghaddam, 2015). Ectoparasites are much affected by water conditions. In contrast, endoparasites need intermediate hosts to complete their life cycle and as a result, their infection process is relatively stable compared to fish ectoparasites (Özer and Krica, 2013). Trichodinids are ciliates belonging to the family Trichodinidae. These ciliates infest many of marine and freshwater fishes in many parts of the world. The genus *Trichodina* could be found on the gills and body surface (Baker, 2007). In the present study, the prevalence of *T. reticulata* was highest in Kiashahr (St2, 77%) and lowest in Anzali (St3, 58%). Such variations could be related to the differences in environmental parameters.

Female fishes have shown more infestations than the males. This may be related to specific physiological conditions of females. In addition, their increased food intake as a result of eggs development process might have exposed them to more contact with the parasites (Omeji et al., 2011). Similar results were

obtained by Holden and Reed (1972), Adebajo (1979) and Emere and Egbe (2006).

There was a positive significant relationship between weight and length of *C. aurata* and prevalence of *T. reticulata*. Thus, the larger fishes were more sensitive compared to the smaller ones. There is significant relation between weight of *C. aurata* and the prevalence of nematodes larvae. This could be a result of covering wider areas in search of food by larger fishes (Omeji et al., 2011). As a result, they susceptible to higher contamination through food rather than the smaller ones.

Parasites infection in different seasons showed contradictory results. In the present study, there was no significant difference between infections caused by parasites and different seasons. Similar result was obtained by Jerônimo et al. (2011). On the other hand, Chanda et al. (2011) reported that the mortality rate was 100% in fish infected by *Ichthyophthiriasis* in low temperature, whilst the number was reduced when temperature increased. Majumdar et al. (2013) and Hossain et al. (2008) reported dependence of the protozoan parasites to seasonal changes in temperature.

Daghigh-Roohi and Sattari (2004) reported three parasite species from Caspian goby consisting of 2 nematodes (*E. excisus* and *Dichelyne minutus*) and one acanthocephalan (*C. strumosum*). In the present study, two parasites, including one nematode larvae (*E. excisus*) and one cestode larvae were found in *N. caspius*, of those, cestode larvae is reported for the first time from this fish in Iran. The genus *Eustrongylides* (Jägerskiöld, 1909) include three species of *E. tubifex*, *E. ignotus* and *E. excisus*, which are found in the gut of aquatic birds and uses oligochaetes as first intermediate hosts, fish as second intermediate hosts and likely amphibians and reptiles are second intermediate/paratenic hosts (Moravec, 1994; Friend and Franson, 1999; Lezama and Sarabia, 2002). Karmanova (1968) reported the roach (*Rutilus rutilus*), Bighead goby (*Ponticola gorlap*) and Round goby (*N. melanostomus*) as obligatory second intermediate hosts for *E. excisus* in estuary of the Volga River. Daghigh-Roohi and Sattari (2004)

reported Monkey goby (*N. fluviatilis*) and Caspian goby (*N. caspius*) as obligatory second intermediate hosts for *E. excisus*. This parasite was also reported from fish families such as Acipenseridae, Cyprinidae and certain Gobiidae (Sattari et al., 2003; Pazooki et al., 2011). Dogiel and Bykhovskiy (1939) stated that transmission of *E. excisus* larvae to acipenserids can lead to severe damages to their muscles. According to Dubinin (1952), *E. excisus* larvae are very pathogenic for fishes, as a result, Gobiidae are important as their intermediate host. Noteworthy, these larvae are found in the abdominal cavity, ovary, testis, under the skin and between the muscles.

In the present study, *E. excisus* was found in *N. caspius* with high prevalence, mean intensity and abundance. The occurrence of cestode larvae and *E. excisus* larvae in females were higher than males which may be related to physiological conditions of females as mentioned in *C. aurata*. There is a significant difference between the abundance of *E. excisus* and different seasons which might be due to the ecological and nutritional conditions in different seasons (Reimchen and Nosil, 2001). Furthermore, seasonal pattern varies with water temperature and dissolved oxygen, so that high prevalence of *E. excisus* may be related to high temperature and low dissolved oxygen. These results are in agreement with findings of Banu et al. (1993), Hossain et al. (1994), Akhter et al. (1997), Chandra et al. (1997) and Steinauer and Font (2003).

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چکیده فارسی

میزان شیوع و شدت آلودگی انگلی ماهی کفال طلایی (*Chelon aurata*) و گاوماهی خزری (*Neogobius caspius*) در بخش جنوبی دریای خزر

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چکیده:

این مطالعه برای بررسی انگل‌های خارجی و داخلی ماهی کفال طلایی (*Chelon aurata*) و گاوماهی خزری (*Neogobius caspius*) در جنوب دریای خزر صورت گرفت. نمونه‌برداری به مدت یک سال در سه منطقه‌ی ساحلی جنوب دریای خزر (استان گیلان، ایران) شامل چمخاله، کیاشهر و انزلی انجام شد. ویژگی‌های زیست‌سنجی ثبت و جنس ماهیان تعیین شدند. سپس برای بررسی انگل‌های خارجی و داخلی مورد آزمایش قرار گرفتند. ۱۵۸ نمونه (۵۸/۳۱٪) از مجموع ۳۳۱ عدد ماهی کفال طلایی و ۶۱ نمونه (۳۵/۸۸٪) از ۱۷۰ عدد گاوماهی خزری آلودگی داشتند. به‌طور کلی ۱۴۵۳ انگل متعلق به ۵ گونه شامل تریکودینا رتیکولاتا، یک پروتوزوای ناشناخته، نوزاد نماتد (در کفال طلایی) و نوزاد استرونیلیدس اکسیسوس (در گاوماهی خزری) یافت شدند. انگل‌های پروتوزوای ناشناخته و نوزاد نماتد در ماهی کفال طلایی و نوزاد سستود در گاوماهی خزری برای اولین بار در ایران گزارش می‌شوند. در این مطالعه، تنوع فصلی، اثرات طول و وزن میزبان و نیز ایستگاه‌ها روی شیوع و میانگین شدت انگل مورد بررسی قرار گرفته است.

کلمات کلیدی: انگل خارجی، انگل داخلی، کفال ماهیان، گاوماهیان.