Occurrence and Intensity Rate of Internal Metazoan Parasites in *Rutilus frisii kutum* and the First Report *Dioctophyma renale* of (Nematoda: Dioctophymidae) in Iran

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**Abstract:** In this study, during October 2007 until April 2008 two hundred specimens of *Rutilus frisii kutum* with an average weight of 1±0.2 kg were parasitologically examined through cutting the body and the abdominal part, digestive tract and other ventral organs. Upon inspection, 148 specimens of fish (74%) were detected to be infected with internal trematodes of *Aspidogaster limacoides* and nematodes. Thus 119 specimens of the fish (i.e 59.5%) were infected with *Asymphylodora kubanicum* which in terms of intensity mean was 12.93. The number of parasites identified in the marine fish were greater than those of river fish. 100 specimens of the fish had also been infected with *Aspidogaster limacoides* with 50% occurrence and the mean intensity of infection was 20.44. Their number in the marine fish exceeded the number of the same parasites in the river fish. Three of the examined fish (1.5%) were infected with *Eustrongylides excisus* larvae with a mean intensity of infection rate of 2.32% in body cavity, followed by 2 fish sample (i.e 0.5%) affected by larvae of *Raphidascaris acus* showing a mean infection intensity rate of 1% in the abdominal area. 5 specimens of fish (2.5% occurrence) were infected with metacercaria *Diplostomum spathaceum* having a mean occurrence intensity of 2.2% in the lens of the marine *R. frisii* kutum, followed by 3 specimens of fish (constituting 1.5%) showing damage by the nematode of *Dioctophyma renale* species that had 1.66 mean intensity of infection in heart pericard and kidneys. The results of the study showed that fish were affected by various worm parasites due to their diverse range of food items. In the study, the *Dioctophyma renale* was recorded for the first time in Iran. Meanwhile, it is for the first time that *Eustrongylides excisus* was found in *Rutilus frisii kutum*.

**Key word:** *Rutilus frisii* kutum • Internal metazoan parasites • Caspian Sea • Iran

**INTRODUCTION**

Caspian sea is a land - locked brackish water lake where zoologically characterized by various aquatic animals and different commercially important fish species.

[1]. The fishes in the Caspian sea and its watershed areas include 78 species and 49 subspecies which belong to 16 fish family. The bulk of Caspian sea fish are anadromous and the major commercially important fish species include the bony fish and a cyprinid fish *Rutilus frisii* kutum.
dwelling mainly along the southern coastal waters of the Caspian sea. The fish reaches maturity at the age of 3 years. In mid March till April when the water temperature ranges between 12-18°C the matured fish migrate to rivers flow into sea for spawning. [2]

They tend to feed on phytoplanktons at larval stage while at later stages they turn to shells, chironomid larvae and polychaete worms. Vosoughee and Mostageer [3] mentioned Rutilus frisii kutum have been subjected to artificial propagation for the past three decades with a view to preserve their stocks. Rutilus frisii kutum fingerling 1-2 g each are reared in earthen ponds which are later released into the rivers running into the sea [2]. Nevertheless, the growing sea water pollutions, diminished river waters, the various species of water birds and aquatic animals and the abundance of parasites hosts have among a negative impact on kutum fish. So far there have been extensive research or reports concerning the parasites affecting Rutilus frisii kutum. Eslami and kohneshahri, (1987) for instance studied the worms in the Rutilus frisii kutum such as anisakis as an invading larval parasites. Mokhayer, (1988) detected certain species of digenent trematode in kutum fish caught from the Sefid-Rood River including Aspidogaster limacoides and Asymphylodora kubanicum [5, 17]. He could also identify Diplostomum spataceum (metacercaria) in his examinations of the Rutilus frisii kutum infected with parasites in the south eastern part of the Caspian sea. Nevertheless, there has so far been no comprehensive research dealing with Rutilus frisii kutum helminthes parasitic fauna in the south west part of the sea. The aim of the current research is to identify the internal parasites of capian roach in the south west part of the Caspian sea. Furthermore, the occurrence and intensity of identified parasites species are presented and species of zoonotic importance are stressed.

**MATERIALS AND METHODS**

This study was carried out during October 2007 - April 2008, for a period of eight months which included examination of 200 specimens of Rutilus frisii kutum fish average 1 kg each These fish were landed from fishing ground situated in south western part of the sea. The fish were caught through beach seine that were later transported to the fish laboratory a live by plastic tubs or tanks equipped with aerator. Upon anesthetization in the laboratory, the fish samples were subjected to biometric analysis (i.e measurement of total body length, weight, Gender and age identification) and the data were recorded in special data forms. Then based on [6] the common procedure of autopsy and parasitology, anatomical studies on abdominal organs, muscles, digestive system and eyes of the sampled fish were carried out with a view to isolate the parasites. Upon separating the parasites they were then rinsed and put in 10% formalin solution which were duely dyed using the Alum carman technique. Lactophenel solution was also applied for making the parasites transparent. It was then followed by examination of the transparent and dyed parasites using the parasite identification keys: [7-9]. Finally, on the base of collected data and collection procedure, they were subjected to computerized data analysis involving Excel software and the one - way analysis of variance (P <0.05) with a view to determine the degree of occurrence, the mean of infection intensity, the dominance index and the diversity of parasites.

**RESULTS**

A total of six metazoan parasite species were detected in examined fish identified to species level. The data related to these parasites such as occurrence, intensity, standard deviation, the range number of parasites (i.e the minimum and maximum number) the prevalence index (dominance) have all been presented in table 1. As shown in table 1, the isolated parasites include trematodes species Diplostomum spathaceum (metacercaria) isolated from the lens (Fig. 1), Aspidogaster limacoides (Fig. 2) and Asymphylodora kubanicum (Fig. 3) from the intestine whereas three nematode species such as Raphidascaris acus (Fig. 4) were identified within the abdominal area. The Larvae of Eustrongylides excisus (Fig. 5-6) were in abdominal section and were

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Infected organ</th>
<th>Occurrence (%)</th>
<th>mean infection intensity ± Standard deviation</th>
<th>Range of parasite number</th>
<th>Dominance index (%)</th>
<th>No of counted parasites</th>
<th>No of infected fish</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raphidascaris acus</td>
<td>Ventricle area</td>
<td>0.5</td>
<td>1 ± 0</td>
<td>1</td>
<td>0.05</td>
<td>2</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>Dioctophyma renale</td>
<td>Auricle and kidneys</td>
<td>1.5</td>
<td>1.66 ± 0.57</td>
<td>1 - 2</td>
<td>0.13</td>
<td>5</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>Eustrongylides excisus</td>
<td>Ventricle area</td>
<td>1.5</td>
<td>2.33 ± 0.57</td>
<td>2 - 3</td>
<td>0.19</td>
<td>7</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>Metacercaria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diplostomum Spathaceum</td>
<td>Eye</td>
<td>2.5</td>
<td>2.2 ± 0.83</td>
<td>1 - 3</td>
<td>0.3</td>
<td>11</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>Asymphylodora kubanicum</td>
<td>Intestine</td>
<td>59.5</td>
<td>12.93 ± 4.93</td>
<td>2 - 35</td>
<td>41.30</td>
<td>1513</td>
<td>119</td>
<td>200</td>
</tr>
<tr>
<td>Aspidogaster limacoides</td>
<td>Intestine</td>
<td>50</td>
<td>20.44 ± 12.19</td>
<td>6 - 86</td>
<td>58.06</td>
<td>2127</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 1: The occurrence rate, mean infection ± standard deviation, prevalence index and range of parasite number and the internal helminths of Rutilus frisii kutum collected from the south western part of the Caspian sea.
attached to the muscle and the nematodes *Dioctophyma renale* (Fig. 7-11) were found in the ventricle section of the heart and kidneys. Among the afore mentioned parasites, *Dioctophyma renale* were detected in the *Rutius frisii kutum* for the first time. The major parasite species detected included the *Dioctophyma renale* with 1.5% occurrence rate and a mean infection intensity of
Fig. 7: Anterior of Dioctophyma renale in (heart)

Fig. 8: Anterior of Dioctophyma renale

Fig. 9: Posterior of Dioctophyma renale in (kidney) in (heart)

Fig. 10: Dioctophyma renale in (heart)

Fig. 11: Dioctophyma renale in (kidney)

1.6% followed by Eustrongylides excisus larvae showed an occurrence rate and mean infection intensity of 1.5% and 2.3% respectively Raphidascaris acus larvae showing an occurrence rate of 0.5% and 1% as the mean infection intensity. Meanwhile Diplostomum spathaceum (metacercra) had an occurrence level and mean infection intensity of 2.5% and 2.2% respectively. In addition, trematode Asymphylodora kubanicum were found to be of 59.5% of occurrence rate and a mean infection intensity of 12.93 whereas Aspidogaster limacoides showed a 50% occurrence level and a mean infection intensity of 20.44% (Table. 1 and Fig. 15). Moreover, parasite infestations caused by Aspidogaster limacoides and Asymphylodora kubanicum were also examined in terms of the distribution percentage in their microhabitate situated in foregut, midgut and hindgut areas. The results of analysis showed the percentage number of Aspidogaster limacoides to be actually 2127 specimens in the three of the above mentioned positions. Of 100 specimens of infected fish, 52% of parasites were concentrated in the foregut area followed by 32% and 16% in the midgut and hindgut areas of the intestines respectively. (Fig. 13) The mean number of parasites detected per fish was 20.44 specimens which were mainly attached within the inner layers of the intestines. It was found that R. frisii kutum caught from the Caspian sea had been far more affected by parasites than those caught in the rivers flowing into it to such an extent that the number of Asymphylodora kubanicum in
Aspidogaster limacoides
Asymphylodora kubanicum
Dioctophyma renale
Raphidascaris acus
Eustrongylides excisus
Diplosiomum spathaceum

Infection percentage of R. frisii kutum in rivers and in the sea (Caspian sea)

Fig. 12: Infection Percentage of *Rutilus frisii* kutum in river and in the Caspian sea.

<table>
<thead>
<tr>
<th>Location</th>
<th>Foregut</th>
<th>Midgut</th>
<th>Hindgut</th>
</tr>
</thead>
<tbody>
<tr>
<td>River</td>
<td>16%</td>
<td>52%</td>
<td>32%</td>
</tr>
<tr>
<td>Caspian Sea</td>
<td>48%</td>
<td>17%</td>
<td>35%</td>
</tr>
</tbody>
</table>

Fig. 13: Showing percentage number of *Aspidogaster limacoides* accumulation in foregut, midgut and hindgut areas earned of *Rutilus frisii* kutum.

Fig. 14: Illustrating percentage number of *Asymphylodora kubanicum* concentrated in the foregut, midgut and hindgut layers of the *Rutilus frisii* kutum intestines layers.

Fig. 15: The infection intensity within the *Rutilus frisii* kutum caused by various internal parasites. The fish were caught in the south west part of the Caspian sea.

The fish coming from the sea was 1513 specimens of parasites from about 119 specimens of *Rutilus frisii* kutum out of which 48% of parasites concentrated on the foregut of the intestines whereas 35% and 17% of the parasites accumulated within the midgut and hindgut areas respectively (Fig. 14). The average number of this parasite per each fish was approximately 12.93 attaching mainly to the deep layers of the intestines. All of the isolated nematodes in this study belonged to the fish caught from the rivers. It means that *Dioctophyma renale* was isolated from the fish caught in khoskrud river whereas *Raphidascaris acus* and *Eustrongylides excisus* larvae were detected among *R. frisii* kutum fish netted in Rood-Sefid River. (Fig. 12)

**DISCUSSION**

The close relationship among the different aquatic animals, the great biodiversity and the land-locked state of Caspian sea provide a suitable condition for the emergence and the subsequent conveyance of various parasitic forms. The presence of various intermediate hosts as shells, Gamarus.crustacians and worms consumed by *Rutilus frisii* kutum as well as the relatively wide range of fish either as the main host or intermediate ones for the parasites, added by the interaction of many seabirds and mammals have all together provided the necessary grounds for a full range of parasites [10]. There have been extensive studies on the Caspian sea fish parasites particularly on *Rutilus frisii* kutum which resulted in the identification of a wide range of parasites living both in the sea and rivers flowing into the Caspian sea. In the present study the kutum fish are examined from prasitological aspect in Caspian sea and the rivers flowing into the sea. The occurrence, the degree of parasite infection, the index of dominance, the range number of parasites as well as the numerical distribution of parasites such as *Aspidogaster limacoides, Asymphylodora kubanicum* in the foregut, midgut and hindgut of kutum
fish intestine were recorded. The average number of these parasites was investigated separately in the fish caught in the river, the sea and the estuaries. The results have been summarized in (Table 1.) Report on the *Philometra karunsis* parasite located in abdominal cavity of Iranian fresh water fish was given by [11, 16]. Whereas *Dioctophyma renale* was recorded for the first in the precardium and kidney of *kutum* fish. In addition the larvae of *Eustrongylides excisus* had already been identified in the abdominal cavity of barboid fish by [12]. However, it is the first time that such a parasite is reported in the abdominal area and attached to muscles of *kutum* fish netted in Sefid-Rood River. Although *anisakis* larvae had been identified by [4] such a parasite was not detected in the present study. In fact, the occurrence rate and the infection intensity of internal parasite worms were found to be variant in the river fish and those caught in the sea and that such a difference was meaningful, (p<0.05) to such an extent that it was in a more limited scale in early autumn but which tended to be more in winter or early spring. The abundance of intermediate host organisms could be cited as one of the reasons for such varying degrees of infection intensity. Since the *Rutilus frisii* kutum is by far, the most popular fish among the coastal inhabitants of the Caspian sea which is consumed in a variety of forms. Parasites such as *Eustrongylides excisus* and *Dioctophyma renale* are highly important in the *kutum* fish because they are capable of infecting carnivorous creatures and people who feed on them(zoonotic) [13]. The results of the present study also suggest measures should be taken by the related health bodies to increase public awareness concerning the consumption of such fishes. The consumption forms and the preparation of the aquatic food could be modified in a way that hazards to human health due to zoonotic parasites could be avoided. The phylogenic characteristics of *Aspidogaster degene* indicate a great affinity among them. Although *Aspidogaster* has a simple life cycle and the degene enjoy a rather complex life cycle (up to four hosts) [14, 15] both parasites detected in the *kutum* fish find their preferred dynamic residence in the one of the anterior part of the intestine. This part is situated in the secretary portion of *kutum* fish intestine [13]. There is therefore no major competition between them over these microhabitats. The intensity of infestation of 33 parasites in each fish plays an undoubtedly crucial role on the *kutum* fish growth rate which in turn calls for further, investigation. *Rutilus frisii* kutum as a highly appreciated fish species in Iran have been subjected to various research works. However study of parasitic fauna has been taken less consideration. This is the most comprehensive research work on helminthes parasites of *Rutilus frisii kutum* which reports six internal helminthes including *Dioctophyma renale* as first record in Iran. Furthermore, *Rutilus frisii* kutum found to be infected by *Eustrongylides excisus* larvae *Dioctophyma renale* as new host very important zoonotic parasite species. The result of the present investigation call very important facts for those conceals to public health (Veterinary organization, Health ministry) and etc. In Iran continued research work on intermediate and definite host ranges of two mention species will clear ecological aspect of found parasites species. Which as result, preventive measures should be drown in the infected regions of Iran.

**ACKNOWLEDGEMENT**

Hereby, I would like to express my thanks and appreciation to the fisheries experts and staff in shahid Ansari fish hatchery complex for their providing me with the required facilities and supports Mana Mobedi, Neda Mirsepahi, Medhat Masoumi and Iadan Rokni.

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