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RESEARCH PAPER

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Infection of European Eel, *Anguilla anguilla* with the Nematode *Anguillicoloides crassus* from Some Estuarine Systems in Turkey

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Abstract

Despite the potential importance of parasitic nematode, *Anguillicoloides crassus* for the survival of eel, only a few studies have addressed this issue on eel stocks of Turkey. The current study reports baseline data on prevalence of parasites and pathological status in naturally infected eels from three important river estuaries (Seyhan River, Göksu River and Manavgat River) in Turkey. In total, 70 individuals of *Anguilla anguilla* from three river estuaries were examined and 46 (65.7%) specimens were infected by *Anguillicoloides crassus.* The nematodes were easily observed together with the excessive hemorrhagic exudate in the swimbladder. The swimbladder of eels were also pathologically examined. The uninfected swimbladder samples were grossly thin and shiny but in infected cases the tissues were detected as opaque, thick, hyperemic and mostly hemorrhagic. Histopathological examination revealed that focal to diffuse inflammation, marked hemorrhagies, erosive-ulcerative changes at the mucosa, edema and hyperemia at the submucosa. In severe cases muscularis mucosa were also affected.

Introduction

The European eel, *Anguilla anguilla* (L.), has been reported to be declining throughout its natural distribution range over the last decades (Guhl, Stürenberg & Santora, 2014; Aalto *et al*., 2016). The causes of eel decline and/or local extinctions track the similar situation as pointed out for other diadromous species and are rarely due to just one factor in their origin, resulting probably from cumulative and/or interplay of several anthropogenic effects (Costa Dias, 2010). The decline in European eel populations might be partly due to infection by *Anguillicoloides crassus* Kuwahara, Niimi and Itagaki 1974 because of its high pathogenicity (Han *et al*., 2008). *A. crassus* is a bloodsucking nematode and native parasite of the Japanese eel *Anguilla japonica.* The parasite firstly reported and described in 1974 and is one of the most common pathogenic parasite of eels (Kuwahara, Niimi & Itagaki, 1974). Researchers reported that *A. crassus* was introduced accidentally into Europe with Japanese eels from Asia at the beginning of 1980s (Koops and Hartmann, 1989; Molnar, Szekely & Baska, 1991).

A. crassus belongs to the Dracunculidae family. The eels are infected via ingested copepod and small fish, which are the intermediate hosts for infective third-stage larvae (Peters and Hartmann, 1986; Evans and Matthews, 1999). After ingesting infected crustacean intermediate hosts, the adult parasites localize to the swim bladder and feed on blood (De Charleroy, Grisez, Thomas, Belpaire & Ollevier, 1990; Polzer and Taraschewski, 1993). If the parasites in greater number,

they can induce increased pathology, including thickening and inflammation of the swim bladder wall, increased anal redness and in some severe cases rupture of the bladder (Evans and Matthews, 1999; Wurtz and Taraschewski, 2000; Crean, Dick, Evans, Elwood & Rosell, 2003).

The European eel is a species of ecological, economic and social importance within the Mediterranean Coast of Turkey. Parasites of the European eel have been intensively studied (Neumann, 1985; Koops and Hartmann, 1989; Koie, 1991; Sures and Streit, 2001; Rodjuk and Shelenkova, 2006). Despite the potential importance of *A. crassus* for the survival of eel, only a few studies have addressed this issue on stocks of Turkey. Considering its economic profitability, studies on pathological damage in European eels caused by parasites are essential because of their perceived importance in the growing fisheries industry in Turkey. Therefore, the current study reports baseline prevalence data for *A. crassus* and pathological status in naturally infected eels from three important river estuaries in Turkey.

Materials and Methods

Sampling Sites

The study was conducted on European eel specimens sampled from three river estuaries [Seyhan River (Tarsus, Mersin); Göksu River (Silifke, Mersin); Manavgat River (Manavgat, Antalya)] located throughout Mediterranean Coast of Turkey between November 2014 to June 2017. Description of sampling sites are given in Table 1.

Fish Sampling and Basic Measurements

At each site, beach seine surveys and fyke nets were used to catch all size classes of eels present in the estuary systems. A total of 70 individuals (22 individuals from Manavgat River; 28 individuals from Göksu River; 20 individuals from Seyhan River) of *A. anguilla* from three river estuaries were examined for swimbladder parasites. Their body size (The length of the eels were measured to the nearest 1.0 mm), and number were recorded. Specimens of *A. anguilla* were sorted into four length classes (<20; 20-39.9; 40-59.9; >60 cm).

Host-Parasite System Examination

Speciemens of European eel were examined for the presence of *A. crassus* immediately after collection. Parasites were removed from the swimbladder lumen of the eels by forceps. Parasites were identified as *A. crassus* following the criteria by Moravec (1994).

Statistical Analysis

Prevalence (Pr%), as the percentage of hosts infected with a particular parasite species or taxonomic group and intensity (Int), as the number of individuals of a parasite species in/on a single infected host was calculated following Bush, Lafferty, Lotz & Shostak (1997). Infection rates and statistical analyses were conducted by using Quantitative Parasitology 3.0 web application (Rozsa, Reiczigel & Majoros, 2000), Excel programme and SPSS 15. The $\chi 2$-test was performed to test for significant differences between the infection rates over the four length classes. A Kruskal-Wallis test was carried out on data to determine differences in infection rates at different locations. Differences were considered to be significant when $p \leq 0.05$.

Histopathological Examination

The swimbladder of the eels was grossly examined and parasites localized in the bladder harvested. Tissue samples were collected from the swimbladder during the necropsy and fixed in a buffered 4% formaldehyde solution. Specimens for histology were processed by an automatic tissue processor equipment (Leica ASP300S, Wetzlar, Germany) and embedded in paraffin. Sections (5µm) were cut by a Leica RM2155 (Wetzlar, Germany) rotary microtome and mounted on glass slides before staining with Hematoxylin and Eosin (H&E). Stained sections were examined under light microscopy (Olympus CX41, Tokyo, Japan). Morphometric evaluation and microphotography was performed using

Table 1. Description of sampling sites

No	Locality	Type	Substrates	Flow velocity	Sampling stations	Coordinates
1	Manavgat, Manavgat (Antalya)	Open river estuary	silt-sand	Fast	I	36º44´15.49" N 31º29´37.09" E
					II	36º45´18.64" N 31º27´49.04" E
					III	36º45´36.92" N 31º27´25.82" E
2	Göksu, Silifke (Mersin)	Open river estuary	silt-sand	Fast	I	36º18´08.16" N 34º02´47.76" E
					II	36º18´43.86" N 34º02´05.90" E
					III	36º20´12.57" N 34º01´38.02" E
3	Seyhan, Tarsus (Mersin)	Open river estuary	silt-sand	Fast	I	36º43´42.77" N 34º54´44.50" E
					II	36º44´29.88" N 34º56´39.64" E
					III	36º45´13.46" N 35º00´09.15" E

the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

Results

The prevalence and intensity values of parasites of estuarine systems are presented in Table 2. In total 70 individuals of *Anguilla anguilla* from three river estuaries were examined and 46 (65.7%) specimens were infected by *Anguillicoloides crassus.* The intensity of *A. crassus* infection in all the eel specimens ranged from 1 to 48 parasites at a mean value of 6.11. Parasite prevalence and mean intensity differed by area. The highest prevalence was 75% in Seyhan River Estuary, followed by Manavgat River Estuary at 63.7% and Göksu River Estuary at 60.7%. The highest mean intensity was 6.67 in Seyhan River Estuary, followed by Göksu River Estuary at 5.94 and Manavgat River Estuary at 5.71.

The prevalence and intensity values of parasites according to the host length classes are presented in Figure 1. The prevalence and intensity levels of infection with *A. crassus* varied with host length. The highest prevalence of infection in both Manavgat and Göksu River Estuaries was observed in the specimens, which are longer than 60 cm. On the other hand, the highest prevalence of infection in Seyhan River Estuary was observed in the speciemens of 40-60 cm length classes. Analysis of intensity indicates that speciemens longer than 60 cm exist the highest infection value in Seyhan and Göksu River Estuaries. But for Manavgat River Estuary, the higgest levels of infection were observed in 40-60 cm length classes. The prevalence and intensity levels of infection among length classes when compared statistically revealed significant differences ($P<0.05$).

At the gross examination of the eels the swim bladders were thin and shiny in not infected cases but they appeared opaque, thick, hyperemic and mostly hemorrhagic in infected cases. When parasite present, the worms were easily visible in the lumen of the swim bladder together with abundant hemorrhagic exudate. Different stages of the larvae (L2, L3 and L4), adults and numerous eggs were present in the exudate. (Figure 2). In addition, lowered elasticity and required increased pressure to swim bladder walls were observed in infected swim bladders. The affected swim bladders showed focal, multifocal, and generally diffuse gross lesions.

At the microscopical examination adult parasites easily identified by ingested blood in their gastrointestinal tracts (Figure 3A). Histopathological examination revealed that focal to diffuse inflammation, marked hemorrhage, erosive-ulcerative changes at the mucosa, edema and hyperemia at the submucosa. In severe cases muscularis mucosae were also affected (Figure 3B-3C). If the parasites in numerous number abnormal papillomatous proliferations commonly observed. The acute reaction was characterized by hyperemic vessels, slight to marked hemorrhages, edema and leukocytes infiltration.

In chronic cases connective tissue proliferations and marked fibrosis together with lymphocytic infiltrations and coagulative necrosis of the epithelial lining were seen. Developing stages of the larvae were commonly seen in the lumen, submucosa, and serosa of the swim bladder. In some cases of these larvae become degenerated. Microscopically, adult worms containing numerous larvae and their blood-filled guts were observed inside the lumen of the swim bladder. Numerous of *A. crassus* larvae were found in the visceral cavity or any organ migration detected.

Discussion

The rapid development of the aquaculture farming industry and transfer of animals has been accompanied by a growth in the number of isolated pathogens and severity of associated diseases. Parasitic invasions are recognized as one of the primary factors responsible for decreasing populations of fish species. One of the most invasive parasite in European eel (*A. anguilla*) is the nematode, *Anguillicoloides crassus* (Popielarczyk, Robak & Siwicki, 2012). It was introduced from south-eastern Asia into western European water bodies as a result of uncontrolled intercontinental transfer of live eels for market purposes (Koie, 1991). It quickly developed in all of Europe in almost 10 years. This parasite was introduced in Turkey; it was cited for the first time at 1996 in a study in the waters of Ceyhan River (Genç, Sahan, Altun, Cengizler & Nevsat, 2005). The eel is an important component of freshwater, Lagoon and River estuarine systems of Turkey. After the first description of *A. crassus* in Turkey several studies have demonstrated that the parasite is widespread in this country and identified as a possible threat to the European eel stock. Genç, Sahan, Altun, Cengizler & Nevsat (2005) reported that *A. crassus* infection was identified in 50 out of 64 European eels collected from the Ceyhan River (Adana, Turkey) in 2002. In other reports, Genç, Sangun, Dural, Can & Altunhan, (2008) reported 11 out of 18 specimens were found infected by the swimbladder nematode collected from the Asi River in 2006. It has been reported that Köyceğiz Lagoon populations of European eel are infected with *A. crassus* with 39.7% percentage (Çolak, Soylu, Erdoğan & Erdoğan, 2012). Infection of *A. crassus* were reported from Göksu, Seyhan, Ceyhan and Asi River Systems (Koyuncu, Kaya, Özer, Barış & Genç, 2017).

This study has shown that *A. crassus* has invaded three big river catchments since its introduction dates. These prevalences are consistent with those obtained by Genç, Sahan, Altun, Cengizler & Nevsat (2005) and Genç, Sangun, Dural, Can & Altunhan (2008) but higher than those obtained by Çolak, Soylu, Erdoğan & Erdoğan (2012).

In other Mediterranean countries, reported infection rates have been reported as follows: 2.3% in Ghar El Melh Lagoon Lake, 15.5% in Bizerte Lagoon Lake

Table 2. Prevelance and mean intensity of infection from three river estuaries

Locality	N*	Ni	P%	Int
Manavgat River	22	14	63.6	5.7
Göksu River	28	17	60.7	5.9
Seyhan River	20	15	75	6.7
Total	70	46	65.7	6.1

*N: number of eel samples, Ni: number of infected eels, P%: prevalence, Int: intensity

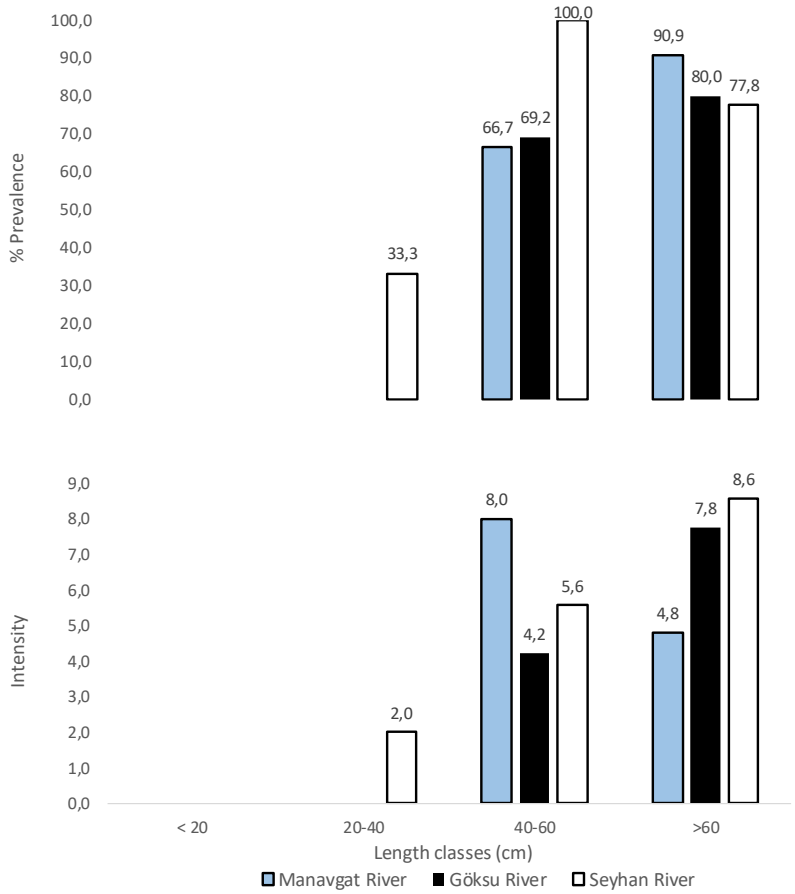

Figure 1. Prevalence and Intensity of infection according to host length.

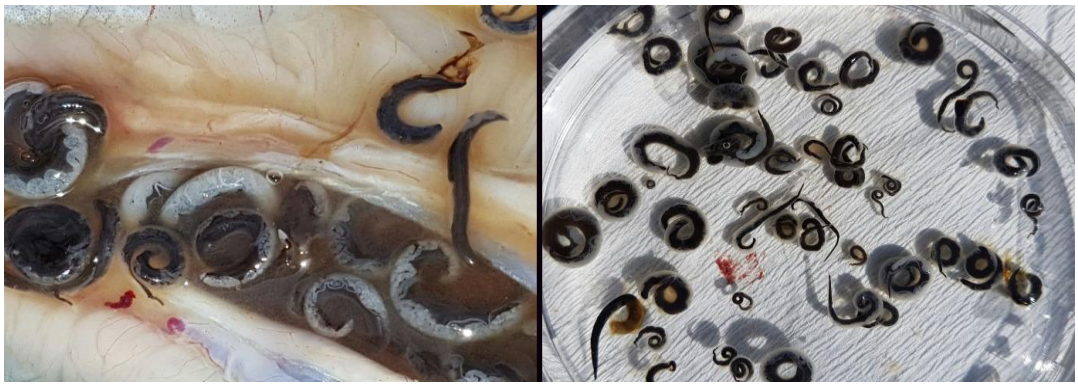

Figure 2. Numerous *Anguillicoloides crassus* nematodes in the swim bladder of a European eel.

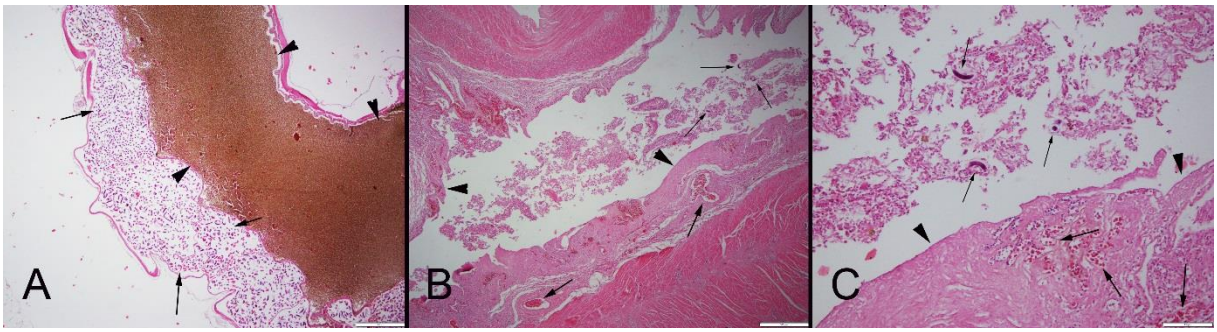

Figure 3. A: Histological appearance of a mature *Anguillicola crassus* section, numerous developmental stages (arrows) and ingested blood (arrow head), HE, Bar= 500µm. **B:** Section of the swim bladder of *Anguillicola crassus* infected eel, numerous developmental stages (thin arrows), hyperemic vessels (thick arrows) and desquamated epithelial layer of the swim bladder (arrow head), HE, Bar= 500µm. **C:** Higher magnification of the swim bladder infected with *Anguillicola crassus* numerous developmental stages (thin arrows), hyperemic vessels (thick arrows) and desquamated epithelial layer of the swim bladder (arrow head), HE, Bar= 100µm.

and 46.3% Ichkeul Lake (Habbechi, Kraiem & Elie, 2012); 3.66% in Ghar El Malh Lagoon Lake (Dhaouadi *et al*., 2014) in Tunisia; 50.44% from Lake Oubeira (Tahri, Crivelli, Panfili & Bensouilah, 2016); 48% Tonga Lake and 50% Mafrag Estuary (Nawel *et al*., 2015) in Algeria; 7,34% from Mar Menor Lagoon Lake in Spain (Martinez Carrosca *et al*., 2011); 10.7% from Eastern Delta (Abdelmonem, Metwally, Hussein & Elsheikha, 2010) and 54.1% from Lake Al Broullus (Abeer, 2016) in Egypt; 61.7% from Vistonis Lake in Greece (Macnamara *et al*., 2014).

The difference in the prevalence is likely due to abiotic parameters of systems and also biotic factors such as host age and size and fish community structure. In this study, *A. crassus* was detected in the majority of length groups sampled (Figure 1). One size classes (< 20 cm) were found to be not infected but sample sizes for these were small. Higher prevalence levels were seen in eels measuring >40 cm size classes. No significant differences were found between prevalence and mean intensity between the estuarine habitats.

Histopathological examination of swimmbladder of the eels that infected with this nematode is rarely reported (Genç, Sahan, Altun, Cengizler & Nevsat, 2005; Sokolowski and Dove 2006; Abdelmonem, Metwally, Hussein & Elsheikha, 2010; Keppel, Dangel & Sures, 2014). In these studies, hemorrhages, edema and inflammatory reaction related with parasites were commonly encountered in swimmbladders. Similar findings were also seen in this study and severity of the lesions were related to the intensity of the parasites. In some cases, all developmental stages of the *A. crassus* larvae and adult forms were found in swimbladder lumen. In severely infected eels, all layer of the swimbladders such as tunica interna, tunica externa, muscularis mucosa, submucosa and vascular connective tissues were affected. In the present study the epithelial changes and deformities detected as pathology in the infected swimbladder including hemorrhages, edema, leukocytes infiltration and coagulative necrosis. In view of this aspects, future studies should be focused on the effects of parasitic nematodes on population dynamics of European eel.

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