

Spatio-temporal dynamics of the parasitic nematode *Anguillicola crassus* in Flanders, Belgium

V. Audenaert¹, T. Huyse¹, G. Goemans², C. Belpaire², F. A. M. Volckaert^{2,*}

¹Laboratory of Aquatic Ecology, Katholieke Universiteit Leuven, Ch. 3000 de Bériotstraat 32, 3000 Leuven, Belgium

²Institute for Forest and Wildlife Management, A. 1560 Duboislaan 14, 1560 Hoeilaart–Groenendaal, Belgium

ABSTRACT: Despite Egusa's earlier warning of the damage that the parasitic nematode *Anguillicola crassus* could inflict on the European eel *Anguilla anguilla*, its introduction in Europe was a fact in the early 1980s. Based on an elaborate dataset on *Anguillicola crassus* infection of 11 river catchments, this paper presents the results of a detailed study on the dispersal of the parasite in Flanders, Belgium, and the host-parasite relationship. In addition, data from 1986 and 1997 are used for comparative purposes, providing a perspective on the temporal infection pattern over 15 yr. The presence of *A. crassus* in Flanders was first discovered in 1985; 2 yr later a survey revealed a prevalence of 34.1% and a mean infection intensity of 5.5, based on adult nematodes only, and 10 yr later the parasite was present at all 11 sites sampled. Prevalence had increased to 62.5% but the mean infection intensity had decreased to 3.9 adults per infected eel. Finally, in the year 2000, a third study revealed that *A. crassus* was present in 139 of 140 investigated sites; a further increase in prevalence to 68.7% and a decrease in mean infection intensity to 3.4 adults per infected eel was observed. When all larval stages were taken into account, mean prevalence amounted to 88.1% and mean intensity to 5.5 adults. The high infection level in Flanders is thought to be the result of restocking with glass eel and yellow eel, both of which are susceptible to *A. crassus*. The general infection parameters were similar in all 11 river catchments. It is possible that in Flanders both prevalence and mean infection intensity are stabilizing due to density-dependent regulation of the parasite infrapopulation. Fibrotic swimbladder walls were observed, mainly in large eels, and 20% of the total number of nematodes consisted of encapsulated larvae in the surveys of 1997 and 2000; 8 cases of swimbladder regeneration were observed.

KEY WORDS: European eel · Exotic species · Host-parasite evolution · Mean intensity · Parasite · Prevalence

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

Anguillicola crassus Kuwahara, Niimi & Itagaki, 1974 (Nematoda, Dracunculoidea, Anguillicolidae) is a parasitic nematode originally found in the swimbladder of the Japanese eel *Anguilla japonica* L. (Egusa 1979). It was accidentally introduced into Europe in the early 1980s through uncontrolled intercontinental transfer of live eels from Taiwan (Paggi et al. 1982, Neumann 1985, Køie 1991). Since then it has rapidly spread among European eel populations (Kennedy & Fitch 1990, Køie 1991, Evans et al. 2001).

This quick expansion is the result of both human-assisted dispersion of the final host and the efficient dispersion mechanisms of the parasite itself (Kennedy & Fitch 1990). The wide range of intermediate and paratenic hosts (De Charleroy et al. 1990, Thomas & Ollevier 1992a, Moravec & Skorikova 1998), high fertility (Kennedy & Fitch 1990), high tolerance, resistance and survivability of the second-stage larvae (Kennedy & Fitch 1990, Thomas & Ollevier 1993) and its capability of infecting eels of all sizes, even glass eels (Nimeth et al. 2000), makes the parasite a very successful and aggressive colonizer (Ashworth & Blanc

*Corresponding author.
Email: filip.volckaert@bio.kuleuven.ac.be

1997). Eels become infected with *Anguillicola crassus* through the food chain. Female nematodes produce a large number of eggs containing first- or second-stage larvae which leave the swimbladder through the pneumatic duct and pass with the faeces into the water. The larvae make use of freshwater cyclopoid copepods, ostracods and calanoids as intermediate hosts in which they develop into third-stage larvae (De Charleroy et al. 1990, Thomas & Ollevier 1992b, Barus et al. 1999). Several fish species, as well as some amphibians and aquatic insects that belong to the eel's diet, might act as paratenic hosts in which the parasite arrests its development (Moravec & Skorikova 1998, Barus et al. 1999). In the final host, the third-stage larvae migrate through the intestinal wall and the body cavity into the swimbladder wall, where they develop into fourth-stage larvae and later, when reaching the lumen of the swimbladder, into adults. At a temperature of 20°C, the development of *A. crassus* eggs into adults can be completed in less than 2 mo (De Charleroy et al. 1990, Kennedy & Fitch 1990).

The genus *Anguilla* (Anguillidae: Teleostei) comprises 15 species, of which only 3 occur in the northern hemisphere: the European (*A. anguilla*), American (*A. rostrata*) and Japanese (*A. japonica*) eels (Watanabe 2003). The natural range of the European eel stretches from the North European to the North African coasts. Most eels in central and eastern Europe are assumed to have been stocked from the coastal areas. Sexually mature European eels migrate to the Sargasso Sea for spawning, after which their larvae migrate back to continental waters (Tesch 1977). Migrating eels display a vertical migration pattern, in which the swimbladder plays a major role (Fricke 1995).

In contrast to the original host of this parasite, the Japanese eel, the European eel could suffer severe damage by *Anguillicola crassus* because of its higher susceptibility (Egusa 1979, Kōie 1991, Nagasawa et al. 1994) and different humoral response (Nielsen 1999). In the blood of European eel, specific antibodies have been found against antigens that are mainly situated in the cuticle of adult nematodes (Knopf et al. 2000a,b). Repeated larval invasion is responsible for oedemic and hyperplastic changes in the swimbladder wall that eventually lead to dead encapsulated larvae as well as disintegrated adult nematodes (Molnár et al. 1993, Wurtz & Taraschewski 2000). The reduction of the basolateral labyrinth of gas-gland cells and the enlargement of the distance of gas-gland cells to capillaries most likely impair the swimbladder function, preventing the fish from migrating (Nimeth et al. 2000). Infected swimbladders can become encapsulated by connective tissue, a condition known as fibrosis, creating a poor basis for reinfection at a severe stage of anguillicolosis (Hartmann & Peters 1989,

Nimeth et al. 2000, Wurtz & Taraschewski 2000). Infection with *A. crassus* can also enhance secondary bacterial infections (Boon et al. 1990), and even cause mass mortalities in dense eel populations in conditions of high temperature and low oxygen content, as in, e.g., Lake Balaton and closed aquatic ecosystems (Molnár et al. 1991, 1993, Barus et al. 1999).

The European eel has long been a popular consumer fish; however, since the 1990s catches have diminished considerably. Infection by *Anguillicola crassus* may partly be responsible for the decrease in the eel population in Europe, although this has never been proved (Moriarty & Dekker 1997). The aim of this paper was to obtain detailed temporal and spatial information on the parasite in order to document the evolutionary pattern of the host-parasite relationship.

MATERIALS AND METHODS

From June 1996 to December 1997, 355 eels were caught at 11 sites in Flanders. From May 2000 to October 2000, 1101 eels were sampled from 140 sites and 11 river catchments (Fig. 1). The sampling aim was the collection of 10 eels in the range of 35 to 45 cm length from each site, within the framework of a survey of bioaccumulation of contaminants in eels throughout Flanders. However, it was not always possible to sample the required number from the targeted length class. The swimbladders were stored in 4% formalin and transferred to 70% ethanol. The adult nematodes were sexed and counted macroscopically. Larvae were identified and counted using a binocular with transmitted light by flattening the swimbladder wall between 2 glass slides. Larvae surrounded by a more dense and opaque tissue, often partially resorbed, were identified as capsules (Fig. 2), and the thickness as well as rupture of the swimbladder wall were registered. The swimbladders were divided into 3 categories according to the thickness of the wall. In the absence of parasitic stages, swimbladders with thin walls (<1 mm) were assumed to be uninfected, whereas those with thick walls (1 to 3 mm) were assumed to be infected in the presence of large amounts of connective tissue and tissue proliferation, and not infected when no histopathological reaction had occurred. Swimbladders with very thick walls (>3 mm), combined with brown fluids in the swimbladder lumen as a result of the disintegration of adult nematodes, pointed towards a former infection. Prevalence, mean intensity of infection, and abundance of *Anguillicola crassus* were estimated at all sites. Prevalence was calculated as the number of infected eels divided by the total number of eels investigated at each site, while mean prevalence was the average prevalence for each basin or for all

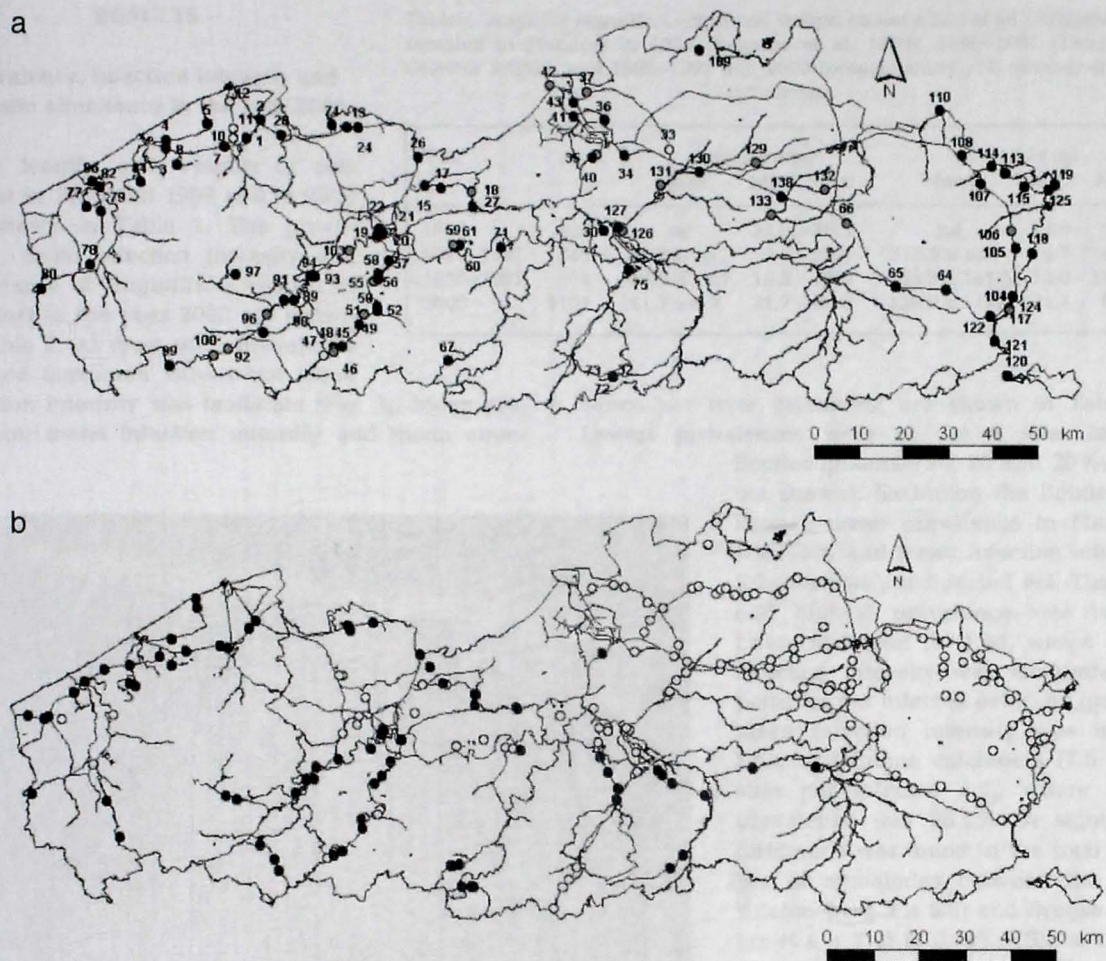


Fig. 1. (a) Sampling sites of European eels *Anguilla anguilla* in Flanders in 2000 and prevalence of parasitic nematode *Anguillicola crassus*: (●) <60%, (◐) 61 to 80%, (○) 80 to 100%. (b) Sites in Flanders restocked with glass (○) or yellow (●) eels in 1997

sites combined. Mean infection intensity represents the total number of nematodes divided by the total number of infected eels. Parasite abundance was calculated as the total number of nematodes per eel, including uninfected specimens. For these calculations, the cut-off value was arbitrarily set at 7 eels per site to avoid loss of information. Data of former studies on these infection parameters in Flanders in 1986, when 424 eels were sampled from 19 sites (Belpaire et al. 1989), and 1990–1991, when 345 eels were collected from a single site over a period of 13 mo (Thomas & Ollevier 1992b), were used for comparative purposes. The mean prevalence and mean infection intensity in 1996 and 1997 and in 2000 were also calculated for adults only to allow a comparison with the results of a survey in 1986 in which no larvae were counted.

Statistical analysis of the data included Tukey's HSD tests for multiple comparisons across 11 basins and 3 categories of swimbladder wall-thickness in the year 2000. A Spearman's rank-correlation test was carried out on the different developmental stages of the parasite as well as on parasite abundance and host length on the data of 1997 and 2000. For each test, the significance level α was set at 0.05. The variance-to-mean ratio (s^2/\bar{x}) of parasite abundance was calculated to provide an index of the degree of dispersion of *Anguillicola crassus* in its host. A ratio >1 indicated overdispersion, a ratio equal to 1 indicated random distribution of the parasite, and a ratio <1 indicated underdispersion. All analyses were carried out with the software packages SAS/BASE[®] and SAS/STAT[®] of the SAS System release 8.1 (SAS Institute)

RESULTS

Prevalence, infection intensity and parasite abundance in the year 2000

The lengths and weights of eels caught in 1996 and 1997 and in 2000 are shown in Table 1. The prevalence, mean infection intensity and abundance of *Anguillicola crassus* in Flanders in the year 2000 are shown in Table 2. At most sites, prevalence reached maximum values but mean infection intensity was moderate (Fig. 3). Mean prevalence, mean infection intensity and mean abun-

Table 1. *Anguilla anguilla*. Length and weight (mean \pm SD) of all European eels sampled in Flanders in 1986 (Belpaire et al. 1989), 1990–1991 (Thomas & Ollevier 1992b), and 1996–1997 and 2000 (present study). N: number of eels; nd: no data

Year	N	Length (cm)			Weight (g)		
		Mean	Min.	Max.	Mean	Min.	Max.
1986	424	nd	23.0	76.5	nd	13.0	905.0
1990–1991	345	47.8 \pm nd	9.5	96.5	318.9 \pm nd	0.7	1426.0
1996–1997	355	41.2 \pm 11.7	19.8	78.0	165.0 \pm 161.5	12.0	1108.0
2000	1101	41.7 \pm 8.2	21.7	81.0	139.9 \pm 114.6	21.4	966.3

dance per river catchment are shown in Table 3. Lowest prevalences were at the 3 sites in the

Boudewijnkanaal (0, 20 and 20%; data not shown). Excluding the Boudewijnkanaal, mean prevalence in Flanders was 95% and mean infection intensity 5.0 parasites per infected eel. The second highest prevalence was in the IJzer catchment (97.1%), where mean infection intensity was moderate (6.9 parasites per infected eel). The greatest mean infection intensity was in the Dijle and Zenne catchment (7.8 parasites per infected eel), where mean prevalence was 86.2%. A significant difference was found in the total number of nematodes between the IJzer catchment (6.2 \pm 8.6) and Brugse Polders (4.4 \pm 7.8) (Tukey's HSD test, df = 10, $p < 0.05$, $R^2 = 0.019$). There was also a significant difference in the number of adult nematodes between the Dijle and Zenne catchment (6.8 \pm 9.6) and the Gentse Kanalen (4.1 \pm 4.6) (Tukey HSD test, df = 10, $p < 0.05$, $R^2 = 0.025$).

The high number of sites at which prevalence and the mean infection intensity were calculated ($n = 101$) enabled us to examine the relationship between these 2 parameters in Flanders (Fig. 4). Prevalence was not normally distributed, so the data could not be modeled parametrically. Since a Spearman's rank correlation test produced no significant result, a plot was made. There appeared to be no clear relationship between prevalence and mean infection intensity, while prevalence and abundance tended to be positively correlated ($r = 0.37$, $p < 0.05$).

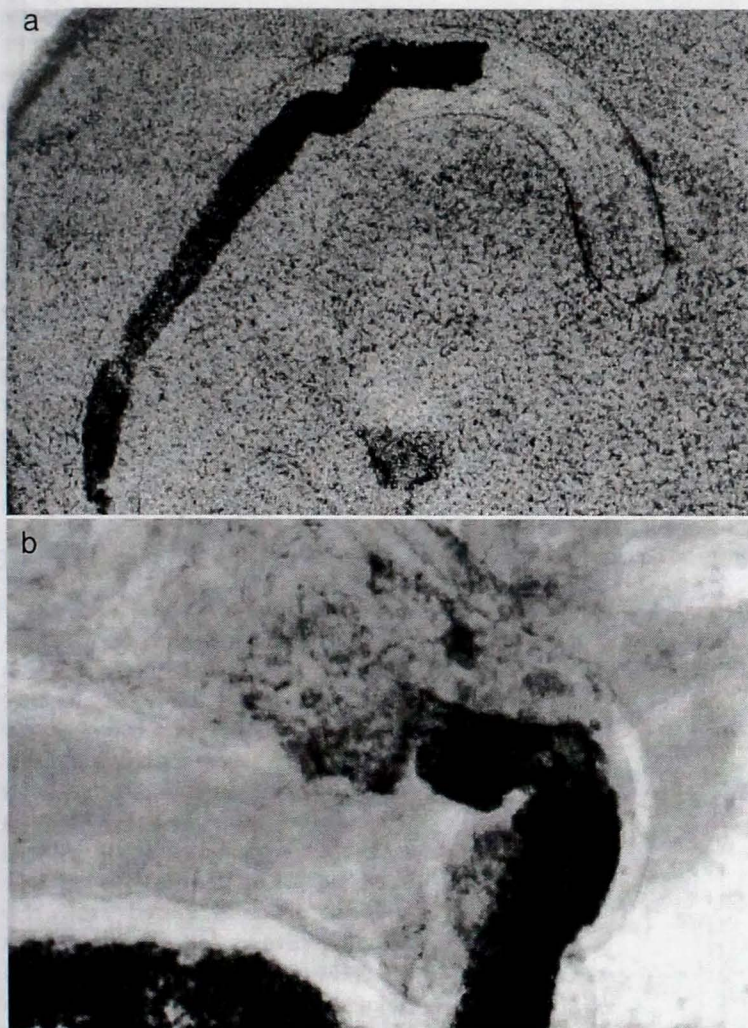


Fig. 2. *Anguillicola crassus*. Encapsulated fourth-stage larvae in 2 stages of absorption. (a) Concentration of host cells around larva with larva still intact; (b) bloodfilled gut of larva in which necrosis has begun

Table 2. *Anguilla anguilla* infected by *Anguillicola crassus*. Prevalence and infection intensity (no. of parasites infected eel⁻¹; mean \pm SD) in European eels over the past 2 decades in Flanders. Data from 1986 from Belpaire et al. (1989). Ns: number of sites sampled, Nsi: number of sites infected; Ne: number of eels collected; Ni: number of eels infected; Np: total number of parasites found; nd: no data

Stage	Ns	Nsi	Ne	Ni	Np	Prevalence (%)		Infection intensity	
						Mean	Range	Mean	Range
1986 (adults)	19	11	424	145	253	34.1 \pm nd	0–100	5.5 \pm nd	1–14.7
1997 (adults)	11	11	355	222	853	62.5 \pm 15.6	21.4–83.3	3.9 \pm 3.5	2.0–6.6
1997 (all stages)	11	11	355	306	2488	86.2 \pm 10.1	64.0–100	7.2 \pm 12.5	3.3–11.7
2000 (adults)	140	139	1101	756	2652	68.7 \pm 13.3	0–100	3.4 \pm 2.9	0–11.0
2000 (all stages)	140	139	1101	970	5666	88.1 \pm 18.1	0–100	5.5 \pm 2.8	0.2–13.3

Temporal evolution of parasite infection

Table 2 also shows the mean prevalence and mean infection intensity of *Anguillicola crassus* in Flanders in 1986, in 1996 and 1997 and in 2000. In the 1996 and 1997 survey, prevalence had increased considerably compared to 1986, while mean infection intensity had begun to decrease. This trend was still present 3 yr later, in 2000, when mean prevalence had doubled (adults only) and mean infection intensity had decreased from 5.5 to 3.4 adults per infected eel (Table 2, Fig. 5). Taking all stages into account, prevalence had increased by 1.9% in the 3 yr period, while mean infection intensity had decreased from 7.2 to 5.5 parasites per infected eel.

Relative proportions of parasite development stages and host reaction

The number of parasites per eel (abundance), calculated for the years 1996 and 1997 and for 2000, and for

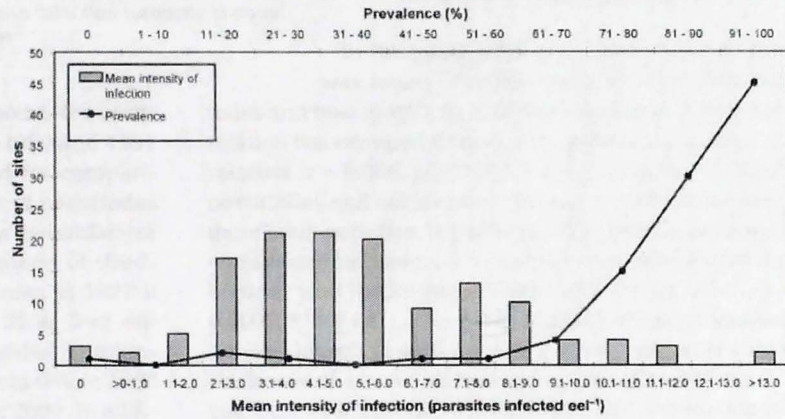


Fig. 3. *Anguilla anguilla* infected by *Anguillicola crassus*. Prevalence and infection intensity in European eels in Flanders in 2000

Natural distribution of parasite

The abundance of *Anguillicola crassus* in European eel displayed a negative binomial distribution in the years 1996 and 1997 and in 2000 (Fig. 6). The variance-to-mean ratio for these data was computed for each developmental stage of the nematode (Table 4). All coefficients were >1 , indicating overdispersion, and the highest values were obtained for third-stage larvae. Most eels were barely infected, while a small number were heavily infected (Fig. 6). In the years 1996 and 1997, 2% of the eels were infected with more than 10 adult parasites, while 50% of the swimbladders were free of adults. In the year 2000 these values amounted to 4.5 and 44% respectively.

Table 3. *Anguilla anguilla* infected by *Anguillicola crassus*. Prevalence, abundance and infection intensity (mean \pm SD) in European eels in Flanders in 2000. Sampling sites were grouped by drainage basin. Ns: number of sites; Ne: number of eels. Abundance presented as number of parasites per eel, infection intensity as number of parasites per infected eel

River catchment	Ns	Ne	Prevalence (%)	Infection intensity	Abundance
IJzer	7	69	97.1 \pm 4.9	6.9 \pm 3.0	6.7 \pm 2.8
Brugse Polders	11	107	72.7 \pm 38.8	4.8 \pm 3.6	3.7 \pm 2.8
Gentse Kanalen	11	104	93.2 \pm 7.1	4.5 \pm 1.9	4.3 \pm 1.8
Benedenschelde	11	103	82.9 \pm 21.9	5.9 \pm 3.3	5.2 \pm 3.7
Leie	11	98	89.2 \pm 11.3	5.8 \pm 2.4	5.2 \pm 2.2
Bovenschedde	14	138	90.0 \pm 15.2	5.7 \pm 2.7	5.1 \pm 2.6
Dender	2	17	100.0 \pm 0.0	7.6 \pm 8.1	7.6 \pm 8.1
Dijle and Zenne	4	42	86.2 \pm 14.1	7.8 \pm 4.0	7.1 \pm 3.8
Demer	3	28	89.3 \pm 10.1	6.7 \pm 1.9	6.2 \pm 1.6
Nete	8	74	83.4 \pm 12.7	5.8 \pm 2.0	4.8 \pm 1.6
Maas	18	174	92.8 \pm 8.1	4.3 \pm 1.9	4.0 \pm 1.7

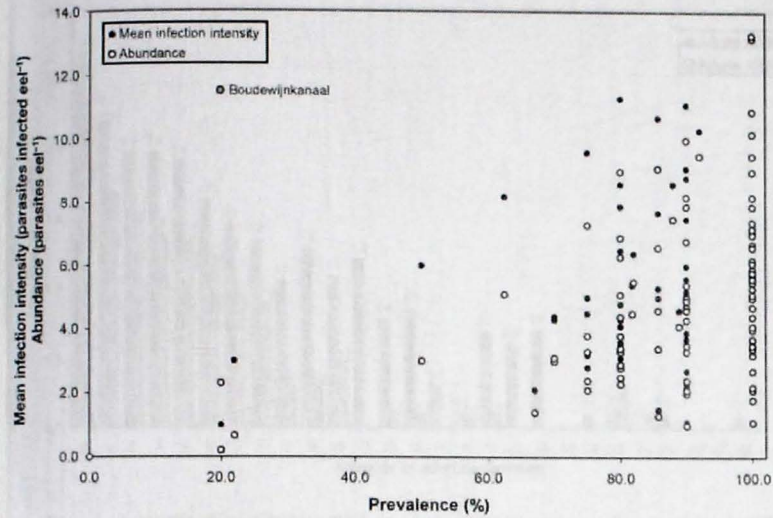


Fig. 4. *Anguilla anguilla* infected by *Anguillicola crassus*. Relationship between prevalence, infection intensity and abundance in European eel in Flanders in 2000. At 100% prevalence the mean infection intensity is equal to the abundance

each developmental stage of the nematode, are summarized in Fig. 7. Data from a survey in 1990 and 1991 (Thomas & Ollevier 1992b) were added for comparison. In all 3 studies, the highest number of nematodes involved larval stages embedded in the swimbladder wall. In 1990 and 1991 the total percentage of third- and fourth-stage larvae was 67%, whereas in 1997 it had decreased to 43% and in 2000 to 33%. The decrease in larval abundance was compensated by an increase in encapsulated larvae (Fig. 2) from 0% in 1990 and 1991 to 20% in 1996 and 1997 and in 2000. In addition, the number of pre-adults increased by 12% and the number of adult nematodes by 6% between 1990 and 2000. The ratio of male to female adults was nearly equal in the 1990 and 1991, 1996 and 1997 and the 2000 studies.

In the year 2000, positive but weak correlations were found between the presence of larvae and capsules (Spearman's rank-correlation test, $r = 0.35$, $p < 0.05$), larvae and adults ($r = 0.29$, $p < 0.05$), and capsules and adults ($r = 0.16$, $p < 0.05$). Similar results were found in 1996 and 1997 for the presence of larvae versus adults, but the number of adults appeared to be negatively correlated with the number of encapsulated larvae ($r = -0.23$, $p < 0.05$). In the year 2000, differences were observed in the total number of nematodes in swimbladders with a wall thickness >3 mm and the numbers in swim-

bladders with a wall thickness <1 mm or 1 to 3 mm (Tukey HSD test, $p < 0.05$, $df = 2$, $R^2 = 0.053$). However, this could also have been due to differences in the methods by which the swimbladder wall was examined. No differences were found between swimbladders with wall thicknesses <1 mm and 1 to 3 mm. There were also differences in the number of adult nematodes ($R^2 = 0.015$), larvae ($R^2 = 0.035$) and encapsulated larvae ($R^2 = 0.032$) between fibrotic swimbladders and the other 2 swimbladder categories (Tukey HSD test, $p < 0.05$, $df = 2$). Fibrotic swimbladders contained significantly less larval stages, capsules and adult nematodes than healthy and less infected swimbladders (Fig. 8).

Relationship between parasite abundance and host length

In the years 1996 and 1997 no correlation was found between number of adult nematodes and host length. In 2000 the Spearman's rank-correlation test revealed a weak but significant positive correlation ($r = 0.089$, $p < 0.01$) between number of adult nematodes and eel length, with larger hosts containing more adult parasites. Significant differences in eel length were found between all 3 categories of different swimbladder wall thicknesses (Tukey's HSD test, $df = 2$, $p < 0.0001$, $R^2 = 0.021$). Eels with a wall thickness <1 mm had a mean length of 40.8 ± 7.5 cm, while eels with a wall thickness of 1 to 3 mm had a mean length of 42.0 ± 7.7 cm. Eels with fibrotic swimbladders had a mean length of 45.3 ± 11.5 cm.

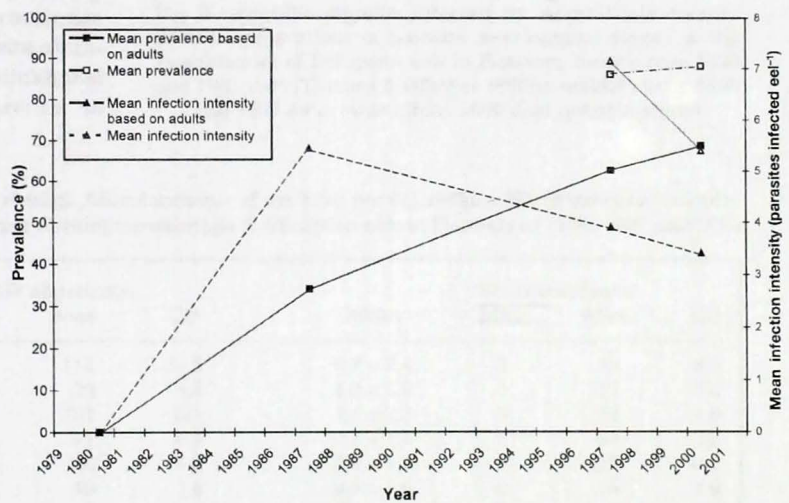


Fig. 5. *Anguilla anguilla* infected by *Anguillicola crassus*. Temporal pattern of parasite infection in Flanders between 1979 and 2001

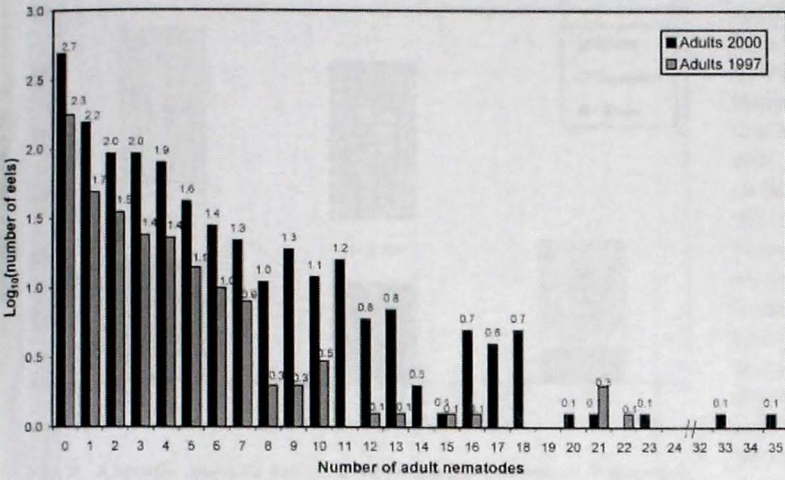


Fig. 6. *Anguilla anguilla* infected by *Anguillicola crassus*. Frequency distribution of adult parasites in European eels in Flanders in 1996-1997 and 2000

abundance between Brugse Polders and the IJzer river catchment, where high prevalence was found. Despite this deviating pattern, statistical analysis showed a general similarity in abundance of *A. crassus* across all river catchments in Flanders. The high prevalence in Flanders is considered to be the consequence of restocking elvers. A map of the restocking sites of glass eel and yellow eel in Flanders in 1997 (Fig.1; data courtesy of the Fisheries Fund) allowed us to examine differences in the infection parameters in the year 2000 in areas restocked with glass or yellow eels. The fact that several river catchments were only restocked with either glass or yellow eels indicates that both eel stages are

DISCUSSION

Spatial distribution of *Anguillicola crassus* in Flanders

This study has shown that *Anguillicola crassus* has invaded all Flemish river catchments since its introduction in 1985. Eel populations were infected at 139 of the 140 sites investigated, with a mean prevalence of 88.1% and a mean infection intensity of 5.5. The large amount of data compensated for the low number of eels per sample. At only 4 of the 140 sites was prevalence less than 25%; 3 of these sites were located along the northwestern part of the Boudewijnkanaal, which belongs to the brackishwater basin of Brugse Polders (conductivity 30 to 36 mS cm⁻¹), and more importantly has not been restocked with eels. Since prevalence seemed to be positively correlated with parasite abundance, the low prevalence in the Boudewijnkanaal might contribute to the significant difference in

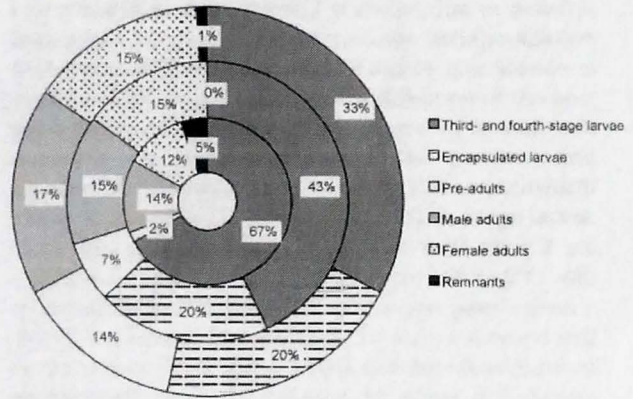


Fig. 7. *Anguilla anguilla* infected by *Anguillicola crassus*. Relative proportions of parasite development stages in the swimbladder of European eels in Flanders. Inner circle: 1990 and 1991 data (Thomas & Ollevier 1992b); middle circle: 1996 and 1997 data; outer circle: 2000 data (present study)

Table 4. *Anguilla anguilla* infected by *Anguillicola crassus*. Abundance (no. of parasites per eel, mean ± SD) of infection and minimum, maximum and coefficient of dispersal (CD) per developmental stage in European eels in Flanders in 1996-1997 and 2000

Parasite stage	1996-1997 abundance				2000 abundance			
	Mean	Min.	Max.	CD	Mean	Min.	Max.	CD
Third-stage larvae	1.3 ± 6.5	0	114	32.5	0.7 ± 2.4	0	54	8.2
Fourth-stage larvae	1.8 ± 3.4	0	29	6.4	1.0 ± 1.9	0	17	3.6
Encapsulated larvae	1.2 ± 2.7	0	21	6.1	1.1 ± 2.3	0	32	4.8
Pre-adults	0.6 ± 1.6	0	11	4.3	0.7 ± 1.5	0	20	3.2
Male adults	0.9 ± 2.0	0	16	4.4	0.9 ± 1.9	0	19	3.9
Female adults	0.9 ± 1.6	0	10	2.8	0.8 ± 1.6	0	15	4.0
Remnants	0.0 ± 0.0	0	0	0	0.04 ± 0.2	0	3	1.0
Total	6.2 ± 11.5	0	176	21.3	5.8 ± 7.3	0	87	9.2

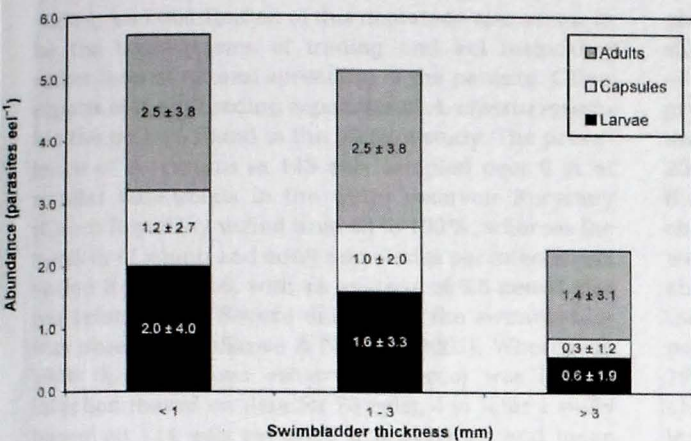


Fig. 8. *Anguilla anguilla* infected by *Anguillicola crassus*. Proportion of various development stages comprising total abundance of nematodes as a function of swimbladder wall thickness of European eels in Flanders in 2000

susceptible to *A. crassus*. This was also reported by Nimeth et al. (2000). It appears that differences in water temperature, salinity and CaCO_3 concentration are not likely explanations, although these parameters are believed to influence parasite abundance. Water temperature is known to be an important external factor influencing hatching, survival and transmission of infective stages of *A. crassus* (Kennedy & Fitch 1990, Thomas & Ollevier 1993, Knopf et al. 1998). Most infections in freshwaters at high latitudes, as in Sweden, are the result of restocking and have lower infection rates than those in lower latitudes (Wickström et al. 1998). Salinity may also influence the infection level of *A. crassus* in the European eel. Data from an observational study of the infection of silver-stage eels in Danish habitats with different salinity levels support this hypothesis (Nielsen 1997). Under experimental conditions, Kirk et al. (2000) observed maximal egg-hatching, survival and infectivity of larvae in freshwaters and a decline with increasing salinity. Nevertheless, adult nematodes were able to survive and reproduce in brackish as well as marine waters. In 1998, T. Huyse (pers. comm.) showed that *A. crassus* larvae could survive in $433 \text{ mg l}^{-1} \text{ Ca}$ for up to 3 wk, and concluded that this concentration of CaCO_3 probably does not affect the parasite's distribution in Flanders.

Temporal and spatial evolution of parasite infection

The first case of anguillicolosis in Flanders was reported in 1985 by Belpaire & De Charleroy (1985). In a batch of *Anguilla anguilla* eels (34.0 to 53.5 cm) originating in The Netherlands and imported for restock-

ing the Nete basin, they found 1 of the 29 eels infected with 18 adult nematodes. The swimbladder was filled with a mucous substance containing numerous nematode larvae. At that time the authors warned against importing infected eels. The report of Belpaire et al. (1989) made clear that eel restocking activities had enhanced the spread of *Anguillicola crassus* throughout Flanders. In 1997, larval stages embedded or encapsulated in the swimbladder wall were also found in infected eels; these stages appeared to have a great influence on the degree of infection, and the increase in the prevalence and mean infection intensity was striking. In the year 2000, a further increase in prevalence was noticed, but mean infection intensity had begun to decrease (Fig. 5). This rise in mean prevalence and decrease in mean intensity was also observed by Ashworth (1995) in a longitudinal study covering 5 yr at 1 site in England. It is possible that in Flanders both the prevalence and

mean infection intensity will stabilize due to density-dependent regulation of the parasite infrapopulation (Ashworth & Kennedy 1999). Parasite abundance is controlled by an overdispersed distribution of the host population, a constant number of gravid female eels regardless of population size of the parasite, and arrested development of the parasite larvae (Ashworth 1995). In the year 1997, third- and fourth-stage larvae were still alive in the swimbladder wall after 3 mo starvation of the host at a temperature of 8 to 9°C with no possibility of re-infection (T. Huyse pers. comm.). This is an unusually long time for larval survival and could represent arrested larval development induced by the adult parasite population. However, it could also be explained by host specific physiological induction in response to starvation. Furthermore, temperature may play an additional role in slowing down the parasite's development (Thomas & Ollevier 1993). At 20 to 22°C, Moravec (1994) observed development of nematode larvae into the adult stage after 6 to 7 wk post-infection. According to Ashworth & Blanc (1997), the tendency towards maximal prevalence and predictable mean infection intensities is present in most European biocoenoses. However, slight annual fluctuations (as observed in the IJsselmeer and the Waddenzee in The Netherlands: Haenen et al. 1994) could occur. The quick rise in prevalence in Flanders has been explained as arising from the restocking of uninfected areas with infected eels, followed by a subsequent natural spread of *A. crassus* through open waters (Belpaire et al. 1989). In the Czech Republic (Barus et al. 1999), Denmark (Boëtius 1989), Ireland (Evans et al. 2001), The Netherlands (Van Banning et al. 1985) and Sweden (Wickström et al. 1998), the

widespread distribution of this nematode also seems to be the consequence of trading and eel restocking rather than of natural spreading of the parasite. Other reports of the spreading capacities of *A. crassus* resemble the pattern found in the present study. The prevalence of *A. crassus* in 143 eels sampled over 6 yr at regular time-points in the water reservoir Korycany (Czech Republic) varied from 43 to 100%, whereas the number of young and adult nematodes per infected eel varied from 1 to 66, with an average of 6.6 nematodes per infected eel. Severe damage to the swimbladder was observed (Palikova & Navratil 2001). Whereas in 1996 the Moulouya estuary (Morocco) was free of infection (based on data for 76 eels), 4 yr later a study based on 114 eels revealed a prevalence and mean infection intensity reaching a maximum of 70% and 5.3 nematodes per infected host respectively (Rahhou et al. 2001). The maximum prevalence and mean infection intensity of *A. crassus* infecting 423 *Anguilla rostrata* in tributaries of the middle and upper Chesapeake Bay (USA) was still lower than in the present study (82% and 9 vs 100% and 13.3 nematodes per infected host respectively). The parasite had spread to North America a decade after invading Europe (Barse et al. 2001). Effects similar to those of *A. crassus* on *A. anguilla* have also been observed for this parasite infecting *A. rostrata*, suggesting an equal susceptibility of both host species to this parasite (Barse & Secor 1999). Therefore, unless precautions are taken in uninfected areas, a further spread of this parasite can be expected.

Reaction of *Anguilla anguilla* to *Anguillicola crassus*

The host's immunological reaction to infection by *Anguillicola crassus* comprises encapsulation of the larvae in the swimbladder wall, inhibiting their development. In this study, the amount of encapsulated larvae was very high compared to that reported in the literature. Furthermore, 10% of the swimbladders showed a thickening of the wall to >3 mm, a condition known as fibrosis that constitutes a poor basis for reinfection (Hartmann & Peters 1989) and could explain the tendency towards decreasing abundance of all stages of the parasite in eels with fibrotic swimbladders. The lower number of adult nematodes in fibrotic swimbladders could also be linked to the considerable reduction in volume of the swimbladder lumen. However, the swimbladders can undergo regeneration (Molnár et al. 1993), although this is considered a rare phenomenon. In 1997 only 2 out of 355 investigated swimbladders, and in 2000 only 6 out of 1101, had developed a new functional gas chamber. The data for the year 2000 indicate that when many larvae were

present in the swimbladder wall, the number of capsules was higher and more parasites developed into adult nematodes. In 1997, however, it seemed that the greater the number of larvae encapsulated, the smaller the number that developed into adults. In the year 2000 there were 10% less larvae than in 1997, while the number of capsules and adults had hardly changed. Abundance of encapsulated larvae in 2000 was independent of the length of the eels, whereas abundance of adults was higher in larger eels (partly through density-dependent regulation of the infestation as explained above; Ashworth & Kennedy 1999). It is not likely that adult nematodes require sizeable swimbladders to develop, since they adapt their length to the available space (Van Banning & Haenen 1990, Moravec 1994). In Flanders in the year 2000, the proportion of specimens with thickened swimbladder walls was higher in bigger eels, similarly to the situation in Lake Balaton, Hungary (Molnár et al. 1994) and the Morava River basin, Czech Republic (Barus et al. 1999). Larger eels are generally older and thus have been in contact with the parasite more often. Furthermore, they consume more heavily infected intermediate and paratenic hosts. The eel's immunological responses to *A. crassus*, i.e. formation of capsules around the larvae and fibrosis, may prevent reinfection; however, the swimbladder loses its functionality in the process. Therefore the large number of helminth-free swimbladders with a fibrotic wall in Lake Balaton (30%: Molnár et al. 1994) and in Flanders in the year 2000 (10%) can hardly be viewed as an increased resistance of the host.

Treatment of anguillicolosis

Regardless of how easy treatment of anguillicolosis may be on a small scale (Taraschewski et al. 1988, Casiraghi et al. 2001), treatment of whole eel populations is far more complicated in ponds, and is virtually impossible in river basins. The best way to deal with such a massive infection is to prevent the parasite from spreading, which implies careful management of the eel stocks. With the exception of glass eels, the restocking of eels in Flanders has ceased since 2000 in response to advice of the Flemish High Council for Fisheries (Belpaire & Coussement 2000). However, a comparison of restocking data and infection data for the year 2000 revealed no difference in the degree of infection between sites stocked with glass or yellow eels. Since glass eels are also susceptible to the parasite (Nimeth et al. 2000), all restocking should be avoided. However, since *Anguillicola crassus* is now present in almost all Flemish waters, it would appear far too late to halt the spreading of this parasite

(EIFAC/ICES 2001). Moreover, the mean intensity has started to decrease, so there may be hope of an eventual ecological and evolutionary equilibrium in the parasite burden similar to that of *A. crassus* in *Anguilla japonica* (Nagasawa et al. 1994, Nielsen 1999).

Acknowledgements. Special thanks are extended to Mia Hubert for statistical advice, and to Christophe Pampoulie, Vincent Van Ginneken and Guido van den Thillart for useful comments. The Ministry of the Flemish Community (AMINAL contract TWOL 2000 AMINAL/BG/IBW/2000-5) funded the project.

LITERATURE CITED

- Ashworth ST (1995) The dynamics and regulation of *Anguillicola crassus* (Nematoda) populations on the European eel. PhD thesis, University of Exeter
- Ashworth ST, Blanc G (1997) *Anguillicola crassus*, a recently introduced aggressive colonizer of European eel stocks. Bull Fr Pêche Piscic 344:335–342
- Ashworth ST, Kennedy CR (1999) Density-dependent effects on *Anguillicola crassus* (Nematoda) within its European eel definitive host. Parasitology 118:289–296
- Barse AM, Secor DH (1999) An exotic nematode parasite of the American eel. Fisheries 24:6–10
- Barse AM, McGuire SA, Vinores MA, Eierman LE, Weeder JA (2001) The swimbladder nematode *Anguillicola crassus* in American eels (*Anguilla rostrata*) from middle and upper regions of Chesapeake Bay. J Parasitol 87:1366–1370
- Barus V, Moravec F, Prokes M (1999) Anguillicolosis of the European eel (*Anguilla anguilla*) in the Czech Republic. Czech J Anim Sci 44:423–431
- Belpaire C, Coussement M (2000) Nota omtrent het uitzetten van paling in de Vlaamse openbare waters. Advies voor de Vlaamse Hoge Raad voor de Riviervisserij (20 maart 2000). IBW.Wb.V.ADV.2000.070. Institute for Forestry and Wildlife Management, Hoeilaart
- Belpaire C, De Charleroy D (1985) Onderzoek naar de gezondheidstoestand van vissen bestemd voor uitzetting. Laboratory for Ecology and Faunal Management, Leuven
- Belpaire C, De Charleroy D, Thomas K, Van Damme P, Ollevier F (1989) Effects of eel restocking on the distribution of the nematode *Anguillicola crassus* in Flanders, Belgium. J Appl Ichthyol 5:151–154
- Boëtius I (1989) Preliminary report on the occurrence of *Anguillicola* in some Danish fresh- and seawater areas. European Inland Fisheries Advisory Commission (FAO) Working Party on Eel, Porto, May/June 1989. FAO, Oporto
- Boon JH, Augustijn H, Cannaerts VMH, Lokin CJA, Machiels MAM, Ollevier F (1990) The suitability of experimental inoculations with infective larvae of *Anguillicola crassus* and their effects on the growth and mortality of the European eel (*Anguilla anguilla*). Aquaculture 87:111–120
- Casiraghi M, Anderson TJC, Bandi C, Bazzocchi C, Genchi C (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 122:93–103
- De Charleroy D, Grisez L, Thomas K, Belpaire C, Ollevier F (1990) The life cycle of *Anguillicola crassus*. Dis Aquat Org 8:77–84
- Egusa S (1979) Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. Rapp PV Réun Cons Int Explor Mer 174:51–58
- EIFAC/ICES (European Inland Fisheries Advisory Commission/International Council for the Exploration of the Sea) Working Group on Eel (2001) ICES Copenhagen
- Evans DW, Matthews MA, McClintock CA (2001) The spread of the eel swimbladder nematode *Anguillicola crassus* through the Erne system, Ireland. J Fish Biol 59: 1416–1420
- Fricke H (1995) Tracking of artificially matured eels (*Anguilla anguilla*) in the Sargasso Sea and the problem of the eel's spawning site. Naturwissenschaften 82:32–36
- Haenen OLM, van Banning P, Dekker W (1994) Infection of eel *Anguilla anguilla* (L.) and smelt *Osmerus eperlanus* (L.) with *Anguillicola crassus* (Nematoda, Dracunculoidea) in the Netherlands from 1986 to 1992. Aquaculture 126: 219–229
- Hartmann F, Peters G (1989) Swimbladder damage in the eel caused by the parasitic nematode *Anguillicola crassa* (Kuwahara, 1974). European Association of Fish Pathologists, Santiago de Compostela
- Kennedy CR, Fitch DJ (1990) Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. J Fish Biol 36: 117–131
- Kirk RS, Lewis JW, Kennedy CR (2000) Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. Parasitology 120: 289–295
- Knopf K, Wurtz J, Sures B, Taraschewski H (1998) Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. Dis Aquat Org 33:143–149
- Knopf K, Naser K, van der Heijden MHT, Taraschewski H (2000a) Humoral immune response of European eel *Anguilla anguilla* experimentally infected with *Anguillicola crassus*. Dis Aquat Org 42:61–69
- Knopf K, Naser K, van der Heijden MHT, Taraschewski H (2000b) Evaluation of an ELISA and immunoblotting for studying the humoral immune response in *Anguillicola crassus* infected European eel *Anguilla anguilla*. Dis Aquat Org 43:39–48
- Køie M (1991) Swimbladder nematodes (*Anguillicola* spp.) and gill monogeneans *Pseudodactylogyrus* spp.) parasitic on the European eel (*Anguilla anguilla*). J Cons Int Explor Mer 47:391–398
- Molnár K, Székely C, Baska F (1991) Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. Bull Eur Assoc Fish Pathol 11:211–212
- Molnár K, Baska F, Csaba G, Glávits R, Székely C (1993) Pathological and histopathological studies of the swimbladders of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). Dis Aquat Org 15: 41–50
- Molnár K, Székely C, Perényi M (1994) Dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection in eels of Lake Balaton, Hungary. Folia Parasitol 41:193–202
- Moravec F (1994) Parasitic nematodes of freshwater fishes of Europe. Kluwer Academic Publishers, Dordrecht
- Moravec F, Skorikova B (1998) Amphibians and larvae of aquatic insects as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea), a swimbladder parasite of eels. Dis Aquat Org 34:217–222
- Moriarty C, Dekker W (1997) Management of the European eel. Fish Bull (Dublin) 15:1–110
- Nagasawa K, Kim YG, Hirose H (1994) *Anguillicola crassus* and *A. globiceps* (Nematoda, Dracunculoidea) parasitic in the swimbladder of eels (*Anguilla japonica* and *A. anguilla*) in East-Asia — a review. Folia Parasitol 41:127–137

- Neumann W (1985) Schwimmblasenparasit *Anguillicola* bei Aalen. Fischer Teichwirt 11:322
- Nielsen ME (1997) Infection status of the swimbladder worm, *Anguillicola crassus* in silver stage European eel, *Anguilla anguilla*, from three different habitats in Danish waters. J Appl Ichthyol 13:195–196
- Nielsen ME (1999) An enhanced humoral immune response against the swimbladder nematode, *Anguillicola crassus*, in the Japanese eel, *Anguilla japonica*, compared with the European eel, *A. anguilla*. J Helminthol 73:227–232
- Nimeth K, Zwerger P, Wurtz J, Salvenmoser W, Pelster B (2000) Infection of the glass-eel swimbladder with the nematode *Anguillicola crassus*. Parasitology 121:75–83
- Paggi L, Orrechia P, Minervini R, Mattiuci S (1982) Sulla comparsa di *Anguillicola australiensis* Johnston et Mawson 1940 in *Anguilla anguilla* del Lago di Bracciano. Parasitology 24:139–144
- Palikova M, Navratil S (2001) Occurrence of *Anguillicola crassus* in the water reservoir Koryčany (Czech Republic) and its influence on the health condition and haematological indices of eels. Acta Veterinaria Brno 70:443–449
- Rahhou I, Melhaoui M, Lecomte-Finiger R, Morand S, Chergui H (2001) Abundance and distribution of *Anguillicola crassus* (Nematoda) in eels *Anguilla anguilla* from Moulouya Estuary (Morocco). Helminthologia 38:93–97
- Taraschewski H, Renner C, Mehlhorn H (1988) Treatment of fish parasites 3. Effects of levamisole, HCL, metrifonate, fenbendazole, mebendazole and ivermectin on *Anguillicola crassus* (nematodes) pathogenic in the air bladder of eels. Parasitol Res 74:281–289
- Tesch FW (1977) The eel. Chapman & Hall, London
- Thomas K, Ollevier F (1992a) Paratenic hosts of the swimbladder nematode *Anguillicola crassus*. Dis Aquat Org 13:165–174
- Thomas K, Ollevier F (1992b) Population biology of *Anguillicola crassus* in the final host *Anguilla anguilla*. Dis Aquat Org 14:163–170
- Thomas K, Ollevier F (1993) Hatching, survival, activity and penetration efficiency of second-stage larvae of *Anguillicola crassus* (Nematoda). Parasitology 107:211–217
- Van Banning P, Haenen OLM (1990) Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. In: Perkins FO, Cheng TC (eds) Pathology in marine science. Academic Press, New York, p 317–330
- Van Banning P, Heermans W, Van Willigen JA (1985) *Anguillicola crassa*, een nieuwe aalparasiet in de Nederlandse wateren. Visserij 38:237–240
- Watanabe S (2003) Taxonomy of the freshwater eels, genus *Anguilla* Schrank, 1798. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel biology. Springer-Verlag, Tokyo
- Wickström H, Clevestam P, Höglund J (1998) The spreading of *Anguillicola crassus* in freshwater lakes in Sweden. Bull Fr Pêche Piscic 349:215–221
- Wurtz J, Taraschewski H (2000) Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. Dis Aquat Org 39:121–134

Editorial responsibility: Wolfgang Körting,
Hannover, Germany

Submitted: August 28, 2002; Accepted: May 19, 2003
Proofs received from author(s): September 8, 2003