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**ORIGINAL ARTICLE**

# The infection rate and pathologic lesions induced by *Proteocephalus osculates* (Goeze, 1782) in European catfish (*Silurus glanis*) from North-west of Iran

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**ABSTRACT**
*Parasitic infection of fish is a serious problem for both natural resources and fish culture farms. It can cause some losses in growth and even mortality of infected fish. This survey was carried out to determine the infection rate and pathologic lesions induced by Proteocephalus osculates cestoda in European catfish (Silurus glanish) fromNorth-west of Iran. A total of 360 S.glanis(120 fish for each dam) were collected from Aras, Mahabad and Zarrineh(shahidkazemi) dam reservoirs in 2011.The seasonal prevalence of infection to Silurus glanish in Aras, Mahabad and Zarrineh were as spring (60,56,59%), summer(70,66.5,69%), Autumn(63.3,66,64%) and winter (45,39,41%) , respectively. Maximum infection intensity (Mean±SE) of P.osculatus was determined in Aras (6.61±0.5), Mahabad (5.82±0.61) and Zarrineh (5.7±0.56) dams in summer. The numbers of this parasite were ranged from 1 to 17 in different seasons. The prevalence and intensity (Mean ±SE) of infection were significantly different in summer and winter with the other seasons ($p<0.05$). Data analysis had demonstrated that infection rate in affected fish were mostly depending on seasons. Also, there was no significant difference in infection to this parasite between male and female European catfish. Also, Pathological study demonstrated that scolex adhesiveness to the intestine of Silurus glanish caused mechanical damages on it. Necrotic bottoms due to impacts of parasite boteria on the intestinal mucus would be appeared where bacteria had impacted on underlying layer. Mucus secretions were not high on adhesive areas, but lymphocytes increasing in mucosal and inner layers of mucus indicated to existence of inflammation. According to the results, in West Azarbaijan, infection of European catfish to Proteocephalus osculates enjoyed the high prevalence and its losses caused irreparable intestinal damages in infected fish.*
*Key words: Proteocephalus osculates , Cestoda, Silurus glanish, European catfish,West Azarbaijan , Iran*

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## INTRODUCTION

Parasitic infection in fish can cause serious problems for both wild and cultured fish such as some losses in growth and even mortality of infected fish. Iran, regarding to its geographical position, and climatic condition with different water resources, enjoys various types of fish species, that each one can be hosted of different kinds of parasites. This makes their parasitic investigation so important. Parasitological studies on fish in Iran have been begun for a few decades, but regarding to variations of fish and different climatic conditions, the variety of fish parasites are expected. Therefore, research on parasitic fauna of different fish in an area can identify new parasites. In Iran, Catfish family (Siluridae) consisted of two species, Silurus glanish, which is native for aquatic territories of Khazar and OrumiaLake, and *Parasilurus triostegus* which is native for Tigris, KaroonRiver [1,2,3]. European catfish enjoys a broad distribution and mostly observed in European water resources, Asia Minor and central Asia. Beside the economical revenue in natural waters, they are also considered as cultured fish and recreational fishing [4]. Also, regarding the shortage of availability for suitable pituitary gland, the pituitary gland of this fish can be used as an artificial spawning inducer in cyprinid fish [17]. Due to nutritional protein values and also export importance of *Silurus glanish*, many investigations are conducted to its artificial propagation and larviculture in pools and other water resources. The aim of this study was to determine seasonal intestinal infection prevalence and pathological effects of *Proteocephalus osculates* in European catfish

(*Silurus glanish*) from West Azarbaijan dams. Also, controlling methods of this parasite are discussed here.

## MATEREALS AND MTHODS

A total of 360 *S.glanis*(120 fish for each area) were collected from Aras, Mahabad and Zarrineh (shahidkazemi) dam reservoirs, during 2011. For parasitological survey the intestine and stomach contents of obtained fish washed in a 100 micron mesh size sieve, and then examined by a stereomicroscope. Parasite scolex was isolated from intestine mucus of fish carefully by two clips. Isolated samples were washed in 6% salt solution according to Fernando et al. [10] and then and fixed by 70% alcohol on the slide glass, carmine stained and mounted by Canadian adhesive. Prepared slides were photographed and measured by microscope equipped with camera Lucida. Parasitic identification carried out considering the scolex shape, existing or lack of bands, and if necessary prepared slides from some pieces of parasite's body such as genital organs , opening parts in genital organs and type of genital organ in bonds according to identifying keys such as Gussev [14], Gibson et al. [17], Jalali (1998) and Busch, et al. [12]. Pathological study was performed after tissue processing and staining samples by H&E. All statistical analyses were performed using SPSS version 17 (SPSS, Inc., Chicago, IL) and, Chi- square, Kruskal-Wallis test ($p < 0.05$).

## RESULTS

Seasonal prevalence of *P.osculatus* infection *of S.glanis* in West Azarbaijan dams determined as follows: Winter (41.6%), spring (58.3%), summer (68.5%) and autumn (64.4%), respectively. The most intensity (Mean ±SE) was due to summer (6.14±0.46) and the least one was observed in winter (4.6±0.55), respectively. Statistical analysis of seasonal prevalence (Mean ±SE) and intensity (Mean ±SE) of parasites in different areas were illustrated in Tables1 and 2, respectively.

| | Spring | | Summer | | Autumn | | Winter | |
|---|---|---|---|---|---|---|---|---|
| Sampling Area | Numb-Infected % | No-Observed % | Infected % | No-Observed % | Infected % | No-Observed % | Infected % | No-Observed % |
| Aras | 60 | 40 | 70 | 30 | 63.3 | 36.7 | 45 | 55 |
| Mahabad | 56 | 44 | 66.5 | 33.5 | 66 | 34 | 39 | 61 |
| Zarrineh | 59 | 41 | 69 | 31 | 64 | 36 | 41 | 59 |

Table 1: Seasonal prevalence (Mean ±SE) of infected *S.glanis* to *Proteocephalus osculates*in different dams

| | Seasons | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spring | | | | | Summer | | | | | Fall | | | | | Winter | | | | |
| | N | Mean | SEM | Minimum | Maximum | N | Mean | SEM | Minimum | Maximum | N | Mean | SEM | Minimum | Maximum | N | Mean | SEM | Minimum | Maximum |
| Aras | 36 | 5.83 | 0.61 | 2 | 13 | 42 | 6.61 | 0.5 | 2 | 17 | 38 | 5.55 | 0.46 | 1 | 14 | 27 | 5.05 | 0.6 | 1 | 10 |
| Mahabad | 34 | 5.62 | 0.59 | 1 | 11 | 39 | 6.13 | 0.46 | 2 | 16 | 36 | 5.31 | 0.44 | 1 | 14 | 25 | 4.64 | 0.55 | 1 | 9 |
| Zarrineh | 34 | 5.41 | 0.57 | 1 | 12 | 40 | 5.69 | 0.43 | 2 | 14 | 33 | 5.08 | 0.42 | 1 | 12 | 28 | 4.27 | 0.50 | 1 | 8 |

Table 2: Intensity (Mean ±SE) of *P.osculatus*in *S.glanis* of 3 dams of West Azarbaijan

No significant seasonal infections were observed within group of the sampling sites (p>0.05).The prevalence (Mean ±SE) and intensity (Mean ±SE) of infected samples in studied dams were significantly different in summer and winter with the other seasons (p<0.05). comparition of infection percentage of *S.glanis* in different sesons in study sites were shown in fig 1.

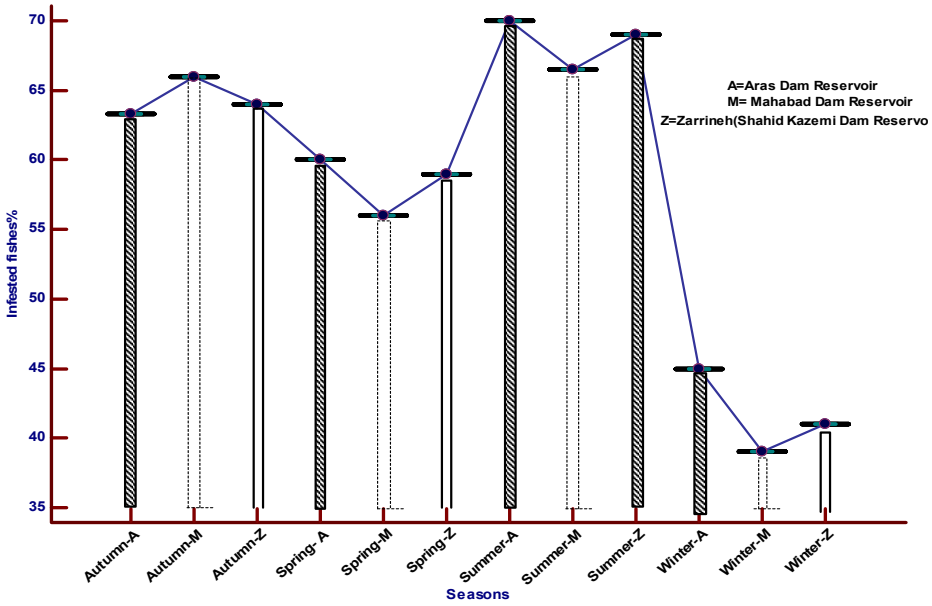


Fig1: comparing infection percentage of *S.glanis* in different sesons in study sites.

The intensity and prevalence of *P.osculatus* in *S. glanis* was high in studied dams (Fig.1). Severe intestine infection of *S.glanis* by *P.osculatus* was illustrated in fig 2.

This parasite contains a scolex with evolved frontal sucker and four clear stabulom whose strobilus has a lot of clear bonds of proglotides that the final bonds are longer than others (Fig.3).

Fig.2: Severe intestine infection of *S.glanis* by *P.osculatus*

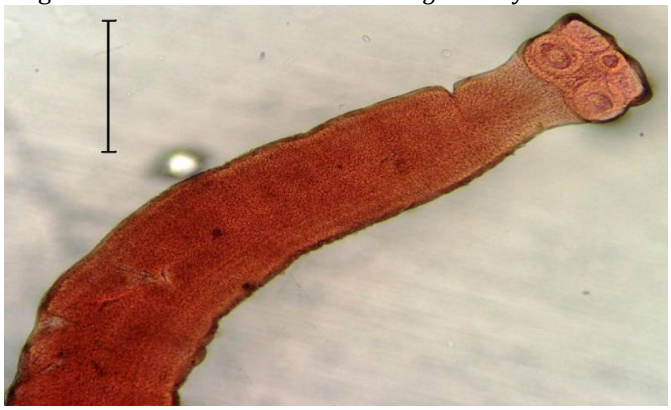

Fig.3: Isolated *P.osculatus* identified by scolex with stablom(Magnified 40×)

Comparing the fish infection by Chi-square test in different seasons revealed a significant difference ($p<0.05$). Comparing the infection percentage among the seasons were showed that spring had a significant difference with summer ($p<0.05$), while were not showed any significant differences with fall and winter seasons ($p<0.05$). Also, summer and fall had not any significant difference ($p<0.05$), while winter showed a significant difference ($p<0.05$), finally, fall and winter were not shown any significant difference ($p<0.05$). Comparing the infection intensity in total fish in different seasons by Kruskalwallis test, showed significant differences ($p<0.05$). Thus, comparing the infection intensity in infected fish by Mann-Whitney test in different seasons, revealed that spring had a significant difference with summer ($p<0.05$), while it had not any significant difference with other seasons ($p<0.05$). Summer had significant difference with fall and winter ($p<0.05$), but fall and winter were not shown any significant difference ($p<0.05$). Furthermore, according to the obtained data there were not any significant differences between male and female *S. glanis* in mentioned parasite infection ($p<0.05$).

From microscopic point of view, when the number of *P.osculatus* was high in intestine, it showed an intense dilution that might cause a blockage in intestine. But from the tissue losses viewpoint, *Proteocephalus* parasite by its boteria that was existed on head of parasite, stacked to fish intestine and each boteria evolved one or two intestine villii. Scolex adhesiveness in to the intestine caused mechanical damages to the intestine tissue. Necrotic bottoms would be appeared where boteria had impacted on underlying layer due to impacts of parasite boteria on intestine mucus. Mucus secretions were not high on adhesive areas, but lymphocytes increasing in mucus layer and under layer of mucus were indicated to inflammation indices. Spot hemorrhages in connecting areas were observed in some cases, but were limited to these areas. Fig 4 and 5 showed the cross section of *S. glanis*intestine invaded by *P.osculatus*.

Fig. 4: Cross section of infected *S. glanis*intestine. Invasion of intestinal mucosal layer by *P.osculatus* adhesion marked with arrow (H&E staining, 40×).

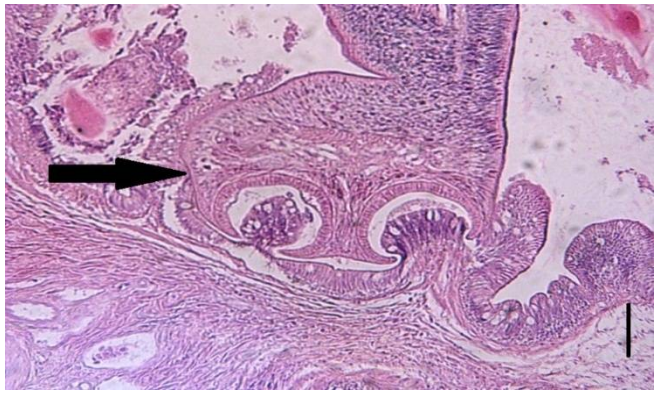

Fig. 5: Cross section of infected *S. glanis* intestine. Scolex boteria of *P.osculatus* stacked in intestine villii (H&E, 100×).

## DISCUSSION

Parasite is an object that lives in the body or on another object named host, feeds from host and shows degrees of compatibility with it, and often do not make any serious damages on it. Therefore, parasite uses the host as a nutritional source and shelter to protect him and often movement [5]. However, in other parasite – intermediate host systems that the continuing life cycle of parasite is depends on death of intermediate host and be eaten the intermediate host by final host, the parasite do its best to kill his host [6]. In natural environments parasites get compatible by their hosts in a way that their existence

would be continued and in other hand hosts life would not be threatened so much. In these situations, the parasites will be dominant that have indirect life cycle with one or two intermediate hosts [7]. In most countries prevalence of parasitic diseases in native fish population and introduced fish to water resources have caused to do researches on their causatives and controlling biologic methods of these diseases that have led to complete recognition of species diversity, life cycle and infectious transmission characteristic studies of parasitic diseases. A lot of studies on fish parasites have been done in Iran and all over the world; however, more valuable studies have done in Russia, Using water resources for fish training and introducing new fish to them which has resulted in prevalence of disease particularly parasitic diseases [8]. Physicochemical variations in water, presence or absence of intermediate hosts of some parasites, and different factors might have roles on appearing these variations [7]. This problem has a significant importance and could be the bases for different parasitological studies on different areas. In Iran in last decade these studies were performed which the investigations about freshwater fish parasites were mostly limited to Ciprinidae species that establish about 70% freshwater fish in Iran. At this juncture, *S.glanis* enjoys a high fisheries value that not only has economic benefits in natural waters, but also is belonged to training and recreational fisheries species category [9]. Due to protein nutritional values of *S. glanis* and its export importance, investigation had been conducted to artificial propagation of *S.glanis* to produce larvae in order to culture in pools and other water resources. *S.glanis* has special importance in neighboring countries particularly northern and west northern countries of Iran and because of this can be considered as an exportable species. The first report about parasite prevalence in European catfish (*Silurus glanish*) was presented by Sefidkar Lankroodi [4] and he reported the *Cuculanuss phaerocephalus* nematode from *Silurus glanish* intestine in Anzali marsh. Then, Mokhayar [16] introduced *Silorodiscoidessilory* parasite from *S.glanis* bronchia of sephidrood. Dechambrieret al. [13] in Sudan reported the infection rate of catfish intestine to *P.osculatus* parasite as 73% and parasite range in 1-10 numbers. According to the data from this study, the infection to *P.oscolatus* in *S. glanis* existed in Iran, also, had a high prevalence (58.3%) and the infection intensity and percentage of parasite in fish were be affected by season and water temperature. The results demonstrated that in summer, fall and winter, the temperature condition for parasite reproduction, proliferation and transmission to the new hosts had gotten more suitable and infection percentage in fish has been increased. As is showed in table 1 and figure 1, in fall and winter the maximum infection was observed and then it was decreased in spring and winter. It is assumed that some long term and general processes form parasitic structure communities but habitation or seasonal effects can cover the effects of these processes or reinforce them and by this way cause some differences among the various populations [10]. Interpreting the data from seasonal changes in infection to *P.osculatus* parasite has demonstrated that this parasite in all seasons presented in *S. glanis* intestine (Table 1) and this could attribute to indirect life cycle of parasite. Understanding these realities, allows to assignment of strategic prevention in intensive culture of these species in artificial pools, and also performing the prevention instructions from parasites such as *Protocephalus*, particularly in winter would be inevitable. Investigating the pathological lesions of *P.osculatus* parasite was done for the first time in Iran and there is little information on this field. Pathogenesis of this parasite in intestine is often related to its Scolex structure and presence of four sucker organs (boteria) in scolex. Also, the size of these organs is determining the pathogenesis intensity of parasite. As showed in pictures 3 and 4, scolex of parasite penetrates into the intestine and each botridy evolves one or two crinkles of intestine and causes inflammation in this area. Following the intestine inflammation, epithelial lesions, hyperplasia of epithelial cells and necrosis was observed in attachment point of parasite to host intestine. These results showed that fish life in storage may be more exposed to parasites (intermediate host) than individual's lives in rivers and natural waters. Also, the relationship between infection intensity and parasitic pathogenesis is very important. In high prevalence of infection, fish growth is decreased due to consumption of the nutrients which are readily uptake by parasite. However, in high infection rates and increasing the numbers of parasites in intestine, intense dilation have been observed. Even it might results in blocking the intestine and maybe makes some holes in it (Fig.1). By developing the disease, intestine mucus completely destroyed and led to fish death.

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