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PREVALENCE AND HISTOPATHOLOGICAL EFFECTS OF PARASITIC COPEPOD *LERNAEA CYPRINACEA* IN ESTUARINE FISHES FROM MEDITERRANEAN REGION OF TURKEY, WITH A NEW HOST RECORD

SUMMARY

Lernaeid copepod *Lernaea cyprinacea* L., 1758 has been reported on the skin and the external surfaces of fins from three freshwater fish hosts: endemic species *Alburnus baliki* Bogutskaya, Küçük & Ünlü, 2000 and *Pomatoschistus anatoliae* Engin & Innal, 2017 and on native species *Chelon ramada* Risso, 1827 sampled from Karpuzçay Creek Estuary, located in the Mediterranean region of Turkey. The highest prevalence of infection (7.5%) was reached in *C. ramada*, and the mean intensity was the same (1 parasite/fish) in all three host species. *P. anatoliae* (Gobiiformes: Gobiidae) was found as new host record for ectoparasite *L. cyprinacea*. Histopathological examination of the sections made in all analyzed fish hosts revealed chronic granulomatous inflammatory reaction and infiltration of lymphocytes, histiocytes and eosinophils around the parasite attachment sites.

Keywords: copepod, estuary, fish host, histopathology, parasite

INTRODUCTION

Most wild fish populations are vulnerable to metazoan parasites (Dezfuli *et al.* 2021). One of the most important enemies of fishes, the parasitic copepods could damage their hosts by the attachment mechanisms and feeding activities (Alaş *et al.* 2015). So far as known, it was determined that the non-host specific copepod *Lernaea cyprinacea* Linnaeus, 1758 or anchor worm can infect native and introduced fish in Turkey, being recorded on freshwater species more in lakes and ponds rather than in river creek and streams (Ahnelt *et al.* 2018; Koyun and Atici 2018).

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L. cyprinacea is the causative agent of lernaeciosis, a disease with serious pathogenic lesions reported in parasitized fish (Innal *et al.*, 2017; Urku and Onalan, 2018). Even edible fish infected with cyclopid parasites are undesirable to anglers and not consumed (Raissy *et al.* 2013; Koyun and Atici 2018; Hua *et al.* 2019).

This ectoparasite has been found infesting over 40 freshwater fishes in Turkish inland waters, predominantly Cypriniformes, including the following species: *Alburnus baliki*, *Barbus xanthos*, *Capoeta capoeta*, *Carassius carassius*, *Carassius gibelio*, *Cyprinus carpio*, *Chondrostoma nasus*, *Chondrostoma beysehirense*, *Cyprinion macrostomus*, *Oxynoemacheilus anaticus*, *Pseudophoxinus egridiri*, *Pseudophoxinus burduricus*, *Pseudorasbora parva*, *Pseudophoxinus zekayi*, *Squalius cephalus* and *Tinca tinca* (Koyun and Atici 2018; Innal, 2020).

The member of Cypriniformes order of teleost fishes, *Alburnus baliki* Bogutskaya, Küçük & Ünlü, 2000 was distinguished as a new species from Manavgat River system in southern Turkey, where is considered regional endemic (Bogutskaya *et al.* 2000; Giannetto and Innal 2021). According to IUCN Red List, it was assessed as endangered species, with a decreasing population trend (Barrios *et al.* 2014; Innal and Giannetto 2020). The Antalya bleak was studied concerning the morphometric measurements (Mangit, 2014), age and growth parameters (Innal and Gülle 2019) or phylogeography (Bektas *et al.* 2020), however with respect to ichthyoparasitofauna, data remain scarce. The copepod *Lernaea cyprinacea* was detected before on *A. baliki* in Karpuzçay Creek (Innal, 2020). Also, the acanthocephalan *Pomphorhynchus laevis* specimens were identified in this host in streams discharging into Antalya Bay (Aydoğdu *et al.* 2011).

Pomatoschistus anatoliae Engin & Innal, 2017 is a gobiid fish described from the estuary of Göksu River on the Mediterranean coast of the Anatolia. To the best of authors' current knowledge, there are no registered information concerning the biometry or parasitofauna of this fish species.

The mugilid host *Chelon ramada* Risso, 1827 syn. *Liza ramada* or the Thinlipp grey mullet was recorded in Beymelek Lagoon, Antalya (Uysal *et al.* 2008) and all coasts of Anatolian brackish waters (Çiçek *et al.* 2015). Grey mullets are recognized as fish species with economic importance because of the high quality of the their flesh (Reis and Ateş 2020). Worldwide, several parasite species of Myxosporea, Monogenea, Digenea, Acantocephala and Crustacea were recorded in this fish species (Merella and Garippa 2001; Ragias *et al.* 2005; Öktener, 2014; Polinas *et al.* 2021). The copepod *Lernaea cyprinacea* was detected before on *Chelon ramada* (<http://www.marinespecies.org>).

Although a lot of parasitological observations were made in fishes inhabiting the freshwater systems, there is still a paucity of data regarding the occurrence of lerneid species in brackish water. Thus, this study was aimed to diagnose the presence of a well-documented crustacean affecting the health of its hosts, namely the opportunistic copepod *Lernaea cyprinacea* among native fish

from Karpuzçay Creek Estuary, one of the brackish water systems of the Mediterranean coast of Turkey.

MATERIAL AND METHODS

Overall, 140 fish individuals belonging to three species were analyzed: 60 *Alburnus baliki* Bogutskaya, Küçük & Ünlü, 2000 (Antalya bleak), 53 *Chelon ramada* Risso, 1827 (Thinlipp grey mullet) and 27 *Pomatoschistus anatoliae* Engin & Innal 2017.

Fish samples were taken between November 2014 and April 2019 using a shore seine net (10 m long and 2 m high; 1.2 x 2 mm mesh size) from Karpuzçay Creek (36°42'56.84" N 31°33'00.95" E; Figure 1), which flows into the Antalya Gulf (Mediterranean Sea, Turkey).



Figure 1. Map of the study site

Immediately after collecting, fish samples were transported in plastic bags to the Burdur Mehmet Akif Ersoy University Ichthyology Laboratory. Each fish individual was measured for total length (TL) to the nearest 0.1 cm, and weighed (W) to the nearest 0.1 g.

Fish species were examined with a dissecting microscope for the presence of ectoparasites. The females of parasitic copepod *L. cyprinacea* were identified according to Bauer (1987). Prevalence and intensity of infection were computed (Bush *et al.* 1997).

The taxonomic names and scientific classification for fish species herein were made in accordance with FishBase (Thomson, 1990; Engin and Innal 2017; Fricke *et al.* 2020).

During the necropsy, samples of fish skin lesions were collected at the site of parasitic infection. For histopathological assessment, the whole body of small fish individuals was transversally cut and fixed in 10% neutral formalin solution. Parasite attachment areas were selected and skin samples were prepared by an automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany). The tissues of fish were embedded in paraffin, and 5 µm serial sections were acquired using a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). Subsequent, histopathological sections were stained with hematoxylin and eosin (HE) and examined under 40X magnification of a light microscope. Morphometric evaluation and microphotography were performed using the Database Manual cellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

RESULTS AND DISCUSSION

The highest prevalence of infection was reached in native species *Chelon ramada*, followed by *Pomatoschistus anatoliae* and *Alburnus baliki*, two fish species endemic to Turkey (Table 1) (7.5%; 3.7% and 3.33%, respectively). The mean intensity recorded was same in all three analyzed species (1 parasite/fish).

The copepod parasites were collected from the skin and external surfaces of the fins belonging to the fish hosts (Figure 2).

Widespread in Turkey, the copepod ectoparasite *Lernaea cyprinacea* is considered a possible threat to the endemic fish stocks, such as *Alburnus baliki* (Innal, 2020).

Table 1. Occurrence of *Lernaea cyprinacea* in host species from Karpuzçay Creek Estuary and biometric values of fish species examined (N=total number of fish individuals, N'=number of infected fish, P=prevalence of infection, I=intensity of infection, O=origin, E= endemic, N=native)

Species	Order	Family	O	Range of TL (cm)	Range of W (g)	N	N'	P(%)	I
<i>Alburnus baliki</i> Bogutskaya, Küçük & Ünlü, 2000	Cypriniformes	Leuciscidae	E	4.2-10.8 (mean 6.4)	0.6-15.7 (mean 2.69)	60	2	3.33	1
<i>Chelon ramada</i> (Risso 1827)	Mugiliformes	Mugilidae	N	1.6-15.9 (mean 4.27)	0.018-40 (mean 2.53)	53	4	7.55	1
<i>Pomatoschistus anatoliae</i> Engin & Innal 2017	Gobiiformes	Gobiidae	E	2.1-4 (mean 2.92)	0.074- 0.558 (mean 0.227)	27	1	3.7	1



Figure 2. *Lernaea cyprinacea* (arrows) on the body hosts: A. *Chelon ramada*; B. *Alburnus baliki*; C. *Pomatoschistus anato liae*

In Karpuzçay Creek Estuary, the highest infection prevalence value was found in *Chelon ramada* (7.5%) and the lowest in *Alburnus baliki* (3.3%). Despite the total number of *P. anato liae* was reduced (27) comparing with the other two sampled fishes, the prevalence of infection was higher than in the case of *A. baliki* represented herein by numerous fish individuals (60). This may suggest that Antalya bleak has a vulnerability to parasitic copepods. In addition, *P. anato liae* represent a new host record for the occurrence of *Lernaea cyprinacea*, therefore future investigations would be necessary for elucidate the aspects of host-parasite interactions.

Difference values for prevalence and intensity of infection depend on various factors, such as season, sample size, feeding habits, sex ratio, length range or physico-chemical parameters (Barson *et al.* 2008; Hossain *et al.* 2018 Innal, 2020; Khalid *et al.* 2021).

In *Lernaea cyprinacea* infection, hemorrhagic and ulcerative skin lesions are common findings (Mirzaei, 2015; Innal *et al.* 2017; Urku and Onalan 2018). The gross pathology noticed in this study were similar to other reports concerning the effects of lerneosis in teleost fishes

Microscopically, severe pathological findings were reported in all analyzed fish hosts, in areas where copepod *Lernaea cyprinacea* was attached. Numerous parasites were commonly localized in the abdominal regions and skeletal muscle (Figure 3).

At the histopathological examination, lesions causing ulcers were also found in the epidermis and dermis of the fish hosts. Characteristic chronic

granulomatous inflammatory reaction was observed in all attachment sites of *L. cyprinacea*.

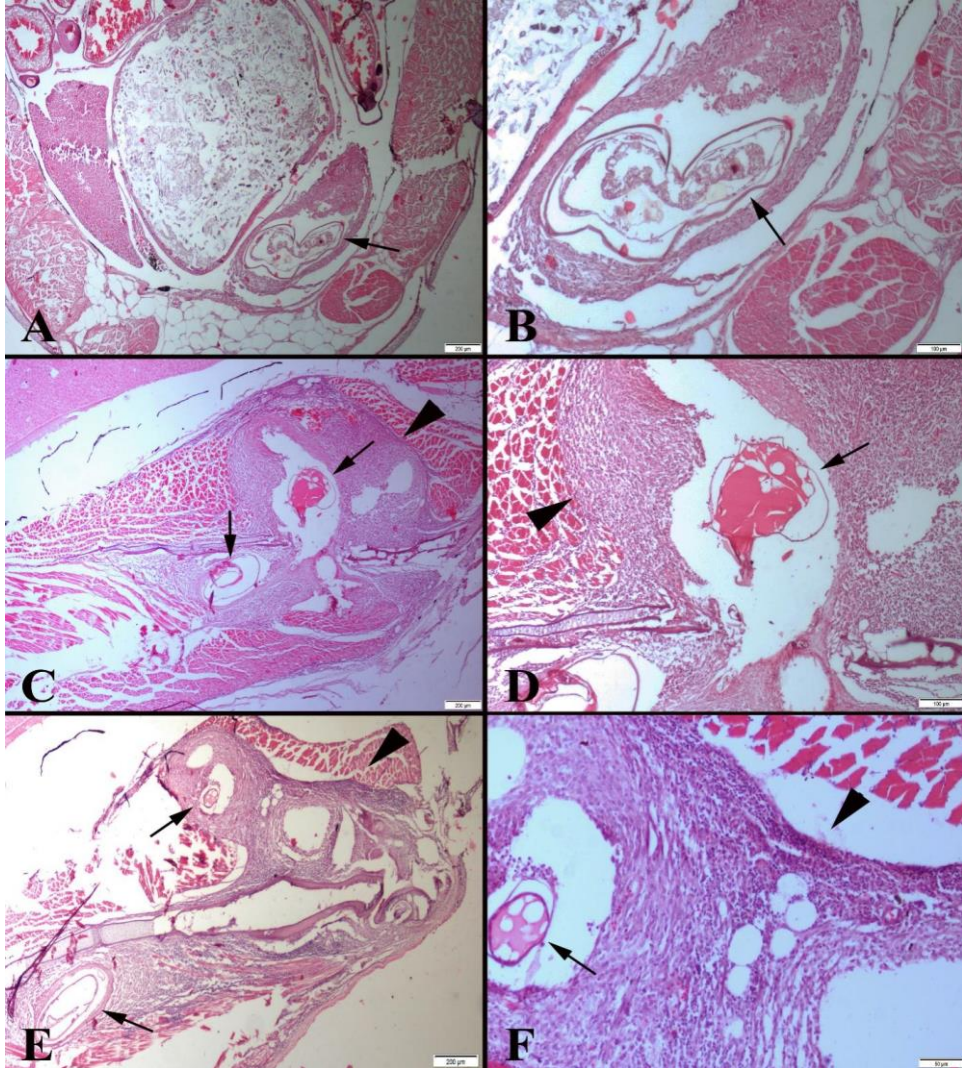


Figure 3: *Lernaea cyprinacea* related histopathological findings in the fish hosts. (A) Granulomatous reaction around the parasite (arrow) localized abdominal cavity, HE, Bar=200 μ m. (B) Higher magnification of the granuloma, HE, Bar=100 μ m. (C) Parasites (arrows) localized skeletal muscle and inflammatory reaction around the parasite (arrow head), HE, Bar=200 μ m. (D) Higher magnification of the granuloma, HE, Bar=100 μ m. (E) Parasites (arrows) localized skeletal muscle covered by diffuse granulomatous reaction (arrow head), HE, Bar=200 μ m. (F) Higher magnification of the inflammatory reaction (arrow head) around the parasite (arrow), HE, Bar=50 μ m.

Mainly lymphocytes, histiocytes, and eosinophils were infiltrated as a response to the parasite presence in affected site. Commonly lesions in abdominal cavity were covered by fibrous tissue. Chronic granulomatous myositis and dermatitis were common findings. In the damaged areas were noticed edema, congestion, and small areas of hemorrhage. Histopathologically parasitic infections generally cause a granulomatous inflammatory response and capsule formation around the parasite, together with melanization and inflammatory cell infiltrations for to isolate and destroy them in fishes (Torres *et al.* 2002; Dezfuli *et al.* 2007; Feist and Longshaw 2008). Sometimes encapsulation can be associated with hemorrhage in the tissues immediately surrounding the encapsulated parasite reported if the excessive number of parasites exists (Ogawa *et al.* 2004). In cases of muscle parasites, migration stages can cause in mechanical destruction of the musculature (Baturo, 1980).

CONCLUSIONS

To the best of our knowledge, this survey reports the first microphotographs of the affected tissues by copepod *Lernaea cyprinacea* in *Chelon ramada*, *Alburnus baliki* and *Pomatoschistus anatoliae*. According to IUCN Red List Status, *C. ramada* is categorized as LC (least concern), *A. baliki* as EN (endangered), while *P. anatoliae* was not yet evaluated (Freyhof 2014; Freyhof and Kottelat 2018). Due to the fact that the Antalya bleak is an endangered species, further studies are needed in order to assess the aspects regarding its epidemiology in natural populations. Moreover, the economic importance of mugilids such as *C. ramada* requires that future research should be focused on assessing the losses induced by lerneid parasites as well as applying good fishery management practices.

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