Survey on the metazoan parasites in *Neogobius* fishes from Southeastern part of the Caspian Sea

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Abstract

In this study a parasitological investigation on 238 samples of Neogobius species from the south-east of Caspian Sea was carried out. The samples including *Neogobius melanostomus* (35), *N. fluviatilis pallasi* (103), *N. kessleri gorlap* (70) and *N. bathybius* (30) were seasonally seined by beach net during one year (summer 2006-spring 2007), fixed in 10 % buffer formalinand transferred to the laboratory. From 231 of examined fishes 235 specimens were infected (97/6 %). The collected parasites were as follows: *Hysterothylacium aduncum, Raphidascaris acus, Cucullanus sphaerocephalus, Dichelyne minutus, Raphidascaroides sp., Cystidicola sp,. Gyrodactylus sp.,* and *Corynosoma strumosum.* Hereby, *N.melanostomus* is introduced as a new host for *Raphidascaroides sp., C. sphaerocephalus* and *Cystidicola sp.. N. fluviatilis pallasi* and *N. kessleri gorlap* for *C. sphaerocephalus, Cystidicola sp.* and *H .adunacum. N. bathybius* for *C. sphaerocephalus, Cystidicola sp., H. aduncum, E.excisus, R. acus* and *C. strumosum. Cystidicola sp.* and *H. adunacum* were reported for the first time from Iran. Among the investigated fishes *N. fluviatilis pallasi* possessed the highest and *N. bathybius* the lowest variety in infection with various species of parasites. The results of this study indicated that there are significant relations between total length (T.L.) and weight in *N. fluviatilis pallasi* with the number of *C. sphaerocephalus, D. minutus* and *E. excises.* Also there are significant relations between total length and weight in *N. melanostomus* and between weights in *N. kessleri gorlap* with the number of *C. sphaerocephalus.*

Keywords: Neogobiidea, Nematoda, Acanthocephala, Monogena, Caspian Sea

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Introduction

Many studies have been conducted on parasite fishes in the Northern part of the Caspian Sea; Bykhovskaya-Pavlovskaya et al. (1962), Lomaki (1970), Gibson (1972), Mikailov (1975), Gussev (1985) and Bauer et al. (2002). In Iran, also, many researches have been done on parasite fishes of the Southern part of the Caspian Sea by Eslami et al. (1972), Mokhayer and Anwaar (1973), Eslami and Kohneshahri (1978), Mokhayer (1983), Ghorogi and Pourgholam (1996), Pazooki and Masoumian (2002), Sattari et al. (2002 , 2003), Khara et al. (2005) and Nik-Syrat et al. (2006). Alltogether 129 species of parasites were reported from Neogobiidae fishes in the Northern and Southern part of the Caspian Sea; 126 species from *N. melanostomus*, 17 from *N. kessleri* and 13 from *N. fluviatilis*. Acipenseridae fishes of the Caspian Sea are very important as commercial fishes. The role of Neogobiidae fishes in the food chain of Acipenseridae, are emphasized. The aims of the research are to investigate parasitic fauna of Neogobiidae fishes in the Southern part of the Caspian Sea, to find out the relations between parasitic infections and length and weight of the examined fishes and also to survey on intensity and prevalence of the infections. The relations between the seasonality and the sex of hosts with infections are discussed as well.

Materials and methods

This study has been done in four seasons from summer 2006 to spring 2007. A total of 238 samples of four different Neogobius species including *Neogobius melanostomus* (35), *N. fluviatilis pallasi* (103), *N. kessleri gorlap (*70) and *N. bathybius* (30) were seined by beach nets. The samples were collected from Southern part of the Caspian Sea, North of Golestan Province, fixed in 10 % buffer formalin, transferred to the laboratory and weighted and measured. Then, they were examined for different parasites. The collected parasites were identified according to Bykhovskaya et al., 1964, Moravec, 1994 and Jalali, 1999. Data analyses were done according to SPSS15 and SPSS9. The percentage of prevalence infections in each fish and season were done as well. Table 1 indicates the sex, length and weight of the examined fishes in each season.

Results

During this study 9 different genera of parasites were detected from 231 fish (97.6%); *Gyrodoctylus, Cucullanus, Dichelyne, Cystidicola, Eustrongylides, Hysterothylacium, Raphidascaris, Raphidascoroides* and *Corynosoma* spp. were detected and only 7 fish specimens (2.4%) were not infected. Table 2 indicates the names of different parasites in their hosts.

Gyrodacylus sp.

Gyrodactylus sp. was found in the gill of *N. fluviatilis pallasi* only in the winter. The dimensions of the parasite were: length 0.36 mm, width 0.102 mm, ir (Inneroot) 0.0243 mm, vb (Ventral anchor) 0.019 and pr (Point recure) 0.016 mm. The prevalence was 10 % (2) (Fig. 1; A, B, C).

Dichelyne minutes (Rudolphi, 1819)**.**

This parasite was only collected from the intestine of the *N. fluviatilis pallasi* in the summer. The body length was 1.76-3.17 mm, width was 0.29-0.51

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mm, length of the pharynx was 0.44-0.64 mm, and the conical tail was 0.071-0.16 mm. The prevalence was 36% (11), the mean intensity 3.5 and the range was 1-7 (Fig. 1; D, E, F). Table 3 indicates infections in different seasons.

Table 1: The sex, length and weights of the examined fishes in each season

Fishes	Sex	No.	Summer 2006	Autumn 2006	Winter 2006	Spring 2007	Total No.	Length Cm	Weigh gr
N. melanostomus	♂	**10**	35	-	-	-	35	4.8-8.9	1.5-10.8
	♀	**25**							
N. fluviatilis pallasi	♂	**53**	30	32	20	21	103	6.9-25.3	4.73-268.8
	♀	**50**							
N. kessleri gorlap	♂	**43**	-	33	19	18	70	16.4-27.9	88-292.6
	♀	**27**							
N. bathybius	♂	**25**	-	-	10	20	30	20.7-30	101.2-296.2
	♀	**5**							

Table 2: The different parasites in 4 *Neogobius* fishes

parasites	*N. melanostomus*	*N. fluviatilis pallasi*	*N. kessleri gorlap*	*N. bathybius*
Gyrodactylus sp.	-	+	-	-
C. sphaerocephalus	+	+	+	+
Cystidicola sp.	+	+	+	-
D. minutus	-	+	-	-
E. excisus	-	+	+	+
H. aduncum	+	+	+	-
R. acus	+	+	+	+
Raphidascarides sp.	+	-	-	-
Corynosoma strusoma	-	+	+	+

***Cucullanus sphaerocephalus* (Rudolphi, 1809)**

This parasite was collected in four seasons and in the intestine of four hosts. The body length was 3.3-20.6 mm and the maximum width was 0.1-0.4 mm. The length of the pharynx and spicules were 0.44-0.64 mm and 0.3-0.5 mm respectively. The genital pole of the females was 6.5-10.3 mm from the posterior end. The conical tail was 0.2-0.5 mm. The prevalence was 36% (11), the mean intensity 3.5 and the range was 1-7

***Hysterothylacium adunacum* (Rudolphi, 1802)**

In this research 14 of these parasites were collected from the intestine of *N. fluviatilis pallasi*, (in summer and winter) and *N. kessleri gorlap* (in autumn, winter and spring). The cysts of parasites were in the intestinal wall. The body length and

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maximum width were 1.7-2.9 mm and 0.07-0.3 mm respectively. The posterior part of the conical tail was covered with spin cuticle and the length was 0.2 mm. The length of the pharynx was 0.1-0.7 mm. The parasites have downward secum, the length of it was 1/3 of the pharynx length. The intestine was dark and direct. (Fig. 2; A, B). Table 3 indicates infections in different seasons.

***Raphidascaris acus* (Bloch, 1770)**

In this research 6 of the parasites were collected from the intestinal wall of *N. melanostomus* and *N. fluviatilis pallasi*, (in autumn), *N. kessleri gorlap* (in autumn and winter) and *N. bathybius* (in winter). The body length was 3.29 mm, the maximum width 0.2 mm, the length of the downward secum 0.45 mm and the pharynx length was 0.357 mm. The nerve ring was 0.153 mm from the interior end of the body. The tail was conical and short (Fig.2; C, D). Table 3 indicates infections in different seasons.

Raphidascaroides sp.

This parasite was found only in the abdominal cavity of two *N. melanostomus* in the summer. The body length and maximum width were 22.5-24 mm and 0.51 mm respectively. The body surface was covered with very thick cuticle. The pharynx length was 3.14 mm and the conical tail 0.188 mm. (Fig. 2; E, F). The prevalence of infections was 5.2% and the mean intensity was 1.

***Eustrongylides excises* (Jägerskiöld, 1909)**

The third larvae stage of this parasite was found only in *N. melanostomus* and *N. fluviatilis pallasi* in autumn, winter and spring. The body length and maximum width were 20-30 mm and 0.3-0.8 mm respectively. The apical end of the body was round with 12 papillae in two row circles. The cuticle was rather thick and the genital pore was opened in the end of the body. The color of these parasites was dark red. The mesentery muscles, intestine and abdominal cavity of fishes were infected by cysts and the larvae of parasites (Fig. 3; A, B). Table 3 indicates infections in different seasons.

Cystidicola sp.

In this study 8 specimens of these parasites were found in the intestine of *N. melanosomus, N. fluviatilis pallasi* and *N. kessleri gorlap* in autumn. The body length, maximum width and the pharynx length were 0.31-0.46 mm, 2.75 mm and 0.15-0.2 mm respectively. The tail was round and the length was 0.105-0.16 mm. The abdomen was elongated and narrow. The nerve ring up to the anterior end was 0.18 mm. The prevalence of infections in *N. melanostomus* (in summer), *N. fluviatilis pallasi* and *N. kessleri gorlap* (in autumn) were 2.8%, 3.1% and 3% respectively (Fig. 3; C, D).

***Corynosoma strumosum* (Rudolphi, 1802)**

This parasite was collected from *N. fluviatilis pallasi, N. kessleri gorlap* and *N. bathybius* in winter and spring. The body length was small, males and females were the same size; 75 mm. The rostrum had 18 torn rows, in each 10 to 12 torn. The fifth and seventh rows were big (Fig.3; E, F). Table 4 indicates infections in winter and spring.

The relation between length and weight of fishes with infection

The results show that there is a significant direct positive relation, between length and weight of *N. melanostomus, N. fluviatilis*

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pallasi and *N. kessleri gorlap* with infections of *Cucullanus sphaerocephalus* (Figs. 4-10). There is also a significant direct negative relation, between length and weight of *N. fluviatilis pallasi* with infections of *Dichelyne minutus* (Figure 10-11). The results also show that there is a significant relation between infections of *C. sphaerocephalus*, *E. excisus*, and *D. minutus* in different seasons (Tables 5, 6).

Table 3: Infections of Nematoda in different seasons*

Seasons	Fs	IF	%	NP	MI	IF	%	NP	MI	IF	%	NP	MI	IF	%	NP	MI
		Cs	*Cs*	*Cs*	*Cs*	*Ha*	*Ha*	*Ha*	*Ha*	*Ra*	*Ra*	*Ra*	*Ee*	*Ee*	*Ee*	*Ee*	*Ee*
Summer	*Nm*	29	83	357	12	1	3	1	1	1	3	1	1	-	-	-	-
2006	*Nfp*	18	60	72	4	4	13	8	2	1	3	2	2	-	-	-	-
Autumn	*Nkg*	23	70	121	5	2	6	2	1	1	3	1	1	25	75	80	3
2006	*Nfp*	28	88	155	6	-	-	-	-	-	-	-	-	20	63	37	2
Winter	*Nkg*	18	95	227	13	1	5	1	1	1	5	1	1	13	68	54	4
2006	*Nfp*	14	70	229	16	1	5	1	1	-	-	-	-	11	55	36	3
	Nb	9	90	136	15	-	-	-	-	1	10	1	1	5	50	5	1
Spring	*Nkg*	12	67	93	8	1	6	1	1	-	-	-	-	11	61	45	4
2007	*Nfp*	10	48	18	2	-	-	-	-	-	-	-	-	10	68	24	2
	Nb	11	55	77	7	-	-	-	-	-	-	-	-	9	45	40	4

*Fs: Fish species, IF: Infected fishes, *C s: Cucullanus sphaerocephalus, Nm: N. melanostomus,* %: Prevalence of infections, *H a: Hysterothylacium adunucum, N f P: N. fluviatilis pallas,* NP: Number of parasites, *Ra: Raphidascaris acus, N kg:N. kessleri gorlap,* MI: Mean intensity, *E e: Eustrongylides excises, N b: N. bathybius*

Table 4: Infections of *Corynosoma strumosum* in different seasons*

Seasons	Fs	IF	%	NP	MI
Winter	*N. k. g*	5	26.3	7	1.4
2006	*N. f. P*	2	10	3	1.5
	N. b	1	10	1	1
Spring	*N. k. g*	3	16.7	4	1.3
2006	*N. f. P*	2	9.5	3	1.5
	N. b	4	20	5	1.2

*Refer to the abbreviations of table 3

Table 5: Difference of infections in different season*

Seasons	Fs	IF	%	NP	MI
Winter 2006	*N. k. g.*	5	26.3	7	1.4
	N. f. P	2	10	3	1.5
	N. b	1	10	1	1
Spring 2006	*N. k. g.*	3	16.7	4	1.3
	N. f. P	2	9.5	3	1.5
	N. b	4	20	5	1.2

*Refer to the abbreviations of table 3

Table 6: Differences between infections in different hosts' sex*

Parasites	Chi-square			df			Asymp.sig		
	N.f.p.	*N.k.g.*	*N.b*	*N.f.p.*	*N.k.g.*	*N.b*	*N.f.p.*	*N.k.g.*	*N.b*
C. sphaerocephalus	1.586	0.102	0.480	1	1	1	0.208	0.750	0.488
E. excisus	0.713	0.347	0.107	1	1	1	0.398	0.556	0.743
D. minutus	0.178	---	---	1	---	---	0.673	---	---
Corynosoma strumosum	0.004	0.498	1.500	1	1	1	0.953	0.480	0.221

*Refer to the abbreviations of table 3

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Figure 1: A; *Gyrodactylus sp*., B; Opistohaptore of *Gyrodoctylus sp*., C; The anterior of *Gyrodactylus sp*.; D; The anterior of *Dichelyne Minutus*, E; The posterior of *D. Minutus*, F; *Cucullanus sphaerocephalus*

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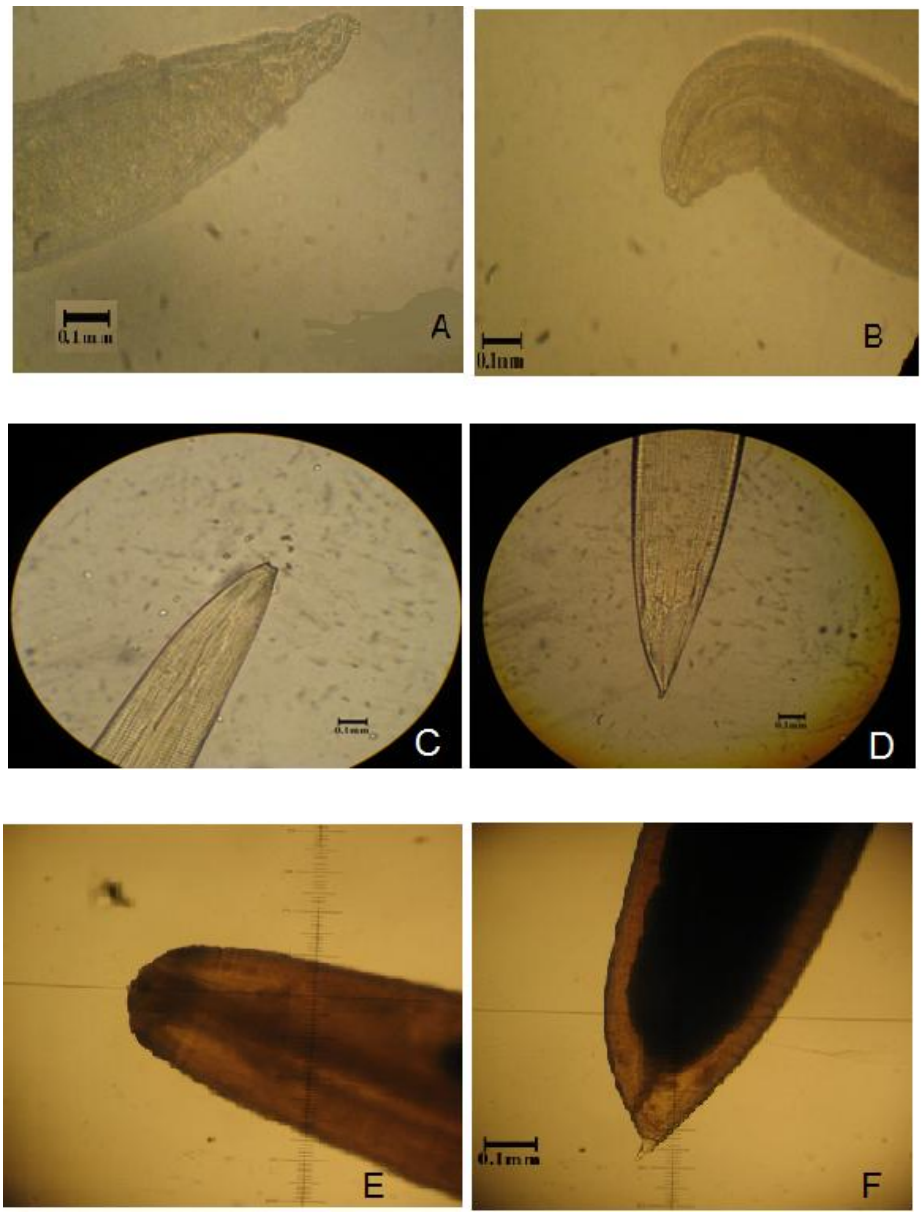

Figure 2: A; The posterior of *Hysterothylacium aduncum*, B; The anterior of *H. aduncum*, C; The anterior of *Raphidascaris acus*, D; The posterior of *R. acus*., E; The anterior of *Raphidascaroides sp*., F; The posterior of *Raphidascaroides sp*.,

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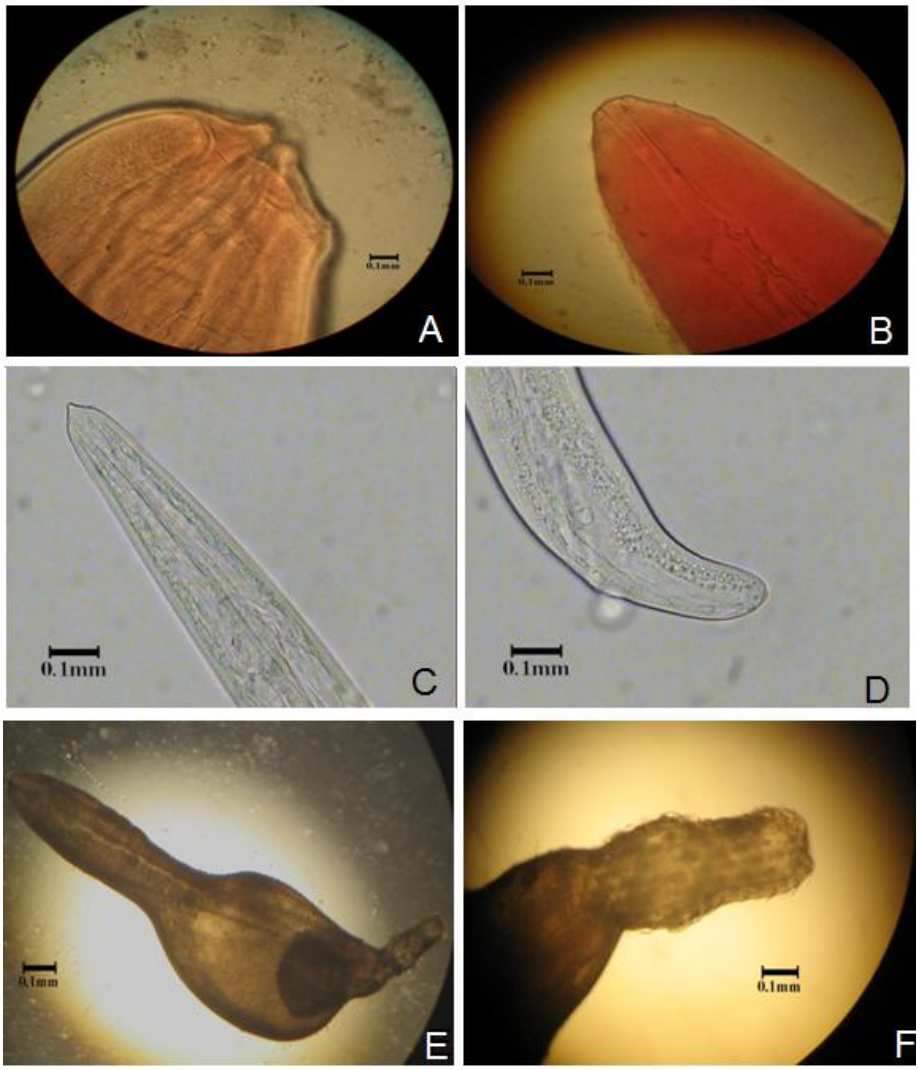

Figure 3: A; The anterior of *Eustrongylides excisus,* B; The posterior of *E.excisus*, C: The anterior of *Cystidicola sp.* D: The posterior of *Cystidicola sp.*, E; *Corynosoma strumosum*, F; The rostrum of *Corynosoma strumosum*

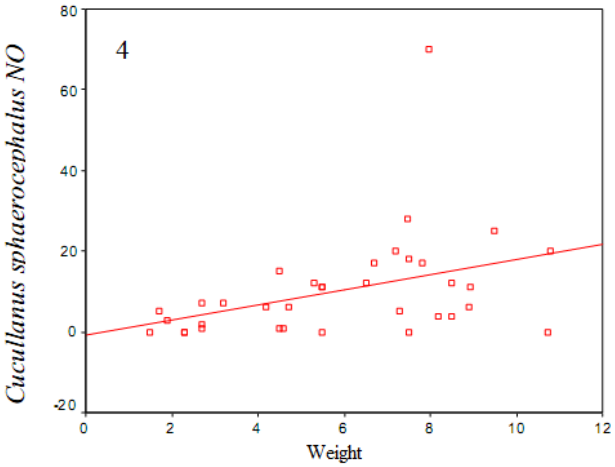

Figure 4: Relationship between weight and infection of *C. sphaerosephalus* in *N. melanostomus*

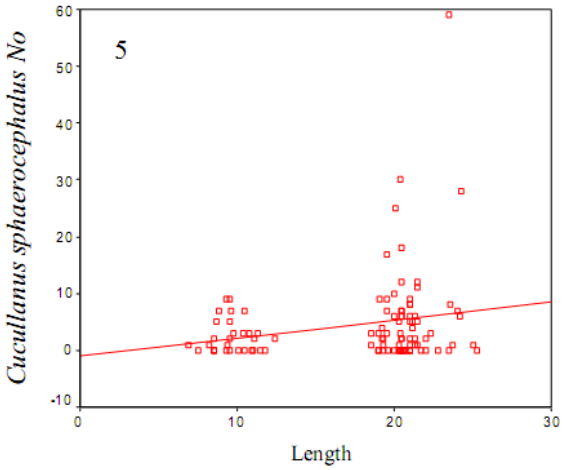

Figure 5: Relationship between length and infection of *C. sphaerosephalus* in *N. fluviatilis pallasi*

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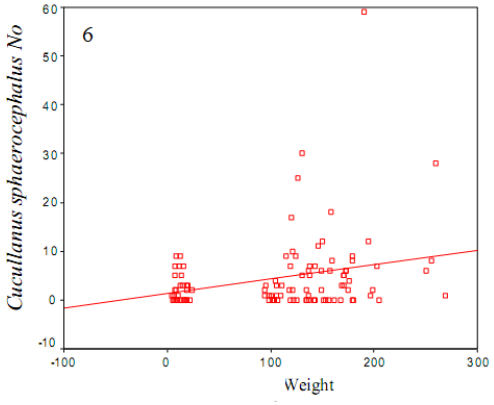

Figure 6: Relationship between weight and infection of *C. sphaerosephalus* in *N. fluviatilis pallasi*

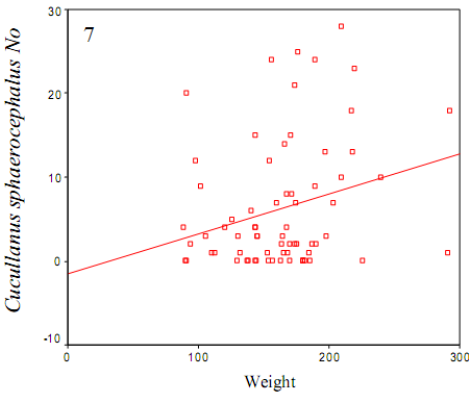

Figure 7: Relationship between weight and infection of *C. sphaerosephalus* in *N. kessleri gorlap*

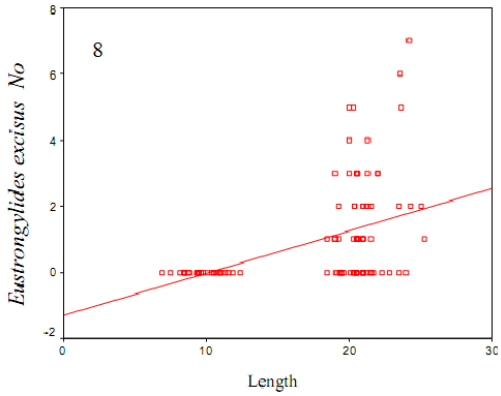

Figure 8: Relationship between length and infection of *E. excisus* in *N. fluviatilis pallasi*

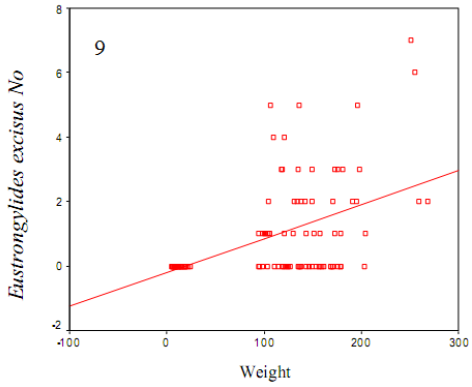

Figure 9: Relationship between weight and infection of *E. excisus* in *N. fluviatilis pallasi*

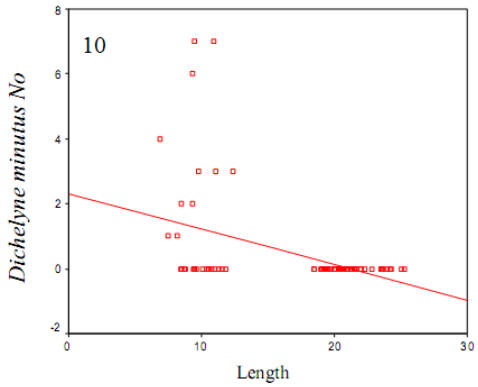

Figure 10: Relationship between length and infection of *D. minutes* in *N. fluviatilis pallasi*

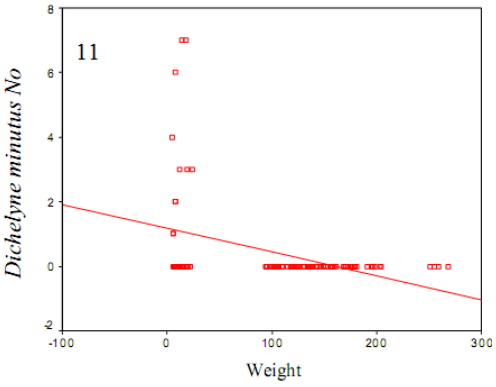

Figure 11: Relationship between weight and infection of *D. minutus* in *N. fluviatilis pallasi*

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Discussion

The fish sampling area is located in the Sarmatian Fauna Region, which is one of the three faunal regions of freshwater fishes of Iran. Neogobiidae fishes are spread through this region. It is expected that the parasitic fauna in the sampling area are the same as the neighboring territories. In Iran only a few researches were done on Neogobiidae parasites (Pazooki and Aghmandi, 1999, Daghigh-Rouhi and Sattari, 2004). The collected parasites found in this study are the same as the ones found in other examinations made in the basin of Northern parts of the Caspian Sea, Black Sea, Azov and other rivers. During this study, *Gyrodactylus* sp. was reported for the first time from the gills of *N. fluviatilis pallasi* in Iran. Before this study only a single *Gyrodactylus* sp., *G. protesrohini* was reported from the Northern part of the Caspian Sea; from *N. melanostomus* in the Russian and Turkish part of the Black Sea and Azov, Danube River, and from *N. kessleri gorlap*, in Danube River (Naydenova, 1974; Gaevskaya et al., 1975; Ondrachova et al., 2005; Oumlzer 2007).

Another parasite *Cucullanus sphaerosephalus*, was collected from 4 species of Neogobiidae. Up to this time only *C. heterochrous* has been reported from *N. melanostomus* from the Black Sea (Naydenova, 1974; Gaevskaya, et al., 1975). This parasite is specific for Acipenseridae and was reported from Europe (Bauer et al., 2002) and from the Iranian part of the Caspian Sea (Mokhayer and Anwar, 1973; Ghoroghi and Pourgholam, 1995; Sattari et al., 2002). Finding this parasite from both Neogobiidae and Acipenseridae shows that, the first fish group has an important role of transferring the parasite to the second one. Because of a significantly positive relation between the length and weight by infections, the bigger infected fishes are more sensitive than the smaller ones.

The next parasite, *Cystidicola sp.* was found in the intestine of *N. melanostomus*, *N. fluviatilis pallasi* and *N. kessleri gorlap*. This parasite was reported from Salmonidae fishes in Europe, North America and Asia (Bykhovskaya et al., 1964). During this study *N. melanostomus***,** *N. fluviatilis pallasi* and *N. kessleri gorlap* were reported as new hosts**.** The only species reported from *N. melanostomus* was *Cystidicola epherneridarum* (Rolbiecki, 2006).

D. minutus has already been reported from different parts of Europe (Moravec, 1994) and Southern parts of the Caspian Sea (Pazooki and Aghmandi, 1999; Daghigh-Roihi and Sattari, 2004). Markowski in 1996 assumed that *Polychaeta nereiis diversicolour* is the intermediate host for the parasites, if this theory is accepted the reason for more infections in the younger (smaller size) hosts is due to the their nutrition diet changes during growth. In this study there were no infections in the large aged fishes and there was a significant negative direction between length and infections (Figs. 7, 8).

Eustrongylides excisus had already been reported from the *N. fluviatilis pallasi* in the Danube River (Ondrackova et al., 2005) and from *N. melanostomus* in the Black Sea (Naydenova, 1974). In Iran this parasite was reported from Acipenseridae,

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Cyprinidae and some Neogobiidae fishes (Sattari et al., 2003; Daghigh-Rouhi and Sattari, 2004).In this study *Eustrongylides excius* is reported for the first time from *N. bathybius*.

Hysterothylacium aduncum was reported from *N. melanostomus* in Poland, Gdansk Gulf and the Black Sea (Rolbiecki, 2006; Kvach and Skora, 2007; Oumlzer, 2007). During this study, *N. fluviatilis pallasi*, *N. kessleri gorlap* and *N. bathybius* were presented as new hosts and this was the first time they were reported from Iran. *Raphidascaris acus* has already been reported from many fish hosts throughout the world (Moravec, 1994) and from Iran (Eslami et al., 1978; Pazooki and Masoumian 2002; Khara et al., 2005). This parasite was reported from *N. fluviatilis pallasi*, *N. kessleri gorlap* and *N. melanostomus* (Ondrackva et al., 2005) but in this study it was also reported from *N. bathybius* for the first time.

N. melanostomus is introduced as a new host for *Raphidascaroides sp.* This parasite was reported from fishes of different parts of the world and Iran (Pazooki and Masoumian 2002, Sattari et al., 2002).

Corynosoma strumosum has already been reported from Acipenseridae, Neogobiidae and Clupeidae in Iran (Sattari et al., 2002; Daghigh-Rouhi and Sattari 2004; Nik-Sirat et al., 2006). Earlier it was presumed that only Clupeidae could transfer this parasite to Acipenseridae fishes but later on it was detected that Neogobiidae fishes can also do so. This parasite was reported for the first time from *N. bathybius* in Iran.

This study revealed that there is a positive direct significant relation between length and weight of *N. fluviatilis pallasi*, *N. kessleri gorlap* and *N. melanostomus* with the number of *C. sphaerocephalus*, (Figs. 4-7). The same correlation is between length and weight of *N. fluviatilis pallasi* with the number of *Eustrongylides excisus* (Figs. 8-11). There is a significant difference between infections of *C. sphaerocephalus*, *Eustrongylides excisus* and *D. minutus* in different seasons (Table 3). The reason was due to the ecological accepts and nutritional behavior in different seasons (Reimchen and Nosil, 2001).

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