Polish Journal of Veterinary Sciences Vol. 15, No. 2 (2012), 253-257

DOI 10.2478/v10181-011-0142-4

Original article

Infection of European eel, *Anguilla anguilla* (L.), with the nematode *Anguillicoloides crassus* (Kuwahara, Niimi et Itagaki, 1974) in Polish waters

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Abstract

The aim of this study was to determine the degree of *Anguillicoloides crassus* infection in European eel inhabiting Polish waters based on selected parasitic descriptors and on anatomical pathology of the swimbladder using macroscopic methods. In all, 154 European eel specimens were sampled from eleven sites in Poland and *A. crassus* was present in the swimbladder of 114 fish. The intensity of *A. crassus* infection in all the eel specimens ranged from 1 to 62 parasites at a mean value of 7.5. High values of mean infection intensity were noted in samples from Pomeranian lakes Bukowo, Łebsko, and Jamno. The health of the swimbladder was evaluated using the swimbladder degenerative index (SDI). The mean value of the SDI for all of the eel examined was 3.3, and extensively degenerated swimbladders were observed mainly in samples in the Szczecin Lagoon and from lakes. According to the individual SDI ratings, 9.1% of the eel specimens did not exhibit pathological symptoms of the swimbladder (SDI-0) and an extremely damaged (SDI-6) swimbladder was noted in 11.7% of the fish examined. In the case of eel infected with *A. crassus*, higher SDI values were reflected in initially increasing shares in subsequent categories. In fish that were not infected with the nematode, only 20% (8 individuals) of the swimbladders showed no symptoms of pathology (SDI-0).

Key words: *Anguillicoloide crassus,* parasite, nematode, infection, European eel, swimbladder degenerative index SDI

Introduction

Parasitic invasions are recognized as one of the primary factors responsible for decreasing populations of European eel (Székely et al. 2009). One of the most invasive parasites in this fish is the nematode *Anguillicoloides crassus*, which parasitizes the swimbladders of eels. This parasite was introduced to Europe in the early 1980s, probably with infected Japanese eel imported from Taiwan (Koops and Hartmann 1989).

The life cycle of the nematode *A. crassus* in Europe has been studied in detail by many authors

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(Kennedy and Fitch 1990, Thomas and Ollevier 1992, Moravec et al. 1994). This parasite is non-pathogenic in Japanese eel, but infections in European eel can lead to deteriorated condition and poor health, and, in extreme cases, death (Egusa 1979, Molnár et al. 1991, Molnár 1993). Pathological studies of the impact of *A. crassus* on the European eel in the wild and on farms indicate that these fish experience acute inflammation, fibrosis, and severe thickening of the swimbladder wall causing its lumen and size to decrease (Molnár et al. 1993).

The development of the parasite comprises several stages during which the occurrence of larval forms (from L1 to L4), pre-adults, and adults is noted (Thomas and Ollevier 1992, Moravec et al. 1994). Sexually mature female *A. crassus* release eggs with the first (L1) and second (L2) larval stages, in which, once swallowed by the intermediate host (planktonic crustaceans including those of the genera *Copepoda* and *Amphipoda*, and the phylum *Ostracoda*) they develop into the invasive stage that afflicts eel – L3 (Kennedy and Fitch 1990). The subsequent stages of the larval nematodes reach the swimbladder wall of the eels, and then enter this organ where they attain sexual maturity (Thomas and Ollevier 1992, Moravec et al. 1994).

The L3 larval stage of the nematode *A. crassus* has been confirmed in many fish species (Rolbiecki 2000) as well as in many species of amphibians, aquatic snails, and larval insects, which are paratenic hosts of the European eel (Moravec and Škoriková 1998). This wide range of paratenic and intermediate hosts in the aquatic environment has enabled *A. crassus* to spread very rapidly throughout Europe (Sures et al. 1999).

The current study aims to determine the degree of *A. crassus* infection in European eel inhabiting Polish waters based on selected parasitic descriptors and on anatomical pathology of the swimbladder using macroscopic methods.

Materials and Methods

The study was conducted on European eel specimens sampled from eleven sites located throughout Poland. In total, 154 specimens were examined with mean lengths from 55 cm to 79 cm and mean weights from 350 g to 1100 g. Before the examinations, the eel were anesthetized Propiscin (3 ml l^{-1}). The swimbladders were excised for anatomical pathology examinations. *A. crassus* that were visible to the naked eye were removed and fixed in 70% ethyl alcohol supplemented with glycerin. In order to analyze the anatomical pathology of the swimbladders, the organs were placed in a mixture of 6% formalin and 0.9% sodium chloride in proportion 1:20. Parasites collected from the swimbladder lumen were identified using the key by Moravec et al. (1994). The number of infected fish was evaluated and the parasitic descriptors defined by Bush et al. (1997) were described. The frequency of occurrence of the nematode *A. crassus* in the swimbladders of the eel examined was determined by identifying the quantitative range. The health of the swimbladder was evaluated using the swimbladder degenerative index (SDI). The categories of the SDI range from 0 (lack of pathological symptoms of infection) to 6 (extremely damaged) with higher values indicating greater degrees of swimbladder degeneration (Lefebvre et al. 2002). Each of the specimens examined was given an individual SDI rating. All of the specimens from given sampling sites were assigned a mean SDI value.

Statistical analyses were performed by calculating the arithmetic means, ranges (max/ min), and standard deviations (SD) using methods in STATISTICA 9.0 (StatSoft).

Results

Of the 154 swimbladders examined, the nematode *A. crassus* was confirmed in 114 (74.1%). The intensity of *A. crassus* infection in all the eel specimens ranged from 1 to 62 parasites at a mean value of 7.5. The parasitic descriptors for European eel sampled in Poland are presented in Table 1.

In total, 868 individuals of *A. crassus* were collected, including 698 pre-adults and 170 adults. Qualitative and quantitative analyses of the nematode *A. crassus* collected from swimbladder lumens of the eel examined are presented in Table 2.

In 62% of the eel examined, the number of parasites ranged from 1 to 4, with 22.8% of these fish infected with one parasite specimen. In the other ranges of 5 to 62 nematodes, the share of infected eel was from 3 to 13%. The share (%) of individual *A. crassus* noted in the swimbladders of the eel examined is presented in Fig. 1.

The mean value of the SDI for all of the eel examined was 3.3 (Table 1). According to the individual SDI ratings, 9.1% of the eel specimens (14) did not exhibit pathological symptoms of the swimbladder (SDI-0). The percentages of fish in subsequent SDI category rankings is as follows: SDI-1 – 10.3% or 16, SDI – 2 – 11.7% or 19, SDI-3 24.7% or 37, SDI-4 – 13.6% or 21, and SDI-5 – 18.8% or 29. An extremely damaged (SDI-6) the swimbladder was noted in 11.7% or 18 of the fish examined. The number and share (%) of eel examined is presented by SDI ranking in Table 3.

Table 1. Values of parasitic descriptors and mean values of SDI of eel infected with the nematode *A. crassus* according to sampling sites.

Sampling site and name	Number of infected eel	Prevalence (%)	Infection intensity		SDI
			Mean ± SD	Range	Mean ± SD
Szczecin Lagoon	26	92.9	10.8 ± 12.4	1 – 49	4.4 ± 1.03
Lake Dąbie	11	64.7	1.9 ± 1.5	1 – 5	1.0 ± 1.55
Lake Bukowo	9	64.2	18.5 ± 17.0	3 – 62	4.1 ± 1.83
Lake Łebsko	5	(100.0)	13.6 ± 20.9	1 – 50	4.8 ± 0.84
Lake Gardno	4	(80.0)	8.0 ± 5.6	3 – 16	4.4 ± 1.52
Lake Resko	12	85.7	7.2 ± 7.8	1 – 23	3.3 ± 0.99
Lake Jamno	10	76.9	11.6 ± 16.1	1 – 55	3.1 ± 2.02
Oder River	5	50.0	3.2 ± 2.5	1 – 7	3.6 ± 1.65
Vistula River	10	71.4	3.4 ± 3.3	1 – 6	1.5 ± 1.43
Węgorapa River	10	62.5	1.7 ± 1.1	1 – 4	3.0 ± 1.90
Drwęca River	12	66.7	2.6 ± 2.0	1 – 7	3.4 ± 1.97
Range	4-26	(50.0 – 100.0)	1.7 – 18.5	1 – 62	1.0 – 4.8
Mean	10	74.1	7.5		3.3
Total	114				

Table 2. Number and share (%) of the nematode *A. crassus* collected from inside eel swimbladders for all sampling sites.

		Mean	Total
Total number of *A. crassus* individuals	number	79	868
Pre-adults	number	64	698
	%	85.5	–
Adults	number	16	170
	%	14.5	–

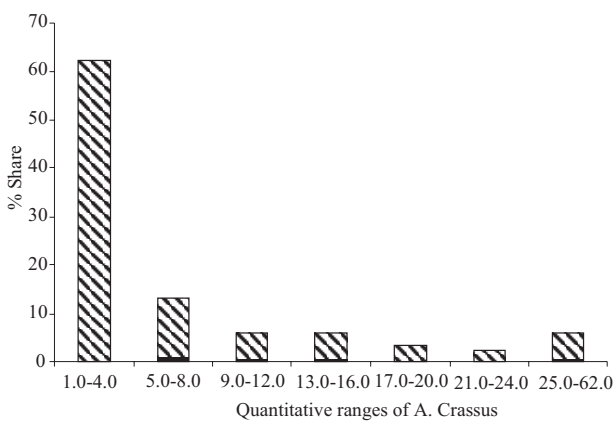

Fig. 1. Quantitative share (%) of *A. crassus* individuals confirmed in the swimbladders of eel examined

Discussion

Analysis of parasitic descriptors is one method of studying the pressure of *A. crassus* on its definitive host (Lefebvre et al. 2002). In the current study, the values of prevalence range from 50-100% and correspond to ranges reported by other authors (Morozińska-Gogol 2005). In other European countries the reported values for this parameter were also high and ranged from 60.7% in Lithuania (Pilecka-Rapacz and Kesminas 2006) to 64.4% in the Czech Republic (Palikowa and Navratil 2001) and 90.2% in Belgium (Thomas and Ollevier 1992). Among the sites analyzed, high values of mean infection intensity were noted in samples from Pomeranian lakes Bukowo, Łebsko, and Jamno. Significantly lower values of this parameter were noted in samples from rivers. The high infection numbers of the eel from Lake Łebsko confirms the results of the study by Morozińska-Gogol (2005). According to various authors (Palikowa and Navratil 2001, Kangur et al. 2002, Pilecka-Rapacz and Sobecka 2004, Rolbiecki 2008), the maximum number of specimens in the lumen of eel swimbladders can range from 1 to as many as 92 individuals. In these studies, the occurrence of more than twenty *A. crassus* individuals was noted in the swimbladders of only a small number of the eels examined.

In some of the samples, pre-adults comprised from 66.4% (Lake Jamno) to 100% (Oder and Węgorapa

Table 3. Share (%) and number of infected and non-infected eel according to swimbladder degenerative index (SDI).

Specimens of European eel		SDI						
		0	1	2	3	4	5	6
Specimens infected with *A. crassus*	number	6	10	9	27	17	27	18
	%	5.3	8.8	7.9	23.7	14.9	23.7	15.8
Specimens not infected with *A. crassusą*	number	8	6	10	10	4	2	0
	%	20	15	25	25	10	5	0
Total	number	14	16	19	37	21	29	18
	%	9.1	10.3	11.7	24.7	13.6	18.8	11.7

rivers) of all the nematodes collected. The greatest share (33.6%) of adult nematodes and their overall maximum number (62) were noted in the samples from Lake Bukowo (tab.2). Lefebvre et al. (2002) conclude that high numbers of parasites in swimbladders might be linked to the age of a given individual since older fish accumulate more parasites than younger ones. According to this author, as eels grow they consume more infected intermediate or paratenic hosts.

Another method which allows the impact of this parasite on eel to be studied is the analysis of changes occurring in the swimbladder (Lefebvre et al. 2002). Earlier studies by other authors (Molnár et al. 1993) point out that changes in the wall of the swimbladder resulting from the presence of various developmental stages can have a substantial impact on host condition while also lowering host resistance. Lefebvre et al. (2002) developed criteria to evaluate the health of this organ with the development of the swimbladder degenerative index. The criteria of this index permit additional, simplified evaluations of the health of eel swimbladders to be made and potential *A. crassus* infections to be confirmed (Lefebvre et al. 2002).

According to individual SDI rankings, 90.9%, or 140, of the eel specimens examined were in category SDI-1 at the least. In the case of eel infected with *A. crassus*, higher SDI values were reflected in initially increasing shares in subsequent categories. The majority of individuals from this group were eels ranked in categories SDI-3 and SDI-5 (27 individuals; 23.7%). In fish that were not infected with the nematode, only 20% (8 individuals) of the swimbladders showed no symptoms of pathology (SDI-0). The majority of individuals (10 individuals; 25%) in this group of fish were ranked in categories SDI-2 and SDI-3. No fish that were not infected with nematodes were categorized as having extensive swimbladder degeneration, which is the criterion for ranking in category SDI-6. In other European countries, the mean SDI in European eel is higher than 2, and in 30% of the population of this species significant pathology, inflammation, and sometimes even total blockage of the swimbladder lumen is noted (Lefebvre et al. 2002. 2003). The mean values for this parameter were high in the preceding analyses, and extensively degenerated swimbladders were observed mainly in samples from lakes and the Szczecin Lagoon.

Symptoms of pathology noted in swimbladders that were free of the nematode *A. crassus* suggest that infection of the eels analyzed is substantially higher than that noted based on parasitic descriptors. The observation by Lefebvre et al. (2002) that the SDI more accurately reflects parasite pressure on fish than does the traditional analysis of parasitic descriptors is therefore confirmed. The various criteria of this parameter offer a simple way to take into consideration the potential negative impact of nematode larval stages on the definitive host, especially when infection is strong and at a high intensity. According to other authors (Molnár et al. 1993), the negative impact of the parasite on the definitive host can significantly degrade host health while also stimulating the development of bacteria, fungi, and other parasitic infections. The high infection of *A. crassus* in European eel confirmed in the present study and by other authors (Rolbiecki et al. 2000. Rolbiecki 2008) indicates that the occurrence of the threats outlined in this paper is a real possibility, and also provides evidence of the rapid spread and permanent presence of this parasite in Polish waters.

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