



A Survey on the Prevalence and Histopathological Findings of *Lernaea* spp. in *Schizocypris altidorsalis* Fish From Chahnimeh Lakes and Hamoun Wetland in Southeast Iran

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Abstract

The aim of the present study was to assess the prevalence and pathology of Lernaeid parasites in *Schizocypris altidorsalis* (Anjak Fish) fish from Chahnimeh lakes and Hamoun wetland in Sistan and Baluchestan, Iran. A total of 1000 *S. altidorsalis* fish were collected from October 2014 to September 2015. The prevalence of *Lernaea* spp. infestation in examined fish was found to be 61.1% (611/1000). Among the examined weight groups, the highest infestation rate (61.7%) was recorded in the weight group of 80-100g, followed by 60.5% and 58.7% in 100-150g and 150-200g weight groups, respectively ($P>0.05$). The frequency of *Lernaea* spp. in winter (75.2%) was higher than other seasons and the lowest prevalence was observed in summer (38%). The association between the season and the prevalence of *Lernaea* spp. infestation was statistically significant ($P<0.001$). The histopathological examination of the sections revealed lesions and ulcers in the epidermis, dermis, and muscles of the fish. Chronic inflammatory reaction and infiltration of inflammatory cells including lymphocytes, plasma cells, macrophages, and eosinophils around the parasite attachment sites were observed. In severe cases, various degrees of degeneration and necrosis were seen in the muscle. The present survey revealed a high prevalence of Lernaeid parasites in *S. altidorsalis* fish and necessitates appropriate control programs.

Introduction

Schizocypris altidorsalis, belonging to the cyprinidae family, is one of the economically valuable fishes. It is a rare native species of the genus *Schizocypris* and belongs to the subfamily Schizothoracinae, which is found in the southeastern regions of Iran, Central Asia and China, India, Pakistan and Afghanistan (Abbaspour et al., 2013). Fish does not only provide valuable protein for people's diet, but also acts as an important source of income for people in the world. Fish provides high quality omega-3 essential fatty acids, vitamins and minerals that are necessary for the proper functioning of the brain, heart and immune system (Ikem et al., 2014).

A wide range of parasites are capable of infecting fish. Among crustaceans, more than 1500 species of copepods are parasitic for fish. *Lernaea* spp, commonly

called 'anchor worms', are important crustacean parasites of freshwater fish and occur in a variety of fishes worldwide (Mirzaei et al., 2014). Because of the direct harm (irritation, hemorrhage, scale damage, ulcers and mortality) and also indirect harm (aesthetic issues), Lernaeosis is of great economic importance (Bhuiyan and Musa, 2008). There are more than 100 fish species, belonging to 25 different families, which are affected with Lernaeid parasites. The parasite feeds on the blood and tissue of the host and causes redness and swelling at attachment site. Secondary infection by bacteria and fungus is also common in fish following the parasite (Mirzaei et al., 2014; Raissy and Ansari, 2012; Woo, 1995). In the life cycle of *Lernaea* species, male dies soon after mating, then females metamorphose and attach to the fish by use of the anterior anchor, and they

embed themselves deeply into the fish and then produce eggs (Nagasawa et al., 2007). Environmental temperature plays a significant role in survival and development of Lernaeid copepods. A water temperature of 26–27°C is ideal for the complete life cycle (Mirzaei et al., 2014; Raissy and Ansari, 2012). The main control methods for Lernaeid copepods involve the application of chemical treatments with different concentration. There are various chemicals such as potassium permanganate (KMnO₄), formalin and malachite green for the treatment of Lernaeid infestation (Idris and Amba, 2011). Moreover, a study by Idris and Amba (2011) indicated that salinity can also be effectively used against *Lernae acyprinacea*. However, the elimination of *Lernaea* species from culture and wild fish is probably impossible (Mirzaei, 2015). Based on our knowledge from the literature review, although fish infestations with *Lernaea* species are common in Iran, there has been little research on Lernaeid copepods infestation. The present study was carried out to survey the prevalence and pathology of Lernaeid parasitic infestation in *S. altidorsalis* and its relationship with fish weights in Southeast Iran.

Materials and Methods

Field Study Area

This study was carried out in Chahnimeh lakes and Hamoun wetland, in the south eastern region of Sistan and Baluchestan province, Iran. Chahnimeh lakes and Hamoun wetland are natural aquatic ecosystems, located in between 61°40' to 61°39' and 60°39' to 61°35' east longitudes and between 30°51'00' to 30°45'59' and 31°15' to 31°32' north latitudes, respectively. They have an average water depth of 8 and 5 m, respectively.

Sample Collection

For this study, a total of 1000 of *S. altidorsalis* were purchased from local fishermen who caught fish from Chahnimeh lakes and Hamoun wetland, during October 2014 to September 2015. Collected specimens were transported to the laboratory and examined for the presence of *Lernaea* spp. Based on their weights; fish were divided into three groups (80-100g, 100-150g and 150-200g). Identification of the *S. altidorsalis* was made according to Coad (1979).

Parasitological Examination

In the laboratory, external surfaces of body, eyes, skin, fins, gills and mouth of fish were carefully investigated for the presence of Lernaeid parasite. Then, the parasites were collected from the host with forceps and preserved in 70% ethanol. They were cleared, stained, mounted and identified following the method of Kabata (1988). Tissue samples were taken and fixed in

10% neutral buffered formalin for histopathological examination. Paraffin sections of 5-µm thickness were stained with, Hematoxylin-Eosin and examined under a light microscope.

Statistical Analysis

Statistical analysis was performed using the Chi-square test. Data were analyzed by SPSS software, version 16 and P<0.05 was considered statistically significant.

Results

Lernaeid copepods were collected from native *S. altidorsalis* fish in four seasons and then were identified at the genus level. Of the 1000 *S. altidorsalis* fish examined in this study, Lernaeid copepods were found in 611 (61.1%) specimens. The highest prevalence of the *Lernaea* spp. (61.7%) was in 80-100g fish which was higher than that of other weight groups. However, there was no significant difference between the host weight and prevalence (P>0.05). The highest seasonal frequency occurred in winter 75.2% (188/250) followed by autumn 72.4% (181/250), while the prevalence rates of 58.8% (147/250) and 38% (95/250) were found in spring and summer, respectively. It was concluded that the season significantly influence the prevalence of *Lernaea* spp. infestation (P<0.001) (Table 1). The Lernaeid parasites were removed from different sites on the body of the fishes and the symptoms of *Lernaea* spp. Infestation consisting of redness, hemorrhage and nodules were seen (Figure 1). Histopathological examination of the sections revealed lesions and ulcers in the epidermis, dermis, and muscles of the fish. Chronic inflammatory reaction and infiltration of inflammatory cells including lymphocytes, plasma cells, macrophages and eosinophils around the parasite attachment sites were observed. In severe cases, various degrees of degeneration and necrosis were seen in the muscle (Figure 2).

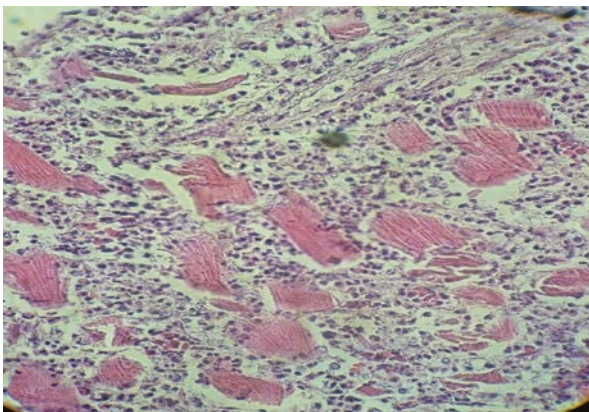
Discussion

In the present study, the prevalence of Lernaeid parasitic infestation in *S. altidorsalis* fish was 61.1%. Results of this study are comparable with those of previous studies on the same parasite in different countries. Kanwal et al. (2012) reported a prevalence of 52.36% for *Lernaea* infestation in Catlacatla in Pakistan. Mirzaei (2015), who studied 3520 ornamental fish in Iran, detected a prevalence of 5.3%. In another study on 200 *Hypophthalmichthys molitrix* in Egypt, Shagar and El-Refae (2012) determined a prevalence of 60%. Rameshkumar and Ravichandran (2012) discovered that 12.3% of *Hemiramphus far* in India are infested with *Lernaea*. Various factors including different climate conditions and parasite host specificity can be the reason for different prevalence rates reported in these studies (Tasawar et al., 2009).

Table 1. Prevalence of *Lernaea* spp. infestation according to the seasonal and weight groups of examined *Schizocypris altidorsalis* fish in Chahnimeh lakes and Hamoun wetland, southeast of Iran.

Fish species	Season	Number of fishes	Number of infested fishes	Prevalence (n/N) (%)	Nr. of infested fishes based on body weight group		
					80-100 g N=652	100-150 g N=256	150-200 g N=92
<i>Schizocypris brucei</i> (1000)	Spring	250	147	58.8	101 (15.4)	32 (12.5)	14 (15.2)
	Summer	250	95	38.0	82 (12.5)	12 (4.6)	1 (1.1)
	Autumn	250	181	72.4	101 (15.4)	62 (24.2)	18 (19.5)
	Winter	250	188	75.2*	118 (18)	49 (19.1)	21 (22.8)
Total		1000	611	61.1	402 (61.7)	155 (60.5)	54 (58.7)

Notes: n, fishes infested with *Lernaea* spp; N, total fishes examined (*P<0.05).

**Figure 1.** Lernaeciosis with redness, hemorrhage and nodules in body surface of *Schizocypris altidorsalis*.**Figure 2.** Inflammatory reaction composed of an admixture of lymphocytes, plasma cells, macrophages, eosinophils with various degrees of degeneration and necrosis in the muscle around the attachment sites of the parasite. H&E, Bar=100 µm.

In this survey, the highest infestation was recorded in fish with lower body weights. However, no significant correlation was found between parasite prevalence and

host weight ($P>0.05$). This finding is in agreement with the observations of Mirzaei et al. (2014) in Iran, while it is in contrary to findings by Kanwal et al. (2012), who performed their study in Pakistan and reported the highest prevalence in fish group with higher body weights. It is very difficult to explain the exact reason for different prevalence rates of Lernaecid infestation in different weight groups of fish; however, it could be due to difference in scale structure of these fishes (Tasawar et al., 2009).

The results of this study showed that the Lernaecid infestation rate was higher in winter than other seasons. This finding is consistent with Bhuiyan and Musa (2008) who reported a significantly higher prevalence of *Lernaea* infestation in winter compared with other seasons. In a study by Mirzaei et al. (2014) the maximum prevalence of Lernaecid infestation was reported in summer. Therefore, climate condition and temperature are important factors that affect the prevalence of *Lernaea* spp. in different seasons (Baker et al., 2008; Bhuiyan and Musa, 2008; Martins et al., 2002).

In this investigation, all the histopathological findings of lernaeciosis including ulceration, chronic inflammatory reaction, infiltration of inflammatory cells and degeneration and necrosis in the muscle were in agreement with previous studies (Abd El-Galil, 2012; Mirzaei, 2015; Roberts, 2012). The embedded parasites induce redness, hemorrhage, nodules and fibrosis around the head of the copepod (Abd El-Galil, 2012; Roberts, 2012).

In summary, the present study gives an update of the prevalence and seasonal diversity of *Lernaea* spp. in *S. altidorsalis* fish in the southeast of Iran. We recommend that further studies are required to estimate the economic losses caused by these parasites in fish, improve management practice and implement well-coordinated control interventions.

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REFERENCES

- Abbaspour, R., Rahbar, M., Karimi, J.M., 2013.** Study of the Fecundity Indices and Diet of *Schizocypris brucei* in Hamoun Wetlands in South East Iran. World Journal of Fish and Marine Sciences 5, 481-485.
- Abd El-Galil, M.A.A., ESSA, M.A.A., Kornil, F.M.M., 2012.** Studies on Lernaeosis and the efficacy of Dipterex as treatment in the Hatchery Reared Fingerlings of Cyprinids. Journal of American Science 8, 574-580.
- Baker, T.G., Pante, E., Levesque, E.M., Roumillat, W.A., de Buron, I., 2008.** *Metamicrocotyla macracantha*, a polyopisthocotylid gill parasite of the striped mullet, *Mugil cephalus*: population dynamics in South Carolina estuaries. Parasitology Research 102, 1085-1088.
- Bhuiyan, A.S., Musa, A.S.M., 2008.** Seasonal Prevalence and Intensity of Infestation by the Ectoparasites in Carps Relating to Physico-Chemical Parameters in Some Ponds of Mymensingh and Bogra Districts of Bangladesh Bangladesh Journal of Scientific and Industrial Research 43, 411-418.
- Coad, B.W., 1979.** A provisional annotated checklist of the freshwater fishes of Iran. Journal of the Bombay Natural History Society J Bombay 76, 86-105.
- Idris, H.B., Amba, M.A., 2011.** A note on *Lernaea cyprinacea* parasitizing the cultured marble goby *Oxyeleotris marmorata* and their control with salinity modification. Advances in Environmental Biology 5, 817-820.
- Kabata, Z., 1988.** Copepoda and Branchiura. In: Margolis L. and Kabata Z. (Eds), Guide to the Parasites of Fishes of Canada. Part II - Crustacea. Fisheries and Oceans, Ottawa 81-96.
- Kanwal, N., Siraj, Z., Tasawar, Z., Mushtaq, F., Lashari, M.H., 2012.** The Lernaeid Parasites of *Catla catla*. Acta Parasitologica Globalis 3, 16-19.
- Ikem, C.O., Abu Simon, J., Obiezue Ndunwanne, N. R., Ofoezieleanyi, E., 2014.** Prevalence and seasonality of parasites of fish in Agulu Lake, Southeast, Nigeria. African Journal of Biotechnology 13, 502-508.
- Martins, M.L., Onaka, E.M., Moraes, F.D., Bozzo, F.R., Paiva, A.M., Gonçalves, A., 2002.** Recent studies on parasitic infections of freshwater cultivated fish in the state of São Paulo, Brazil. Acta Scientiarum 24, 981-985.
- Mirzaei, M., Khedri, J., Ghashghaei, O., 2014.** Survey of Lernaeid in *Shizothorax zarudnyi* from Chahnimeh lakes in Sistan, Iran. Journal of Parasitic Diseases 40 505-509.
- Mirzaei, M., 2015.** Prevalence and histopathologic study of *Lernaea cyprinacea* in two species of ornamental fish (*Poecilia latipinna* and *Xiphophorus helleri*) in Kerman, South-East Iran. Turkish Journal of Parasitology 39, 222-226.
- Nagasawa, K., Inoue, A., Myat, S., Umino, S., 2007.** New host records for *Lernaea cyprinacea* (Copepoda), a parasite of freshwater fishes with a checklist of the Lernaeidae in Japan (1915-2007). Journal of the Graduate School of Biosphere Science 46, 21-33.
- Raissy, M., Ansari, M., 2012.** Parasites of Some Freshwater Fish from Armand River, Chaharmahal va Bakhtyari Province, Iran. Iranian Journal of Parasitology 7, 73-79.
- Rameshkumar, G., Ravichandran S., 2012.** *Lernaeenicussprattae* (Crustacea:Copepoda) on *Hemiramphus far*. Middle-East Journal of Scientific Research 11, 1212-1215.
- Roberts, R.J., 2012.** Fish Pathology, 4th ed., Wiley-Blackwell.
- Shagar, G.E., El-Refae, A.M.E., 2012.** Studies on cultured silver carp (*Hypophthalmichthys molitrix*) diseases induced by some bacterial, fungal and parasitic pathogens in Sharkia Governorate. Journal of the Arabian Aquaculture Society 7, 221-238.
- Tasawar, Z., Naz, F., Lashari, M. H., Hayat, C. S., Ali, B. S. H., Naeem, M. Shaheen, R., 2009.** Incidence of lernaeid parasite in *Catla catla* on a fish farm. Sarhad Journal of Agriculture 25, 285-289.
- Woo, P.T.K., 1995.** Fish diseases and disorders. Vol 1: protozoan and metazoan infection. CAB. International.