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New data on the biology of the introduced exotic nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 in the eel *Anguilla anguilla* in Lake Wdzydze (Polish waters)

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Abstract

Anguillicola crassus Kuwahara, Niimi et Itagaki, 1974 is an Asian parasitic nematode that was introduced to Europe and inhabits the swim bladder of eels. In the 2002-2005 period, 237 eels, *Anguilla anguilla* (Linnaeus, 1758), from Lake Wdzydze were examined for the occurrence of the nematode. 79.3% of the fish were infected, at a mean intensity of 7.2 nematodes per fish. Morphological measurements of the observed *A. crassus* are presented. This is the first published report of *A. crassus* in eels from Lake Wdzydze.

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INTRODUCTION

Anguillicola crassus Kuwahara, Niimi et Itagaki, 1974 is a pathogenic nematode of the swim bladder of eels. Previously it inhabited only the Japanese eel *Anguilla japonica* Temminck & Schlegel, 1847 in East Asia. However, in 1982 it was reported in Europe (Germany), in the European eel *Anguilla anguilla* (Linnaeus, 1758) (cf. Neumann 1985). Neither the origin of this parasite nor its vector route to Europe are known for certain. It seems most probable that it was introduced with Japanese eels from either Taiwan (Køie 1991) or in re-exported European eels from Japan, or other East Asian countries (China or Taiwan) (Scholz 1999). In Europe, *A. crassus* has turned out to be a typical invasive species that rapidly attacked cultivated and natural European eel populations (Kirk 2003, Taraschewski 2006). It has also been reported in North Africa; in Egypt (Koops and Hartmann 1989), Morocco (El Hilali et al. 1996), and Tunisia (Maamouri et al. 1999), and has been introduced to North America where it infects the American eel *Anguilla rostrata* (Lesueur, 1817) (cf. Barse and Sector 1999). During the *A. crassus* life-cycle, there are intermediate hosts, primarily copepods (De Charleroy et al. 1990, Moravec et al. 1993), and paratenic hosts, mainly small fish (Thomas and Ollevier 1992a, Székely 1995, Rolbiecki 2002b). The eel (final host) becomes infected by feeding on invertebrates and small fish in which the invasive stage III larvae lives.

The results presented here supplement data on the biology and distribution of *A. crassus* in eels in Poland.

Materials And Methods

During the 2002-2005 period, a total of 237 swim bladders from eels (of lengths 500-935 mm) caught in Lake Wdzydze were examined. Due to technical reasons, the majority of the material (82%) was frozen prior to analysis. In order to test any relationship between the occurrence of *A. crassus* and eel length, the fish were divided into four length classes: 500-600 mm (57 fish), 601-700 mm (89 fish), 701-800 mm (59 fish), and >800 mm (32 fish).

In order to confirm the presence of the parasite, the swim bladder lumen and walls were viewed under a stereomicroscope and then under a microscope. The adult nematode specimens were collected and the defrosted larvae were fixed and preserved in 70% ethanol. Live larvae were sacrificed in hot 70% ethanol and then cleared in lactophenol; glycerin jelly preparations were made with some of the larvae (Rolbiecki 2002a).

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Results

The prevalence of infection by the nematode *A. crassus* (in all stages) in the examined eels was 79.3%, at a mean intensity of 7.2 individuals (ind.) per eel, and a range of intensity of 1-46 ind. Eggs with stage II parasite larvae or hatched larvae (Fig. 1) were noted in the majority of infected fish swim bladders (87.3%). Due to their high number they were not included in the infection parameters.

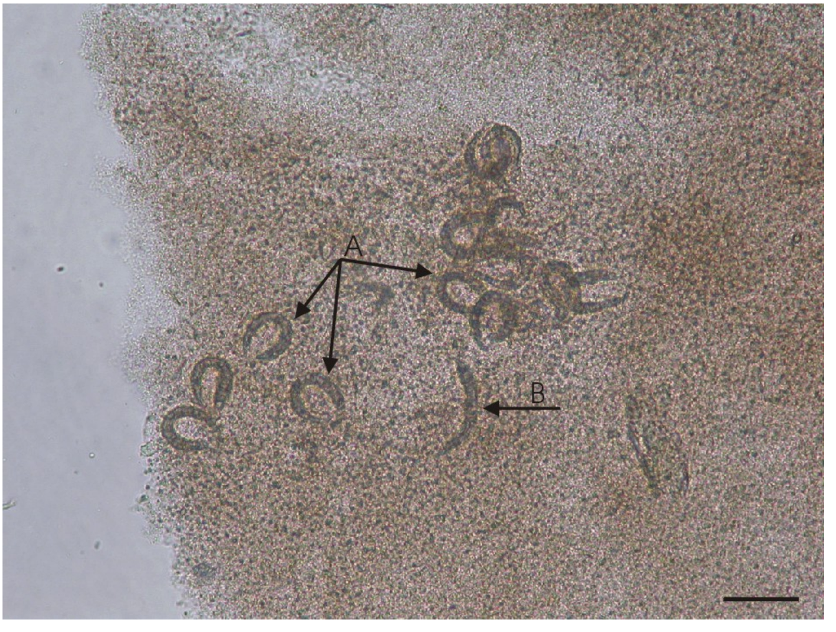

Fig. 1. *Anguillicola crassus* second stage larva. A. larva inside the egg, B. freshly hatched larva. Scale bar: 100 μm.

Most of the nematodes collected were adult (Fig. 2), with a slightly higher proportion of females (581 ind.) than males (540 ind.), stage IV larvae (168 ind., Fig. 3), and stage III larvae (71 ind., Fig. 4). All of the larvae in stage III and some in stage IV were observed in the bladder wall, while the remaining nematodes and eggs were seen in the swim bladder lumen.

Higher prevalence values (all nematode stages) were noted in eels of the 701-800 mm length class (86.4%), while the highest intensity (mean and range) were seen in fish from the >800 mm length class (8.1 ind. and 2-46 ind., Tab. 1).

Fig. 2. *Anguillicola crassus adult in eel swimbladder.* Scale bar: 1 cm.

Fig. 3. *Anguillicola crassus* fourth stage larva. Scale bar: 1 mm.

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Fig. 4. *Anguillicola crassus* third stage larva. Scale bar: 100 µm.

Table 1

Level of infection by *Anguillicola crassus* in different eel size classes.

Length [mm]	Prevalence [%]	Mean intensity [ind.]	Density [ind.]	Range of intensity [ind.]
500-600	77.2	6.6	5.1	1-16
601-700	75.3	7.1	5.3	1-25
701-800	86.4	7.5	6.5	2-43
above 800	81.3	8.1	6.6	2-46
Total	**79.3**	**7.2**	**5.7**	**1-46**

Descriptions of nematodes (measured fresh before fixing, measurements in mm):

Male (n=20): body 6-26 × 0.511-2.3 (mean 15.3 × 1.11), buccal capsule 0.019-0.027 × 0.031-0.046 (mean 0.023 × 0.045), oesophagus 0.528-0.755 × 0.091-0.287 (mean 0.654 × 0.209), length ratio of oesophagus and body 1 : 9.2 - 36.8 (mean 1 : 26.9), distance between the nerve ring and the anterior extremity 0.151 - 0.347 (mean 0.203), distance between the excretory pore (not always visible) and the anterior extremity 0.528 - 0.878 (mean 0.665), prominent cloacal process 0.055 - 0.146 (mean 0.100), rectal glands 0.088 - 0.416 × 0.088 - 0.307 (mean 0.232 × 0.169), tail 0.153 - 0.255 (mean 0.203).

Female (n=20): body 8 - 35 × 0.401 - 4.1 (mean 23.6 × 2.8), buccal capsule 0.018 - 0.029 × 0.044 - 0.066 (mean 0.023 × 0.053), oesophagus 0.604 - 0.895 × 0.181 - 0.301 (mean 0.735 × 0.224), length ratio of oesophagus and body 1 : 13.2 - 39.1 (mean 1 : 25.7), distance between the nerve ring and the anterior extremity 0.151 - 0.226 (mean 0.204), distance between the excretory pore (not always visible) and the anterior extremity 0.511 - 0.885 (mean 0.701), distance between vulva and the posterior extremity 0.945 - 8.118 (mean 4.873), tail 0.199 - 0.302 (mean 0.241), rectal glands 0.242 - 0.574 × 0.124 - 0.528 (mean 0.428 × 0.351), eggs without larvae 0.044 - 0.058 × 0.029 - 0.036 (mean 0.051 × 0.034), eggs with larvae 0.051 - 0.088 × 0.036 - 0.073 (mean 0.058 × 0.054).

L4 larvae (n=10): body 3.998 - 4.121 × 0.196 - 0.257 (mean 4.308 × 0.222), oesophagus 0.491 - 0.559 × 0.121 - 0.272 (mean 0.526 × 0.173), distance between the nerve ring and the anterior extremity 0.204 - 0.263 (mean 0.241), rectal glands 0.095 - 0.12 × 0.08 - 0.091 (mean 0.109 × 0.085), tail 0.146 - 0.263 (mean 0.19).

L3 larvae (n=10): body 0.998 - 2.914 × 0.063 - 0.091 (mean 1.818 × 0.071), oesophagus 0.196 - 0.277 × 0.018 - 0.029 (mean 0.221 × 0.022), distance between the nerve ring and the anterior extremity 0.099 - 0.131 (mean 0.115), tail 0.069 - 0.098 (mean 0.084).

L2 larvae (n=20): body 0.136 - 0.181 × 0.011 - 0.016 (mean 0.157 × 0.014), oesophagus 0.023 - 0.031 × 0.004 - 0.006 (mean 0.028 × 0.005), tail 0.019 - 0.031 (mean 0.025).

Eggs from the lumen of swimbladders (n=20): size 0.058 - 0.085 × 0.027 - 0.055 (mean 0.062 × 0.046).

DISCUSSION

A. crassus is currently the most dangerous European eel nematode parasite, and has turned out to be more of a threat to the European eel than to its Japanese relative (typical host). In Japan this nematode has been reported as occurring in a maximum of 40% of eels in a population. Although in those infections *A. crassus* inhabit the swim bladder and feed on blood, they do not cause significant damage to their host. In the course of its evolution, the Japanese eel has become immune to the harmful effects of this parasite (Egusa 1979). However, prevalence of infections in European eel populations have been reported to be as high as 100%, with intensity of even several tens of adult nematodes in one fish. These infections have an impact on the condition of the host fish. Blood feeding nematodes (Fig. 5) have been reported to cause damage

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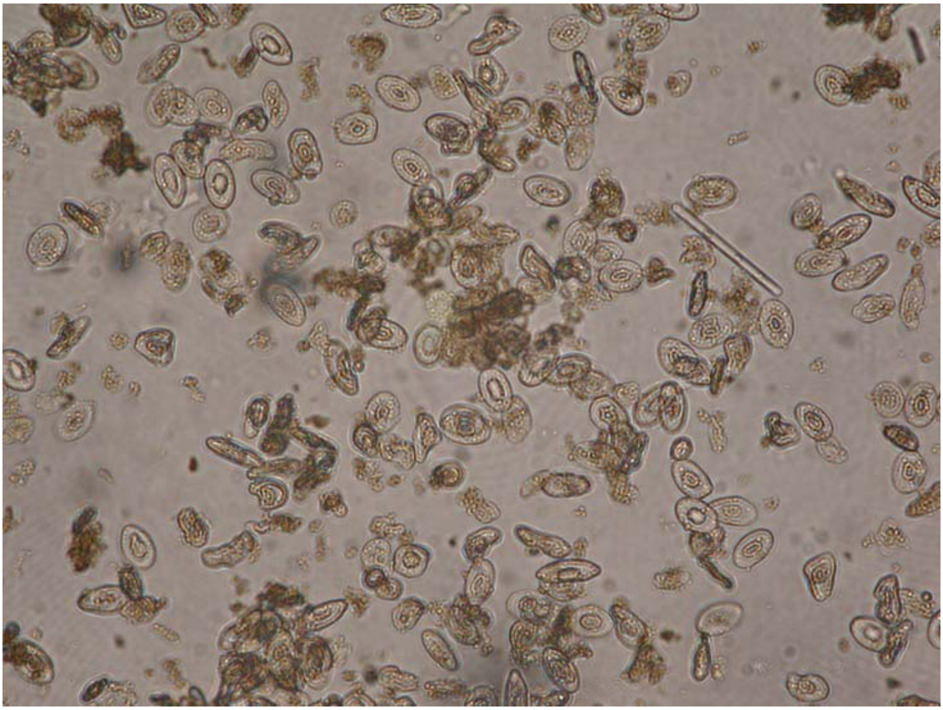

Fig. 5. Erythrocytes from the lumen of *Anguillicola crassus* intestine.

to the swim bladder (e.g. Molnar et al. 1993, Würtz and Taraschewski 2000), and cause the serious disease known as anguillicolosis. Larvae that are migrating in the direction of the swim bladder also pose a threat as they damage other internal organs. Eels with anguillicolosis are easier prey for larger predators (Barse and Secor 1999). Additionally, Køie (1991) maintains that infected eels may have an impaired ability to migrate to the west Atlantic to spawn. In cases where there are additional stressors, for example bacterial infection, low water oxygen concentrations, or high fish densities and transport, eels can die (Molnar et al. 1991, Baruš 1995).

The dimensions of the nematodes observed in this study were often different to those previously reported by other authors (e.g. Kuwahara and Niimi 1974, Moravec and Taraschewski 1988, Blanc et al. 1992, Moravec et al. 1993, Moravec 1994, Rolbiecki 2004), possibly indicating a wider range of morphological variability than previously thought. Additionally, researches usually report of nematode dimensions on a small sample of *A. crassus* individuals. It should also be borne in mind that nematodes can present different dimensions in waters of different salinity. *A. crassus* originates from fresh waters, in which it is supposed that they might exhibit optimum conditions, including, for example, larger dimensions compared to individuals from brackish or saline waters. In the case of third and fourth stage larvae, differences in dimensions were observed, apparently dependent on the host species. Further, methods applied during analyses, including the media used to fix, preserve or clear nematodes, as well as the type of material examined (fresh, frozen, or chemically treated), can impact measurements.

A. crassus was first reported in wild eels in Poland in 1988 (Własow 1991, Grawiński 1994, Rolbiecki et al. 1996); it was also found in eels imported from Sweden and Germany that same year (Własow et al. 1991). Additionally, nematods of the genus *Anguillicola* were reported in Sweden in 1987 (Hellstrom et al. 1988) in eels bought in Poland (which were destined for export to Germany). Also, German researchers (Koops and Hartmann 1989) reported that in 1988 10% of eels purchased in Poland were infected with *Anguillicola* spp. Most data regarding occurrences of *A. crassus* in eels from Poland come from lakelands, coastal areas, and the gulfs and lagoons of the Southern Baltic Sea (e.g. Własow et al. 1997, Bystydzieńska et al. 2005, Pilecka-Rapacz and Sobecka 2004, Sobecka and Piasecki 2002, Rolbiecki and Rokicki 2006).

The level of infection of the eels examined in this study by the *A. crassus* was relatively high (79.3%, 7.2 ind.) in comparison with earlier studies of fish in Poland. For example, one study reported the prevalence of infected eels in the Gulf of Gdańsk as being 41.9%, at an intensity 3.0 ind. (Rolbiecki et al. 2000). In other reports, infections in the Vistula Lagoon were seen to range from 63.3-100% of fish, with mean intensity of 4.2-10.3 ind. (i.e. Rolbiecki et al. 1996, Własow et al. 1997, Bystydzieńska et al. 2005, Rolbiecki and Rokicki 2006), in Lake Łebsko 84% at 7.6 ind. (Morozińska-Gogol 2005), and in Lake Miedwie 100% at 3-44 ind. (Sobecka and Piasecki 2002). Apparently, outside of Poland in other European countries, infection rates of fish are high with, as a rule, more than half of the eel population infected. For example, eel infection levels have been reported as follows: England (River Thames) 48-52%, 2.3-3.9 ind. (Norton et al. 2005), Estonia (Lake Võrtsjärv) 51-86%, 4.0-12.6 ind. (Kangur et al. 2002), Spain (River Tea) 55.5%, 5.5 ind. (Aguilar et al. 2005), Germany (Elbe River) 60.6%, 7.4 ind. (Möller et al. 1991), Turkey (River Ceyhan) 72.4-82.8%, 3.2-3.3 ind. (Genç et al. 2005), and in Belgium (Kolenhaven, Albercanal) 90.2%, 17.0 ind. (Thomas and Ollevier 1992b). The introduction of a new host in the life cycle of a parasite can cause disturbances in natural ecosystems. This phenomenon can intensify if the parasite is new from the point of view of the components of the parasite-host system. In consequence, environmental destabilization often causes a parasitic "demographic explosion" that is

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reflected in infection parameters, something that is particularly evident in *A. crassus*.

It should be added that during stocking in Lake Wdzydze and the Vistula Lagoon in August and October 2006, 50 eels were examined (Rolbiecki et al. 2008), and the eel fry imported from Denmark were found to be already infected with *A. crassus* (August: 4.8%, 1.0 ind. and October: 20.7%, 2.5 ind.). Further, another invasive species was observed, namely the monogenean *Pseudodactylogyrus anguillae* (August: 54.2%, 4.0 ind. and October: 17.2%, 1.2 ind.). Since the current study addressed only infection of the swim bladder, it is not possible to report whether the monogenean also occurs in Lake Wdzydze.

The results presented here show that the infection of eels by *A. crassus* were high in all fish length classes (Tab. 1). Higher prevalence levels were seen in eels measuring 701-800 mm (86.4%) than in the other size classes, while the highest intensity of infections per fish was observed in fish over 800 mm long (8.1 ind. and 2-46 ind.). The varied rates of infection of the eels may result from changes in food composition as they grow, an observation made by Möller et al. (1991). It should be borne in mind, however, that eel diets vary depending on the basin inhabited, as dictated by trophic conditions and the availability of food. Another significant issue is parasite-induced mortality. As has been observed by various authors (Molnar et al. 1991, Baruš 1995), *A. crassus* can be fatal to eels, especially when other stressors are present. However, it is difficult to confirm fish deaths under natural conditions. As reported by Lester (1984), changes in the infection parameters (primarily prevalence) in correlation with fish age (length) may indicate that the cause of death was parasite infection.

It is apparent that *A. crassus* is increasing its range of occurrence in Poland at a high level of infection. It is highly probable that the area of occurrence of *A. crassus* in Poland corresponds directly to the distribution of its final host, the eel. It should also be added that *A. crassus*, in a similar manner to other parasites, is moved and introduced to new aquatic basins by the hosts themselves, both final and paratenic. An important factor in this process, and hence the increasing occurrence of parasites, is human activity, including uncontrolled fish stocking. This refers in particular to fish that are the hosts of dangerous invasive parasite species. The introduction of new components disturb the balance of given ecosystems, and often lead to serious biological, and, as in the current case, financial consequences.

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