Parasite fauna of Chub, *Squalius turcicus* De Filippi, 1865 (Teleostei: Cyprinidae) from some rivers of the Southern Caspian Sea basin in Iran

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Abstract: This study was carried out to investigate the parasite fauna of Chub, *Squalius turcicus* De Filippi, 1865 from the Siah, Neka and Haraz rivers of the Southern Caspian Sea basin in Iran, during 2015. Individuals of *S. turcicus* were caught by nets during the spring to winter of 2015. Fish were transferred to specific laboratory alive and then were used for parasitological investigations. Total percentage of parasites infection of Chub in the Haraz, Siah and Neka rivers were 48.7, 71.8 and 57.4%, respectively. Different parasite species including monogenean (*Gyrodactylus mutabilitas, Dactylogyrus vistulae* and *Paradiplozoon*), protozoa (*Ichthyophthirius multifilis* and *Trichodina* sp.) and myxozoa (*Myxobolus muelleri*) were identified in the fish gill, intestine and skin. Significant differences were observed in the parasites’ prevalence among the rivers and seasons. Such differences may be due to impact of environmental conditions, especially water quality, on fish parasite fauna. This is the first report of *M. muelleri* in the intestine of *S. turcicus* in Iran.

Keywords: Fish, Disease, Monogenean, Protozoa, Myxozoa.


Introduction

The Caspian Sea is the largest closed body of water on the Earth, which its southern part is located in the north of Iran with 864 small and large rivers (Zakeri 1997). The Southern Caspian Sea basin as a part of a highly diverse area of Paratethys basin (Naseka & Bogutskaya 2009), shows high ichthyological diversity with 119 species belong to 63 genera, 18 families, 16 orders and two classes (Esmaeili et al. 2014). Thus, several studies have focused on fish parasites of this region (Noei et al. 2015; Mirnetegh et al. 2017; Taheri Mirghaed 2017).

Chub, *Squalius turcicus* is a freshwater and brackish fish species belonging to Cypriniformes, the most diverse order of the Southern Caspian Sea ichthyofauna (37.81%; Esmaeili et al. 2014). This species is widely distributed in Europe, Eurasia and Asia, especially the rivers of the Caspian Sea basin (Freyhof 2014; Khaefi et al. 2016; Esmaeili et al. 2017). The Caspian Sea populations had already been considered as *S. cephalus* (see Esmaeili et al. 2014, 2016; Khaefi et al. 2016).

Chub is tolerant to poor water quality and physical habitat degradation gradients, prefers slow-flowing waters and commonly lives in small rivers (Maceda-Veiga & De Sostoa 2011). This fish mainly feeds on aquatic invertebrates such as terrestrial insects as well as plant material along the whole water column (Balestrieri et al. 2006). There are no known World Organization of Animal Health (OIE) notifiable...
diseases for this species. However, different types of infectious diseases were scientifically reported in chub, including parasitic infestations. Among all parasites, *Dactylogyrus vistulae*, *Bucephalus polymorphus*, *Paradiplozoon ergensi*, *Acanthocephalus anguillae*, *Pomphorhynchus laevis*, *Myxobolus* sp., *Chilodonella hexastica*, *Diplodstromum spathaceum*, *Lernaea cyprinacea*, *Ichthyophthirius* sp., *Rhabdochona* sp., *Caryophyllaeus brachycollis* and larval stages of *Tylodelphys clavata* have been reported in chub (Galli et al. 2001; Pazooki & Masoumian 2012; Daghigh Roohi et al. 2015; Shokrolahi et al. 2016). Previous parasitological studies in the Southern Caspian Sea basin described a wide spectrum of parasites for different species of Gobiidae (Youssefi et al. 2016), Acipenseridae (Pazooki & Masoumian 2004) and Cyprinidae (Pazooki et al. 2007) families, however, data on the parasite fauna of *S. turcicus* from main water habitats is scarce.

Parasites may have a considerable impact on some aspect of host biology such as behavior and growth performance of wild fish populations as well as the survival by the influence susceptibility of the host fish to secondary infections (Scholz 1999; Woo 1999; Barber & Dingemanse 2010). Moreover, parasite fauna of wild populations may have influenced by fish host characteristics (size, sex, genetic, food habit, density and life mode) as well as the environmental parameters specially, water quality (Rózsa et al. 2000; Galli et al. 2001; Marcogliese et al. 2006; Chapman et al. 2015).

As chub inhabits in the Southern Caspian Sea basin and every changes in its parasitic fauna may be have direct and indirect influences on fish culture of this area, this study was carried out to investigate the parasite fauna of chub from the Haraz, Siah and the Neka rivers of the southern Caspian Sea basin in Iran, during 2015.

**Materials and Methods**

**Study Area:** Present investigation was conducted in three major rivers of southeast of the Caspian Sea basin, north of Iran, including six sampling sites at the Siah River (Site 1: 36°29’17.8”N, 52°53’29.0” E; Site 2: 36°37’20.0”N, 52°54’54.1”E), Haraz River (Site 1: 36°37’11.02”N, 52°26’29.29”E; Site 2: 36°29’28.3”N, 52°22’10.4”E), and Neka River (Site 1: 36°38’46”N, 53°18’33”E; Site 2: 36°37’55”N, 53°20’50.9”E). Details of sampling areas are shown in GIS map (Fig. 1).

**Fish samples:** Chub specimens were caught by nets at all sampling sites during the spring to winter of 2015. Samples of the live fish (weigh of 42±3g and length of 15±1.3cm) were transported to the Diagnostic Laboratory, Veterinary Diagnostic Center (Department of Aquatic Health and Diseases at University of Tehran, Iran) for parasitological examinations.

**Parasitological examination:** The fish were euthanized under supervision of regulatory issued by Animal Ethical Committee of University of Tehran. The fish samples were dissected carefully and skin, gills, fins, eyes as well as gastrointestinal tracts and internal organs were examined for parasites. The isolated parasites were fixed and stained (Fernando et al. 1972; Gussve 1983), and then were observed under a light microscope (Labovital 4) equipped with a digital camera (Sony, SSC-DC80P). The identification of parasites was performed according to Bychovskaya-Pavlovskaya (1962), Gussve (1985),...
Statistical analysis: The difference between prevalence of parasites between groups were carried out by One-way ANOVA and Tukey complementary test with SPSS software version 20.0 (SPSS Inc., Chicago, USA). The level of significance was considered $P \leq 0.05$.

Results
During this study, parasite fauna of *S. turcicus* (n=748) in different seasons was investigated from three rivers of Caspian Sea basin in Iran. Total percentage of parasites infection of Chub in Haraz River, Siah River and Neka River were 48.7%, 71.8% and 57.4%, respectively (Table 1). Different parasites, including, two species of protozoa (*Ichthyophthirius multifiliis* and *Trichodina* sp.), three species of monogenean (*Gyrodactylus mutabilitas*, *Dactylogyrus vistulae* and *Paradiplozoon*), and one species of myxozoa (*Myxobolus muelleri*) were isolated from gills, intestine and skin of the examined fish (Table 2). Morphologies of the isolated parasites are shown in Figure 2.

Siah River: Prevalence of identified parasites in live *S. turcicus* from Siah River in different seasons is shown in Table 3. Among the identified parasites *Paradiplozoon* (25.4%) was the most common followed by *Trichodina* sp. (23.8%), *I. multifiliis* (23.4%), *D. vistulae* (16.6%), *G. mutabilitas* (14.2%), and *M. muelleri* (3.9%). However, the rate of infection was increased with increase in temperature as the highest and the lowest prevalence were observed in the summer and winter season, respectively. The prevalence of *I. multifiliis* at winter had significant difference with spring and summer ($P \leq 0.05$). *Gyrodactylus mutabilitas* and *M. muelleri* were significantly higher at summer and winter, respectively ($P \leq 0.05$). The *Trichodina* sp. had no significant difference at summer and winter ($P > 0.05$). The prevalence of *D. vistula* was significantly higher in autumn and winter ($P > 0.05$).

Haraz River: Results of the identified parasites in live *S. turcicus* from the Haraz River are shown in Table 4. In Haraz River, *G. mutabilitas* was the most abundant and found on the gill as well as the skin of 29.2% and 10.8% of examined fish, respectively, followed by *M. muelleri* (10.8%), *Trichodina* sp. (7.9%), *Paradiplozoon* (5%) and *D. vistulae* (1.6%). However, *I. multifiliis* was not found in this river. The prevalence of *G. mutabilitas* isolated from gill
was significantly higher at the autumn (38.3%) (P>0.05). The Trichodina sp. (28.3%) and G. mutabilitas isolated from skin (27%) had significantly higher prevalence at spring and summer, respectively (P≤0.05). There was no significant difference on prevalence M. muelleri and D. vistulae and Paradiplozoon at different seasons (P>0.05).

**Neka River:** Similarly, the protozoan and monogenean species observed in S. cephalus from the Haraz and Siah rivers were also identified in fish samples from Nekar River, however, M. muelleri was not observed in none of the fish samples (Table 5). The protozoan, I. multifiliis (28.2%) was the most common, and the monogenean D. vistulae (7.8%) was least common. Moreover, the highest and the lowest prevalence were observed in the summer and winter, respectively. The prevalence of I. multifiliis (39.71%) and Trichodina sp. (38.24%) in spring and Paradiplozoon (39%) in summer were significantly

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**Fig.2.** Morphology of the isolated parasites from live S. turcicus from Haraz, Nekarood and Siahrood Rivers of Southern Caspian Sea basin in Iran. Gyrodactylus mutabilitas (A); Ichthyophthirius multifiliis (B); Trichodina sp. (C); Paradiplozoon (D); Myxobolus muelleri (E); Dactylogyrus vistulae (F).

**Table 3.** Parasite’s prevalence in live Squalius turcicus during different seasons in the Siah River. Lowercase letters show significant difference in parasite’s prevalence among different seasons. No: Number of positive samples; %: Percent of positive samples.

<table>
<thead>
<tr>
<th>Parasites / Positive samples</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Ichthyophthirius multifiliis</td>
<td>17</td>
<td>26.9a</td>
<td>25</td>
<td>40ab</td>
<td>13</td>
</tr>
<tr>
<td>Trichodina sp.</td>
<td>26</td>
<td>41.2abc</td>
<td>0</td>
<td>0bf</td>
<td>7</td>
</tr>
<tr>
<td>Myxobolus muelleri</td>
<td>0</td>
<td>0a</td>
<td>0</td>
<td>0a</td>
<td>0</td>
</tr>
<tr>
<td>Dactylogyrus vistulae</td>
<td>4</td>
<td>6.34bd</td>
<td>3</td>
<td>5cd</td>
<td>23</td>
</tr>
<tr>
<td>Gyrodactylus mutabilitas</td>
<td>4</td>
<td>6.4a</td>
<td>29</td>
<td>46ab</td>
<td>0</td>
</tr>
<tr>
<td>Paradiplozoon</td>
<td>23</td>
<td>36.5ac</td>
<td>32</td>
<td>51ab</td>
<td>6</td>
</tr>
</tbody>
</table>

Sample No. (each season) | 63 | 63 | 63 | 252
The results conform to results of Noorbakhsh et al. (2014) and Darvishi et al. (2015) respectively. The results confirm the correlation between the parasites fauna and water quality (Kennedy 1997; Galli et al. 2001; Dias et al. 2017). Taheri Mirghaed et al. (2017) reported the unsuitable qualitative condition of these rivers. The NSFWQI (Water quality index of America’s national health organization) average value of the Siah, Neka and Haraz rivers were 37, 38.6 and 38.7, respectively, suggesting that the water quality of the rivers reduced due to entrance of industrial, urban and agricultural waste.

Also, the highest infection observed in the summer (i.e. *I. multifiliis, G. mutabilitas* and *Paradiplozoon* in Siah and Neka rivers) may have been due to seasonal variations in feeding and behavior of *S. turcicus* as well as water temperature that further confirm the correlation between the parasites fauna and water quality (Kennedy 1997; Galli et al. 2001; Tavares-Dias et al. 2017). In this study monogeneans (*G. mutabilitas, D. vistulae* and *Paradiplozoon*) and protozoans (*I. multifiliis* and *Trichodina* sp.) were the most abundant parasites followed by Myxozoa (*M. muelleri*). Taheri Mirghaed et al. (2017) reported higher than other seasons (*P*≤0.05). There was no significant difference on prevalence of *D. vistulae* at different seasons (*P*>0.05). The parasite, *G. mutabilitas* had significantly higher prevalence in summer (*P*≤0.05).

**Discussion**

Fish populations act as direct or indirect hosts for different parasite species with varied strategies in their life cycle which diversity and prevalence of parasites is mainly depend on the host species and environment, especially the quality level of the water body in which they complete their life cycles (Kennedy 1997; Galli et al. 2001).

Based on the present results, the percent of parasitic infestation of *S. cephalus* in Siah, Neka and Haraz rivers were 71.8% and 57.4% and 48.7%, respectively. The results conform to results of Noorbakhsh et al. (2014) and Darvishi et al. (2015) reporting the unsuitable qualitative condition of these rivers.

Table 4. Parasite’s prevalence in live *Squalius turcicus* during different seasons in the Haraz River. Lowercase letters show significant difference in parasite’s prevalence among different seasons. No: Number of positive samples; %: Percent of positive samples.

<table>
<thead>
<tr>
<th>Parasites / Positive samples</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Total (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td><em>Gyrodictyum mutabilitas</em> (S)*</td>
<td>12</td>
<td>20 a</td>
<td>19</td>
<td>31.6 a</td>
<td>23</td>
</tr>
<tr>
<td>Trichodina sp.</td>
<td>17</td>
<td>28.3 ab</td>
<td>2</td>
<td>3 b</td>
<td>0</td>
</tr>
<tr>
<td>Myxobolus muelleri</td>
<td>1</td>
<td>2 a</td>
<td>8</td>
<td>13 a</td>
<td>7</td>
</tr>
<tr>
<td>Dactylogyrus vistulae</td>
<td>0</td>
<td>0 a</td>
<td>2</td>
<td>3 a</td>
<td>2</td>
</tr>
<tr>
<td><em>Gyrodictyum mutabilitas</em> (G)</td>
<td>5</td>
<td>8.33 b</td>
<td>16</td>
<td>27 ab</td>
<td>5</td>
</tr>
<tr>
<td>Paradiplozoon</td>
<td>3</td>
<td>5 a</td>
<td>0</td>
<td>0 a</td>
<td>6</td>
</tr>
</tbody>
</table>

*G: Gills; S: Skin*

**Table 5. Parasite’s prevalence in live *Squalius turcicus* during different seasons in the Neka River. Lowercase letters show significant difference in parasite’s prevalence among different seasons. No: Number of positive samples; %: Percent of positive samples.**

<table>
<thead>
<tr>
<th>Parasites / Positive samples</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Total (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td><em>Ichthyophthirius multifiliis</em></td>
<td>27</td>
<td>39.71 ab</td>
<td>24</td>
<td>38 a</td>
<td>17</td>
</tr>
<tr>
<td>Trichodina sp.</td>
<td>26</td>
<td>38.24 ab</td>
<td>2</td>
<td>3 b</td>
<td>7</td>
</tr>
<tr>
<td>Dactylogyrus vistulae</td>
<td>6</td>
<td>9 a</td>
<td>3</td>
<td>5 a</td>
<td>7</td>
</tr>
<tr>
<td><em>Gyrodictyum mutabilitas</em></td>
<td>5</td>
<td>7.35 ab</td>
<td>21</td>
<td>33 abcd</td>
<td>1</td>
</tr>
<tr>
<td>Paradiplozoon</td>
<td>12</td>
<td>18.7 b</td>
<td>25</td>
<td>39 ab</td>
<td>6</td>
</tr>
</tbody>
</table>

*No: Number of positive samples; %: Percent of positive samples*
D. vistulae from the gill of S. turcicus at one of rivers of the Mazandaran Province.

Chub sampled from the Siah River had the most species-rich parasite fauna, hosting 6 parasite species, while, the sampled fish from the others rivers had 5 species as I. multifiliis and M. muelleri were not identified from Haraz and Neka rivers, respectively.

The protozoan found in this study were Trichodina sp. and I. multifiliis. The percent of infection with Trichodina sp. in the fish sampled from the Siah, Haraz and Neka rivers were 23.8%, 7.9% and 17.6 %, respectively. Moreover, 23.4% and 28.2% of the sampled fish from the Siah and Neka rivers were infected by I. multifiliis. Out of 748 sampled chubs, 347 were infected with monogenean species, including G. mutabilitas, D. vistulae and Paradiplozoon.

Short-living species combined with a direct life cycle and high reproduction rates such as protozoan and monogenean parasites can react to the environmental conditions of the host (Lester 1990), which this can explain the high infection percentage in chub populations and significant variations in prevalence of these parasites between the studied rivers and different seasons.

Gyrodactylus sp. is indicated as a living probe for assessment of aquatic environmental quality. The incidence of this parasite has a direct correlation with lowering of water pH (Biswa & Pramnik 2016). Similarly, different species of protozoan and monogenean have been identified in S. cephalus from some polluted and unpolluted rivers in northern Italy (Galli et al. 2001), as well as from Alborz Dam (Shokrolahi et al. 2016), and Zayandeh River in central Iran (Mehdipour et al. 2004). Therefore, these results confirm that the monogeneans and protozoans, beside their typical abilities on infecting fish skin and gills can be considered as bioindicators of water quality and subsequently fish health condition (Kennedy 1997; Galli et al. 2001; Tavares-Dias et al. 2017).

The phylum Myxozoa is a highly diverse taxon of microscopic endoparasites that the genus Myxobolus itself alone includes more than 751 species (Eiras et al. 2005). Infection with these parasites may result in severe diseases and can affect the growth, flesh quality and fecundity of their fish hosts (Gómez et al. 2014). The highest prevalence of M. muelleri was recorded at Haraz River which contains the large number of rainbow trout farms. By influencing performance of growing fishes, M. muelleri can have a large economic impact for industrial fish farms.

Masoumian & Pazooki (1999) have reported two species of myxozoa (M. minutus and M. muelleri) in the muscles of S. turcicus from Tajan River, north of Iran. In this study, we also identified M. muelleri in the intestine of this fish from Haraz and Neka rivers, which is the first report of this parasite in the intestine of this fish in Iran.

In conclusion, this study investigated the parasite fauna of S. turcicus from Siah, Neka and Haraz rivers of Southern Caspian Sea basin in Iran. Monogeneans (G. mutabilitas, D. vistulae and Paradiplozoon) and protozoans (I. multifiliis and Trichodina sp.) were the most abundant parasites followed by Myxozoa (M. muelleri). M. muelleri is reported for the first time from the intestine of S. cephalus in Iran. Several indictors such as season could influence diversity of parasitic fauna. This study found the more positive samples at the spring and summer. Different factors may affect the parasitic infestation in these seasons such as water temperature fluctuation, reproductive cycle, etc. Further studies are needed to record water quality parameters and its influence on parasite life cycle at Southern Caspian Sea basin in Iran.

Conflict of interest: Authors declare that they do not have any conflict of interest.

Acknowledgments
This research was supported by a grant from the Research Council of the University of Tehran.

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مقاله پژوهشی
بررسی فون انگلی ماهیان سفید رودخانه‌ای (Squalius turcicus) آبریز جنوب دریای خزر در ایران

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چکیده: مطالعه حاضر با هدف بررسی فون انگلی ماهیان سفید رودخانه‌ای (Squalius turcicus) در رودخانه‌های جنوب دریای خزر در سال 1395 انجام شد. ماهیان سفید رودخانه‌ای در فصل‌های بهار تا زمستان با استفاده از تور صید شدند. ماهیان صید شده به صورت زنده به آزمایشگاه انتقال داده شده و تحت آزمایشات انگل‌شناسی قرار گرفتند. میزان کل نمونه مثبت انگلی در رودخانه‌های جنوب دریای خزر، نکروز و سیاه به ترتیب 7/48، 8/71 و 4/57 درصد بود. انگل‌های متعددی از پوست، روده و آبشش ماهیان برداری شدند که شامل مونوجن ژیروداکتیلوس موتابیلیتس، داکتیلوژیروس ویستوله و پارادیپلوزون و پروتوزوآ (ایکتیفتریوس مولتی فیلیس و گونه تریکودینا) و میکسوزوآ (میکسوبولوس مولری) بودند. تفاوت معنی‌داری در میزان شیوع انگل‌ها در رودخانه‌های جنوب دریای خزر تحت تاثیر محیطی آب در فصول مختلف مشاهده شد که می‌تواند ناشی از تأثیر شرایط محیطی آب در فصول مختلف باشد. این اولین گزارش از میکسوبولوس مولری و میکسوزوآ در رودخانه‌های جنوب دریای خزر ایران است.

کلمات کلیدی: ماهی، بیماری، انگل‌های منوزن، تک‌اختلگان، میکسوزوآها.