Anguillicola crassus (Nematoda, Dracunculoidea) infections of European eel (*Anguilla anguilla*) in the Netherlands:

epidemiology, pathogenesis and pathobiology

23 FEB. 1995
UB-CARDEX

Olga Haenen

CENTRALE LANDBOUWCATALOGUS
0000 0611 3746

ISN 90349240951

Promotor : **Dr. W.B. van Muiswinkel,**
Hoogleraar in de Zoölogische Celbiologie
Landbouwuniversiteit, Wageningen

Co-promotor : **Dr. F.H.M. Borgsteede,**
hoofd sectie Parasitologie
DLO-Instituut voor Veehouderij en Diergezondheid,
Lelystad

NN08201, 1905.

Anguillicola crassus (Nematoda, Dracunculoidea) infections of European eel (*Anguilla anguilla*) in the Netherlands:

epidemiology, pathogenesis and pathobiology

Olga Liduina Maria Haenen

Proefschrift

ter verkrijging van de graad van doctor
in de Landbouw- en Milieuwetenschappen,
op gezag van de rector magnificus,
Dr. C.M. Karssen
in het openbaar te verdedigen
op vrijdag 10 maart 1995
des namiddags te vier uur in de aula
van de Landbouwuniversiteit Wageningen

ISN: 903492

Omslag: Olga Haenen

Fotografie: Fred van Welie
Joop Iedema

Vormgeving: Herma Daus-Ooms

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Haenen, Olga Liduina Maria

Anguillicola crassus (Nematoda, Dracunculoidea) infections of European eel (*Anguilla anguilla*) in the Netherlands : epidemiology, pathogenesis and pathobiology / Olga Liduina Maria Haenen ; [ill. van de auteur]. - [S.l. : s.n.] - Ill.
Thesis Landbouw Universiteit Wageningen. - With ref.
With summary in Dutch.
ISBN 90-5485-354-9
Subject headings: Anguillicola crassus / parasitic nematode / eel.

BIBLIOTHEEK
LANDBOUWUNIVERSITEIT
WAGENINGEN

The study described in this thesis was performed at the DLO-Institute for Animal Science and Health, Lelystad, The Netherlands.

aan mijn ouders

NN08201, 1905

Stellingen

1. De snelle vestiging en verspreiding van *Anguillicola crassus* in Nederland is mede te danken aan het brede scala van tussengastheren en paratenische gastheren (dit proefschrift).

2. Bij het vergelijken van de *Anguillicola crassus*-infectie in wilde paling ten opzichte van experimenteel geïnfecteerde paling is de rol van stress bij palingexperimenten niet te onderschatten (dit proefschrift).

3. Het ongeremd importeren van ongekeurde pootvis vanuit andere werelddelen kan leiden tot faunavervalsing (dit proefschrift).

4. Het lange termijn effect van *Anguillicola crassus* op de Nederlandse palingstand is moeilijk te voorspellen (dit proefschrift).

5. Met het woord '*zwermblaaswormpje*' geeft Van Dale's Groot Woordenboek der Nederlandse Taal ongewild via een drukfout al het grote verspreidingsvermogen van *Anguillicola* aan.

6. Vis wil zwemmen, maar aal is al vloeibaar.

7. Het gebruik van chemische middelen ter bestrijding van visziekten herbergt tal van risico's voor de visteeltsector.

8. Indien muziek en exacte wetenschap hand in hand gaan is er sprake van synergisme.

9. E-mail is een gouden systeem voor het onderhouden van formele en informele contacten.

10. Bij het opzetten en handhaven van een kwaliteitssysteem is motivatie van de uitvoerenden vele malen belangrijker dan de precieze tekst van de kwaliteitsdocumenten.

11. Het verspreiden van varkensmest op kleigrond versterkt het negatieve imago van de varkenshouderij.

12. Bij de aanvang van een groot project zijn durf om iets te ondernemen en vertrouwen om juiste beslissingen te nemen belangrijker dan het volledig overzien van het project: het VOC spiegelretourschip 'de Batavia' te Lelystad is hier een prachtig voorbeeld van.

Stellingen behorend bij het proefschrift "*Anguillicola crassus* (Nematoda, Dracunculoidea) infections of European eel (*Anguilla anguilla*) in the Netherlands : epidemiology, pathogenesis and pathobiology", Olga L.M. Haenen. Wageningen, 10 maart 1995.

Contents

Chapter 1

General Introduction

General Introduction

Before 1980, the parasite *Anguillicola crassus* was unknown to Europe, but now it can be regarded as a common swimbladder parasite of European eel, *Anguilla anguilla*. Since its introduction into Europe, infections with *A. crassus* have caused severe pathological changes in eels.

This thesis focuses on the observed rapid spread of the infection with *A. crassus* in European eels in the years after introduction of the parasite into The Netherlands. The epidemiology, pathogenesis, and pathobiology are described and discussed in eight chapters. Chapter 2 summarizes investigations on the pathobiology and epidemiology of the infection that were made in eels from freshwater lakes and eel farms shortly after the introduction of the parasite into Dutch eel populations. Chapter 3 describes aspects of the epidemiology and pathogenesis : investigations were made of freshwater fish species that can act as reservoirs or paratenic hosts for the parasite. Chapter 4 describes pathogenetic investigations in eels via experimentally induced infections of eel with *A. crassus* larvae isolated from smelt and ruffe to prove that these fish species can transmit the infection to the eels, and that they are real paratenic hosts for *A. crassus*. Chapter 5 describes induced infections of eels to test the pathogenesis and temporal pattern of the life cycle of the parasite. Chapter 6 analyses data from 1986 to 1992 on the eels naturally infected with *A. crassus* in Dutch lakes and the Waddenzee, and of *A. crassus* in freshwater smelts in these lakes, to study the epidemiology and pathobiology of the parasite in these fish species. Chapter 7 describes a method for producing infective *A. crassus* larvae to be used in infection trials. Chapter 8 describes the pathobiological aspects of the infection : the effects of various doses of *A. crassus* on eels, that were artificially infected. Moreover, chapter 8 describes experiments to test for an antibody response against *A. crassus* and to determine whether the decreased infection in naturally infected eels in The Netherlands during the past few years is due to an increased number of eels developing an immune response to the parasite. In chapter 9 the results of the previous chapters are discussed, and general conclusions are drawn.

This chapter 1 is a general introduction to the biology of eels, eel culture and fisheries, and eel parasites. The biology of *A. crassus*, its geographical distribution, its significance for eels, and the responses of eels against parasites are briefly described.

Biology of the eel. According to Tesch (1983) *Anguilla* Shaw (Gen. Zool. 4,15, 1803) is a genus of fishes, belonging to the superclass Gnathostomata, class Osteichtyes, subclass Actinopterygii, infraclass Neopterygii, subdivision Teleostei, order Anguilliformes, suborder Anguilloidei, and family Anguillidae. The order Anguilliformes consists of 26 recent and fossil families. Tesch (1983) mentions that there are at least 18 eel species within the genus *Anguilla*, which differ from each other by the length of the dorsal fin, the presence of a marble-like skin, and the design of the jaw, including the location of the teeth. The American eel (*A. rostrata*), European

eel (*A. anguilla*), and Japanese eel (*A. japonica*) are economically important in fisheries and aquaculture.

The life span of the eel was described hypothetically by Schmidt (1912) and reviewed by Tesch (1983). The spawning grounds of the European eel (*A. anguilla*) and the American eel (*A. rostrata*) are situated in the Sargasso Sea (30°N.W., 65°W.L.) and probably also elsewhere (Boëtius and Harding, 1985). Exactly how and where the eels spawn is still unknown. However, at these locations many eel larvae, so-called *Leptocephali* of *A. anguilla*, can be found from January to March. The *Leptocephali* of *A. rostrata* migrate in the direction of the American East coast and Greenland. The *Leptocephali* of *A. anguilla* migrate both passively and actively for about 2 years via gulf streams in the direction of the European and North African coast. From June to October the *Leptocephali* metamorphose to elvers near the coast. At that time, the elvers are still transparant and about 7 cm long. The entrance of elvers into The Netherlands takes place yearly around the end of February, after physiological adaptation of the elvers to freshwater environments. They first gather in front of the sluices. Subsequently, they enter the fresh water. Pigmentation starts at this point, little by little, the elvers becoming yellow eel (in Dutch : rode aal, bronsaal, groene aal, De Nie, 1987). The eels are called stocking eels up to a length of 28 cm. Eels up to 30 cm are sexually not yet determined, but above this length they differentiate sexually. After at least 5 years the eels change to silver eels (Dutch : schieraal). The eye pupils enlarge, the fins enlarge, and the eels develop a silver colour; they finally transform into sea fish, able to swim at great dephts. It is still not known if they ultimately swim back to the Sargasso Sea to spawn, because no mature European eels have been captured outside the European continent. Tucker (1959) postulated, that *A. rostrata* and *A. anguilla* are the same species, but are different oeco-phenotypes.

Economic importance of eel. Eel is one of the freshwater fish species which is cultured on a large scale for consumption. Eel culture in Japan and Europe was reviewed by Heinsbroek (1991). In this study a total production of *Anguilla* species was calculated for 100,000 to 110,000 tons in 1987 (fisheries and fish culture), most of which (70%) was consumed in Japan, and nearly all of which was produced in Japan and Taiwan. The remaining 20 to 30% was consumed in Europe, and about half of this amount (3,000 to 4,000 tons per year) was produced in eel culture.

In Japan eel is cultured mainly in greenhouse ponds; 10 to 20% is cultured in open ponds. Japan has about 1550 eel farms with a mean production of 23 to 26 tons per year (range : 4 to 100 tons per farm), which results in a total production of 36,000 to 40,000 tons per year. Between 95 to 98% of the eels raised in culture are *A. japonica*, which are grown out of elvers caught in Japan; between 2 to 5% are *A. anguilla*, grown out of elvers imported from Europe or domestic precultured fingerlings (eels of 3 to 5g weight), from imported elvers. Japanese eel are cultured at a water temperature of 20 to 32°C, and European eel in Japan at 12-22°C, whereas European eels in Europe are cultured at 23-25°C (Heinsbroek, 1991).

The artificial reproduction of eels in captivity has not yet been successful, although much research has been done already in this area by Boëtius and Boëtius (1980) and

others. If the artificial reproduction succeeds, elvers will no longer be a limiting factor in eel culture.

Eel in The Netherlands : fisheries and culture. Since ancient times the European eel *A. anguilla* has been fished in the fresh waters of The Netherlands and sold for consumption. Today eels are still fished commercially in lakes like the IJsselmeer and Markermeer, and thousands of fyke nets are used to catch them. However, in recent years, the eel catches in these lakes have declined : 687 tons in 1986, 473 tons in 1987, 399 tons in 1988, around 550 tons/year in 1989-1992, 375 tons in 1993, and 260 tons in 1994 (Dekker and van Willigen, 1993; Dekker, 1995). At first the decreases from 1986 to 1988 were attributed to eel-eating cormorants, but after analysis of the food chain, it was concluded that heavy commercial fishing was a more important reason for the decline (Buijse et al., 1990). The low catches in 1993 and 1994 were attributed to declines in numbers of glass eels (Dekker, 1995).

Commercial eel culture in The Netherlands has been developing since 1979 (Kamstra, 1994). Starting with elvers or stocking eels (<28 cm) larger eels are intensively produced in indoor eel farms, which use recirculated tap water. Between 1986 and 1993, eel farming rapidly grew to a production of approximately 1250 tons. There are now approximately 20 eel farms in The Netherlands. These farms are expected to expand and increase production to 1600 tons of eel per year.

Until 1987-1988 Dutch eel farms stocked their tanks with undersized eels from the IJsselmeer and Markermeer. When stocking eels were scarce, live eels were also imported from other countries.

Nematode parasites of the European eel. General findings on number of metazoan parasite species and nematode species related to eels are listed in Table 1. According to Tesch (1983) there are 44 metazoan parasite species of European eel, 11 of which are nematode species, not including *A. crassus* and *A. australiensis* (Peters and Hartmann, 1986). The latter species was reportedly introduced into Italy in the early 1980s, when imported Australian eels (*Anguilla australis*) from New Zealand were stocked (Paggi et al., 1982). This parasite was later retyped and renamed to *A. novaezelandiae* sp.nov. (Moravec and Taraschewski, 1988). In Table 1, maximally, 13 nematode species of eel were found (Køie, 1988b), of which 3 were dracunculoid nematodes of the swimbladder, namely *Philometra ovata, Daniconema anguillae* (Moravec and Køie, 1987), and *A. crassus*. The two nematode species found by Hubert and Justine (1990) were *A. crassus* and *Paraquimperia* species.

The following ectoparasites play a role at Dutch eel farms : the protozoans *Trichodina* spp., *Ichtyobodo* spp., *Chilodonella* spp., and *Dermocystidium* spp. and the monogenean trematode *Pseudodactylogyrus* spp. of the gills (unpublished observations). From 1986 to 1988, the most important endoparasite of eels was *A. crassus*, after which farms prevented themselves against the parasite.

Table 1. *Data from the literature on numbers of metazoic parasite and nematode species found in the European eel,* ***Anguilla anguilla.***

Year	Number of eels checked	Number of parasite species	Number of nematode species	Country/ Region	Reference
1966	116	5	1[a]	Netherlands	Borgsteede, 1966
1971	83	12	4	Poland	Seyda, 1973
<1980[b]	?[c]	24	6	Europe	Reichenbach-Klinke, 1980
<1980	26	4	2[a]	E-England	Williams & Bolton, 1985
<1983	?	44	11	?	Tesch, 1983
1986-1988	370	42	13[d]	Denmark	Køie, 1988b
1988	129	6	2[a]	N-Portugal	Saraiva & Chubb, 1989
1990	22	3	2	France	Hubert & Justine, 1990
1993	100	6	0	Netherlands	Borgsteede, unpublished

a) in the gastro-intestinal tract
b) before 1980
c) not given in reference
d) only in brackish and fresh waters.

The biology of *Anguillicola crassus*

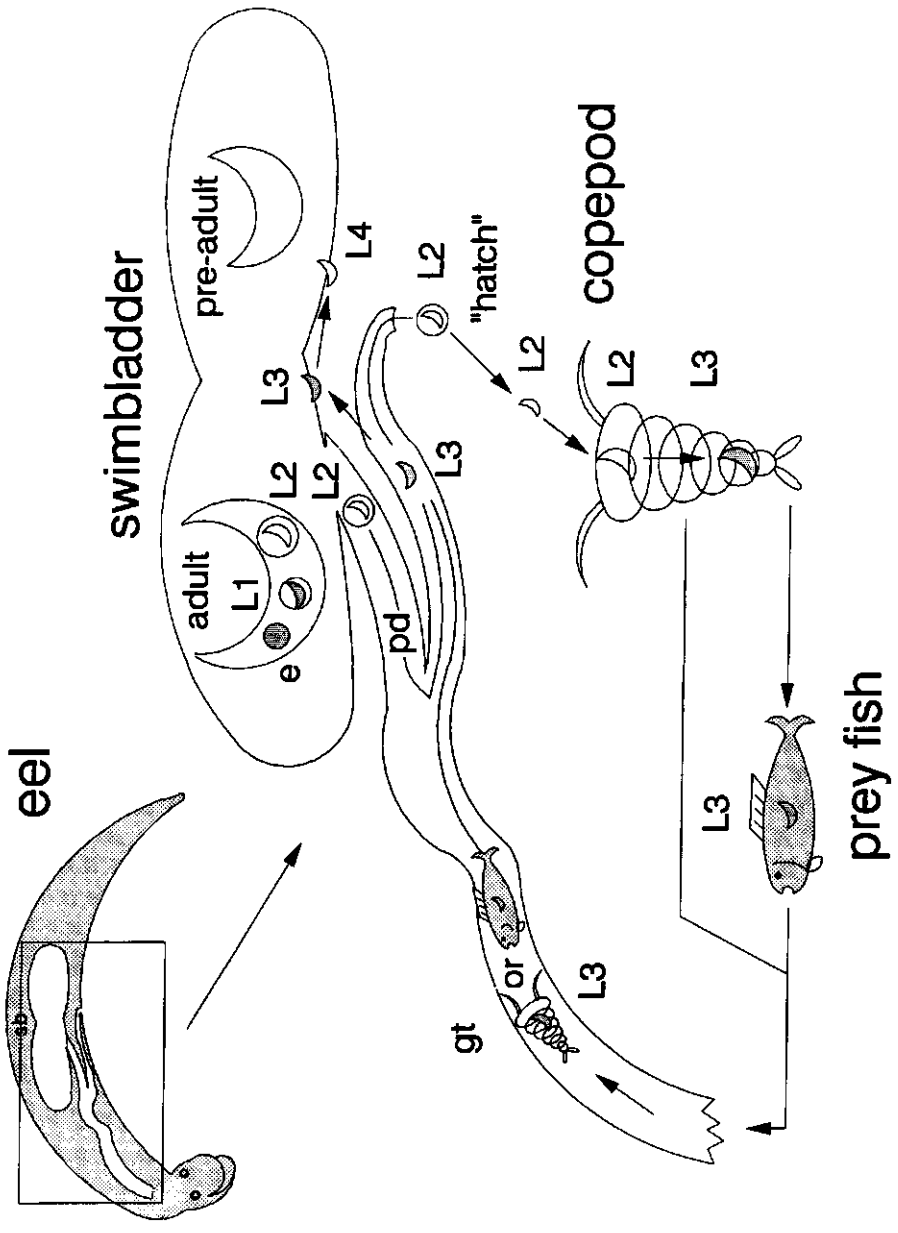
Taxonomy

The genus *Anguillicola* Yamaguti, 1935 consists of five species, which are all parasites of eel swimbladders. *A. crassa* Kuwahara, Niimi and Itagaki, 1974 (Nematoda, order Dracunculoidea, family Anguillicolidae) is originally a swimbladder parasite of Japanese eel *Anguilla japonica*. Moravec and Taraschewski (1988) revised the genus *Anguillicola*, revising the name *A. crassa* to *A. crassus* and placing this group in a new subgenus *Anguillicoloides*.

Life cycle

The indirect life cycle of *Anguillicola* species was described by Egusa (1979), Puqin and Yuru (1980), and Wang and Zhao (1980). Since *A. crassus* was introduced into Europe, many authors have described its life cycle in relation to European eels (for example, Haenen et al., 1989; Petter et al., 1989; De Charleroy et al., 1990; Thomas, 1993). The life cycle is schematically presented in Figure 1. Adult *A. crassus* reside in the

Fig. 1. Schematic illustration of the indirect life cycle of *Anguillicola crassus* in European eel, *Anguilla anguilla* via copepods and prey fish. sb = swimbladder, gt = gastro-intestinal tract, pd = pneumatic duct; e = parasite egg, L1 = first stage larva, L2 = second stage larva, L3 = third stage larva, L4 = fourth stage larva of *A. crassus*.


eel
swimbladder
pre-adult
adult
L1
L2
L3
L4
e
pd
gt
or
"hatch"
copepod
prey fish

swimbladder lumen of eel, where they feed on eel blood. The female produces eggs, which are fertilized after copulation. These are released through the vulva or by rupture of the female parasite. First stage (L1) larvae moult into second stage (L2) larvae, still within the egg sheat. The L2 larvae are passively transported via the pneumatic duct to the oesophagus. Subsequently, they pass through the digestive tract into the environment. The ensheated L2 larvae hatch within a few hours after entering the freshwater environment. They move actively, attach to substrates, and can be eaten by copepods, which serve as intermediate hosts. Inside the copepod, the L2 larvae migrate to the hemocoel and moult into L3 larvae after about 10 days. This L3 stage is the phase in which the parasite can infect various fish species. The eels eat the copepods and become infected. The L3 larva then migrates directly through the wall of the digestive tract (oesophagus, stomach, and intestine) to the swimbladder wall where it moults after a few weeks to the fourth stage (L4). The L4 larvae start to feed on eel blood, migrate to the swimbladder lumen and become pre-adults, and ultimately adults.

The total life cycle of *A. crassus* in Japanese eel was estimated to take one year (Egusa, 1979; Puqin and Yuru, 1980). However, the life cycle of *A. crassus* in European eel in Europe took only two months (De Charleroy et al., 1990). Moreover, in Europe various freshwater fish species were found to contain larval stages of *A. crassus* in their swimbladder, thereby acting as intermediate host (paratenic hosts (Haenen and Van Banning, 1990, 1991; Höglund and Thomas, 1992; Thomas and Ollevier, 1992; Székely, 1994)). No paratenic hosts were known from Southeast Asian literature related to *A. crassus*. Because European eels prey upon some of the paratenic hosts, such as smelt (De Nie, 1987; Buijse et al., 1990), *A. crassus* may be transmitted in this way to the eels (Haenen and Van Banning, 1991). Although *A. crassus* is a freshwater parasite, free L2 larvae can also survive in brackish water for about three weeks (De Charleroy et al., 1989; Reimer et al., 1994) and even in sea water for a few days (Kennedy and Fitch, 1990). Moreover, infective L3 larvae may be transmitted to sea water inside brackish water fish species that act as paratenic hosts, such as the black goby, *Gobius niger* (Höglund and Thomas, 1992; Reimer et al., 1994).

Geographical Distribution. *A. crassus* is widely distributed throughout Japan (Egusa et al., 1969, Kuwahara et al., 1974, Hirose et al., 1976), Korea (Kim et al., 1989), probably Taiwan (Køie, 1988b, 1991) and southeast China (Wang and Zhao, 1980), and especially affects the Japanese eel, *Anguilla japonica*. There is much eel culture in these areas. The parasite was introduced into the communities of European eel *A. anguilla* in Europe at the start of the 1980s, probably through the import of infected eels from Taiwan to Germany (Køie, 1988b, 1991). Since then it has spread quickly over most of Europe (Germany: Neumann, 1985; The Netherlands: Van Banning et al., 1985; Italy: Canestri-Trotti, 1987; France: Dupont and Petter, 1988; Sweden: Hellström et al., 1988; Denmark: Køie, 1988a; Belgium: Belpaire et al., 1989; Germany: Koops and Hartmann, 1987; United Kingdom: Kennedy and Fitch, 1990; Hungary: Székely et al., 1991; Portugal: Cruz et al., 1992; Czechia: Moravec, 1992; Baltic Sea: Reimer et al., 1994; Norway: Mo and Steien, 1994).

Significance of *Anguillicola crassus* for Japanese and European eel. *A. crassus* is a common parasite of the Japanese eel, but was not causing lesions (Egusa, 1979). The prevalences (%-age of infected eels) in Japanese eel ranged in open waters from 17.5% (Kim et al., 1989) to 56.3% (Wang and Zhao, 1980). The intensities (number of *A. crassus* per infected eel) ranged from 1 to 11 (Wang and Zhao, 1980). In European eel imported into southern Asia, the prevalence could reach 100% and the intensity more than 30 parasites per infected eel (Egusa, 1978, 1979).

Egusa (1979) suggested that European eels are much more sensitive to *A. crassus* than Japanese eels. This was clear, after the parasite was introduced into Europe. Soon thereafter, the prevalence of the *A. crassus* infection was above 80% (Van Banning and Haenen, 1990) and intensities mounted to 73 adult specimens per swimbladder (Molnár et al., 1993). The growth of infected eels was reduced under experimental circumstances (Boon et al., 1990) and likewise their swimming speed (Sprengel and Lüchtenberg, 1991). Moreover, *A. crassus* caused various lesions in the swimbladder and surrounding tissues, often containing dead encapsulated larvae and adults of *A. crassus* (Van Banning and Haenen, 1990; Molnár et al., 1993; Haenen et al., 1994). Under pressure of proliferative inflammations of the swimbladder, L2 larvae may loose their egg sheat (Van Banning and Haenen, 1990). Lesions combined with a lack of oxygen caused mass mortalities of eels in Lake Balaton (Hungary) in 1991 (Molnár et al., 1991; Molnár, 1993). Athough in 1993 the prevalences of *A. crassus* in European eel in the IJsselmeer and Markermeer were still above 80%, the intensities decreased after 1989 from about 14 to 16 *A. crassus* per eel towards 4.5 to 5 per eel in 1993 (Haenen et al., 1994). A similar decrease in infection was also found in eels from Lake Balaton (Molnár et al., 1994).

At Dutch eels farms secondary bacterial infections have occurred in swimbladders of eels heavily infected with *A. crassus*, resulting in mortalities of 10-20% (Liewes and Schaminee-Main, 1987; Van Banning and Haenen, 1990). Various anthelminthics against *A. crassus* have been tested (Taraschewski et al., 1988; Hartmann, 1989; Kamstra, 1990; Geets et al., 1992), but they have not been used on a large-scale at eel farms because of cost aspects and residues (unpublished observations). *A. crassus* infections are no longer a problem at eel farms, since only uninfected elvers or *A. crassus*-free fingerlings, raised on *A. crassus*-free eel farms, are used.

Responses of fish to parasites. It is known that fish have an immune system (Van Muiswinkel, 1995 in press). Woo (1992) reviews the immunological responses of fish to parasitic organisms. The responses can be innate, acquired, or both, and sometimes complement activation plays a role. Three major types of host responses of fish to parasites are described (Van Muiswinkel and Jagt, 1984) : Parasites can be encapsulated in host tissue after a nonspecific tissue response, sometimes parasites cause an inflammatory response of the host characterized by leukocyte activity, and/or parasites may cause a specific immune response involving lymphoid cells and antibody production. Some mechanisms of escape of parasites from host immune responses are known. Sometimes, parasites are thought to adsorb host antigens on their body surface to evade the host immune system, like with some Cestodes (Woo,

1992). Some sporozoans can show mimicry to host antigens, and thereby escape from the hosts response; *Cryptobia* (flagellate) in rainbow trout is thought to develop cyclic antigenic changes, when the host has just build up enough controlling factors (Van Muiswinkel and Jagt, 1984). In general there is very little known about reactions of fish to nematode infections (Woo, 1992).

Eels can respond innate to parasitic infections, like *Eimeria anguillae*, Apicomplexa (Benajiba et al., 1994) and the acanthocephalan, *Paratenuisentis ambiguus* (Hamers et al., 1992). Molnár and Moravec (1994) describe a response of adherent macrophages to L3 larvae of the dracunculoid nematode *Daniconema anguillae* in subcutaneous eel tissue. Eels can also develop acquired resistance to infections with ectoparasitic gill monogeneans of the genus *Pseudodactylogyrus* (Slotved and Buchmann, 1993). Specific immunological responses of eels against the nematode *A. crassus* have been studied by Buchmann et al. (1991), and Höglund and Pilström (1994a, in press, 1994b). They found an acquired specific antibody response of eels against antigens of adult *A. crassus*.

References

Belpaire, C., De Charleroy, D., Grisez, L. & Ollevier, F. (1989) Spreading mechanisms of the swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla*, and its distribution in Belgium and Europe. European Inland Fisheries Advisory Commission (FAO). Working Party on Eel. Porto, Portugal, 7 p.

Benajiba, M.H., Marques, A., Lom, J. & Bouix, G. (1994) Ultrastructure and sporogony of *Eimeria* (syn. *Epieimeria*) *anguillae* (Apicomplexa) in the eel (*Anguilla anguilla*). *J. Euk. Microbiol.* **41(3)** : 215-222.

Boëtius, J. & Boëtius, I. (1980) Experimental maturation of female silver eels, *Anguilla anguilla*. Estimates of fecundity and energy reserves for migration and spawning. *Dana* **1** : 1-28.

Boëtius, J. & Harding, E.F. (1985) A re-examination of Johannes Schmidt's eel investigations. *Dana* **4** : 129-162.

Boon, J.H., Augustijn, H., Cannaerts, V.M., Lokin, C.J., Machiels, M.A. & 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.

Borgsteede, F.H.M. (1966) De parasitofauna van enkele vissoorten uit het IJsselmeer. De samenstelling en de invloed ervan op de vissen. RIVO-rapport apr-dec 1966, 28 pp.

Buchmann, K., Pedersen, L.O. & Glamann, J. (1991) Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Dis. aquat. Org.* **12** : 55-57.

Buijse, A.D., van Eerden, M.R., Dekker, W. & van Densen, W.L.T. (1990) A trophic model for IJsselmeer (The Netherlands), a shallow eutrophic lake. ICES C.M. 1990/L : 71 (poster).

Canestri-Trotti, G. (1987) Occurrence of the nematode *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 in eels from the Po Delta, Italy. *Bull. Eur. Ass. Fish Pathol.* **7(5)** : 109-111.

Cruz, P., Silva, E., Grazina Freitas, M.S. & Carvalho-Varela, M. (1992) First report of *Anguillicola crassus* in the European eel in Portugal. *Bull. Eur. Ass. Fish Pathol.* **12(5)** : 154-156.

De Charleroy, D., Thomas, K., Belpaire, C., & Ollevier, F. (1989) The viability of the free living larvae of *Anguillicola crassus*. *J. appl. Ichthyol.* **5(3)** : 154-156.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. & Ollevier, F. (1990) The life cycle of *Anguillicola crassus*. *Dis. aquat. Org.* **8** : 77-84.

Dekker, W. & van Willigen, J.A. (1993). De aalvisserij in het IJsselmeer; Evaluatie van de toestand van het visbestand tot 1992. RIVO intern rapport no. 93.011, 29 pp.

Dekker, W. (1995). Visstand en visserij op het IJsselmeer en Markermeer: toestand in 1994. RIVO intern rapport no. 95.001, 33 pp.

De Nie, H.W. (1987) Food, feeding and growth of the eel (*Anguilla anguilla* L.) in a Dutch eutrophic Lake. Thesis Agricultural University, Wageningen, The Netherlands, 129 pp.

Dupont, F. & A.J. Petter, A.J. (1988) *Anguillicola*, uni épizooti plurispécifique en Europe. Apparition de *Anguillicola crassa* (Nematoda, Anguillicolidae) chez l'anguille Européenne (*Anguilla anguilla*) en Camargue, Sud de la France. *Bull. Fr. Peche Piscic.* **308** : 38-41.

Egusa, S. (1978) Anguillicolosis of eels. In : Infectious diseases of fishes, Koseisha Koseikaku, Tokyo, pp. 501-506. (In Japanese.)

Egusa, S. (1979) Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. In Thurow, F., 1979. *Eel research and Management. Rap. Proc. Verb. Reun. Cons. Int. Exp. Mer* **174** : 51-58.

Egusa, S., Kira, K. & Wakabayashi, H. (1969) On the occurence of *Anguillicola globiceps* Yamaguti, a swimbladder roundworm, in pondcultured eels. *Fish Pathol.* **4** : 52-58. (in Japanese).

Geets, A., Liewes, E.W. & Ollevier, F. (1992) The efficacy of some anthelmintics against the swimbladder nematode *Anguillicola crassus* of eel *Anguilla anguilla* under saltwater conditions. *Dis. aquat. Org.* **13** : 123-128.

Haenen, O.L.M. & van Banning, P. (1990) Detection of larvae of *Anguillicola crassus* (an eel swimbladder nematode) in freshwater fish species. *Aquaculture* **87** : 103-109.

Haenen, O.L.M. & van Banning, P. (1991) Experimental transmission of *Anguillicola crassus (Nematoda, Dracunculoidea) larvae from infected prey fish to the eel Anguilla anguilla. Aquaculture* **92** : 115-119.

Haenen, O.L.M., 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 1986-1992. *Aquaculture* **126** : 219-229.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C. & Ollevier, F. (1989) Experimentally induced infections of the European eel, *Anguilla anguilla* L., with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. aquat. Org.* **7** : 97-101.

Hamers, R., Lehmann, J., Stürenberg, F.-J. & Taraschewski, H. (1992) In vitro study of the migratory and adherent responses of fish leucocytes to the eel-pathogenic acanthocephalan *Paratenuisentis ambiguus* (van Cleave, 1921) Bullack et Samuel, 1975 (Eoacanthocephala : Tenuisentidae). *Fish Shellfish Immunol.* **2** : 43-51.

Hartmann, F. (1989) Investigations on the effectiveness of Levamisol as a medication against the eel parasite *Anguillicola crassus* (Nematoda). *Dis. aquat. Org.* **7** : 185-190.

Heinsbroek, L.T.N. (1991) A review of eel culture in Japan and Europe. *Aquaculture and Fisheries Management* **22** : 57-72.

Hellström, A., Ljungberg, O. & Bornstein, S. (1988) *Anguillicola*, en ny alparasit i Sverige. *Svensk Veterinartidning* **40** : 211-213.

Hirose, H., Sekino, T. & Egusa, S. (1976) Notes on the egg deposition, larval migration and intermediate host of the nematode *Anguillicola crassa* parasitic in the swimbladders of eels. *Fish Pathology* **11** : 27-31 (Japanese; English summary).

Höglund, J. & Pilström, L. (1994a, in press) Mechanical isolation and characterization of antigens for adult *Anguillicola crassus. Fish Shellfish Immunol.*, in press.

Höglund, J. & Pilström, L. (1994b) Purification of adult *Anguillicola crassus* whole-worm extract antigens for detection of specific antibodies in serum from the European eel (*Anguilla anguilla*) by ELISA. *Fish Shellfish Immunol.* **4** : 311-319.

Höglund, J. & Thomas, K. (1992) The black goby *Gobius niger* as a potential paratenic host for the parasitic nematode *Anguillicola crassus* in a thermal effluent of the Baltic. *Dis. aquat. Org.* **13** : 175-180.

Hubert, V. & Justine, J.-L. (1990) Étude préliminaire des parasites des poissons de la région Parisienne. *Bull. de la Société Française de Parasitologie* **8(2)** : 283-288.

Kamstra, A. (1990) *Anguillicola* in Dutch eel farms; current state. *Int. Revue ges. Hydrobiol.* **75(6)** : 867-874.

Kamstra, A. (1994) Kijken in een kristallen bol gevuld met recirculatiewater. *Aquacultuur* **9** (1) : 24-26.

Kennedy, C.R. & Fitch, D.J. (1990) Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in eel, *Anguilla anguilla*, in Britain. *J. Fish Biol.* **36** : 117-131.

Kim, Y.-G., Kim, E.-B., Kim, J.-Y. & Chun, S.-K. (1989) Studies on a nematode, *Anguillicola crassa* parasitic in the air bladder of the eel. *J. Fish Pathol.* **2** : 1-18. (in Korean with English summary.)

Køie, M. (1988a) Parasites in eels, *Anguilla anguilla* (L.) from eutrophic lake Esrum, Danmark. *Acta Parasit. Pol.* **33** : 89-100.

Køie, M. (1988b) Parasites in European eel *Anguilla anguilla* (L.) from Danish freshwater, brackish and marine localities. *Ophelia* **29(2)** : 93-118.

Køie, M. (1991) Swimbladder nematodes (*Anguillicola* spp.) and gill monogeneans (*Pseudodactylogyrus* spp.) parasitic on the European eel (*Anguilla anguilla*). *J. Cons. int. Mer* **47** : 391-398 (1991).

Koops, H., & Hartmann, F. (1987) Infection of eels from different regions with *Anguillicola*. Rep. EIFAC (FAO) Working Party on Eels : Bristol, UK, 13-16 April '87, 7 p.

Kuwahara, A., Niimi, A., Itagaki, H. (1974) Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Phimetridae, Anguillicolidae). *Jap. J. Parasit.* **23**, 5 : 275-279.

Liewes, E.W. & Schaminee-Main, S. (1987) Onderzoek naar de effecten van de parasiet *Anguillicola crassa* op de ontwikkeling van de paling (*Anguilla anguilla*) in een zoutwater palingmesterij. Intern report Texvis BV, Texel, The Netherlands.

Mo, T.A. & Steien, S.H. (1994) First observation of the eel swimbladder nematode *Anguillicola crassus* in Norway. *Bull. Eur. Ass. Fish Pathol.* **14** : 163-164.

Molnár, K. (1993) Effect of decreased oxygen content on eels (*Anguilla anguilla*) infected by *Anguillicola crassus* (Nematoda : Dracunculoidea). *Acta Veterinaria Hungarica* **41 (3-4)** : 349-360.

Molnár, K. & Moravec, F. (1994) Third-stage larvae of *Daniconema anguillae* (Nematoda: Dracunculoidea) in the subcutaneous tissue of eel *Anguilla anguilla*. *Folia Parasitol.* **41** : 215-219.

Molnár, K., Székely, Cs. & Baska, F. (1991) Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bull. Eur. Ass. Fish Pathol.* **11(6)** : 211-212.

Molnár, K., Baska, F., Csaba, Gy., Glávits, R. & Székely, Cs. (1993) Pathological and histopathological studies of the swimbadder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Dis. aquat. Org.* **15** : 41-50.

Molnár, K., Székely, Cs. & Perényi, M. (1994) Dynamics of *Anguillicola crassus* (Nematoda : Dracunculoidea) infection in eels of Lake Balaton, Hungary. *Folia Parasitol.* **41** : 193-202.

Moravec, F. (1992) Spreading of the nematode *Anguillicola crassus* (Dracunculoidea) among eel populations in Europe. *Folia Parasitologica* **39** : 247-248.

Moravec, F. & Køie, M. (1987) *Daniconema anguillae* gen. et sp. n., a new nematode of a new family Daniconematidae fam. n. parasitic in European eels. *Folia Parasitol.* **34, 4** : 335-340.

Moravec, F. & Taraschewski, H. (1988) Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda, Anguillicolidae) of the swimbladder of eels, including descriptions of two new species *A. novazelandiae* sp.n. and *A. papernae* sp.n.. *Folia Parasitol.* **35** : 125-146.

Neumann, W. (1985) Swimmblasenparasit *Anguillicola* bei Aalen. *Fischer und Teichwirt* **11**: 322.

Paggi, L. Orecchia, P., Minervini, R. & Mattiucci, S. (1982) Sulla comparsa di *Anguillicola australiensis* Johnston e Mawson, 1940 (Dracunculoidea : Anguillicolidae) in *Anguilla anguilla* del Lago di Bracciano. *Parassitologia* **24** : 139-144.

Peters, G. & Hartmann, F. (1986) *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. *Dis. aquat. Org.* **1** : 229-230.

Petter, A.J., Fontaine, Y.A. & Le Belle, N. (1989) Etude du developpement larvaire de *Anguillicola crassus* (Dracunculoidea, Nematoda) chez un Cyclopidae de la region Parisienne. *Ann. de Parasitologie Humaine et Comparee* **64, 5** : 347-355.

Puqin, W. & Yuru, Z. (1980) Observations on the life history of *Anguillicola globiceps* (Nematoda, Anguillicolidae). *Acta Zool.Sin.* **26** : 243-249.

Reichenbach-Klinke, H.-H. (1980) Krankheiten und Schädigungen der Fische. 2nd ed. Ed. Gustav Fischer Verlag, Stuttgart, Duitsland, 472 pp.: 417-418.

Reimer, L.W., Hildebrand, A., Scharberth, D. & Walter, U. (1994) *Anguillicola crassus* in the Baltic Sea : field data supporting transmission in brackish waters. *Dis. aquat. Org.* **18** : 77-79.

Saraiva, A. & Chubb, J.C. (1989) Preliminary observations on the parasites of *Anguilla anguilla* (L.) from Portugal. *Bull. Eur. Ass. Fish Pathol.* **9(4)** : 88-89.

Schmidt, J. (1912) Danish researchers in the Atlantic and Mediterranean on the life-history of the freshwater eel (*Anguilla vulgaris* Turt.). *Int.Revue ges. Hydrobiol.* **5**: 317-342.

Slotved, H.C. & Buchmann, K. (1993) Acquired resistance of the eel, *Anguilla anguilla* L., to challenge infections with gill monogeneans. *J. Fish Dis.* **16** : 585-591.

Seyda, M. (1973) Parasites of eel *Anguilla anguilla* (L.) from the Szczecin Firth and adjacent waters. *Acta Ichthyologica et Pescatoria* **3 (2)** : 67-76.

Sprengel, G. & Lüchtenberg, H. (1991) Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla anguilla*. *Dis. aquat. Org.* **11** : 31-35.

Székely, Cs. (1994) Paratenic hosts for the parasitic nematode *Anguillicola crassus* in Lake Balaton, Hungary. *Dis. aquat. Org.* **18** : 11-20.

Székely, Cs., Lang, M. & Csaba, Gy. (1991) First occurence of *Anguillicola crassus* in Hungary. *Bull. Eur. Ass. Fish Pathol.* **11 (4)** : 162-163.

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.

Thomas, K. (1993) The life cycle of the eel parasite *Anguillicola crassus* (Nematoda : Dracunculoidea). Thesis Katholieke Universiteit Leuven, België, Faculteit Wetenschappen, Departement Biologie, Laboratorium voor Ecologie en Aquacultuur, 157pp.

Thomas, K. & Ollevier, F. (1992) Paratenic hosts of the swimbladder nematode *Anguillicola crassus*. *Dis. aquat. Org.* **13** : 165-174.

Tesch, F.-W. (1983) Der Aal. Biologie und Fischerei. 2nd edition. Ed. Paul Parey Verlag, Hamburg, Deutschland, 340 pp.

Tsukamoto, K. (1992) Discovery of the spawning area for Japanese eel. *Nature* **356** : 789-791.

Tucker, D.W. (1959) A new solution to the Atlantic eel problem. *Nature* **183** : 495-501.

Van Banning, P. & Haenen, O.L.M. (1990) Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In : Pathology in marine science, Ed. F.O. Perkins & T.C. Cheng, 1990. Academic Press, Inc., New York. Proc. 3rd Int. Coll. on Path. in Marine Aquaculture, Gloucester Point, Virginia : 317-330.

Van Banning, P., Heermans, W. & van Willigen, J.A. (1985) *Anguillicola crassa*, een nieuwe aalparasiet in Nederlandse wateren. **Visserij 38** : 237-240. (In Dutch).

Van Muiswinkel, W.B. (1995 in press) Piscine immune system : innate and acquired immunity. In : Fish Diseases and Disorders, Vol. I. Ed. P.T.K. Woo, C.A.B. International, Oxon, UK (in press).

Van Muiswinkel, W.B. & Jagt, L.P. (1984) Host-parasite relationships in fish and other ectothermic vertebrates. *Devel. Comp. Immunol.* Suppl. **3** : 205-208.

Wang, P. & Zhao, Y. (1980) Observations on the life history of *Anguillicola globiceps* (Nematoda : Anguillicolidae). *Acta Zool. Sinica* **26** : 243-249. (In Chinese with English abstract.)

Williams, I.C. & Bolton, P.A. (1985) The helminth parasites of the European eel, *Anguilla anguilla* (L.) from the Driffield Canal, North Humberside. *Naturalist* **110** : 31-36.

Woo, P.T.K. (1992) Immunological responses of fish to parasitic organisms. *Ann. Review Fish Dis.* **2** : 339-367.

Yamaguti, S. (1935) Studies on the helminth fauna of Japan. Pt.9. Nematodes of fishes, I. *Jap. J. Zool.* **6** : 337-386.

Chapter 2

Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*

P. van Banning[1] *and O.L.M. Haenen*[2]

[1] DLO-Netherlands Institute for Fishery Investigations, P.O.B. 68, 1970 AB IJmuiden, The Netherlands
[2] DLO-Central Veterinary Institute, P.O.B. 65, 8200 AB Lelystad, The Netherlands

In: "Pathology in Marine Science", Ed. F.O. Perkins & T.C. Cheng, 1990. Academic Press, inc., New York. Proceedings of the 3rd International Colloquium on Pathology in Marine Aquaculture, Gloucester Point, Virginia, Oct. 2-6, 1988 : 317-330.

Abstract

The nonindigenous swimbladder nematode *Anguillicola crassus* has been introduced into the inland waters of the Netherlands, probably via importation of live European eel, *Anguilla anguilla*, for commercial purposes. The nematode has spread very quickly through the Dutch wild eel populations and by 1987 it had a prevalence of 80-100% for most inland waters. The high infection levels, together with the increasing mortalities in infected cultured eel, has lead to questions about the effects of this nematode on eel. Wild young eel (length: 6-15 cm) from a fresh water lake and farmed young eel (8-10 cm and 25-40 cm) were studied by direct observations of squash preparations and by histology of the swimbladder and other organs. It was observed that eel can be infected very soon after immigration of the elvers into fresh water. The nematode may even reach the adult phase in such small eels, adapting their size to the restricted lumen of the small swimbladder.

The observed effects on the swimbladder wall were dilation of blood vessels, inflammation, and rupture. The swimbladder of eels surviving these acute stages has fibrotic walls and often shows adhesion to the surrounding organs. Such non-functional swimbladders restrict survival and reproductive potential of the nematode.

Introduction

The increasing demand for eel for commercial and aquaculture purposes in western Europe has lead to world-wide importation of live eel into some countries, including the Netherlands. Such a situation carries the risk of introducing nonindigenous parasites and pathogens into fish farms and the environment. This is demonstrated by recently made observations of parasites of Asiatic and North American origin in wild and farmed eel in Europe. Køie (1987) described in Denmark three parasites introduced from Asia : the monogeneans *Pseudodactylogyrus anguillae* and *P. bini*, and the nematode *Anguillicola crassa*. The latter has now been renamed *A. crassus* by Moravec and Taraschewski (1988). Both this nonindigenous eel parasite (Neumann, 1985; Peters and Hartmann, 1986) and the North American acanthocephalan *Paratenuisentis ambiguus* (see Taraschewski et al., 1987) have recently been recorded in Germany. In the Netherlands, the introduction and widespread settlement of *A. crassus* in the wild eel populations was most obvious (Fig. 1). Although *A. crassus* was first recorded in 1985 (van Banning et al., 1985), its widespread distribution in different eel populations in the Netherlands at that time suggests that introduction could have already taken place 2 or 3 years earlier. The prevalence increased rapidly in 1986 and 1987, up to levels of 94% (Dekker and van Willigen, 1987).

The introduction of *A. crassus* into the Netherlands became a matter of concern because of the predicted impact on the condition and survival of wild and farmed eel. Although *A. crassus* does not cause serious effects in its original host, the Japanese eel, *Anguilla japonica*, it has been reported that the parasite causes pathological effects on the European eel, *Anguilla anguilla*, in eel farming in Japan (Egusa, 1979). Furthermore, it was observed that an increased mortality rate of 10-20% and a

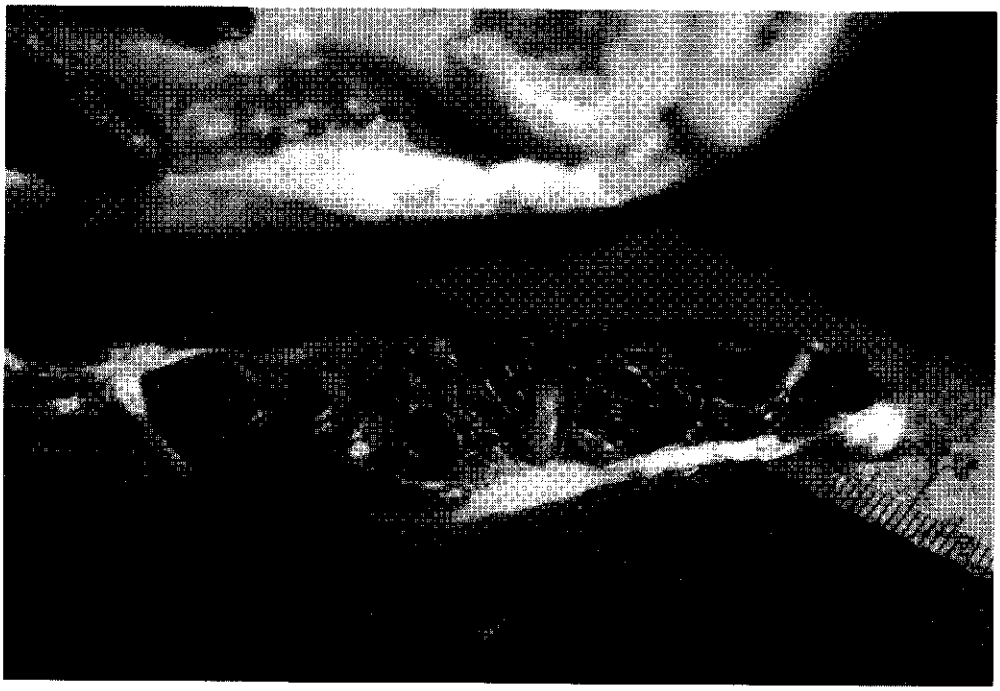


Fig. 1. Swimbladder taken out of an eel and opened to show the burden of *A. crassus* adults in the lumen.

retardation of growth of 20-30% could occur in infected farm eels (Liewes and Schaminee-Main, 1987). Although most of the questions concerning the effects of *A. crassus* on eel are directed to the fresh water phase in order to examine the parasite's biological cycle and mode of infection, it can also affect marine eel farming as these are stocked with fresh water eel, which can already be infected with *A. crassus*.

Wild eels of 6-15 cm length were selected to gain insight into the primary infection process and prevalence in small eel. Further, it was expected that this length class would offer a better possibility for studying the first effects of the infection. Young eels of 6-7 cm length have just left the marine elver stage and recently entered the fresh water environment. It can be assumed that such small eels have not been previously infected with *A. crassus* and are free from any effect of the infection because this nematode is not infectious in the marine environment. Therefore, the pathogenesis of the nematode on the eel can be studied from the very initial period of stay following initial entry into the freshwater environment. For comparison of the effects, a study was also made of farmed eel.

Materials and methods

Sampling and examination of wild eels. Wild eels, *Anguilla anguilla*, with *A. crassus* infection were obtained in June and October, 1987, with fine-mesh beam trawling from

the IJsselmeer, a fresh water lake situated in the northern part of the Netherlands. For practical and histological reasons, a maximum length of 15 cm of the eel was sampled. The eels were transferred alive to the laboratory. Within 20 hr after capture, the effects of *A. crassus* infection were studied by macroscopical observation and squash preparations of the swimbladder and other internal organs for the presence and the condition of the parasites (abnormal = dead or not well developed; normal = alive and active). For this purpose the eels (N=25) were anaesthetized with 2-phenoxy-ethanol (0.2-0.5 ml/liter). For histological studies, other samples (N = 20, distributed over all length ranges) of the same catches were taken. The eels were fixed in 10% buffered formalin, paraffin wax embedded, sectioned transversally at 4µm, and stained with haematoxylin and eosin.

Sampling and examination of farmed eels. Samples of farmed eel, *Anguilla anguilla*, were provided by a fresh water eel farm and a marine eel farm (salinity 30 g/kg) in the Netherlands. The samples were taken at random (length class 8-10 cm) or selected (length class 25-40 cm) as eels infected with *A. crassus* by the eel grower. The farmed eel of 8-10 cm length had been artificially infected 5 months before at the freshwater farm. Farmed eels could have a different infection history and other stress conditions compared to wild eels. So, for farmed eels other parameters were also taken into account, such as secondary bacterial infections. For the bacteriological study, swabs from liver, spleen, and kidney were taken and grown on TSA- and HIS-agar plates at 22°C. When growth was observed, the bacteria were differentiated using morphological and biochemical tests according to Cowan (1977) and Bergey (1984).

Larval stages. For this study, the larval stages of the nematode were defined according to data from wild eel (Kuwahara et al., 1974; Hirose et al., 1976; Puqin and Yuru, 1980) and from experimental observations (De Charleroy et al., 1988a,b; Haenen et al., 1988). The following life cycle was proposed : within the egg, larval stage 1 (L_I) develops, which molts into larval stage 2 (L_{II}), still covered by the egg sheath; L_{II} leaves the eel, hatches in the water and is eaten by the first intermediate host (fresh water copepods) in which the L_{II} molts to L_{III}; the infected copepod is eaten by the eel and L_{III} migrates from the intestine towards the swimbladder wall, where it molts to L_{IV}; ultimately, L_{IV} migrates to the swimbladder lumen and becomes pre-adult and then adult.

Results

Wild eel. *Anguillicola crassus* was found to be already abundant in the small eels sampled in June. In this month, such small eel have lived in the freshwater environment for only a short period (approximately 6-8 weeks), which can thus be also considered as the infection period. In view of such a short infection period, it was remarkable that *A. crassus* already showed a prevalence of 80% in June (Table 1). Furthermore, all stages of the nematode were already present in the swimbladder of the young eel from 8.5 cm upwards, including egg producing adults. The adults in these small eel were in the range of 3-22 mm length (Table 2).

Table 1. *Observations of presence of **Anguillicola crassus** in squash preparations of organs of young eel, sampled in June, 1987 in the IJsselmeer. Prevalence of infection: 80%. Prevalence of swimbladders with imflammatory reactions: 60%. No presence of fibrotic swimbladders.*

Length of eel in cm	Number of L_{III} in stomach, liver, intestine	Number of $L_{III/LIV}$ in swimbladder wall	Number of worms in swimbladder lumen		Presence of L_I/L_{II} in swimbladder lumen	Presence of inflammatory reactions and dilations of blood vessels
			pre-adult	adult		
6.5	0	0	0	0	-	-
7.0	0	0	0	0	-	-
7.4	1	0	0	0	-	-
7.5	0	0	0	0	-	-
7.7	0	0	0	0	-	-
8.5	0	3	1	2	+	+
8.8	0	4	2	0	-	+
9.0	0	0	1	1	+	+
9.0	0	0	0	1	-	+
9.5	0	0	0	2	+	+
9.5	2	1	0	1	-	-
9.5	0	4	1	1	-	+
9.8	2	4	0	2	+	+
9.8	0	2	0	4	+	+
9.8	0	1	0	3	+	+
9.8	0	1	0	4	+	+
10.2	0	8	1	1	-	+
10.3	0	1	1	2	+	-
10.5	0	1	2	0	-	-
11.2	0	0	0	0	-	-
11.2	0	2	1	2	-	+
11.3	0	1	1	2	+	+
12.1	1	0	0	3	+	-
12.6	1	2	0	2	+	+
16.5	1	1	0	2	+	+

+ = present
- = not present

The presence of *A. crassus* could be detected with squash preparations showing L_{III} in stomach, intestine, swimbladder, liver, and kidney, and L_{IV}, adults, L_I, and L_{II} in the swimbladder. The latter organ is considered to be the target organ for the effects of the activities of *A. crassus*. Acute inflammatory reactions, including dilation of blood vessels, formation of connective tissue, and rupture of the swimbladder were observed (Tables 1,3). The presence of inflammatory reactions and connective tissue formation in the swimbladder wall of the small eel were clearly linked to the presence of *A. crassus*, especially when adults are present together with L_I and L_{II}. In the initial infection period (represented by the June sampling) the total infection prevalence in

Table 2. *Lengths in mm of different stages of* ***Anguillicola crassus*** *as observed in young eel of 6-15 cm, sampled in June, 1987 from the IJsselmeer.*

Stage	n	Length min. -max. range	Length x ± SD
L_{IV} in swimbladder	36	0.5 - 2.8	1.1 ± 0.7
pre-adult	10	3 - 6	3.9 ± 1.1
adult female	18	6 - 22	10.1 ± 3.6
adult male	19	3 - 8	5.3 ± 1.8

young eel was 80%, 60% showed inflammatory reactions in the swimbladder wall but thickened swimbladders were not detected macroscopically (Table 1). In the subsequent period (represented by the October sampling) these figures changed to 100%, 28%, and 80%, respectively (Table 3). In the squash preparations, eels with thickened or occluded swimbladders had fewer L_{III}, L_{IV}, and adults of *A. crassus*. In these adults, nonfunctional gonads were shown by the absence of eggs and L_I. Therefore, it seems that fibrotic thickened swimbladders are not suitable for further settlement and survival of *A. crassus*.

The presence of larvae, pre-adults, and adults of *A. crassus* was also detected in histological eel sections (Table 4). In both sampling periods (June and October) the first occurrence of adult parasites was found in 11 cm long eel. The digestive system of the worms was filled with eel erythrocytes, indicating that they had actively fed on the blood of the eel. No parasites were found in sections from eels measuring 7-9 cm.

The histological findings as to the effects of the parasite on swimbladder and organs of young eel are summarized in Figures 2,3,4 and Table 4. The following microscopical lesions were found: inflammatory reactions; tunnels in the swimbladder wall; dilation of blood vessels of the swimbladder wall; fibrosis and fibrotic conglomerates of the swimbladder, gut, and other organs; stenosis of intestine by shrinkage of scar tissue; and enteritis.

A few cases of larval presence (L_I and L_{III}) in muscular tissue were observed in squash preparations and histological sections, but most of these larvae were dead or encapsulated. Because of the rarity and the inactivated situation of such larvae in muscular tissue, they were considered as cases showing aberrant migration and, therefore, not representative of the normal behavior of *A. crassus* larvae within eel.

Farmed eel. Farmed eel heavily infected with *A. crassus* show loss of apetite, abnormal behaviour by hanging near the surface, the presence of open skin ulcers in the caudal part of the belly, and a red and swollen anus. The swimbladders of such diseased eels always contain *A. crassus* adults with high production of eggs, L_I, and L_{II}. Macroscopically, their swimbladders frequently show hemorrhagic characteristics and ruptures, resulting in the presence of free adult worms in the body cavity. In general,

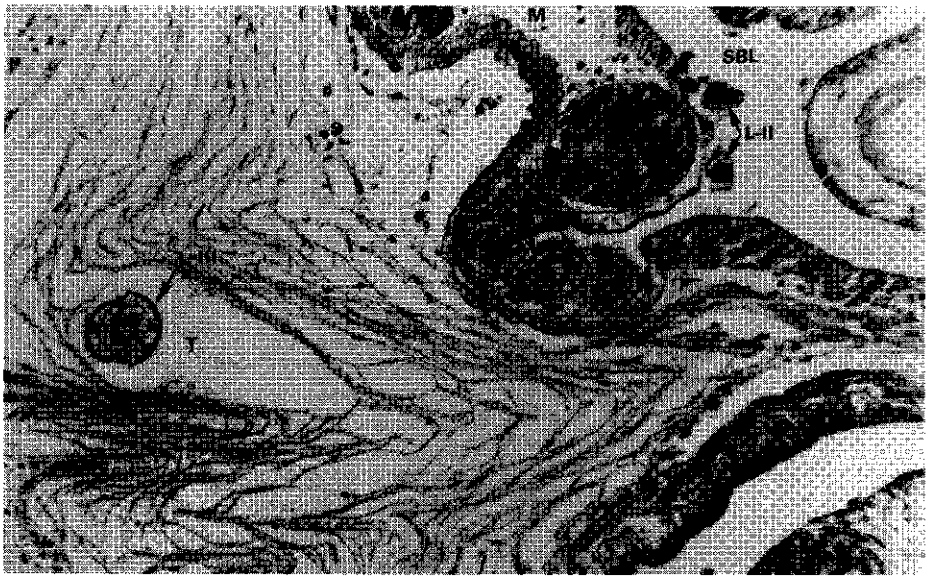


Fig. 2. Cross section of the swimbladder of wild eel (12 cm in length), infected with *Anguillicola crassus*. L_{II} larvae are attached to the thickened swimbladder wall. The mucosa (M) shows dilation of blood vessels (D). An L_{III} larva can be found in a tunnel (T) within the subserosa (S). H&E; 123x. SBL = swimbladder lumen.

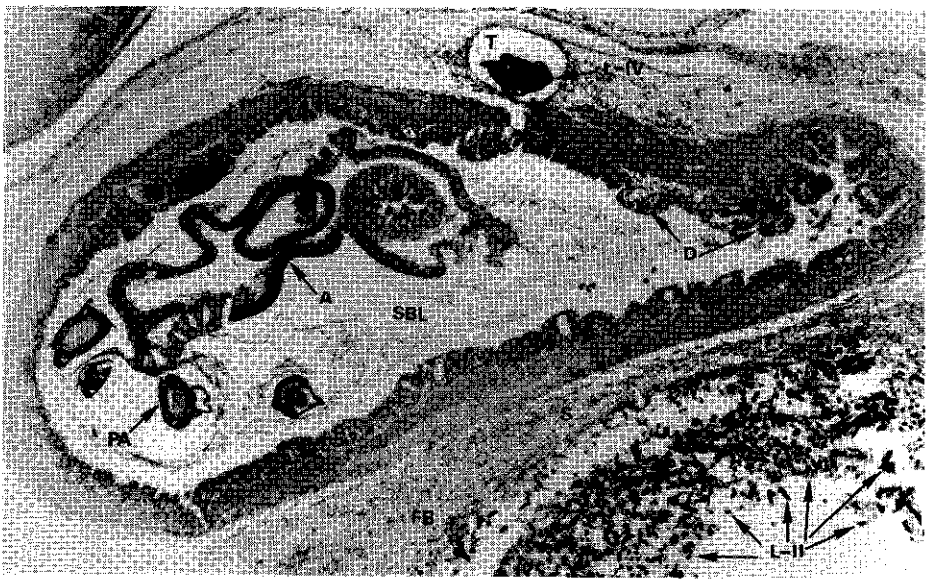


Fig. 3. Cross section of swimbladder of wild eel (14 cm in length) infected with *Anguillicola crassus*. L_{II} larvae are numerous in the fibrotic bulb (FB) of the subserosa (S) of the swimbladder. An L_{IV} larva has formed a tunnel (T) in this subserosa. Dilation of blood vessels (D) is seen in the mucosa (M) of the swimbladder wall. Pre-adult (PA) and adult (A) parasites can be found in the swimbladder lumen (SBL). H&E; 24.6x.

Table 3. *Observations of presence of **Anguillicola crassus** in squash preparations of organs of young eel sampled in October, 1987 in the IJsselmeer. Prevalence of infection: 100%. Prevalence of swimbladders with inflammatory reactions: 28%. Prevelance of eels with fibrotic swimbladders: 80%.*

Length of eel in cm	Number of L_{III} in stomach, liver, intestine		Number of L_{III}/L_{IV} in swimbladder wall		Number of worms in swimbladder lumen				Presence of L_I/L_{II} in swimbladder	Presence of inflammatory reactions	Presence of fibrotic swimbladder wall
					pre-adult		adult				
	A	N	A	N	A	N	A	N			
7.3	0	0	1	3	0	0	1	0	-	+	+
8.4	1	0	1	0	0	0	0	0	-	-	+
8.6	0	0	3	1	0	0	0	0	-	+	-
8.7	1	0	0	3	0	0	0	0	-	-	+
8.9	0	0	4	0	0	0	0	0	-	-	+
9.1	0	0	4	4	0	0	1	2	-	+	+
9.8	0	0	2	1	0	0	0	0	-	-	+
9.8	0	0	1	2	0	0	0	0	-	-	+
10.2	0	0	1	2	0	0	1	0	-	-	+
10.2	0	0	0	0	0	0	2	0	-	-	+
10.3	0	0	1	0	0	0	0	0	-	-	+
10.4	0	1	3	0	0	0	0	0	-	-	+
10.5	0	1	0	1	1	0	0	0	-	-	+
10.5	0	0	1	1	0	0	2	0	-	-	+
10.5	0	0	1	0	0	0	0	0	-	-	+
10.8	0	0	1	2	0	0	2	0	-	-	+
10.9	0	0	0	18	0	0	0	3	+	+	-
11.1	1	0	0	0	0	0	0	0	-	-	+
11.3	0	0	1	0	0	0	0	0	-	-	+
11.3	0	0	3	2	0	0	0	0	-	-	+
11.8	0	0	4	0	0	0	2	0	-	-	+
11.9	0	0	0	6	0	0	0	3	+	+	-
12.0	0	0	4	1	0	0	3	0	-	-	+
12.3	0	0	0	6	0	1	0	3	+	+	-
13.0	0	0	0	2	0	3	0	6	+	+	-

A = abnormal (dead or not well developed)
N = normal
\+ = present
\- = not present

A. crassus infection strongly favors (secondary) bacterial infections in farmed eel. Bacterial typing showed that the infections were caused by *Aeromonas hydrophila*, and, additionally, in the marine farmed eel, by *Pseudomonas* spp. Such bacteria are to be considered as general opportunistic pathogens that enter the eel through the lesions caused by *A. crassus*. These secondary bacterial infections can result in an increase of mortality up to 10-20% in eel farms (Liewes and Schaminee-Main, 1987). Farmed eel that survive the *A. crassus* infection can also show a thickened fibrotic swimbladder as observed in wild eel.

Table 4. *Histological findings of presence of **Anguillicola crassus** in sections of young eel, sampled in 1987 from the IJsselmeer and from eel farms.*

	Wild eel										Farmed eel	
											f.w.	sea
	J	O	J	O	J	O	J	O	J	O	farm	farm
(cm)	7-8	7-8	9-10	9-10	11-12	11-12	13-14	13-14	15-16	15-16	8-10	> 25
L_I present	-		-	-	+	+	+	-	-	-	+	+
L_{II} in s.b.wall/lumen	-	-	-	-	+	+	+	+	+	+	+	+
L_{II} in body cavity	-	-	-	-	-	-	+	+	+	-	-	+
L_{III} in intest. wall/ lumen	-	-	-	+	-	+	-	-	-	+	+	+
L_{III} in body cavity	-	-	-	+	-	-	-	-	-	-	+	-
L_{III} in s.b. wall/lumen	-	-	-	-	+	-	+	+	-	-	+	-
L_{IV} in s.b. wall	-	-	-	-	-	-	+	+	-	-	-	+
Pre-adult in s.b.lumen		-	-	-	+	+	+	+	+	-	-	++
Tunnels in s.b.wall	-	-	-	-	+	+	+	+	+	+	+	+
Inflammation in s.b.wall	-	-	-	-	+	+	+	+	+	+	+	+
Dilation bloodv. s.b.wall	-	-	-	-	+	+	+	+	+	+	-	+
Fibrosis of s.b.wall	-	-	-	-	+	+	+	+	+	+	+	+
Fibrosis of intestine	-	-	-	+	+	+	+	+	-	+	-	+
Fibrosis of kidney	-	-	-	+	+	+	+	+	-	+	-	+
Bacterial infection	-°	-°	-°	-°	-°	-°	-°	+	-°	-°	+	+

The results of bacteriological tests are given in the bottom row for reference.
+ = positive, - = no indication; ° = not tested; s.b. = swimbladder; f.w. = freshwater; J = June, 1987; O = October, 1987

Histological sections of farmed eel showed the following :
1) The first group consisted of eels 8-10 cm long from a freshwater farm. These had been fed 5 months previously with copepods infected with *A. crassus* larvae. Pre-adult and adult parasites were found in the swimbladder lumen. These small eels also showed L_{II} in the intestinal lumen and L_{III} were found in the fibrotic swimbladder wall and in the swimbladder lumen.
2) The second group consisted of eels 25 cm or more in length, originating from a marine farm. These eels must have already been infected for a long time, at least since their introduction from fresh water for growth in the marine farm. L_{IV} was found in the swimbladder wall. Pre-adults and adults occurred in the swimbladder lumen. An adherent, fibrotic complex of swimbladder, kidney, and intestine had formed, filling up the body cavity. L_{II} were found in this complex. In one eel, L_{II} were observed in the ventral abdominal musculature.

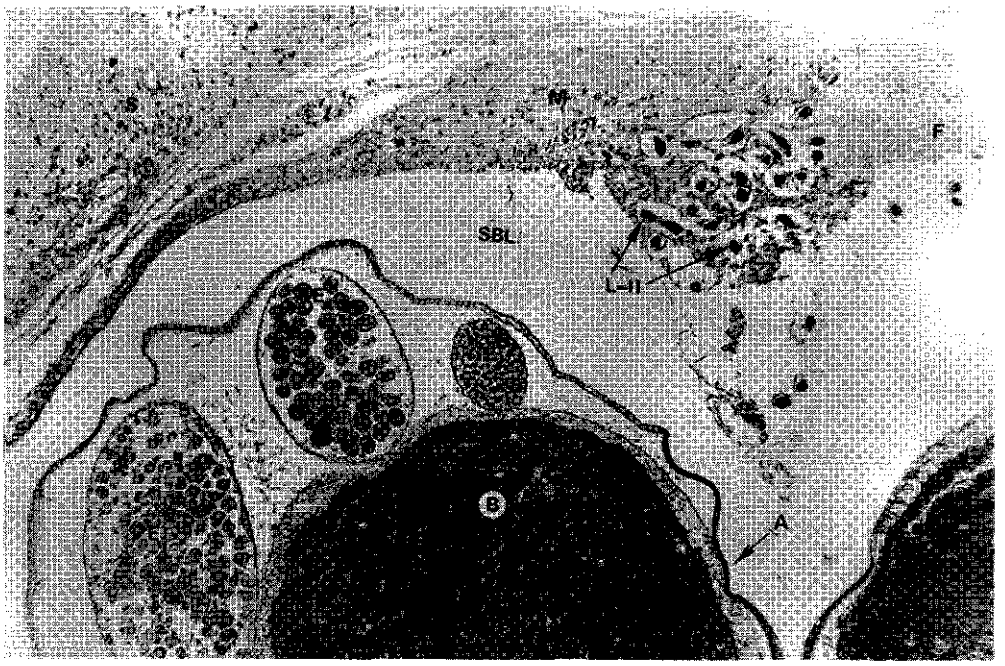


Fig. 4. Cross section of an *Anguillicola crassus* adult female (A) situated in the swimbladder lumen (SBL) of wild eel (12 cm in length). Different egg stages (E) can be seen. The parasite's gut is filled with erythrocytes of the eel (B). L_{II} larvae can be seen within the mucosa (M) of the swimbladder wall causing fibrosis (F). Fibrosis of the serosa (S) can also be observed. H&E; 61.6x.

Discussion and conclusions

As Egusa (1979) has stated, *A. crassus* causes pathological changes in the European eel. The infection route and life cycle were defined by Hirose et al. (1976), Kuwahara et al. (1974), Puqin and Yuru (1980), De Charleroy et al. (1988a,b), and Haenen et al. (1988). Our results support and enhance the findings presented in the earlier studies.

The observed length of adult worms ranges of 3-22 mm in young eel (Table 2). This differs from those observed in larger eel by Kuwahara et al. (1974) and Taraschewski et al. (1987), which measured 20.5-71.5 mm and 5.7-36.7 mm, respectively. This indicates the ability of *A. crassus* to adapt the size of the adult stage to the available space in the swimbladder.

In eel, L_{III} were found in the intestinal wall, body cavity, swimbladder wall, and swimbladder lumen, which confirms the hypothesis that there is direct migration of L_{III} from the intestinal lumen to the swimbladder. L_{IV} were found in the swimbladder wall. Pre-adults and adults were found only in the swimbladder lumen.

A severe reaction to the parasite in the swimbladder was found both in wild and farmed eel: tunnel formation, acute inflammation with dilation of blood vessels, and ultimately fibrosis of the swimbladder wall. Finally, the swimbladder adheres totally to

surrounding organs, such as kidney and intestine. In this complex, L_{II} tend to migrate and may get stuck, or lost, as in the case of incidental findings of L_{II} in the muscle. Furthermore, it seems that this phase of migration of L_{II}, with the accompanying irritation and inflammation of the tissues surrounding the swimbladder, is the most harmful phase of the parasite in the eel host. Whether eels with heavy fibrosis of the swimbladder cannot be reinfected or are less suitable for survival of the parasite needs further study.

Lesions in European eel caused by *A. crassus* appear to be much more severe than in the Japanese eel. In addition, aberrant migration routes have been found in the European eel. This may reflect the fact that European eel is not a natural host for *A. crassus*.

Secondary bacteriological infections were found in farmed eel. These were absent or very rare in wild eel, but it must be kept in mind that the dead or very diseased eels are not caught by fishing, whereas in eel farms they are. It is thus difficult to compare the losses in farmed and wild eels that are primarily due to *A. crassus*. Despite this, it will be interesting to monitor the pathological effects of the parasite in wild and farmed eels over the coming years. Furthermore, the prediction of a prevalence stabilization of the infection as a result of an increasing number of eels with thickened swimbladders in eel populations should be checked.

A. crassus is causing severe pathological effects in both wild and farmed European eel, *Anguilla anguilla*, in the Netherlands. In farmed eel, risk factors may facilitate a secondary bacterial sepsis of the *A. crassus*-infected eel. The general changes are acute inflammation followed by fibrosis of the swimbladder, which ultimately become incorporated into surrounding tissues and become nonfunctional. A survey of the wild stocks over the coming years is needed to determine the long-term effect of the introduced *A. crassus* on the recruitment and condition of young European eel.

Literature cited

Bergey, D.H. (1984). *"Bergey's Manual of Systematic Bacteriology"*, Vol. 1 (N.R. Krieg and J.G. Holt, eds.). Williams and Wilkins, Baltimore.

Cowan, S.T. (1977). *"Manual for the Identification of Medical Bacteria"*. Cambridge University Press, Cambridge, England.

De Charleroy, D., Thomas, K. & Belpaire, C. (1988a). Notes on the biology and diagnostical methods of *Anguillicola crassa*, a swimbladder nematode in the European eel (*Anguilla anguilla* L.). *Rept. Zool. Instit. Univ. Leuven*, Belgium.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. & F. Ollevier (1988b). The life cycle of *Anguillicola crassa* (Kuwahara, Niimi and Itagaki). Proc. F.H.S./A.F.S. Intl. Fish Health Conference, Vancouver (Abstract).

Dekker, W. & van Willigen, J. (1987). Short note on the distribution and abundance of *Anguillicola* in the Netherlands. Euro. Inland Fisher. Adv. Comm. (FAO), *Report on Eel Working Group*, Bristol, April, 1987.

Egusa, S. (1979). Notes on the culture of the European eel (*Anguilla anguilla*) in Japanese eel-farming ponds. In: "Eel Research and Management" (F. Thurow, ed.). *Rap. Proc. Verb. Reun. Cons. Int. Exp. Mer*, **174**, 51-58.

Haenen, O.L.M. , Grisez, L., De Charleroy, D., Belpaire, C. & Ollevier, F. (1988). Artificial infection of the European eel, *Anguilla anguilla* L., with L_{III} larvae of *Anguillicola crassus* (Nematoda, Dracunculoidea). Proc. F.H.S./A.F.S. Intl. Fish Health Conference, Vancouver.*

Hirose, H., Sekino, T. & Egusa, S.(1976). Notes on the egg deposition, larval migration and intermediate host of the nematode *Anguillicola crassa* parasitic in the swimbladder of eels. *Fish Pathol.*, **11**, 27-31 (In Japanese; English summary).

Køie, M. (1987). Eel parasites in natural waters and aquaculture in Denmark. In: "Parasites and diseases in natural waters and aquaculture in Nordic Countries" (A. Stenmark and G. Malmberg, eds.). Zoo-tax, Naturhistoriska Riksmuseet, Stockholm, Sweden.

Kuwahara, A., Niimi, A., & Itagaki, H. (1974). Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Philometridae, Anguillicolidae). *Japan. J. Parasitol.*, **23**, 275-279.

Liewes, E.W. & Schaminee-Main (1987). Onderzoek naar de effecten van de parasiet *Anguillicola crassa* op de ontwikkeling van de paling (*Anguilla anguilla*) in een zoutwater palingmesterij. Intern Report Texvis BV, Den Burg, Texel, The Netherlands.

Moravec, F. & Taraschewski, H. (1988). Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda: Anguillicolidae) of the swimbladder of eels, including descriptions of two new species, *A. novaezelandiae* sp.n. and *A. papernai* sp.n. *Folia Parasitol.***

Neumann, W. (1985). Schwimmblasenparasit *Anguillicola* bei Aalen. *Fischer und Teichwirt* **11**, 322.

Peters, G. & Hartmann, F. (1986). *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. *Dis. Aquat. Org.* **1**, 229-230.

Puqin, W. & Yuru, Z. (1980). Observations on the life history of *Anguillicola globiceps* (Nematoda: Anguillicolidae). *Acta Zool. Sin.*, **26**, 243-249.

Taraschewski, H., Moravec, F., Lamah, T. & Anders, K. (1987). Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and *Paratenuisentis ambiguus* (Acanthocephala, Tenuisentidae). *Dis. Aquat. Organ.*, **3**, 167-176.

Van Banning, P., Heermans, W. & Van Willigen J.A. (1985). *Anguillicola crassa*, een nieuwe aalparasiet in Nederlandse wateren. *Visserij*, **38**, 237-240.

* Published: J. Aquatic Animal Health 1991, 3:263-265.

** Published: 1988, 35:125-146.

Chapter 3

Detection of larvae of *Anguillicola crassus* (an eel swimbladder nematode) in freshwater fish species

O.L.M. Haenen[a] *and P. van Banning*[b]

[a] DLO-Central Veterinary Institute, P.O.B. 65, 8200 AB Lelystad, The Netherlands
[b] DLO-Netherlands Institute for Fishery Investigations, P.O.B. 68, 1970 AB IJmuiden, The Netherlands

Haenen, O.L.M. and Van Banning, P., 1990. Detection of larvae of *Anguillicola crassus* (an eel swimbladder nematode) in freshwater fish species. *Aquaculture*, 87: 103-109.

Abstract

The question of whether small freshwater fish are infected by *Anguillicola crassus*, a parasitic swimbladder nematode normally found in eels, was investigated. Various small freshwater fish that are preyed upon by eels were examined for larvae of *A.crassus*. Freshwater smelt *(Osmerus eperlanus)*, ruffe *(Gymnocephalus cernuus)*, roach *(Rutilus rutilus)*, bream *(Abramis brama)*, perch *(Perca fluviatilis)*, zander *(Stizostedion lucioperca)*, and three-spined stickleback *(Gasterosteus aculeatus)* were caught from a freshwater lake in The Netherlands. Examination revealed that, except for roach and bream, all the fish were infected with these nematodes. We suggest that in addition to nematode-infected crustaceans, which are the known route of infection for eels, these small fish may constitute another route of infection.

Introduction

Since 1985, the nonindigenous parasitic nematode *Anguillicola crassus* (Kuwahara et al., 1974; Moravec and Taraschewski, 1988) has been found in the European eel *Anguilla anguilla* L. that inhabits the inland waters of The Netherlands. The nematode originated from eastern Asia where it infects the Japanese eel *Anguilla japonica* (Egusa, 1979), but has spread rapidly through western Europe (Peters and Hartmann, 1986; Dekker and Van Willigen, 1987; Taraschewski et al., 1987; Køie, 1987, 1988; Belpaire et al., 1989). By 1986 *A. crassus* had infected up to 94% of the eels in the inland waters of The Netherlands (Dekker and Van Willigen, 1987).

The pathological effects of *A. crassus* on European eels were described by Egusa (1979). Van Banning and Haenen (1989) studies these effects in detail in wild eels and detected acute inflammatory reactions and fibrosis of the swimbladder.

In studying the life cycle of *Anguillicola* spp. and *Anguillicola globiceps*, respectively, Egusa (1979) and Puqin and Yuru (1980) noted that planktonic copepods act as intermediate hosts for second- and third-stage nematode larvae. The ingestion of the second-stage nematode larvae by crustaceans was studied in detail by Hirose et al. (1976), De Charleroy et al. (1988) and Haenen et al. (1988, 1989). De Charleroy et al. (1988) also found third-stage (L-III) *A. crassus* larvae in the body cavities of fish such as carp and ide. They suggested that these fish may act as an intermediate host for the nematode. Tesch (1983) and De Nie (1987) have reported that European eels of 34 cm in length or more prey on young fish such as smelt, roach, perch, three-spined stickleback, and elvers.

In the present study, we examined these species together with young bream, ruffe, and zander for the presence of *A. crassus* larvae.

Although larvae were not found in roach and bream, larvae of various stages were detected in the swimbladder of freshwater smelt, ruffe, perch, zander, and three-spined stickleback.

Materials and methods

Young freshwater smelt (*Osmerus eperlanus*), ruffe (*Gymnocephalus cernuus*), roach (*Rutilus rutilus*), bream (*Abramis brama*), perch (*Perca fluviatilis*), zander (*Stizostedion lucioperca*), and three-spined stickleback (*Gasterosteus aculeatus*) were caught in the IJsselmeer, a freshwater lake in The Netherlands, by means of a fine-mesh beam trawl. We selected the smallest fish, that is, those most likely to be preyed upon by large eels. Fish were immediately transported to the laboratory and examined whitin 24 h for nematodes. The fish were measured and dissected, and the intestinal tract and swimbladder were removed. A light microscope was used to examine fresh preparations of the intestinal tract and swimbladder. Nematodes were counted and measured. Larvae that had been collected from eels were similarly counted and measured, and data from both examinations were compared. The gross pathological effects of *A. crassus* on the swimbladders were noted.

Results

In June 1988 a small group of smelt was examined in a pilot study. Third-stage larvae (L-III) of *Anguillicola crassus* were found in the swimbladders of all fish examined (Table 1) (Fig. 1). Some larvae were dead and one was encapsulated. None of the intestinal tracts or body cavities of the fish contained nematodes. Larvae from various fish species were identified as *A. crassus* by comparing their morphological features with those of *A. crassus* larvae found in eels (Table 2). The infection rate was 100%.

In October 1988 numerous smelt, ruffe, roach, bream, perch and zander were screened for nematodes (Table 3). The swimbladders of smelt, ruffe, perch, and zander

Table 1. *Number of **A. crassus** larvae (L-III) recovered from freshwater smelt (**Osmerus eperlanus**) caught in the IJsselmeer, June 1988*

Fish	Length (cm)	No. of larvae in swimbladder		Total no. of larvae
		Dead	Live	
1	11.5	6	10	16
2	11.8	0	1	1
3	9.0	0	1	1
4	10.5	1 (encapsulated)	2	3
5	10.0	0	1	1
Mean ± s.d. (n=5)	10.3 ± 3.20	1.4 ± 2.60	3.0 ± 3.93	4.4 ± 6.50

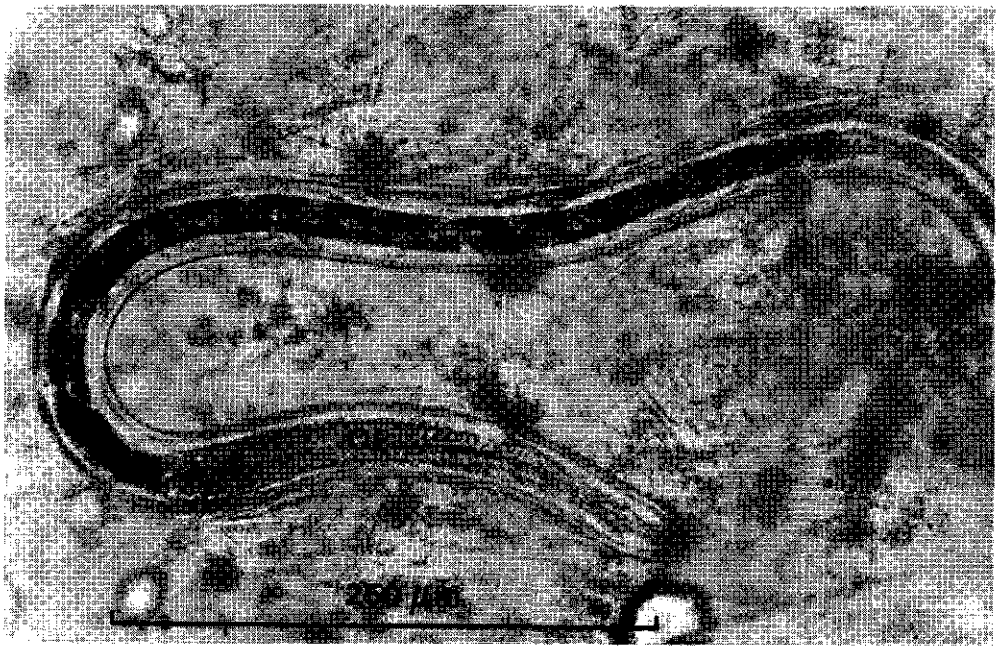


Fig. 1. Live third-stage larva of *Anguillicola crassus* in the swimbladder of a freshwater smelt caught in the IJsselmeer in June 1988: OE=oesophagus, IT=intestinal tract.

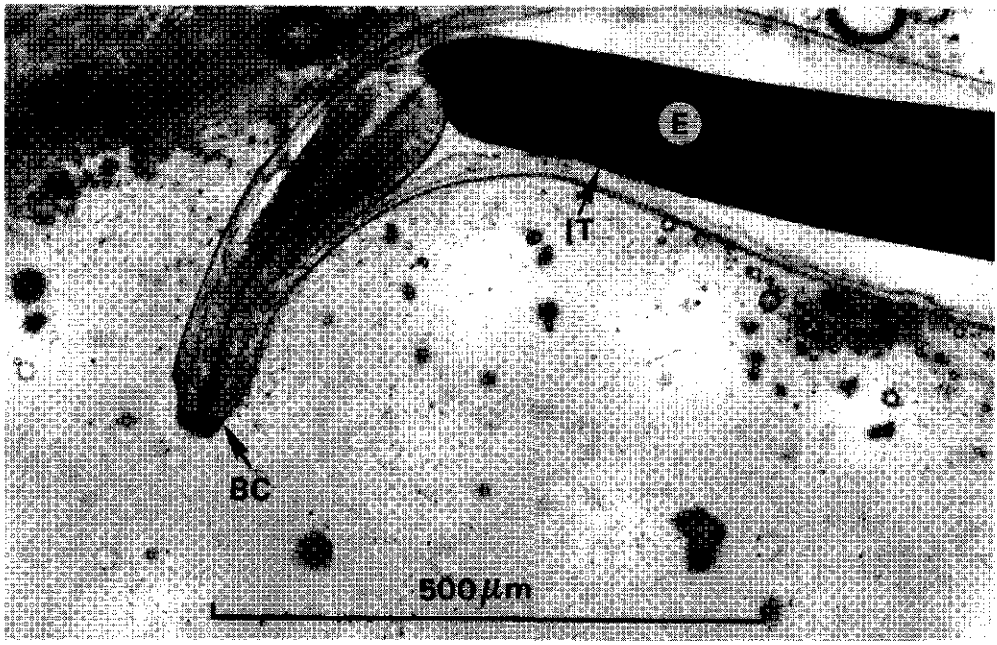


Fig. 2. Head of live pre-adult *Aguillicola crassus* in the swimbladder of perch, caught in the IJsselmeer in October 1988: BC=buccal cavity; OE=oesophagus, IT=intestinal tract filled with erythrocytes (E) of the host.

Table 2. *Comparison of nematode larvae detected in the swimbladders of various freshwater fish with the larvae of **A. crassus** in eels*

Species	No. of Larvae	Length range (mm) of *A. crassus*	
		Larvae (mean ± s.d.)	Oesophagus[a] (mean ± s.d.)
Smelt, ruffe, perch, zander	15	0.6-0.8 (0.68 ± 0.04)	0.19-0.22 (0.21 ± 0.01)
Eels	20	0.6-0.8 (0.69 ± 0.06)	0.19-0.22 (0.21 ± 0.01)

[a] All larvae had the typical strong muscular oesophagus with broad base.

Table 3. *Characteristics of **A. crassus** detected in the swimbladders of various freshwater fish caught in the IJsselmeer, October 1988*

Fish species	Length in cm (mean ± s.d.)	% Fish infected	No. of *A. crassus* larvae detected in fish swimbladders				
			L-III[a] larvae (mean ± s.d.)	Encapsulated L-III larvae	Unencapsulated L-III larvae	L-IV[b] larvae	Pre-adult larvae
Smelt (*n*=49)	7.7 ± 1.39	85.7	1-10 (3.4 ± 3.96)	1-2 (9 x)[c]	0	2 (2 x)	0
Ruffe (*n*=45)	7.7 ± 1.23	80.0	1-12 (2.5 ± 2.68)	1 (2 x)	1 (3 x)	0	2 (1 x)
Roach (*n*=45)	6.5 ± 0.64	0	0	0	0	0	0
Bream (*n*=45)	7.4 ± 1.44	0	0	0	0	0	0
Perch (*n*=45)	6.8 ± 1.12	82.2	1-7 (1.2 ± 6.61)	0	0	0	1 (1 x)
Zander (*n*=45)	11.4 ± 3.59	36.0	1-2 (0.4 ± 0.57)	0	0	0	0

[a] L-III= third stage.
[b] L-IV= fourth stage.
[c] x = no. of times detected.

Table 4. *Characteristics of A. crassus detected in the swimbladders of various freshwater fish caught in the IJsselmeer, November 1988*

Fish species	Length in cm (mean ± s.d.)	% Fish infected (no. checked)	No. of *A. crassus* larvae detected in fish swimbladders			No. of thickened swimbladders
			L-III[b] larvae (mean ± s.d.)	L-IV[c] larvae	Pre-adult larvae	
Smelt (*n*=53)[a]	6.6 ± 0.92	60 (*n*=40)	1- 4 (1.0 ± 1.11)	0	0	0
Smelt (*n*=50)	8.2 ± 1.28	80 (*n*=10)	1- 6 (2.5 ± 2.06)	0	0	0
Ruffe (*n*=50)	6.5 ± 0.79	100 (*n*=10)	1-38 (6.9 ±12.09)	13 (1 ×)[d]	0	0
Perch (*n*=50)	7.5 ± 0.71	70 (*n*=10)	1-23 (3.5 ± 7.18)	1-6 (5 ×)	0	0
Zander (*n*=33)	14.0 ± 1.61	60 (*n*=10)	1-2 (0.5 ± 0.70)	0	0	0
Three-spined stickleback (*n*=50)	5.0 ± 0.28	68 (*n*=50)	1- 4 (1.0 ± 1.19)	1-2 (7 ×)	1 (1 ×)	1

[a] Presample, caught 1 week before other groups.
[b] L-III = third stage.
[c] L-IV = fourth stage.
[d] × = no. of times detected.

contained numerous L-III larvae. Dead L-III larvae (encapsulated or unencapsulated) were detected in the swimbladders of smelt and ruffe only. Fourth-stage larvae (L-IV) were detected in swimbladders of the smelt only. Some pre-adult nematodes were detected in the swimbladders of ruffe and perch (Fig. 2). None of the intestinal tracts of the fish contained nematodes. Roach and bream contained no nematodes. The infection level of the infected groups varied from 36 to 85%. Larvae were identified as described above.

In November 1988 smelt, ruffe, perch, zander, and three-spined stickleback were collected and their simbladders were examined (Table 4). The infection level of these fish was 60% or more. The swimbladders of all fish contained third-stage larvae of *A. crassus*; those of ruffe, perch, and three-spined sticklebacks contained fourth-stage larvae as well as a few pre-adult *A. crassus*. Nematodes were identified as described above. The only pathological effect of the infection was a thickened swimbladder found in a three-spined stickleback, with three L-III larvae. Smelt, perch, and ruffe had the most *A. crassus* larvae per swimbladder (cf. Tables 3 and 4).

Discussion

Most of the swimbladders of fish examined in this study were infected with third-stage larvae of *A. crassus*, which until now was thought to infect swimbladders of eels but not those of other fish species. In an earlier study on eels, we demonstrated that L-III larvae of *A. crassus* migrate to the swimbladder, and remain there alive for some time before passing into the fourth and pre-adult stages, when they begin to feed on the blood (Haenen et al., 1989). In the present study, fourth-stage larvae were detected in the swimbladders of smelt, ruffe, perch, and three-spined stickleback; pre-adult nematodes were found only in ruffe, perch, and threespined sticklebacks. Nematodes in the fourth and pre-adult stage were observed feeding on blood and were filled with erythrocytes of the host fish (Fig. 2).

Because *A. crassus* is infective during its third larval stage (Egusa, 1979; Puqin and Yuru, 1980; De Charleroy et al., 1988; Haenen et al., 1989), small prey fish containing these larvae may, when eaten, transfer the infection to eels. Our results show that the larvae are nonspecific in choosing their hosts, and suggest further that larvae may be transferred from one host to another when eaten. The results of Scott (1954) and Smith (1974) give support to this possibility because they reported that other nematode species that infect fish are transferred in this way.

Although in an earlier study (Van Banning and Haenen, 1989) we found that *A. crassus* caused pathological effects in eels, in the present study we detected no such effects in the fish species examined. Only one three-spined stickleback had a thickened swimbladder.

Remarkably, roach and bream contained no nematodes whatsoever. This held true even though third-stage larvae of *A. crassus* appear to be nonspecific in their host preference, and even though roach and bream prey on small crustaceans, which can be infected with the larvae.

In conclusion, small freshwater fish can be infected with the larvae of *A. crassus*. These findings lead us to believe that these fish, when eaten, may transfer *A. crassus* infections to eels.

References

Belpaire, C., De Charleroy, D., Thomas, K. and Van Damme, P., 1989. Effects of restocking eels on the distribution of the nematode *Anguillicola crassus* in Flanders, Belgium. *J. Appl. Ichthyol.*, 5(3):151-154.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. and Ollevier, F., 1988. The life cycle of *Anguillicola crassus* (Kuwahara, Niimi and Itagaki). Proc. F.H.S./A.F.S. International Fish Health Conference, Vancouver, *J. Aquatic Anim. Health*, in press.*

Dekker, W. and Van Willigen, J., 1987. Short note on the distribution and abundance of *Anguillicola* in the Netherlands. EIFAC Report Eel Working Group, Bristol, April 1987 (mimeogr. rep.), 9 pp.

De Nie, H.W., 1987. Food, feeding periodicity and consumption of the eel *Anguilla anguilla* (L.) in the shallow eutrophic Tjeukemeer (The Netherlands). *Arch. Hydrobiol.*, 209(3): 421-443.

Egusa, S., 1969. Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. In: F. Thurow (Editor), Eel Research and Management. *Rap. Proc. Verb. Reun. Cons. Int. Exp. Mer*, **174**: 51-58.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, D. and Ollevier, F., 1988. Artificial infection of the European eel, *Anguilla anguilla* L., with L-III larvae of *Anguillicola crassus* (Nematoda, Dracunculoidea). Proc. F.H.S./A.F.S. International Fish Health Conference, Vancouver. *J. Aquatic Anim. Health*, in press.**

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C. and Ollevier, F., 1989. Experimentally induced infections of the European eel, *Anguilla anguilla* L., with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of the larvea. *Dis. Aquat. Org.*, **7**: 97-101.

Hirose, H., Sekino, T. and Egusa, S., 1976. Notes in the egg deposition, larval migration and intermediate host of the nematode *Anguillicola crassus* parasitic in the swimbladders of eels. *Fish Pathol.*, **11**: 27-31 (in Japanese with English summary).

Køie, M., 1987. Eel parasites in natural waters and aqua culture in Denmark. In: A. Stenmarck and G. Malmberg (Editors), Parasites and Diseases in Natural Waters and Aquaculture in Nordic Countries. Zoo-tax, Naturhistoriska Riksmuseet, Stockholm, Sweden, pp. 38-45.

Køie, M., 1988. Parasites in eels, *Anguilla anguilla* (L.) from eutrophic Lake Esrum, Denmark. *Acta Parasitol. Pol.*, **33**: 89-100.

Kuwahara, A., Niimi, A. and Itagaki, H., 1974. Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Phimetridae, Anguillicolidae). *Jpn. J. Parasitol.*, **23**(5): 275-279.

Moravec, F. and Taraschewski, H., 1988. Revision of the genus *Anguillicola* Yamaguti 1935 (Nematoda: Anguillicolidae) of the swimbladder of eels, including descriptions of two new species, *A. novaezelandiae* sp.n. and *A. papernae* sp.n. *Folia Parasitol.*, **35**: 125-146.

Peters, G. and Hartmann. F., 1986. *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. *Dis. Aquat. Org.*, **1**: 229-230.

Puqin, W. and Yuru, Z., 1980. Observations on the life history of *Anguillicola globiceps* (Nematoda, Anguillicolidae). *Acta Zool. Sin.*, **26**: 243-249.

Scott, D.M., 1954. Experimental infection of Atlantic cod with a larval marine nematode from smelt. *J. Fish. Res. Board Can.*, **11**: 894-900.

Smith, J.W., 1974. Experimental transfer of *Anisakis* sp. larvae (Nematoda: Ascaridida) from one fish host to another. *J. Helminthol.*, **48**: 229-234.

Taraschewski, H., Moravec. F., Lamah, T. and Anders, K., 1987. Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and *Paratenuisentis ambiguus* (Acanthocephala, Tenuisentidae). *Dis. Aquat. Org.*, **3**: 167-176.

Tesch, F.W., 1983. Der Aal. Biologie und Fischerei. Paul Parey, Hamburg, 340 pp.

Van Banning, P. and Haenen, O.L.M., 1989. Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. In: F.O. Perkins (Editor), PAMAQ III Symposium 1988, Gloucester Point, U.S.A. *Academic Press*, in press.***

* Published in 1990: Dis. Aquat. Org. 8: 77-84.

** Published in 1991, Vol. 3: 263-265.

*** Published in Pathology in Marine Science, 1990, (see chapter 2 of this thesis).

Chapter 4

Experimental transmission of *Anguillicola crassus* (Nematoda, Dracunculoidea) larvae from infected prey fish to the eel *Anguilla anguilla*

O.L.M. Haenen[a] *and P. van Banning*[b]

[a] DLO-Central Veterinary Institute, P.O.B. 65, 8200 AB Lelystad, The Netherlands
[b] DLO-Netherlands Institute for Fishery Research, P.O.B. 68, 1970 AB IJmuiden, The Netherlands

Haenen, O.L.M. and Van Banning, P., 1991. Experimental transmission of *Anguillicola crassus* (Nematoda, Dracunculoidea) larvae from infected prey fish to the eel *Anguilla anguilla*. *Aquaculture*, **92**: 115-119.

Abstract

Infected swimbladders with presumptive larvae of the nematode *Anguillicola crassus* were removed from freshwater smelt (*Osmerus eperlanus* L.) and ruffe (*Gymnocephalus cernuus* L.) and were fed to European eels *Anguilla anguilla* L. to test whether the larvae can infect eels via this route. The nematode larvae migrated to the swimbladder of eels and developed into adult *A. crassus*. It was concluded that smelt and ruffe can transmit *A. crassus* to European eels.

Introduction

Anguillicola crassus, a parasitic nematode that infects the swimbladder of eels, has recently been introduced into Europe where it has rapidly infected the European eel, *Anguilla anguilla* (Peters and Hartmann, 1986; Dekker and Van Willigen, 1987; Køie, 1987; Taraschewski et al., 1987). The nematode can be pathogenic for eels, especially in aqua- culture (Van Banning and Haenen, 1990), where it can cause ruptures, secondary inflammation, and fibrosis of the swimbladder. Several studies have been done on the infection cycle of the nematode in the European eel (Hirose et al., 1976; Egusa, 1979; Haenen et al., 1989; De Charleroy et al., 1990). De Charleroy et al. (1990) were able to infect carp and ide with *A. crassus* by feeding them infected copepods. They also suggested that other fish might act as reservoir hosts for the nematode.

In a recent study, Haenen and Van Banning (1990) showed the presence of presumptive *A. crassus* larvae in the swimbladders of several species of wild freshwater fish that are preyed upon by eels (De Nie, 1987). The larvae were presumed to be *A. crassus* on the basis of morphological features.

To determine if European eels can become infected with *A. crassus* by eating infected fish, the eels were fed swimbladders infected with *A. crassus* larvae collected from fresh- water smelt (*Osmerus eperlanus)* and ruffe (*Gymnocephalus cernuus*). Because both eel groups developed *A. crassus* infections in their swimbladders, it was concluded that smelt and ruffe, and possibly other prey fish, can transmit *A. crassus* to European eels.

Materials and methods

The 90 European eels used in the experiment were each 36 cm long and originated from a Dutch eel farm that was considered to be free of *Anguillicola crassus*. After the eels arrived at the laboratory, 30 eels were anaesthetized (2-phenoxyethanol 1 mg/l) and killed, and their swimbladders were examined to confirm that they contained no *A. crassus*. Before and during the experiment, the eels were kept in 30-litre flow-through aquaria at 18-24°C. The eels were fed pelleted food. Eels were allotted to three groups: a control group (9 eels), a group (25 eels) that was fed infected smelt swimbladders (smelt-fed group) and a group (25 eels) that was fed infected ruffe swimbladders (ruffe-fed group).

Forty smelt (length = 7.7 ± 0.5 cm) and 40 ruffe (length = 8.6 ±1.6 cm) were caught by means of a fine-mesh beam trawl in June 1989 in the IJsselmeer, a freshwater lake in The Netherlands. The fish were immediately transported to the laboratory in an aerated tank at 4°C. The smelt died immediately after being caught. Within 24 h of being caught, the ruffe were killed, and ruffe and smelt were measured, and dissected. Their swimbladders were removed and fresh tissue preparations were examined by light microscopy for parasites.

The swimbladders of the ruffe and smelt contained only one type of nematode larva. The larvae were morphologically similar to those found in our earlier study, and their oesophagi were characteristically muscular and broad at the posterior end (Haenen and Van Banning, 1990). Therefore, the larvae were considered to be *A. crassus*. The nematodes were counted while still in the swimbladders, and the infected swimbladders were cut into fragments with scissors. The fragments were immersed in buffered saline (pH 7.2).

The eels were anaesthetized with 2-phenoxyethanol (0.5 mg/l) for approximately 15 min. A blunt syringe (bovine milk catheter) was used to administer orally approximately 1-2 ml of buffered saline to the control eels and approximately 1-2 ml of the swimbladder- nematode mixture in buffered saline to the other two groups. The smelt-fed group received a total of 51 third-stage larvae of *A. crassus* and the ruffe-fed group received a total of 78 third-stage larvae of *A. crassus*. After 8 weeks, the swimbladders of 4 eels from the control group, 11 eels from the smelt-fed group and 14 eels from the ruffe-fed group were examined for nematodes. The eels were anaesthetized in 2-phenoxyethanol (1 mg/l) for 15 min, and then the swimbladders were removed. A light microscope was used to examine fresh swimbladder preparations (squash) for nematodes. Fourteen weeks after the inoculation, the remaining eels were killed and examined in the same way.

Results

Of the 156 smelts and 46 ruffe examined, 23.7% and 55.3% were infected with *Anguillicola crassus* larvae, respectively. After the larvae-infected swimbladders of these fish were fed to the eels, 8% of the smelt-fed group and 36% of the ruffe-fed group became infected (Table 1). In the infected eel groups, first- and second-stage larvae, pre-adults, and adult nematodes were detected (Table 2). None of the control eels became infected. Pre-adult and adult nematodes were removed from the swimbladders of the infected eels, and their body lengths and widths were measured (Table 3). The morphological features of the nematodes were characteristic of *A. crassus* (Kuwahara et al., 1974; Taraschewski et al., 1987). Eight weeks after the infection, the swimbladders of eels from the smelt-fed group contained no *A. crassus*. In contrast, nearly half of the eels from the ruffe-fed group contained first- and second-stage larvae, pre-adults, and adults of *A. crassus*. Fourteen weeks after the inoculation, when the remaining eels were examined, both groups of eels contained adult nematodes, but only the smelt-fed group had first- and second-stage larvae at this point. Third- and fourth-stage larvae were not detected in any of the eels.

Table 1. *Percentage of eels infected with **Anguillicola crassus** after experimentally induced infection. Eels were randomly selected*

Eel groups[a]	% Infected eels (no. tested)		
	At 8 weeks	At 14 weeks	Mean total
Group S[b]	0 (11)	14.3 (14)	8.0 (25)
Group R[c]	42.9 (14)	27.3 (11)	36.0 (25)

[a] Control group (9 eels) did not become infected.
[b] Eels were inoculated orally with infected smelt swimbladders.
[c] Eels were inoculated orally with infected ruffe swimbladders.

Table 2. *Presence of several stages of **Anguillicola crassus** in swimbladders of eels after experimentally induced infection*

Eel groups	No. pre-adult and adult worms recovered (no. eels)			L-I or L-II[a] larvae observed at	
	At 8 weeks	At 14 weeks	Total	8 weeks	14 weeks
Group S[b]	0 (11)	3 (14)	3 (25)	-	+
Group R[c]	11 (14)	3 (11)[d]	14 (25)	+	-[e]

[a] L-I = first-stage larvae: L-II = second-stage larvae.
[b] See footnote[b]. Table 1.
[c] See footnote[c]. Table 1.
[d] Only female parasites.
[e] Only unfertilized worm eggs were observed.

Discussion

After the infection the only nematodes found in the swimbladders of the eels were *Anguillicola crassus*. Because the smelt died immediately after being caught, third-stage larvae in the dead smelt were less active than those in the ruffe, which were still alive at the beginning of the experiment. Moreover, the ruffe-fed eels ingested 50% more larvae than the smelt-fed eels. Consequently, these two differences may have contributed to the much higher rate of infection for the ruffe-fed eels (36%) than for the smelt-fed eels (8%).

It was concluded that infected ruffe, smelt, and possibly other prey fish can transmit *A. crassus* to eels.

Table 3. *Morphological characteristics of* ***Anguillicola crassus*** *specimens recovered from swimbladders of eels 8-14 weeks after experimentally induced infection*

Eel group	Sex	Stage of maturation	Length (mm)	Max. width (mm)	Buccal capsule *lxw* (mm)	Length of oesophagus (mm)	No. of caudal papillae in male
Smelt-fed	male	adult	10.4	1.4	0.020x0.040	0.62	6
	female	adult	36.5	3.0	0.020x0.050	0.80	
	female	adult	38.5	3.2	0.025x0.050	0.68	
Ruffe-fed	male	pre-adult	6.8	0.47	0.025x0.040	0.64	6
	male	adult	8.6	0.84	0.025x0.040	0.62	6
	female	pre-adult	12.1	1.1	0.025x0.050	0.66	
	female	adult	17.1	2.1	0.025x0.050	0.62	
	female	adult	36.2	3.0	0.025x0.060	0.80	

References

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. and Ollevier, F., 1990. The life cycle of *Anguillicola crassus. Dis. Aquat. Org.* **8**: 77-84.

Dekker, W. and Van Willigen, J., 1987. Short note on the distribution and abundance of *Anguillicola* in The Netherlands. European Inland Fishery Advisory Commission (FAO), *Report Eel Working Group*, Bristol, April 1987 (mimeogr. rep.), 9 pp.

De Nie, H.W., 1987. Food, feeding periodicity and consumption of the eel *Anguilla anguilla* (L.) in the shallow eutrophic Tjeukemeer (The Netherlands). *Arch. Hydrobiol.*, **109** (3): 421-443.

Egusa, S., 1979. Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. In: F. Thurow (Editor), Eel Research and Management. *Rap. Proc. Verb. Reun. Cons. Int. Exp. Mer*, **174**: 51-58.

Haenen, O.L.M. and Van Banning, P., 1990. Detection of larvae of *Anguillicola crassus* (an eel swimbladder nematode) in freshwater fish species. *Aquaculture*, **87**: 103-109.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C. and Ollevier, F., 1989. Experimentally induced infections of the European eel, *Anguilla anguilla* L., with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of the larvae. *Dis. Aquat. Org.*, **7**: 97-101.

Hirose, H., Sekino, T. and Egusa, S., 1976. Notes on the egg deposition, larval migration and intermediate host of the nematode *Anguillicola crassus* parasitic in the swimbladders of eels. *Fish Pathol.*, **11**: 27-31 (in Japanese with English summary).

Køie, M., 1987. Eel parasites in natural waters and aquaculture in Denmark, In: A. Stenmark and G. Malmberg (Editors), Eels Parasites and Diseases in Natural Waters and Aquaculture in Nordic Countries. *Zoo-tax, Naturhistoriska Riksmuseet*, Stockholm, Sweden pp. 38-45.

Kuwahara, A., Niimi, A. and Itagaki. H., 1974. Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Phimetridae, Anguillicoli- dae). *Jpn. J. Parasitol.*, **23** (5): 275-279.

Peters, G. and Hartmann, F., 1986. *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. *Dis. Aquat. Org.*, **1**: 229-230.

Taraschewski, H., Moravec, F., Lamah, T. and Anders, K., 1987. Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and *Paratenuisentis ambiguus* (Acanthocephala, Tenuisentidae). *Dis. Aquat. Org.*, **3**: 167-176.

Van Banning, P., and Haenen, O.L.M., 1990. Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In: F.O. Perkins and T.C. Cheng (Editors), Pathology in Marine Science: Proc. 3rd Int. Coll. on Pathology in Marine Aquaculture, Gloucester Point, USA, Oct. 1988. *Academic Press*, New York, NY, pp. 317-330.

Chapter 5

Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae

O.L.M. Haenen[1], L. Grisez[2], D. De Charleroy[2], C. Belpaire[2], F. Ollevier[2]

[1] DLO-Central Veterinary Institute, PO Box 65, 8200 AB Lelystad, The Netherlands
[2] Zoological Institute, Naamsestraat 59, Leuven 3000, Belgium

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C., and Ollevier., F., 1989. Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Diseases of Aquatic Organisms*, **7**: 97-101.

Abstract

Migration pattern of third-stage *Anguillicola crassus* larvae, and pathogenesis of the lesions induced by third-stage larvae, was investigated in European eel *Anguilla anguilla* L. Young elvers (1 g) were fed infected *Paracyclops fimbriatus* (Copepoda). Eel samples were collected and examined histologically at varying intervals during the 6 mo post-infection period. Third-stage larvae (L-III) migrated directly through the intestinal wall and body cavity to the swimbladder within 17 h post-infection. L-IV larvae were detected 3 mo post-infection, and immature adults were detected within 4 mo post-infection. The parasites occasionally showed aberrant migration paths. Pathological effects caused by the parasite were less severe after experimentally induced infections than those detected in some natural infections.

Introduction

The parasitic nematode *Anguillicola crassus* (Kuwahara et al. 1974, Moravec & Taraschewski 1988) originates from eastern Asia where it infects the Japanese eel *Anguilla japonica*, but does not cause serious pathological changes (Egusa 1979). In contrast to Japanese eels, European eels *Anguilla anguilla* L. develop pathological effects from *A. crassus* infections (Egusa 1979, Liewes & Schaminee-Main 1987, van Banning & Haenen 1989).

The life cycle of *Anguillicola* spp. in Japanese eels has been described by Egusa (1979) and Puqin & Yuru (1980). The adult resides in the swimbladder lumen of the eel. After the female has copulated, the fertilized eggs are released through the vulva and, according to Egusa (1979), also by rupture of the female parasite. First-stage larvae (L-I) moult into second-stage larvae (L-II) while still within the egg. The eggs pass via the pneumatic duct through the digestive tract and out of the eel into the water. After hatching, the L-II larvae are eaten by copepods which serve as intermediate hosts. Inside the copepod the L-II larvae migrate to the haemocoel and moult into L-III larvae in 10 d. When these copepods are eaten by eels the L-III larvae migrate through the wall of the digestive tract to the swimbladder wall, where, according to Puqin & Yuru they moult into L-IV larvae 4 to 5 mo later. Immature adult and adult nematodes reside in the swimbladder lumen and feed actively on eel blood. The total life cycle of *Anguillicola* spp. in the Japanese eel has been estimated at 1 yr (Egusa 1979, Puqin & Yuru 1980).

The life cycle of *Anguillicola crassus* in European eels was studied recently by De Charleroy et al. (1989), who demonstrated that, under optimal conditions, the life cycle of *A. crassus* in European eels takes less than 2 mo.

In 1980 Puqin & Yuru proposed a direct migration route of third-stage larvae of *Anguillicola globiceps* through the intestinal wall and body cavity into the swimbladder wall of Japanese eel *Anguilla japonica*.

This report describes the migration of L-III larvae of *Anguillicola crassus* in the European eel and the pathological effects induced by these parasites in an experimentally induced infection.

Materials and methods

Eggs of *Anguillicola crassus*, containing L-II larvae, were collected from the swimbladder fluid of an infected eel. These eggs were released into fresh water at 20°C, where they hatch within a few hours (De Charleroy et al. 1989). The intermediate host, the copepod *Paracyclops fimbriatus*, was cultured at 20°C in the laboratory and fed with the newly hatched L-II larvae (estimated equal numbers of larvae and copepods). The mean infection level of the copepods, after 9 d infection, was 1.2 larvae copepod^{-1}. Three hundred unparasitised European elvers, each weighing ca 1g, were fed with the infected copepods (1 exposure of about 4 times as many copepods as eels). Afterwards, the elvers were kept in water at 20°C and fed with commercial pellet food, at a rate of 2% of body weight per day.

After this single infection, 60% of the eels were found to be infected with *Anguillicola crassus* larvae (infection level varying from 1 up to more than 20 larvae; dependent on the individual feeding behaviour of the eels).

At 28 different time intervals, samples of 10 eels were collected, anaesthetized and fixed in Bouin Hollande for histological examination (t = 0 and 1 h post-infection [p.i.]; every 4 h during the first 3 d; at 4, 7 and 8 d p.i.; and at 1, 2, 3, 4 and 6 mo p.i.). Histological sections of 4 µm were stained with hematoxylin and eosin or trichrome, according to the method of Pollack (1944).

Results

One hour after feeding the eels with the infected copepods, L-III larvae of *Anguillicola crassus* were detected in the stomachs of the eels. The swimbladder was still uninfected (Fig. 1). At 5 h p.i., L-III larvae were detected in the different layers of the digestive tract, especially in the submucosa, and in the body cavity. Tunnels were detected in the wall of the digestive tract (Fig. 2), as well as haemorrhages with numerous mono- nuclear phagocytes (Fig. 3). Until 17 h p.i. this situation did not change. At 17 h p.i., L-III larvae were detected for the first time in the swimbladder wall (Fig. 4); they were situated in the subserosa and had not yet fed on eel erythrocytes. This situation remained unchanged until 3 mo p.i. At 3 mo p.i. L-IV larvae were detected for the first time, in the swimbladder wall; eel erythrocytes were detected within the parasite's intestine (Fig. 5). At 4 mo p.i. immature adults, full of eel erythrocytes and developing gonads, were detected within the swimbladder lumen (Fig. 6). At 6 mo p.i. this was again seen. No adult parasites were found at all. A summary of the results is given in Table 1. No pathological effects, such as inflammations or fibrosis of the swimbladder, were detected.

Occasionally, L-III larvae were detected migrating aberrantly, for instance in the ventral musculature (Fig. 7).

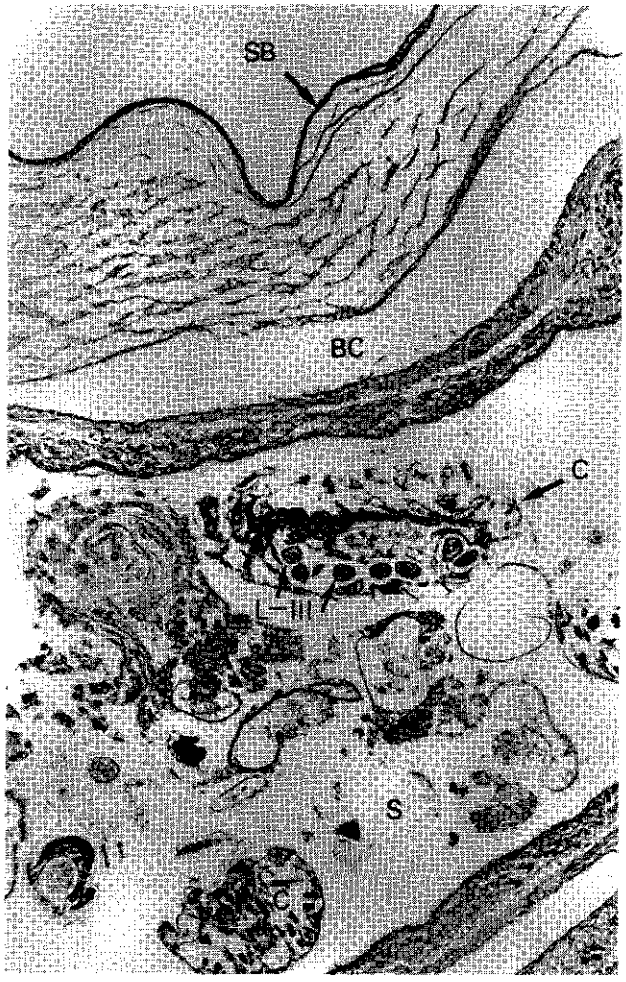


Fig. 1.
Anguilla anguilla. Copepods (C) containing L-III larvae of *Anguillicola crassus* in the stomach (S) of eel, 1 h p.i. The swimbladder (SB) is not yet infected. BC: body cavity. H&E, 100 x, cross section.

Table 1. ***Anguillicola crassus* in *Anguilla anguilla*.** *Location of parasite larvae in the European eel after experimentally induced infection.*

Location	Time p.i.				
	1 h	5 h	17 h	3 mo	4 mo
Intestinal lumen	L-III	L-III	L-III	L-III	L-III
Intestinal wall		L-III	L-III	L-III	L-III
Body cavity		L-III	L-III	L-III	L-III
Swimbladder wall			L-III	L-III & L-IV	L-III & L-IV
Swimbladder lumen					IA[a]

[a] IA = immature adult.

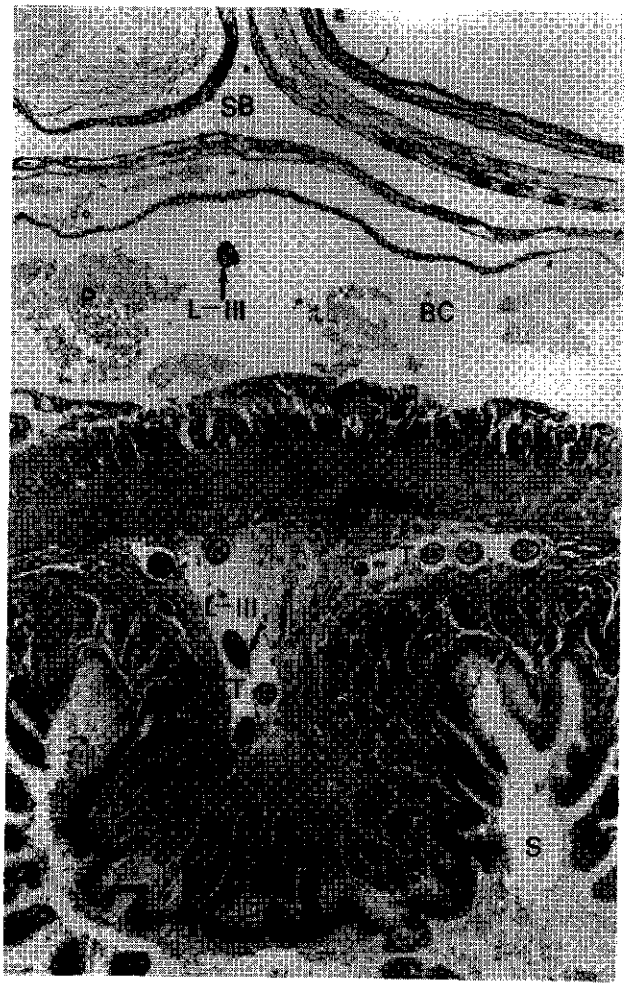


Fig. 2. *Anguilla anguilla.* L-III larvae of *Anguillicola crassus* migrating directly through the submucosa (SM) of the stomach (S) to the body cavity (BC) and causing tunnels (T), 5h p.i. Protein (P), a sign of haemorrhages, is visible in the body cavity. The swimbladder (SB) is not yet infected. Trichrome, 100 x, cross section.

Discussion

In this study the artificially induced infection of *Anguilla anguilla* with *Anguillicola crassus* was successful. We could detect the migration patterns of the parasitic larvae.

The 17h period between feeding with infected copepods and the first appearance of L-III larvae in the swimbladder wall is remarkably short. The L-III larvae were detected mostly in the submucosa of the digestive tract, where they apparently reside some time before passing the denser muscularis. What attracts the larvae to the swimbladder is not known. Some larvae migrated aberrantly, but most of the L-III larvae migrated to the swimbladder.

L-IV larvae were detected in the swimbladder wall at 3 mo p.i., earlier than was reported for Japanese eels (Puqin & Yuru 1980). These larvae had already been feeding on eel erythrocytes.

Since immature adults were detected in the swimbladder at 4 mo p.i., the life cycle of the nematode in European eels is considerably shorter than that described for

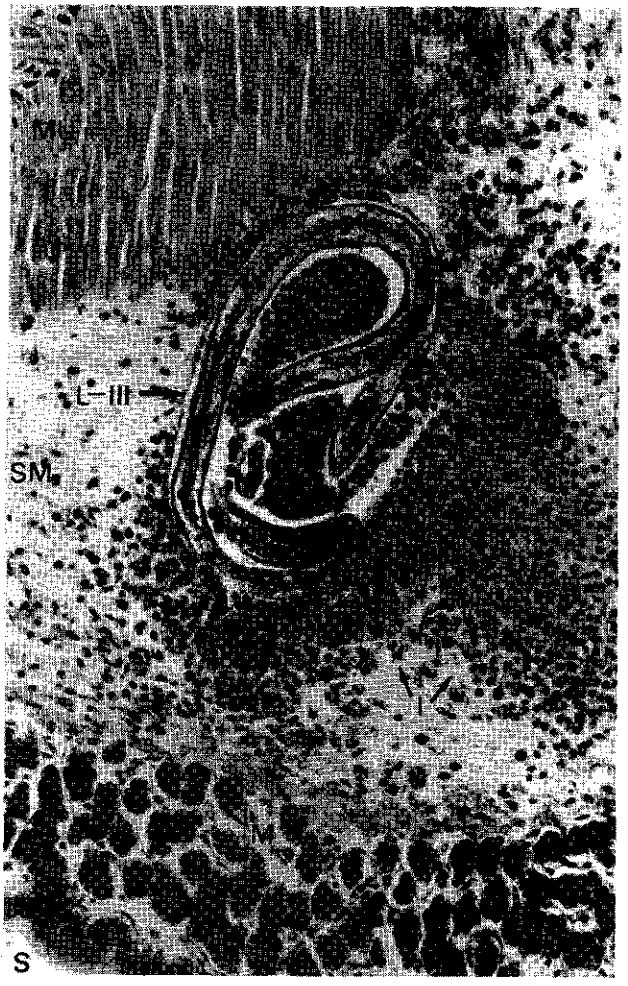


Fig. 3.
Anguilla anguilla. Longitudinal section of L-III larvae of *Anguillicola crassus* situated in the submucosa (SM) of the eel stomach (S). Inflammatory cells (I) (mainly mononuclear phagocytes) and erythrocytes are visible.
M: mucosa; MU: muscularis.
Trichrome, 200 x.

Japanese eels (1 yr) (Egusa 1979, Puqin & Yuru 1980). The life cycle seems longer however, than that reported for European eel by De Charleroy et al. (1989), of 2 mo. This is probably related to the different detection methods used in the experiments. De Charleroy et al. examined whole swimbladders of fresh eels for *Anguillicola crassus*, whereas we only examined a few histological sections. Therefore, eels in our experiments may actually have contained older larval stages at earlier times, which we missed.

Although we are aware of no reports that *Anguillicola crassus* migrates aberrantly in the Japanese eel, our study revealed that the nematode does on occasion migrate aberrantly in the European eel.

In a previous study of naturally occurring infections (van Banning & Haenen 1989), we demonstrated that *Anguillicola crassus* caused pathological changes in eels. In contrast, the infections experimentally induced in the present study did not cause severe pathological changes. Eels living under natural conditions may be continuously exposed to nematode infections and thus may suffer more severe lesions than the

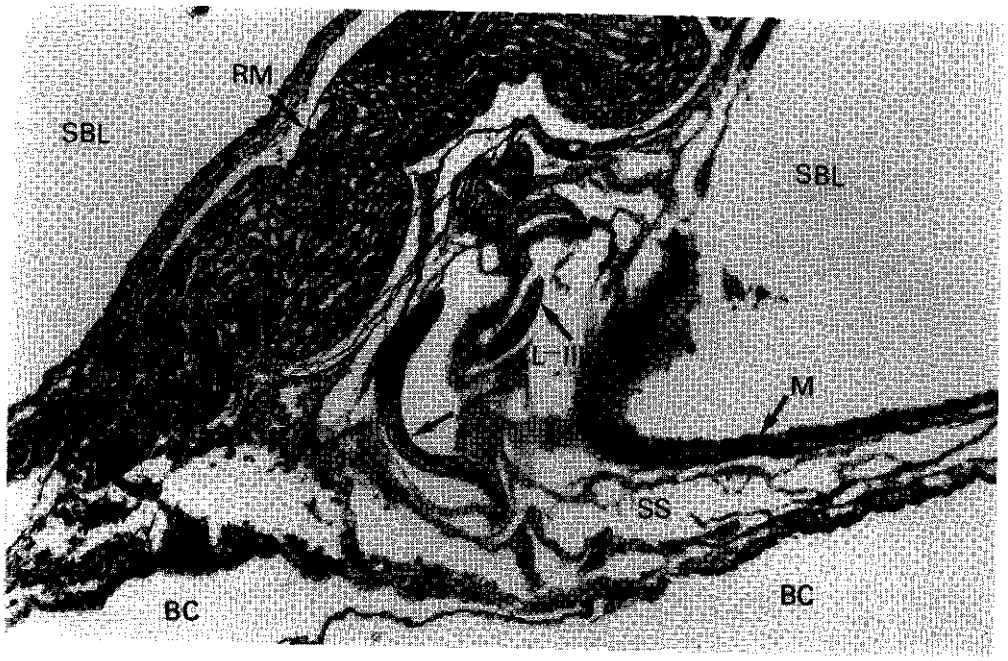


Fig. 4. *Anguilla anguilla.* Longitudinal section of eel showing L-III larvae of *Anguillicola crassus* situated in the swimbladder subserosa (SS), 17 h p.i. M: mucosa; RM: rete mirabile, gas organ; SBL: swimbladder lumen; BC: body cavity. Trichrome, 100 x.

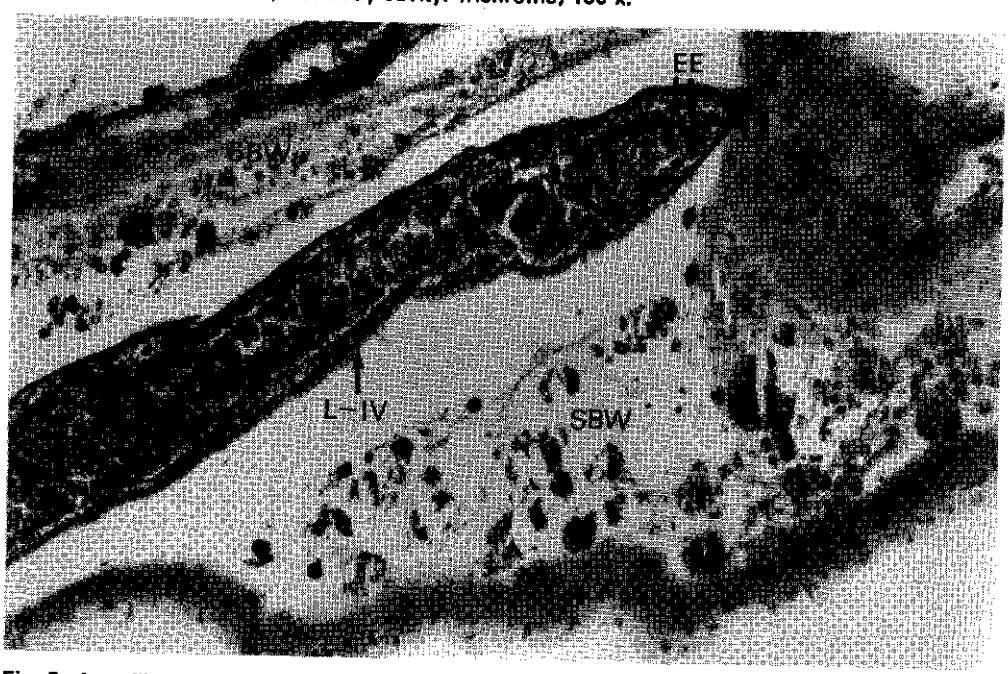


Fig. 5. *Anguilla anguilla.* Detail of L-IV larva of *Anguillicola crassus* situated in the swimbladder wall (SBW), 3 mo p.i. Eel erythrocytes (EE) are visible within the parasite. Trichrome, 200 x.

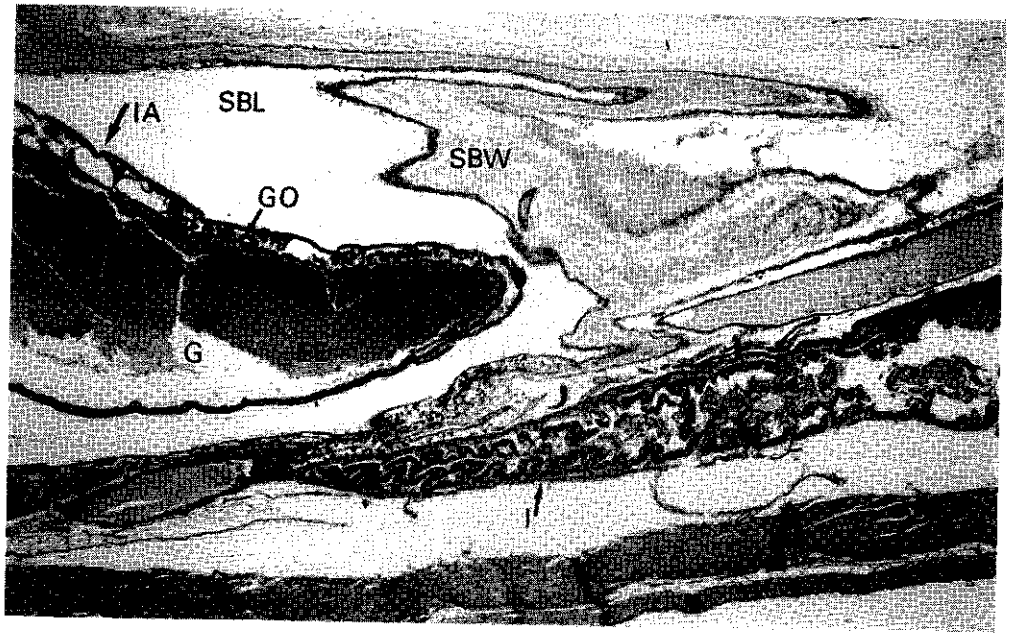


Fig. 6. *Anguilla anguilla.* Immature adults (IA) of *Anguillicola crassus* situated in the swimbladder lumen (SBL) of the eel, 4 mo p.i. The gut (G) of the parasite is filled with eel erythrocytes (EE). GO: Early development stage of gonads; SBW: swimbladder wall; I: intestine. Trichrome, 20 x.

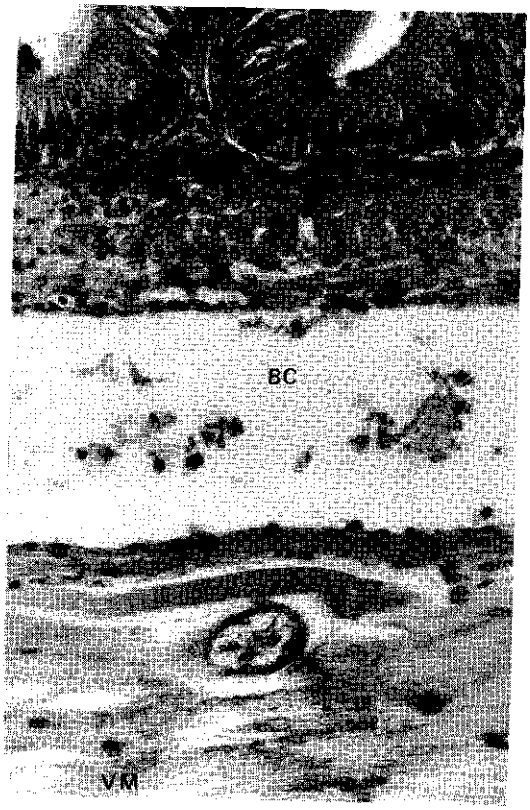


Fig. 7. *Anguilla anguilla.* L-III larva of *Anguillicola crassus* aberrantly migrating through the ventral musculature (VM) of the eel. BC: body cavity. H&E, 400 x.

experimentally infected eels, which were exposed only once to the parasites. Therefore, future studies on the pathological changes induced by *A. crassus* on European eels should include repeated experimentally induced infections.

Literature cited

Banning, P. van, Haenen, O.L.M. (1989). Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In: Perkins, F.O. (ed.) Proceedings PAMAQ III-Conference, Gloucester Point, USA. *Academic Press*, New York.*

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., Ollevier, F. (1989). The life cycle of *Anguillicola crassa* (Kuwahara, Niimi and Itagaki). Proceedings F.H.S./A.F.S. Inter- national Fish Health Conference, Vancouver, *J. Aquat. Anim. Health.***

Egusa, S. (1979). Notes on the culture of the European eel (*Anguilla anguilla*) in Japanese eel farming ponds. In: Thurow F. (ed.) Eel research and management. *Rapp. Proc. Verb. Réun. Cons. Int. Explor. Mer* **174**: 51-58.

Kuwahara, A., Niimi, A., Itagaki, H. (1974). Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n. sp. (Phimetridae, Anguilli- colidae). *Jap. J. Parasit.* **23** (5): 275-279.

Liewes, E.W., Schaminee-Main, S. (1987). Onderzoek naar de effecten van de parasiet *Anguillicola crassa* op de ontwikkeling van de paling (*Anguilla anguilla*) in een zout water palingmesterij. *Internal report, Texvis B.V.,* Den Burg, Texel, The Netherlands.

Moravec, F., Taraschewski, H. (1988). Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda: Anguillicolidae) of the swimbladder of eels, including descriptions of two new species, *A.novaezelandiae* sp. n. and *A. papernai* sp. n. *Folia Parasitol.* (Prague) **35**: 125-146.

Pollack, O.J. (1944). A rapid trichrome stain. *Arch. Path.*, **37**: 294.

Puqin, W., Yuru, Z. (1980). Observations on the life history of *Anguillicola globiceps* (Nematoda, Anguillicolidae). *Acta zool. sin.* **26**: 243-249.

* Published 1990, pp. 317-330. See chapter 2 of this thesis.

** Published 1990: Dis. aquat. Org. 8: 77-84.

Chapter 6

Infection of eel *Anguilla anguilla* (L.) and smelt *Osmerus eperlanus* (L.) with *Anguillicola crassus* (Nematoda, Dracunculoidea) in the Netherlands from 1986-1992

O.L.M. Haenen[a], *P. van Banning*[b], *and W. Dekker*[b]

a DLO-Institute for Animal Sciences and Health, P.O. Box 65, 8200 AB Lelystad, The Netherlands

b Netherlands Institute for Fisheries Research, P.O. Box 68, 1970 AB IJmuiden, The Netherlands

Haenen, O.L.M., 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 1986-1992. *Aquaculture* **126**: 219-229.

Abstract

Dutch wild eels *Anguilla anguilla* (L.) and smelts *Osmerus eperlanus* (L.) from freshwater and saltwater areas in the Netherlands were collected from 1986-1992 and their swimbladders were examined for *Anguillicola crassus* (Nematoda, Dracunculoidea) and for parasite-related pathological changes. Throughout the 6-year sampling period, young eels (up to 17 cm) showed severe pathological changes due to the parasite. The prevalence of infection in larger eels (23-34 cm) showed the highest prevalence between 1987-1988, and the highest intensity (i.e. number of parasites per infected fish) between 1988-1989. After 1989 the prevalence of the parasite decreased, and the lesions became less severe. Larger eels (23-34 cm) from the Waddenzee (salt water), which is close to the IJsselmeer, showed a high prevalence of the parasite from 1987-1990, although the intensity of infection decreased from 1987 onwards, as did the percentage of fibrotic swimbladders after 1988. Smelt, which is a paratenic host for larvae of *A. crassus* and which is a prey for large eels, showed a sharp decrease in prevalence of the parasite shortly after 1988. Thereafter the prevalence stayed rather constant at about 40% of the smelt population. No pathological changes were found in the smelt.

Introduction

The parasitic nematode *Anguillicola crassus* (Moravec and Taraschewski, 1988), originally described as *A. crassa* (Kuwahara et al., 1974), resides in the swimbladder of the eel *Anguilla anguilla*. It was introduced into Europe in the early 1980s (Neumann, 1985; Van Banning et al., 1985), and has since then spread rapidly among European eel populations (Peters and Hartmann, 1986; Canestri-Trotti, 1987; Køie, 1987; Taraschewski et al., 1987; Dupont and Petter, 1988; Hellström et al., 1988; Belpaire et al., 1989; Dekker and van Willigen, 1989; Koops and Hartmann, 1989; Van Willigen and Dekker, 1989; Kennedy and Fitch, 1990; Möller et al., 1991; Székely et al., 1991; Cruz et al., 1992; Moravec, 1992).

Immediately after the parasite was introduced into Europe, both farmed and wild eel showed high intensities of infection and severe swimbladder lesions (Neumann, 1985; Dekker and van Willigen, 1988; Van Banning and Haenen, 1990; Køie, 1991; Molnár et al., 1991; Cruz et al., 1992; Thomas and Ollevier, 1992; Molnár et al., 1993).

After the parasite was found in Dutch wild eels, an investigation was conducted from 1986-1992 in the freshwater lakes IJsselmeer and Ketelmeer, and in the sluice-connected waters Markermeer and Waddenzee (from 1987-1990) (Fig. 1). This article reports the findings of the investigation, the purpose of which was to determine trends in the presence of the parasite in eels of different sizes as well as its pathological effects. Smelt, *Osmerus eperlanus*, which are preyed upon by eel and which act as paratenic host for *A. crassus* larvae (Haenen and van Banning, 1990, 1991), were also sampled.

Material and methods

Figure 1 shows the sites at which fish were caught for the study.

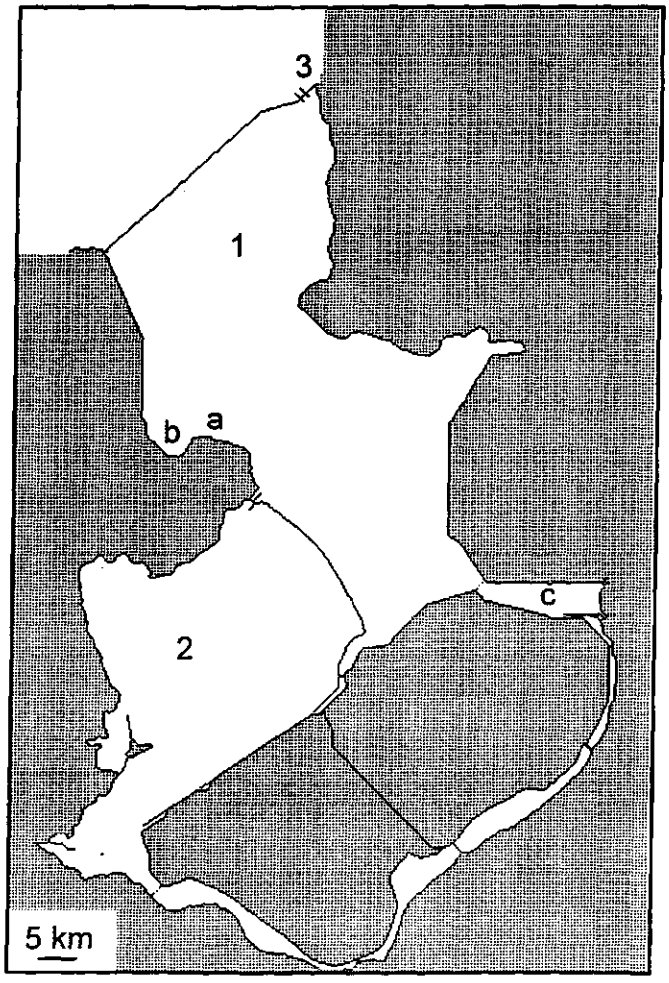


Fig. 1. Sampling sites of eels and smelts in the Netherlands.
1 = IJsselmeer
2 = Markermeer
3 = Waddenzee
a = Wagenpad
b = Medemblik
c = Ketelmeer.

Sampling and examination of small eels from the IJsselmeer. Small eels (up to 17 cm) were caught with an electrified fine meshed beam trawl in spring (May-June) and autumn (October-November) around the site Wagenpad, located in the western part of the IJsselmeer. The eels were taken alive to the laboratory to be examined. The eels were anaesthetized with metomidate (20 mg/l) or 2-phenoxy ethanol (0.2-0.5 ml/l), and measured. The swimbladders were dissected and examined with a light microscope for *A. crassus* (number and stages of development). A few fish, depending on the

sample sizes were transversally cut, and the pieces were fixed in 10% buffered formalin, embedded in wax, sectioned transversally at 4 µm, and stained with haematoxylin and eosin (H&E) for histopathological examination.

Larger eels from the IJsselmeer and Markermeer (freshwater). Larger eels (23-34 cm) were caught from the IJsselmeer and Markermeer with an electrified fine-meshed beam trawl at different sites in spring and autumn from 1986-1992. In 1987 no samples were collected from the Markermeer. The swimbladders of all eels were examined macroscopically for the number of *A. crassus* and for lesions, but were not examined histopathologically.

Larger eels from the Waddenzee (salt water). Larger eels (23-34 cm) were caught from the Waddenzee from 1987-1990 between mid September and mid November. Fyke nets were left in place for 3-5 days per catch along the coastline, close to Kornwerderzand sluice. This site is located at the eastern end of the Afsluitdijk, a dyke which separates the Waddenzee from the IJsselmeer. A random sample of about 50 eels per catch was taken alive to the laboratory. The eels were anaesthetized as described earlier, measured, and examined in the same way as large eels taken from the IJsselmeer and Markermeer.

Smelts. In the spring and autumn of 1988, 1989, 1991 and 1992, we also collected smelts, (*Osmerus eperlanus*) from different sites in the IJsselmeer, in the same way described for the small eels. The smelts died immediately after they were caught. They were put into a plastic bag, and transported under cool conditions to the laboratory, where they were stored at 4°C. The next morning 50 fish were taken randomly selected from each group, measured, and necropsied. A fresh tissue preparation was made of each swimbladder and was examined under a light microscope for the number and stages of development of *A. crassus* specimens.

The Generalized Linear Model (McCullagh & Nelder, 1989) was used to statistically analyze the prevalence and intensity of infection per year in the large freshwater eels. For smelts, Analysis of Variance (Genstat 5 Committee, 1987) was used to statistically analyze the prevalence and intensity of infection per year and site, and the length of the fish in relation to the intensity of the infection.

Results

Small eels from the IJsselmeer. Table 1 presents quantitative data about the infection of small eels with *A. crassus*. The prevalence of the infection varied between 42-91% over the years, with no apparent trend. Swimbladders showed the following lesions: haemorrhages (0-70% of the eels), congestion of blood vessels (14-60% of the eels), and pigmentations (3-30% of the eels), also without a trend.

The percentage of eels with fibrotic swimbladders varied between 45 and 90%. The small sized eels showed severe lesions of the swimbladder, caused by the parasite, like haemorrhages, congestion of blood vessels and thickening of the swimbladder. As

Table 1. ***Anguillicola crassus** infections in small sized eels (<18 cm) from the IJsselmeer in the period 1987-1992 (macroscopic and microscopic examinations).*

Date	No. of eels	Lenght range (cm)	Site[a)]	Prevalence of infection (%)	Fibrotic swimbladders (%)
June '87	18	7-15	Wag	56	44
Oct '87	8	9-16	Med	88	75
June '88	16	6-15	Med	81	69
Oct '88	10	9-15	Ket	90	90
June '89	19	7-16	IJs	42	53
Oct '89	16	9-16	IJs	69	56
Sept '90	17	8-16	IJs	65	47
May '91	11	9-16	Wag	73	46
Oct '91	35	9-16	Wag	91	60
May '92	47	6-16	Wag	81	70
Nov '92	29	9-17	Wag	86	90

a) Wag = Wagenpad
Med = Medemblik
Ket = Ketelmeer
IJs = central IJsselmeer

Table 2. ***Anguillicola crassus** infections in larger eels (23-34 cm) from the Waddenzee between 1987-1990. Mean prevalence (%) and intensity (number of parasites per infected eel) of **A. crassus**.*

Year	Number of eels	Prevalence (%)	Range[a] (%)	Intensity[b]	Range[a]
1987	378	86	80-96	7.7	6.0-8.6
1988	500	85	77-96	6.0	4.5-9.0
1989	399	87	82-94	4.8	4.2-5.9
1990	291	90	86-94	4.8	4.0-5.5

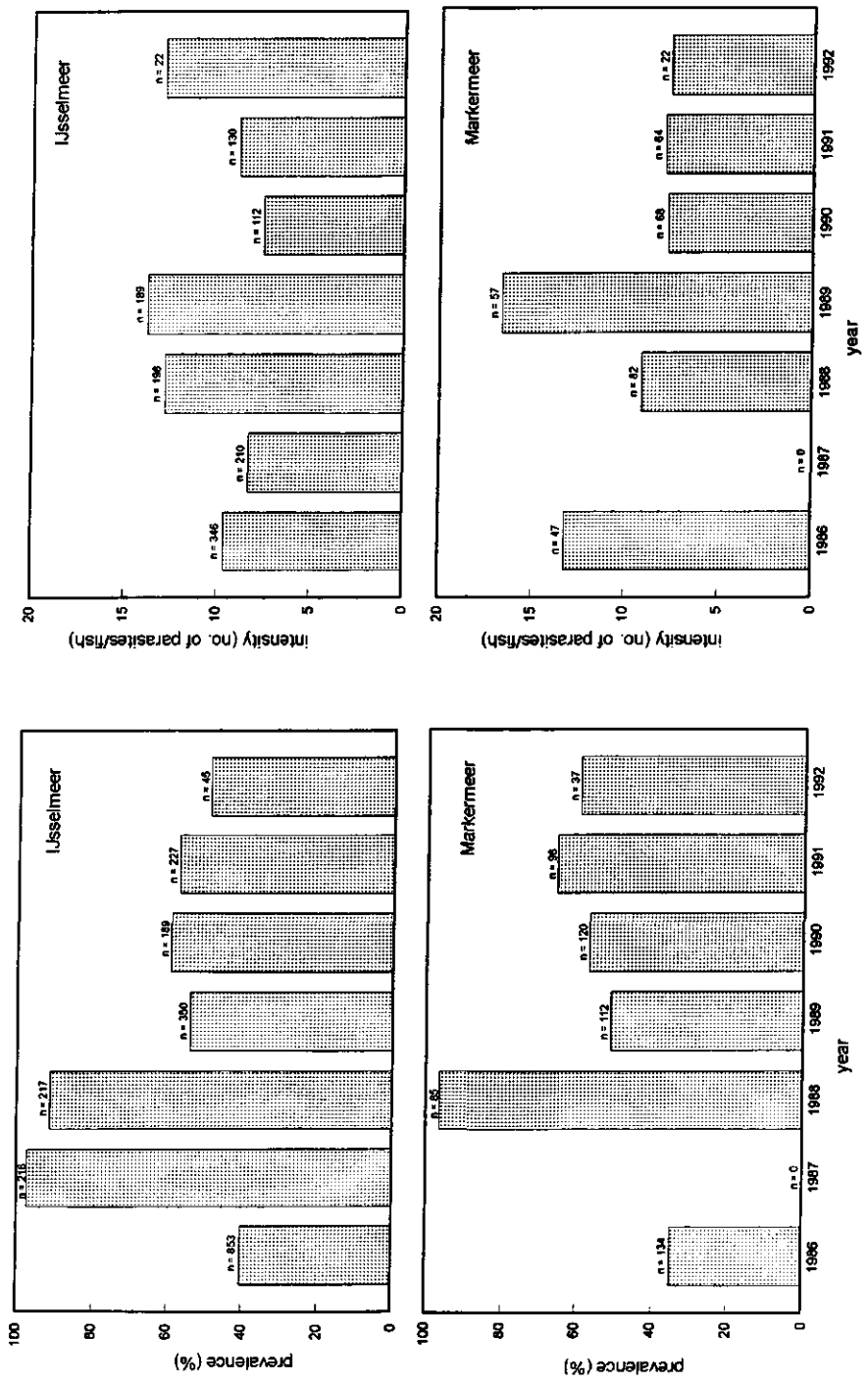
[a] Per group of about 50 eels.
[b] Intensity = mean number of parasites per infected eel.

early as 1987 (Fig. 2), parasites were found encapsulated in the fibrotic swimbladder wall, which was often connected to the intestine by fibrotic tissue. Mostly L2- and L3-larvae were found in these complexes, as well as dead pre-adults and adults. Tunnels showing the migration pathway of L3-larvae were clearly visible in the fibrotic tissue. The kidney was also sometimes covered with a fibrotic layer, in which *A. crassus* L2- and L3-larvae could be found. Often many lymphocyte-like cells were seen in the fibrotic swimbladder wall.

A. crassus was found only in eels larger than 8 cm (we checked 12 eels from 6-8 cm).

Fig. 2. Cross section of the swimbladder of European eel (IJsselmeer, 1987; 14 cm) with a chronic infection of *Anguillicola crassus*. Severe fibrosis (F) of the swimbladder wall with many encapsulated L1 and L2 larvae of the parasite (P) surrounded by mononuclear phagocytes (M), congestion of blood vessels (C) and haemorrhages (H) (40x).

Larger eels (23-34 cm) from the IJsselmeer and Markermeer. The mean yearly prevalence (%) of *A. crassus* in the eels is shown in Figure 3. For the IJsselmeer the peak years were 1987 and 1988 with a maximum of 97.2% (n=216) in 1987. For the


IJsselmeer
Markermeer
prevalence (%)
intensity (no. of parasites/fish)
year
1986
1987
1988
1989
1990
1991
1992
n = 853
n = 218
n = 217
n = 350
n = 189
n = 227
n = 45
n = 134
n = 0
n = 85
n = 112
n = 120
n = 98
n = 37
n = 346
n = 210
n = 196
n = 189
n = 112
n = 130
n = 22
n = 47
n = 0
n = 42
n = 57
n = 68
n = 84
n = 22

Markermeer a similar trend was seen, with a maximum in 1988 of 96.4% (n=85). No data were recorded in 1987, however.

The intensity of infection (i.e., mean number of *A. crassus* per infected eel) is also shown in Figure 3. The mean intensities were 10.3 parasites per infected eel for both the IJsselmeer and the Markermeer.

From 1987 onwards fibrotic swimbladders were found in 3-20% of the eels from the IJsselmeer. Fibrotic swimbladders were not detected until 1989 onwards in eels from the Markermeer (2-36%).

Larger eels (23-34 cm) from the Waddenzee. Table 2 shows the mean prevalence and intensity of *A. crassus* infections from 1987-1990. Although the prevalence of infection was high and was fairly constant during the sampling period (September until November), no change was observed. In contrast, the intensity of the infection decreased during the study. The mean percentage of eels with fibrotic swimbladders peaked in 1988 at 34.5% (range: 10-33% per sample catch) and decreased thereafter.

Smelts from the IJsselmeer. Table 3 shows the percentage and intensity of *A. crassus* infections in smelt from 1988-1992. Smelt from the Ketelmeer showed a high prevalence of infection (88%) in 1988, but this figure is based on only one sample catch. No trend in the prevalences or intensity was observed in smelt caught at the Wagenpad site from 1991-1992.

Most infected smelts contained L3-larvae of *A. crassus* and sometimes L4-larvae were found. Dead or encapsulated L3-/L4-larvae were found only rarely in the swimbladder lumen. No other stages of the parasite were found in the swimbladders, and swimbladders showed no pathological changes.

Statistical analysis. According to the Generalized Linear Model analysis, there was no significant difference ($P>0.05$) between the prevalence (logistic model) or the intensity (loglinear model) of the infection in larger eels (23-34 cm) from the IJsselmeer and the Markermeer. The residuals around the prevalence were considered to be distributed binomially, and the intensity was considered to be distributed according to the Poisson model. To test if there were yearly effects, the data of eels taken from the two waters were pooled (Table 4) for an analysis with *t*-values of pairwise differences between years. The prevalence of *A. crassus* in larger eels in 1987 and 1988 did not differ significantly, but was significantly higher in 1987 and 1988 than in 1986 and 1989-1992. The intensity of *A. crassus* was highest in 1988 and 1989, with no significant difference between the two years. The intensity measured in this period was significantly higher than in 1986-1987 and 1990-1992 ($P>0.05$).

Wagenpad, which was sampled several times did not differ significantly from the other sites in the IJsselmeer for the prevalence or intensity of the infection in smelts.

Fig. 3. Prevalence and intensity of *Anguillicola crassus* infections in larger eels (23-34 cm) in the Netherlands (IJsselmeer and Markermeer) in 1986-1992. n = number of eels analyzed.

Table 3. *Anguillicola crassus infections in smelt from different sites of the IJsselmeer between 1988-1992.*

Date	Site	No. of smelts examined	Prevalence of infection (%)	Intensity[a]
19 Oct '88	Ketelmeer	34	88	not recorded
18 Oct '89	Kornwerderzand	25	48	2.16
30 May '91	Wagenpad	50	18	1.88
30 May '91	Staverse Geul	50	42	1.42
28 Oct '91	Wagenpad	50	38	1.42
18 May '92	Wagenpad	50	38	1.21
18 May '92	Kreupel	50	30	1.20
11 Nov '92	Wagenpad	50	42	1.52

[a] mean no. of *A. crassus* larvae per infected fish.

Table 4. *Estimated mean prevalence and intensity of **A. crassus** infection in large eels (23-34 cm) from the IJsselmeer and Markermeer between 1986-1992.*

Year	Prevalence (%)	Intensity (no. of parasites/infected eel)
1986	39.8 (a)[a]	6.78 (a)
1987	97.2 (b)	6.59 (a)
1988	92.7 (b)	6.93 (b)
1989	53.2 (c)	7.14 (b)
1990	58.2 (c)	6.49 (a)
1991	59.7 (c)	6.62 (a)
1992	53.7 (c,a)	6.80 (a)

[a] a, b and c are significant different groups ($P < 0.05$).

The estimated mean prevalences were based on logistic regression, and the intensities on loglinear regression.

Also, there was no correlation between the number of *A. crassus* larvae per swimbladder and the length of the smelts. No significant differences in prevalence and intensity was found from 1988-1992 ($P > 0.05$).

Discussion

In small eels, severe lesions, such as those described by Van Banning and Haenen (1990) and Molnár et al. (1993) were seen during the whole period. Chronic

swimbladder inflammation (Molnár et al., 1993) was often found, with dead encapsulated remains of adult worms within the heavy fibrosis.

The fact that no trend in the infection of small eels was observed means that the infectivity of the parasite for small eels did not change. Moreover, the fact that no trend in the infection-related lesions of small eels was seen makes us conclude that *A. crassus* did not evolve to become less pathogenic for the eels. Every year new elvers arrive in the IJsselmeer (Dekker et al., 1992) and get their first infection with *A. crassus*. Our results show, that the small eels stayed susceptible for the parasite in the last few years. Therefore, genetical selection of less susceptible eels would not be the case. This selection might occur on the long term.

The explanation for the decrease of the infection in larger eels might be an immunological one. The development of a humoral and nonspecific immunological response against *A. crassus* in eels has been suggested by Van Willigen and Dekker (1989), Höglund et al. (1992) and Molnár et al. (1993). Buchmann et al. (1991) demonstrated a humoral response of eels to the parasite. Whether the immunological response is protective is not known. Protective immunity is known to occur in some fish species towards protozoan parasites, for example against *Ichtyophthirius multifiliis* in carp (Houghton, 1987). Metazoan parasites, like *Diphyllobothrium dendriticum* elicit a nonspecific and a humoral immune response in rainbow trout (Sharp et al., 1992). However, in metazoan parasitic infections of fish, the precise role of the immune response has yet to be determined (Woo, 1992). This aspect should receive more attention in future studies.

The *A. crassus* infection developed rapidly towards high prevalences, both in IJsselmeer and Markermeer, and the Waddenzee. In 1985 IJsselmeer eels had a prevalence of *A. crassus* infection of only 28-45%, the Waddenzee eels (near Harlingen) only 2% (Van Banning et al., 1985). By 1986 prevalences were as high as 37.5-50% for the IJsselmeer and Markermeer and 0.5-25% for the Waddenzee (Dekker and van Willigen, 1989). In 1987, thickened swimbladders without a lumen, so-called collapsed swimbladders, were first detected in infected eels from the IJsselmeer (Dekker and van Willigen, 1988). This condition indicates the chronic form of infection (Molnár et al., 1993).

The prevalence of *A. crassus* infection in the IJsselmeer rose to a peak of 97% in 1987, according to our results. In 1988 91% of the IJsselmeer eels and 97% of the Markermeer eels were still infected. After these years the prevalences of infection decreased. This tendency in decrease was already suggested by Dekker and van Willigen (1988). However, the prevalence of infection continued to fluctuate: even in 1993 81% (n=116 eels) of the 23-34 cm eels from the IJsselmeer and 84.6% (n=65) from the Markermeer were infected. However, the intensity of infection in these eels was lower than in earlier years: 5.06 (n=94) for the IJsselmeer eels and 4.40 (n=55) for the Markermeer eels. We suggest that these values will probably not stabilize for a few years.

The effects of annual variations of weather on the population dynamics of zooplankton (intermediate hosts, often copepods) and the role of different fish species acting as paratenic hosts and prey for eels have not been taken into account as

possible causes of the infection fluctuations in this study. However, our study shows, that possible fluctuations in the infection of copepods with *A. crassus* larvae did not give rise to changes in the infection of small eels and smelts (after 1988) during our investigation.

The larger eels from the Waddenzee in our study had been caught with fyke nets near the sluice Kornwerderzand. This sluice allows contact between salt water and fresh water from the IJsselmeer. Eels can be transferred between the two waters by the strong current every time the sluice opens, the water flowing mostly in the direction of the Waddenzee (Dekker, unpublished). The infected Waddenzee eels, which may originate from the IJsselmeer (Dekker and van Willigen, 1989) showed a higher prevalence of infection than the IJsselmeer eels. This could be the result of heavily infected eels swimming more slowly (Sprengel and Lüchtenberg, 1991) and being weaker because of decreased haematocrit and plasma proteins (Boon et al., 1990), and they are therefore less capable of swimming back against the strong current in the sluice. Also, quick transfer of eels between fresh water and salt water might induce stress and therefore immuno-suppression (Ellis, 1981).

The intensity of infection (number of parasites per infected eel) was high in both the IJsselmeer (7-14) per eel and Markermeer (7-13) per eel from 1986-1992. These numbers were not as high as those found by Molnár et al. (1991), who often detected 30-50 *A. crassus* per swimbladder in lake Balaton (Hungary), directly after the introduction of the infection. Dekker and van Willigen (1989) found 0.14% of the eels carrying more than 20 *A. crassus* in 1986 in the Netherlands (both salt and fresh water). In Belgium, Thomas and Ollevier (1992) found a mean intensity of *A. crassus* of 17 parasites in 1990-1991, higher than the rate found in the Netherlands in that period. They counted the parasites not only macroscopically, like we did, but also microscopically, which enabled them to detect also larval stages, thus more parasites. They also caught the eels in a different way, by using fyke nets and by harvesting eels trapped in intake screens of a power plant.

Prevalences or intensity of *A. crassus* infection in eels did not differ statistically in our study, in the IJsselmeer and Markermeer. These waters are separated by a dike and two sluices, which are opened more than 20 times a day. The Ketelmeer is directly connected with the IJsselmeer.

Many fish species are known as paratenic hosts (De Charleroy et al., 1990, Haenen and Van Banning, 1990, Thomas and Ollevier, 1992), of which some are known to be preyed upon by eels (De Nie, 1987). Smelt are preyed upon by eels and may transmit the L3 larvae of *A. crassus* to the eel (Haenen and Van Banning, 1990, 1991). In examining smelt, we sometimes also detected L4-larvae, but no pre-adult or adult parasites. Many other fish species preyed upon by eels can be paratenic hosts. Large eels also consume infected copepods (Kennedy et al., 1992) and are thereby directly infected. These facts might explain the rapid development of the *A. crassus* infection in eels. Although dead or encapsulated larvae were sometimes found in the smelt, the parasite is not pathogenic to the smelt.

Acknowledgements

We thank the Organization for Improvement of Inland Fisheries for providing us with the eels from the Waddenzee. We also thank the "Stern" crew and J. van Willigen for catching the eels and smelts, H. van Lieshout and R. Buijs for making the microscopic slides, drs. H.J.C. Huipen for assisting with the statistical analysis, and N. Kuiper and R. Spies for helping with the manuscript.

References

Belpaire, C., D. De Charleroy, L. Grisez and F. Ollevier, 1989. Spreading mechanisms of the swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla*, and its distribution in Belgium and Europe. European Inland Fisheries Advisory Commission (FAO). *Working Party on Eel.* Porto, Portugal, May 29th-June 3rd 1989.

Boon, J.H., V.M. Cannaerts, H. Augustijn, M.A. Machiels, D. De Charleroy and F. Ollevier, 1990. The effect of different infection levels with infective larvae of *Anguillicola crassus* on haematological parameters of European eel (*Anguilla anguilla*). *Aquaculture* **87**: 343-253.

Buchmann, K., L.O. Pedersen and J. Glamann, 1991. Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Dis. Aquat. Org.* **12**: 55-57.

Canestri-Trotti, G., 1987. Occurrence of the nematode *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 in eels from the Po Delta, Italy. *Bull. Eur. Ass. Fish Pathol.* **7**(5): 109-111.

Cruz, P., E. Silva, M.S. Grazina Freitas and M. Carvalho-Varela, 1992. First report of *Anguillicola crassus* in the European eel in Portugal. *Bull. Eur. Ass. Fish Pathol.* **12**(5): 154-156.

De Charleroy, D., L. Grisez, K. Thomas, C. Belpaire and F. Ollevier, 1990. The life cycle of *Anguillicola crassus. Dis. aquat. Org.* **8**: 77-84.

Dekker, W., L. Schaap and J. van Willigen, 1992. Aanwas van jonge vis in het IJsselmeer. RIVO-DLO report BINVIS 92-04, IJmuiden, the Netherlands, 18 pp (in Dutch).

Dekker, W. and J. van Willigen, 1988. Abundance of *Anguillicola crassa* in Dutch outdoor waters and the reaction of its host *Anguilla anguilla*. ICES C.M. 1988/M:13, 6 pp. mimeo.

Dekker, W. and J.A. van Willigen, 1989. Short note on the distribution and abundance of *Anguillicola* in The Netherlands. *J. Appl. Ichthyol.* **1**: 46-47.

De Nie, H.W., 1987. Food, feeding periodicity and consumption of the eel *Anguilla anguilla* (L.) in the shallow eutrophic Tjeukemeer (The Netherlands). *Arch. Hydrobiol.* **109** (3): 421-443.

Dupont, F. and A.J. Petter, 1988. *Anguillicola*, une épizootie plurispécifique en Europe. Apparition de *Anguillicola crassa* (Nematoda, Anguillicolidae) chez l'anguille Européenne *Anguilla anguilla* en Camargue, Sud de la France. *Bull. Fr. Peche Piscic.* **308**: 38-41.

Ellis, A.E., 1981. Stress and the modulation of defence mechanisms in fish. In: Stress and Fish ed. A.D. Pickering, London: *Academic Press*, pp. 147-169.

Genstat 5 Committee, 1987. Genstat 5, Reference manual, Clarendon Press, Oxford.

Haenen, O.L.M. and P. van Banning, 1990. Detection of larvae of *Anguillicola crassus* (an eel swimbladder nematode) in freshwater fish species. *Aquaculture* **87**: 103-109.

Haenen, O.L.M. and P. van Banning, 1991. Experimental transmission of *Anguillicola crassus* (Nematoda, Dracunculoidea) larvae from infected prey fish to the eel *Anguilla anguilla*. *Aquaculture* **92**: 115-119.

Hellström, A., O. Ljungberg and S. Bornstein, 1988. *Anguillicola*, en ny alparasit i Sverige. *Svensk Veterinartidning* **40**: 211-213.

Höglund, J., J. Andersson and J. Hardig, 1992. Haematological responses in the European eel, *Anguilla anguilla* L., to sublethal infestation by *Anguillicola crassus* in a thermal effluent of the Swedish Baltic. *J. Fish Diseases* **15**: 507-514.

Houghton, G., 1987. The immune response of carp (*Cyprinus carpio* L.) to *Ichthyophthirius multifiliis* (Fouquet, 1876). PhD thesis, Plymouth Polytechnic, Plymouth, England.

Kennedy, C.R. and D.J. Fitch, 1990. Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in eel, *Anguilla anguilla*, in Britain. *J. Fish Biol.* **36:** 117-131.

Kennedy, C.R., P. Nie, J. Kaspers and J. Paulisse, 1992. Are eels planktonic feeders? Evidence from parasite communities. *J. Fish Biol.* **41:** 567-580.

Køie, M., 1987. Eel parasites in natural waters and aquaculture in Denmark. In: Parasites and diseases in natural waters and aquaculture in nordic countries. Ed. A. Stenmarck and C. Malmberg Zoo tax Naturhistorisk riksmuseet. Stockholm, Sweden, 38-45.

Køie, M., 1991. Swimbladder nematodes (*Anguillicola* spp.) and gill monogeneans (*Pseudodactylogyrus* spp.) parasitic on the European eel (*Anguilla anguilla*). *J. Cons. Int. Mer,* **47:** 391-398 (1991).

Koops, H. and F. Hartmann, 1989. *Anguillicola* infestations in Germany and in German eel imports. *J. Appl. Ichthyol.* **1:** 41-45.

Kuwahara, A., A. Niimi and H. Itagaki, 1974. Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Phimetridae, Anguillicolidae). *Jap. J. Parasit.* **23, 5:** 275-279.

McCullagh, P. and J.A. Nelder, 1989. Generalized Linear Models (2nd Ed.). Chapman and Hall, London.

Möller, H., S. Holst, H. Lüchtenberg and F. Petersen, 1991. Infection of eel *Anguilla anguilla* from the river Elbe estuary with two nematodes, *Anguillicola crassus* and *Pseudoterranova decipiens*. *Dis. Aquat. Org.* **11:** 193-199.

Molnár, K., F. Baska, Gy. Csaba, R. Glávits, and Cs. Székely, 1993. Pathological and histopathological studies of the swimbladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Dis. Aquat. Org.* **15:** 41-50.

Molnár, K., Cs. Székely and F. Baska, 1991. Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bull. Eur. Ass. Fish Pathol.* **11:** 211-212.

Moravec, F., 1992. Spreading of the nematode *Anguillicola crassus* (Dracunculoidea) among eel populations in Europe. *Folia Parasitologica* **39:** 347-348.

Moravec, F. and H. Taraschewski, 1988. Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda, Anguillicolidae) of the swimbladder of eels, including descriptions of two new species *A. novazelandiae* sp.n. and *A. papernae* sp.n.. *Folia Parasitol.* **35:** 125-146.

Neumann, W., 1985. Swimmblasenparasit *Anguillicola* bei Aalen. *Fischer und Teichwirt* **11,** 322.

Peters, G. and F. Hartmann, 1986. *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. *Dis. Aquat. Org.* **1:** 229-230.

Sharp, G.J.E., A.W. Pike and C.J. Secombes, 1992. Sequential development of the immune response in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) to experimental plerocercoid infections of *Diphyllobothrium dendriticum* (Nitzsch, 1824). *Parasitology* **104:** 169-178.

Sprengel, G. and H. Lüchtenberg, 1991. Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla anguilla*. *Dis. Aquat. Org.* **11:** 31-35.

Székely, Cs., M. Lang and Gy. Csaba, 1991. First occurence of *Anguillicola crassus* in Hungary. *Bull. Eur. Ass. Fish Pathol.* **11** (4): 162-163.

Taraschewski, H., F. Moravec, T. Lamah and K. Anders, 1987. Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and *Paratenuisentis ambiguus* (Acanthocephala, Tenuisentidae). *Dis. Aquat. Org.* **3:** 167-176.

Thomas, K. and F. Ollevier, 1992. Population biology of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Dis. Aquat. Org.* **14:** 163-170.

Van Banning, P. and O.L.M. Haenen, 1990. Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In: Pathology in marine science, Ed. F.O Perkins & T.C. Cheng, 1990. Academic Press, inc., New York. Proc. 3rd Int. Coll. on Path. in Marine Aquaculture, Gloucester Point, Virginia, Oct 2-6, 1988: 317-330.

Van Banning, P., W. Heermans and J.A. van Willigen, 1985. *Anguillicola crassa*, een nieuwe aalparasiet in Nederlandse wateren. *Visserij* **38** (6-7): 237-340.

Van Willigen, J. and W. Dekker, 1989. 1988 Update on *Anguillicola* in Dutch outdoor waters. EIFAC, Working Party on Eel, Oporto, Portugal, June 1989. 8pp. mimeo.

Woo, P.T.K., 1992. Immunological responses of fish to parasitic organisms. *Ann. Rev. of Fish Diseases*: 339-366.

Chapter 7

An improved method for the production of infective third-stage juveniles of *Anguillicola crassus* (short communication)

O.L.M. Haenen[#], T.A.M. van Wijngaarden[#] and F.H.M. Borgsteede[~]

DLO-Central Veterinary Institute, Section Fish Diseases[(#)], Section Parasitology[(~)], P.O. Box 65, 8200 AB Lelystad, The Netherlands

Haenen, O.L.M., van Wijngaarden, T.A.M., and F.H.M. Borgsteede, 1994 An improved method for the production of infective third-stage juveniles of *Anguillicola crassus*. Short communication. *Aquaculture* 123:163-165.

Abstract

A method was developed for the production of infective third-stage juveniles of *Anguillicola crassus*. Third-stage (L3-) juveniles, were isolated from the intermediate hosts (copepods) with a potter method, two weeks after infection with L2-juveniles. The L3-juveniles were separated from the debris by migration over a filter and stored at 4°C in sterile RPMI for at least one week before use.

Short communication

The nematode *Anguillicola crassus* of European eel *Anguilla anguilla* has gained much attention since its introduction into Europe in the early eighties (van Banning, 1985; Neumann, 1985). In several studies, artificially induced infections of eel with third-stage juveniles (L3-juveniles) were performed. In most experiments infected copepods were used as infective material and force-fed to the eels (De Charleroy et al., 1990b; Haenen et al., 1989). In other studies, L3-juveniles were isolated from young eels (Boon et al., 1990) or other fish species acting as paratenic hosts, such as ide and carp (De Charleroy et al., 1990a, 1990b). When using copepods as a source of infection, the infection dose can only be estimated. Isolation of L3-juveniles from the body cavity of young artificially infected eels or paratenic hosts is laborious and time consuming. In this study we developed a method to produce a clean suspension of L3-juveniles based on techniques described earlier by Muller (1972) and Brandt and Eberhard (1990) for the isolation of L3-juveniles of *Dracunculus insignis* from infected copepods. This allows easy counting of the L3-juveniles.

Collection of copepods. Cyclopoid copepods were isolated from the biological filter of an indoor eel farm free of *Anguillicola crassus*. The filter rings were rinsed in buckets with tap water of about 20°C, and the suspensions taken to the laboratory at room temperature. The coarse debris was allowed to sediment for at least 1 h. The supernatant was then filtered through a 300 µm sieve, and a 160 µm sieve respectively. Copepods suitable for artificially induced infection with L2-juveniles were washed from this 160 µm sieve. These were suspended in flasks filled with 1:1 tap water and sterilized pond water. The copepods were maintained at about 20°C in aerated flasks and fed with ground fish feed pellets once in every 3-4 days.

Collection of second stage juveniles of *A. crassus*. Wild eels about 25-50 cm long, caught in the IJsselmeer, were kept in aquaria at 20°C. For isolation of *A. crassus* L2-juveniles, 6 eels were anaesthetized with metomidate (2 ml/l of a 50 mg/l solution), and dissected. The swimbladder was removed, put in a Petri dish and carefully opened lengthwise. The contents of the swimbladder were examined for adult parasites and L1/L2-juveniles. When L1/L2-juveniles were seen adult female parasites were directly transferred to another Petri dish with buffered salt solution (PBS). The swimbladder was rinsed with PBS and the fluid added to the dish as well. The Petri dish with adult

females and juveniles was kept at room temperature for 1-5 h, allowing the L2-juveniles to hatch.

Experimentally induced infection of copepods. The concentrations of both copepods and the L2-juveniles were estimated by counting a sample under the binocular light microscope at a magnification of 25 to 40 times. Copepods and juveniles were put together in a ratio of 1:2 in Petri dishes filled with 1:1 tap water and sterilized pond water. The dishes were placed in an incubator at 20°C for 1 day, to allow the copepods to eat the juveniles. The contents of the dishes were then rinsed with a few mls of tap water and poured into glass jars of 400 ml. The jars were filled up to 400 ml each with 1:1 tap water and sterilized pond water, covered, and placed in the incubator for 2 weeks. The copepods were fed once every 3 to 4 days with ground trout pellet (Trouvit®).

Harvesting the L3-juveniles. The contents of the jars with the copepods were cleaned through a 100 µm sieve. The copepods on the sieve were resuspended in a small volume of Roswell Park Memorial Institute (RPMI)-1640 medium (GIBCO BRL, Breda, the Netherlands). The narrow bottom part (5 ml) of an all-glass Dounce tissue potter of 7 ml (Wheaton, Millville, NJ USA, cat.no. 357542, type B) was filled with this suspension. The pestle of the potter was gently moved up and down twice only, as described by Brandt and Eberhard (1990). The pottered suspension was poured onto an Ederol paper filter (no. 261, J.C. Binzer), placed on a small 38 µm sieve on 2 glass bars in a large Petri dish filled with RPMI. The L3-juveniles were allowed to migrate through this sieve for at least 1.5 h. The migrated number of juveniles were counted. The required number of L3-juveniles could easily be collected with a Pasteur pipette. In case the pottered suspension contained too much debris and micro-organisms the suspension was first poured into a Baermann glass with fresh RPMI 1640. The juveniles were allowed to sediment for at least 1.5 h, and after removing the supernatant they were resuspended in fresh RPMI 1640. If necessary, this sedimentation process was repeated. Sedimentation in a Baermann glass could also be an alternative way of collecting the juveniles. In that case they were counted directly after sedimentation and resuspension. The juveniles could be stored in sterile RPMI 1640 at 4°C for at least 1 week, without loss of viability.

Acknowledgements

The authors thank J.B.J.W. Cornelissen for his advice. N.S.C. Kuiper and R. Spies are thanked for typing the manuscript.

References

Boon, J.H., H. Augustijn, V.M. Cannaerts, C.J. Lokin, M.A. Machiels and F. Ollevier, 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.

Brandt, F.H., and M.L. Eberhard, 1990. *Dracunculus insignis* in ferrets: comparison of inoculation routes. *J. Parasitol.* **76**: 93-95.

De Charleroy, D., V. Cannaerts, H. Augustijn, L. Grisez, J.H. Boon and F. Ollevier, 1990a. An improved method for artificial infection of the European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea). *J. Appl. Techn.* **6**: 182-188.

De Charleroy, D., L. Grisez, K. Thomas, C. Belpaire and F. Ollevier, 1990b. The life cycle of *Anguillicola crassus*. *Dis. aquat. Org.* **8**: 77-84.

Eberhard, M.L., E. Ruiz-Tiben, and S.V. Wallace, 1988. *Dracunculus insignis*: experimental infection in the ferret, *Mustela putorius furo*. *J. Helminthology* **62**: 265-270.

Haenen, O.L.M., L. Grisez, D. De Charleroy, C. Belpaire and F. Ollevier, 1989. Experimentally induced infections of the European eel, *Anguilla anguilla* L., with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. Aquat. Org.* **7**: 97-101.

Muller, R., 1972. Maintenance of *Dracunculus medinensis* (L.) in the laboratory and observations on experimental infections. *Parasitology* **64**: 107-116.

Neumann, W., 1985. Swimmblasenparasit *Anguillicola* bei Aalen. *Fischer und Teichwirt* **11**: 322.

Van Banning, P., W. Heermans and J.A. van Willigen, 1985. *Anguillicola crassa*, een nieuwe aalparasiet in Nederlandse wateren. *Visserij* **38** (6-7): 237-240.

Chapter 8

Effects of experimental infections with different doses of *Anguillicola crassus* (Nematoda, Dracunculoidea) on European eel (*Anguilla anguilla*)

O.L.M. Haenen[a], T.A.M. van Wijngaarden[b], M.H.T. van der Heijden[c], J. Höglund[d], J.B.J.W. Cornelissen[a], L.A.M. van Leengoed[a], F.H.M. Borgsteede[a], W.B. van Muiswinkel[b]

[a] DLO-Institute for Animal Science and Health, P.O. Box 65, 8200 AB Lelystad, the Netherlands
[b] Agricultural University, Department of Experimental Animal Morphology and Cell Biology, P.O. Box 338, 6700 AH Wageningen, the Netherlands
[c] Agricultural University, Department of Fish Culture and Fisheries, P.O. Box 338, 6700 AH Wageningen, the Netherlands
[d] National Veterinary Institute, Laboratory of Parasitology, P.O. Box 7073, S-750 07 Uppsala, Sweden

(submitted for publication)

Abstract

To study the effects of various doses of *Anguillicola crassus* in primary infections parasite-free European eels were orally infected with doses of 0, 1, 5, 10, 20, or 40 third-stage (L3) larvae. The eels were either killed and examined for parasites and lesions after 56 days, or reinfected with 20 L3 larvae to study the effect of primary infection on resistance. Reinfected eels were killed and examined at day 112. Blood samples were collected weekly to determine haemoglobin (Hb) and haematocrit (Hct) levels, and the samples were examined in an enzyme-linked immunosorbent assay (ELISA) to detect antibodies against adult parasite cuticula antigen. The mean percentage of *A. crassus* recovered from eel swimbladders ranged between 14-20% at day 56 and 9-26% at day 112. At both dates, results showed that the higher the dose, the more parasites were recovered. Furthermore, the higher the dose, the more severe were lesions, such as thickening of the swimbladder, haemorrhages, congestion of blood vessels, and pigment spots in the swimbladder. However, reinfecting eels had no effect on the total number of parasites recovered, nor did it effect the severity of lesions. The levels of Hct and Hb decreased significantly in all eels, including controls, regardless of dose size or reinfection, but this was attributed to the weekly collection of blood samples. Results of the ELISA showed that the eels developed no detectable antibody response against *A. crassus*. We concluded that under experimental conditions, although eels develop pathological signs after primary and secondary infection with *A. crassus*, they do not develop an antibody response or resistance.

Introduction

The swimbladder nematode *Anguillicola crassus* (Kuwahara, Niimi and Itagaki, 1974; Moravec and Taraschewski, 1988) of Japanese eel *Anguilla japonica* and European eel *Anguilla anguilla* was recently introduced into Europe (Van Banning et al., 1985; Peters and Hartmann, 1986). Many studies have been conducted (Nagasawa et al., 1994 in press) on the pathogenesis (Hirose et al., 1976; Haenen et al., 1989; De Charleroy et al., 1990b; Haenen and Banning, 1991; Moravec et al., 1993; Thomas, 1993) and pathobiology (Boon et al., 1990a, b, c; Van Banning and Haenen, 1990; Höglund et al., 1992; Molnár et al., 1993) of this parasite. In freshwater lakes in The Netherlands the prevalence and intensity of infection among wild eels have been decreasing since 1988, and the prevalence and intensity of lesions have been decreasing since 1988 (Haenen et al., 1994a).

These observations suggest that *A.crassus* gradually has adapted to its new definite host and/or that the eels have developed some kind of resistance. The hypothesis is that young wild eels become infected soon after entering freshwater rivers and lakes, but survive the infection because they develop an acute nonspecific response, produce specific antibodies directed against the parasite in a later phase, and ultimately become resistant to reinfection.

Previous experimental infections of eels with *A. crassus* have been carried out, mainly on a qualitative basis, in order to study the parasite's life cycle and pathogenic

effects (Boon et al., 1990a,c; De Charleroy et al., 1990a). In this study we investigated quantitative and qualitative effects of induced infections of eels with well defined numbers of *A. crassus* larvae. Parasite recoveries, body weights, and swimbladder lesions were investigated. Additionally, blood samples from eels were tested for haemoglobin and haematocrit levels, and eel sera were tested for specific antibodies against cuticula antigen of adult *A. crassus* (Höglund and Pilström, 1994a in press) in an enzyme-linked immunosorbent assay. Specific binding of the eel sera to *A. crassus* antigens was characterized in Western blots.

Materials and methods

Experimental design

Eels

Table 1 shows the experimental design. Seven groups (A through G) of 12 eels weighing 80-120 g were obtained from an eel farm that is free of *A. crassus*. The eels were individually numbered (tattoo under sedation with metomidate 0.1 mg/l, Janssen Pharmaceutica B.V.), and placed in seven aquaria (A through G) of 25 l each. The water temperature during the experiment varied between 18-20°C.

Blood sampling and weighing

At day 0 (see Table 1) the eels were sedated with metomidate (see above), and a blood sample of 1 ml was taken from each eel from the caudal vein, and divided over two vacuum containers (Venoject) of 5 ml each: one with no additives for serum isolation, the other with 0.05 ml 0.38 mol/l EDTA. The untreated blood samples were allowed to clot for at least 3 h at room temperature, and then were centrifuged 10 min at 480x*g* at room temperature. The sera were stored at -20°C until they were tested in the ELISA. The EDTA treated blood samples were analyzed within 3 h after sampling for haematocrit (Hct, blood erythrocyte volume fraction = B-EVF) percentages, determined after 5 min of haematocrit microcentrifugation (Autocrit II). Haemoglobin (Hb) concentrations (mmol/l) were determined according to the method of Helleman et al., 1975. Eels were weighed (grams) on an electronic balance (Sartorius) at the start of the experiment and then again every 14 days (Table 1).

Culture of L3 larvae of Anguillicola crassus and infection

Third stage (L3) larvae of *A. crassus* were cultured by the method described by Haenen et al. (1994b). Briefly, second stage (L2) larvae isolated from naturally infected eels from the IJsselmeer were fed to freshwater copepods, collected from a bio-filter at an *A. crassus* free eel farm. Two weeks later L3 larvae were harvested from the copepods by the potter method (Haenen et al., 1994b) and counted. Two batches of L3 larvae were prepared, one for the primary infection and one for the reinfection.

The L3 larvae were suspended in 2 ml RPMI-1640 medium (Roswell Park Memorial Institute, Gibco) per well in a round bottom 48-well plate. The eels were sedated (metomidate 0.1 mg/l). The larvae suspension was drawn into a syringe with a blunt

Table 1. *Experimental design of the artificial infection of eels with L3 larvae of* ***Anguillicola crassus.***

Group	Dose at day 0/day 56 (no. L3 per eel)	Handling at day: 0	7	14	21	28	35	42	48	56	63	70	77	84	91	98	105	112
A-I*	n/n	b,w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	b,w,s
A-II	n/20	b,w	-	-	-	-	-	-	-	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
B-I	0#/0	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
B-II	0/20	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
C-I	1/n	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s								
C-II	1/20	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
D-I	5/n	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s								
D-II	5/20	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
E-I	10/n	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s								
E-II	10/20	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
F-I	20/n	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s								
F-II	20/20	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s
G-I	40/n	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s								
G-II	40/20	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w	b	b,w,s

n = no inoculation; b = blood sampling; w = weighing; s = slaughter

*) each subgroups consisted of 6 eels; until day 56 subgroups -I and -II were together in one aquarium

#) dose 0 means : orally inoculated with RPMI medium without *A. crassus* larvae

needle (bovine milk catheter) and orally administered into the oesophagus of the eels. Each well was washed once with 0.1 ml fresh RPMI-1640, and this medium was additionally administered. The wells were checked afterwards for remained L3.

Group A was not orally inoculated. Group B was orally inoculated with RPMI-1640 medium only. Group C, D, E, F, and G were inoculated with 1, 5, 10, 20, and 40 L3 larvae (Table 1).

Parasite recovery and lesions

At day 56 six eels from the groups C through G were killed by an overdose of metomidate (≥ 0.3 mg/l), weighed, bled as described for day 0, and dissected. Their swimbladders were examined macro- and microscopically (magnification 20-100x) for numbers and stages of parasites and for associated lesions (see below). The remaining six eels of the groups C to G were each orally reinfected, with a suspension of 20 L3 larvae per eel. Six eels of groups A and B were also infected with 20 L3 larvae per eel. These eels were stocked into two new aquaria. At day 112 all eels were killed and examined essentially as described for day 56.

The following lesions were noted : thickened swimbladder, haemorrhages in the swimbladder, congestion of blood vessels of the swimbladder, and pigment patches on the swimbladder. Each lesion was scored 0-4 (0 = not present, 1 to 4 = weak to severe). The mean score for each lesion per group was calculated by dividing the total score of each lesion by the number of eels examined.

Statistical analysis. The individual recoveries at day 56 and 112 (number of *A. crassus* recorded in the swimbladder divided by the number of L3 larvae administered) were analyzed with one-way analysis of variance (ANOVA), and unweighed least squares linear regression was calculated (Sokal and Rohlf, 1981). The scores of the lesions and mean changes of weight were analyzed, in relation to size of infection dose, for day 56 and 112, by using Kruskal-Wallis nonparametric test and unweighed least squares linear regression (Sokal and Rohlf, 1981). These parameters were also each compared between day 56 and 112 with the above described methods. The titers of sera from naturally infected eels were related with ANOVA to the total number of *A. crassus* per swimbladder, as above.

Preparation of antigens for the indirect ELISA and protein analysis. Cuticula antigen of adult *A. crassus* was essentially produced according to the method of Höglund and Pilström (1994a in press). Briefly, adult males and females of *A. crassus* were isolated from the swimbladders of eels caught in Dutch lakes, washed in 0.01M Phosphate buffered saline pH 7.2 (PBS) and then incubated overnight in 500 ml PBS at 4°C. The cuticula of each live adult worm was dissected with a pair of forceps, put into fresh PBS on ice, and carefully washed with PBS. The mixture was centrifuged at 1200x*g* for 10 min at room temperature, and the supernatant was removed. The pellet was ground with a glass Wheaton-Dounce tissue potter of 7 ml (Wheaton, Millville, NJ USA, cat.no. 357542, type B), mixed with fresh PBS, sonicated (MSE sonicator, England) on ice for 3 min and then centrifuged at 4000x*g* for 10 min at room temperature. The protein

concentration of the supernatant which was used as antigen was determined by the method of Lowry et al. (1951), using bovine serum albumin (BSA, Fraction V, Sigma, USA) as standard, and was stored at -70°C until use.

This method was also used essentially for the production of somatic adult female and male antigens of *A.crassus*. PBS-A (PBS (see above) supplemented with 0.1 I.U. Sodium Pennicillin G, 250 mg Streptomycin, 100,000 I.U. Nystatin (BDH Chemicals Ltd.), 50 mg TPCK (N,Tosyl L Phenyl analyl chlormethan, Merck), 25 mg TLCK (N, Tosyl L Lysyl Chlormethanhydrox chlorid, Merck) per liter) was used instead of PBS. Additionally, the adult somatic antigen suspensions were dialysed overnight against PBS as standard, and filtered through a 0.2 m filter to obtain the antigen preparations. Rotofor antigen was produced by J. Höglund (Höglund and Pilström, 1994b). Höglund cuticula antigen from adult *A. crassus* was produced by J. Höglund (Höglund and Pilström, 1994a, in press). L3 somatic antigen was produced out of L3 larvae of *A. crassus* which were isolated from infected copepods, according to the method of Haenen et al., 1994b. The production of this antigen was further essentially analogous as described above for the somatic adult female and male antigen preparations.

Sampling of positive sera from naturally infected eels. To test, whether the cuticula antigen of *A. crassus* binds to Dutch eel sera we collected sera (n=38) from naturally infected eels from two Dutch freshwater lakes, the IJsselmeer and the Markermeer. Eels were caught in May and November 1993 by the method of Haenen et al., (1994a). The sera were collected and the eels were then slaughtered and examined for *A. crassus* and related lesions, as described for the eels that were experimentally infected.

Sampling of negative eel blood. As a negative control, eel blood from uninfected eels was sampled for protein testing, with a heparinized syringe. The sample was taken from the caudal vein and put into EDTA-treated vacuum containers (described earlier). The blood sample was frozen at -20°C overnight, thawned, and mixed before further use.

Enzyme Linked Immuno Sorbent Assay (ELISA). Each well of the micro-titer plates (Greiner nr. 655061, the Netherlands) was coated with cuticula antigen (0.1 µg protein/50 µl in 0.05M carbonate buffer, pH 9.6-9.8), covered with plastic tape and incubated at 4°C overnight. The surplus of antigen was removed and the wells were blocked with 100 µl 5% w/v low fat milk powder (Semper, Sweden) in 1 mM PBS with 0.5% Tween 20 (PBS-T) for 1 h at room temperature. Between each of the following additions the plates were washed three times with PBS-T. The negative control serum (NES) was pooled serum from *A. crassus* negative eels, the infected eel serum (IES) pooled serum from Swedish naturally infected eels with *A. crassus* (Höglund and Pilström, 1994b). The other eel sera were diluted in 1% w/v milk powder in PBS-T. Twofold serial dilutions of sera were tested, starting with 1:25 (v/v) in 50 µl per well and the sera were incubated 1.5-2 h at room temperature. The monoclonal antibody WEI 2, directed against a light chain of eel immunoglobulin (Van der Heijden et al.,

1994 in press) was diluted 1:200 in 1% w/v milk powder in PBS-T, and 50 µl of this dilution was added per well and incubated 1.5 h at room temperature. Then 50 µl of the conjugate rabbit anti-mouse Ig-HRPO (P260, DAKO Immunoglobulins A/S, Denmark) diluted 1:5000 in 1% w/v milk powder in PBS-T was added per well and incubated for 1 h at room temperature. Finally, 50 µl substrate (Tetramethylbenzidine (1 mg/ml) and H_2O_2 (0.005%) in 0.1M Na-acatate and 0.1M citric acid buffer (pH 6.0)) was added to each well of the plate. Exactly 10 minutes later the reaction was stopped by adding 50 µl 0.5M H_2SO_4 per well. Plates were read with a spectrophotometer (Easyreader, SLT, Vienne) at 450 nm. The reciprocal of a serum dilution with 50% of the maximum OD of the positive control serum (IES) was determined as the titer.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins in various *A. crassus* antigen preparations were analyzed by SDS-PAGE. The antigen samples (5-10 µl) were analyzed in a Bio-Rad minigel apparatus at 15 mA constant current through a 4% acryl/bisacryl pH 8.8 stacking gel and a 13% acryl/bisacryl (29.2/0.8 w/v) pH 6.8 resolving gel by the discontinuous buffer system of Laemmli (1970). Prestained low-range molecular mass standards (5 µl)(Bio-Rad Laboratories, Veenendaal, the Netherlands, cat. no. 161-0304) were run simultaneously to determine the approximate molecular masses of samples. The gels were run at 15mA and 200V for the stacking gel and at 20mA and 200V for the resolving gel. After the run, gels were washed once in demineralized water for 5 min, and silver stained with a silver stain kit (Merck, cat.no. 5197, Darmstadt, Germany).

Western blotting. After the SDS-PAGE was completed, proteins of unstained gels were electrophoretically transferred for 1 h at 100 V in 20 mM Tris-192mM glycine-20% methanol (pH 8.3) to nitrocellulose membranes (Bio-Rad, cat.no. 170-3932, Veenendaal, the Netherlands) by using a Bio-Rad Miniblot unit (Mini Protein II Cell, Bio-Rad). Protein binding sites on the nitrocellulose membranes were blocked by overnight incubation in 5% (v/v) normal horse serum (NHS) in Tris buffered saline (TBS; 20 mM TRIS, 500 mM NaCl; pH 7.5) at 4°C. The membranes were cut into strips, which were separately incubated for 2 h at room temperature in eel sera diluted 1:10 to 1:100 (v/v) in TBS-H (TBS with 5% (v/v) NHS). Subsequently, the strips were washed in TBS, three times for 5 min at room temperature. Monoclonal WEI 2 (Van der Heijden et al., 1994 in press) diluted 1:200 (v/v) in TBS-H was added and incubated for 1h at room temperature, and then strips were again washed three times, as above. Subsequently, the strips were incubated for 1 h at room temperature in rabbit anti-mouse Ig-HRPO (P260, Dako Immunoglobulins A/S, Denmark) diluted 1:500 (v/v) in TBS-H. Finally, the strips were washed again three times in TBS (see above), and one time in demineralized water. The substrate DAB (1.5% (w/v) 3'3' diaminobenzidine in TBS) and 0.02% (v/v) H_2O_2 was added. The strips were stained for 5 min (max 10 min) at room temperature, washed in TBS, and dried.

Results

Parasite recovery. The higher the dose, the more *Anguillicola crassus* specimens (larvae and adults) were recovered per eel swimbladder (Figures 1a for day 56, and 1b for day 112). The mean number (%) of *A. crassus* specimens recovered from eel swimbladders are presented in Table 2. Statistical analysis showed, that at both days recoveries were significantly positive related to the infection dose ($P<0.05$), without a significant difference between day 56 and day 112. At day 48 one eel from group G died and at day 85 the other eels of group G-II died because of water quality problems in their tank.

When the swimbladders of the eels were examined at various days of slaughter, the following pattern of development of *A.crassus* was seen. At day 48 post infection L4

Table 2. *Mean percentages of **Anguillicola crassus** recovered from swimbladders of European eels **Anguilla anguilla** out of total number of L3 larvae administered per group after single (day 0 or day 56) or double (day 0 and 56) oral infection with different doses of the parasite.*

Group	Dose[a] at day 0/56	Mean %-age of *A. crassus* recovered		Range (%) of *A. crassus* recovered
		day 56	day 112	
A-I[b]	n/n		-[c]	-
A-II	n/20		10	0-30
B-I	0/0		-	-
B-II	0/20		26	0-45
C-I	1/n	16.7		0-100
D-I	5/n	20		0-60
E-I	10/n	16.7		10-40
F-I	20/n	14.2		0-35
G-I	40/n	14.6		2.5-30
C-II	1/20		16.7	4.8-28.6
D-II	5/20		11.3	0-32
E-II	10/20		14.4	3.3-20
F-II	20/20		8.8	0-17.5
G-II	40/20		11.7[d]	5-21.7

a) no. of L3 larvae of *A. crassus* given per eel
b) each group consisted of 6 eels
c) no parasites recovered
d) at day 48 one and at day 85 the other eels died because of water quality problems

n = no inoculation

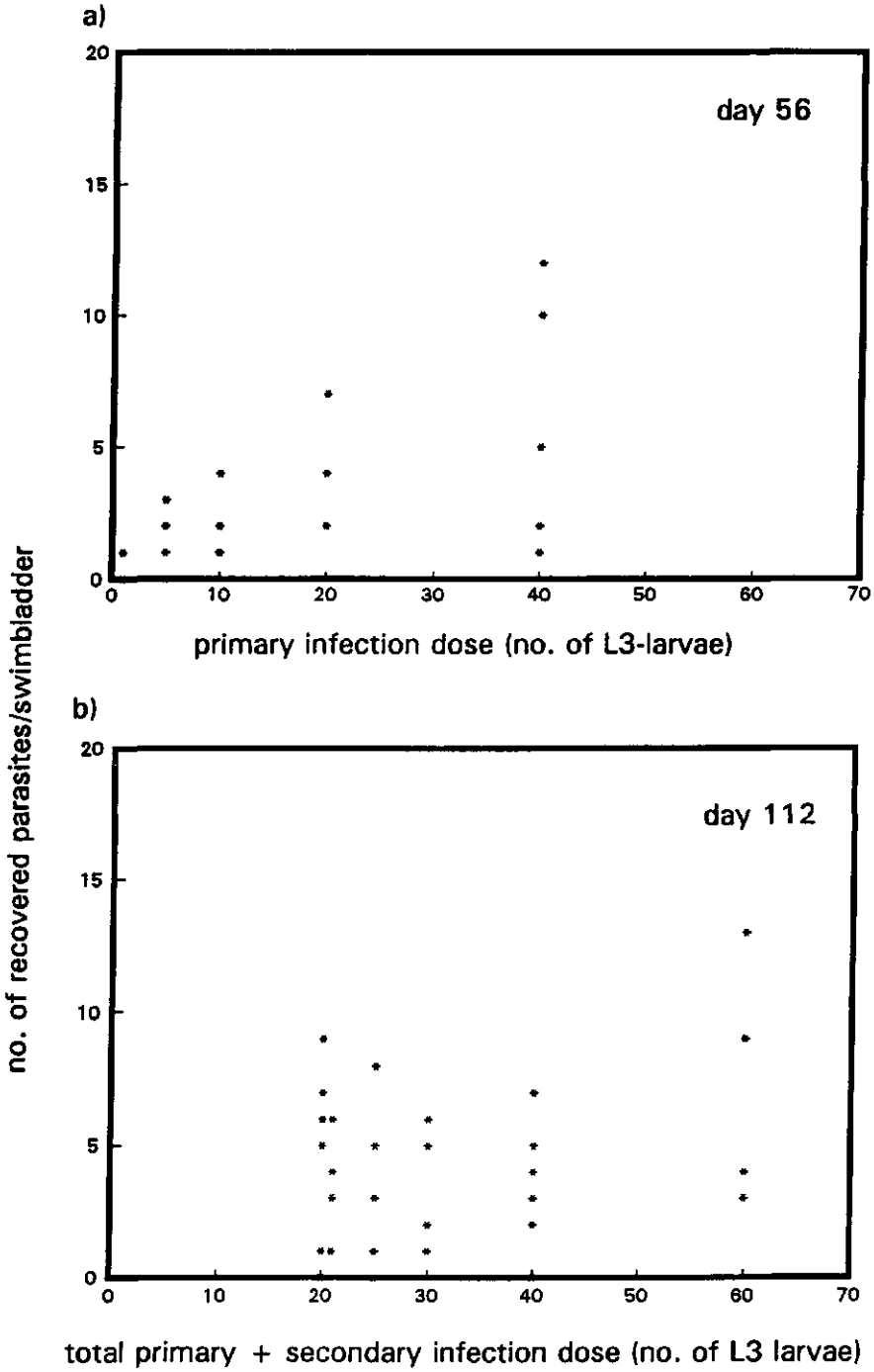


Fig. 1. Individual absolute recoveries of *Anguillicola crassus* specimens in the swimbladder of European eel related to oral infection dose with L3-larvae of *A. crassus*, at day 56 (a) and day 112 (b). The total dose in Figure b consist of a primary dose of 0, 1, 5, 10, 20, or 40 L3 at day 0 plus a secondary dose of 20 L3 at day 56.

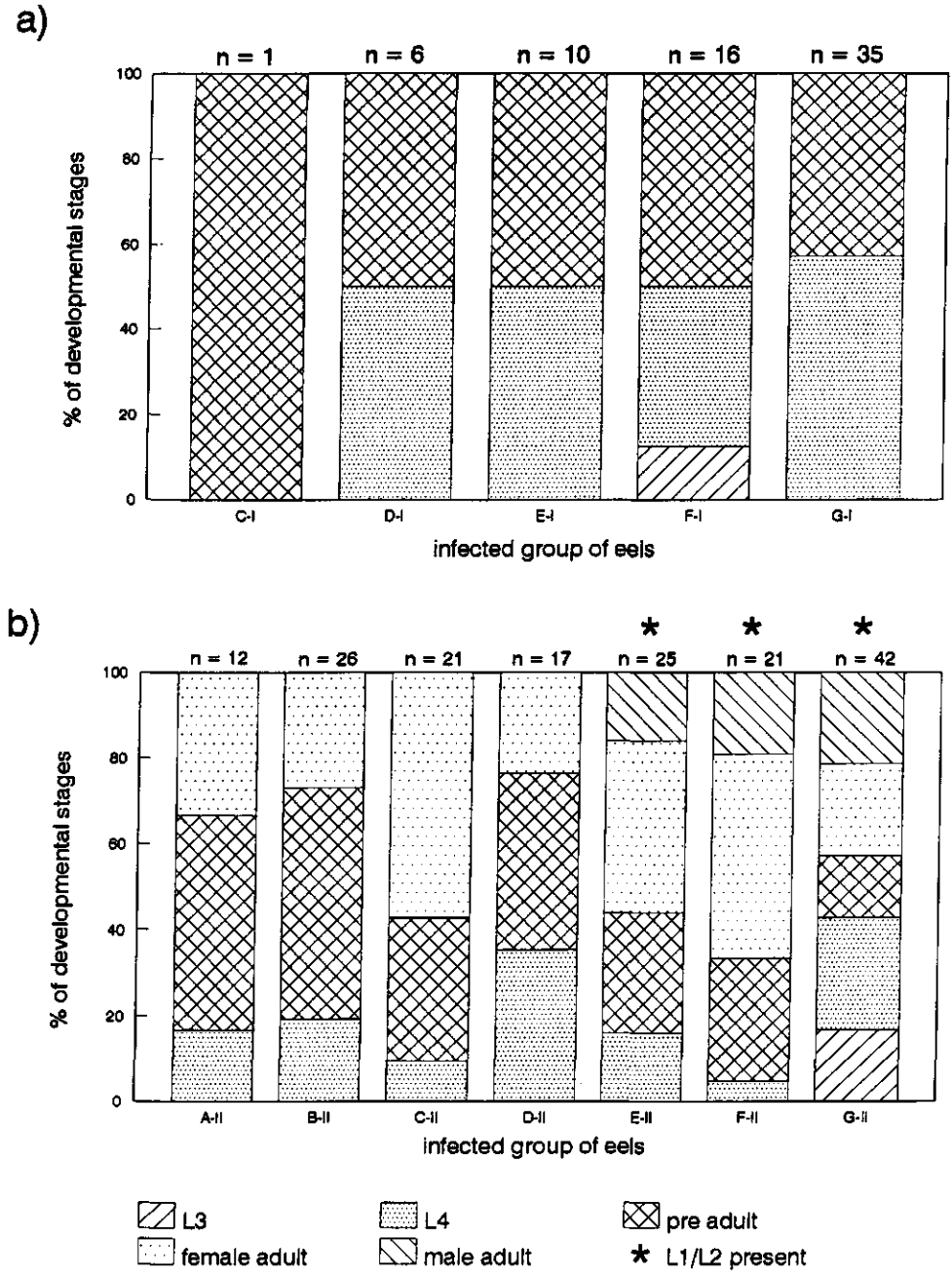


Fig. 2. Developmental stages (%) of total number of *A. crassus* specimens recovered in the swimbladder of European eel, related to total infection dose. a) at day 56 post infection; b) at day 112 post infection (G-II at day 85 post infection). Total no. of *A. crassus* recovered per group of eels is given above each bar. Group C-I (dose at day 0/day 56 = 1/-), D-I (5/-), E-I (10/-), F-I (20/-), G-I (40/-), A-II (-/20), B-II (0/20), C-II (1/20), D-II (5/20), E-II (10/20), F-II (20/20), G-II (40/20).

larvae and pre-adult *A. crassus* were detected in one eel, and at day 56 the same stages were still found. Then, at day 85 (29 days after the second infection) all stages of the parasite were detected. At day 112 the eels which had been infected only 56 days before had only L4 larvae, pre-adults and female adults. At day 112, however, the reinfected groups had also L1/L2 larvae and male adults. Figure 2 shows the pattern of developmental stages of recovered *A. crassus* specimens observed in the eel swimbladder of the various eel groups at days 56 and 112. In summary, at day 29, L4 larvae and pre- adults had developed; at day 56, female adults had developed; and at day 85, both female and male adults and L1/L2 larvae had developed.

Table 3. *Mean scores and regression analysis of swimbladder lesions in European eel after oral infection with* ***Anguillicola crassus.***

Group	Dose[b] at day 0/56	Mean score[a]			
		Thickened swim-bladder	Haemorrhages in swimbladder	Congestion of blood vessels	Pigment patches
at day 56:					
C-I[c]	1/n	0	0.2	0.2	0.2
D-I	5/n	0	0.2	0.25	0
E-I	10/n	0	0.1	0.6	0
F-I	20/n	0	0.4	0.7	0.25
G-I	40/n	0	1.2	1.1	0.3
at day 112:					
A-I	n/n	0	0.1	0.25	0
B-I	0/0	0	0	0	0
A-II	n/20	0.25	0.6	0.7	0.2
B-II	0/20	0.4	0.3	0.85	0.2
C-II	1/20	1.2	1.3	1.5	0.4
D-II	5/20	0.4	0.1	0.7	0.1
E-II	10/20	0.9	0.9	0.8	0.1
F-II	20/20	1.5	1.3	1.5	0.2
G-II[d]	40/20	0.7	2.7	2.2	0.8
Regression analysis ($P<0.05$):					
R^2		0.293	0.381	0.400	0.187
x-coefficient		0.019	0.034	0.030	0.009
intercept		0.0005	0.078	0.106	0.006

a) score 0 = no lesions, score 4 = severe lesions
b) no. of L3 larvae of *A. crassus* given per eel
c) 6 eels per group
d) at day 48 one and at day 85 the other eels died because of water quality problems

n = no inoculation

Lesions. The mean scores and the regression analysis of the four types of lesions observed per group, related to the various dosages are given in Table 3. The frequency of thickened eel swimbladders, haemorrhages in the swimbladder, congestion of blood vessels, and pigment patches was significantly correlated with the infection dose ($P<0.05$), although there were no significant differences in effects between day 56 and 112.

Blood parameters. The mean Hct and Hb at day 112 in the blood samples of the noninfected controls as percentage of those of day 0 were for group A-I (only sampled at days 0 and 112) : 108%±26.5 and 109%±20.2 (n=3 eels), for group A-II (sampled weekly from day 56) : 62%±22.1 and 68%±9.4 (n=5), and group B-I (sampled weekly from day 0) : 11%±5.3 and 23%±5.7 (n=4). At day 56 these percentages varied in the other groups between 22-61% (Hct) and 41-73% (Hb), and at day 112 between 7-38% (Hct) and 22-50% (Hb), all with very high standard deviations. These differences in Hct and Hb between the eel groups A-I, A-II, and B-I respectively were considered to rather be an effect of the frequent blood sampling than a result of the feeding activities of the parasite. Therefore, these values were not statistically analyzed for their relation with infection dose.

Body weight. All eel groups, the noninfected included, lost weight during the experiment at an average of 0.11-0.20 g per eel per day. The individual changes in weight of the eels were statistically not dependent on the infection dose, for both day 56 and day 112, nor were there any significant differences between the mean changes of weight per eel per day until day 56 or 112 ($P<0.05$).

ELISA and *A. crassus* protein analysis. The protein concentration of the cuticula antigen for the ELISA was 550 µg/ml. Figure 3 shows the extinction curve of the ELISA with the positive (IES) and negative (NES) control sera. All eel sera from the experiment had extinctions comparable with or even below the maximum of the negative control serum. It is concluded, that the experimental sera were lacking antibody activity to this antigen. The extinction values of sera from the naturally infected eels were highly related to the positive control serum (IES). Therefore, the extinction factor (extinction of naturally infected serum/extinction of IES) was taken at a certain dilution, and correlated to total number of *A. crassus* specimens per swimbladder (Figure 4). There was no significant correlation between the total number of *A. crassus* and the extinction factor ($P>0.05$).

The results of the SDS-PAGE of various *A. crassus* antigen preparations are shown in Figure 5. The cuticula antigens (lanes 1 and 6) show protein bands at about 40 kD, which are absent in the other antigens and eel blood (lane 4). A 43 kD band was found in adult *A. crassus*, eel blood, rotafor, and cuticula Höglund, but not in cuticula (lane 1) and somatic L3 (lane 7). Around 66 kD a band was found in cuticula Höglund (lane 6) and somatic L3, but not in the other lanes. A band of approximately 90 kD was found in *A. crassus* female (lane 2) only.

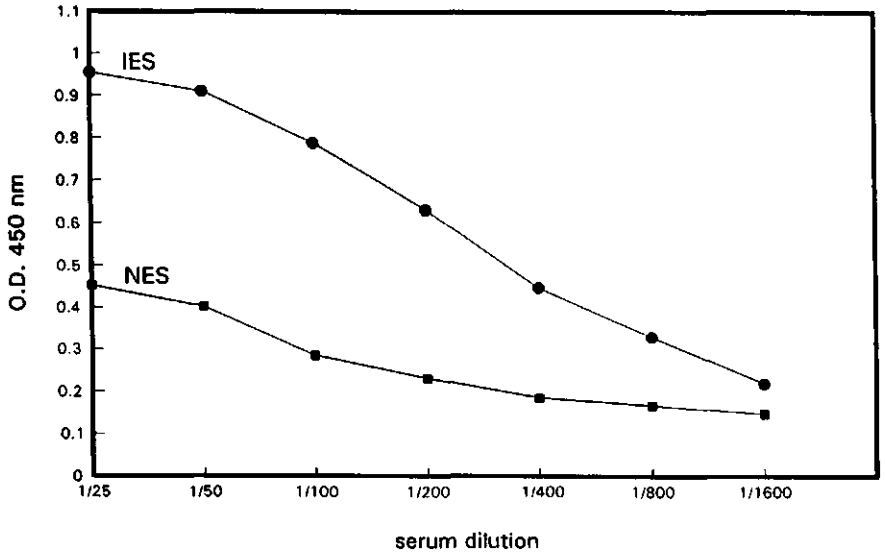


Fig. 3. ELISA control graph: Optical Density (OD) at 450 nm of control sera (NES = pooled serum from *Anguillicola crassus* negative eels, IES = pooled serum from *A. crassus* positive eels) (Höglund and Pilström, 1994b) at different serum dilutions.

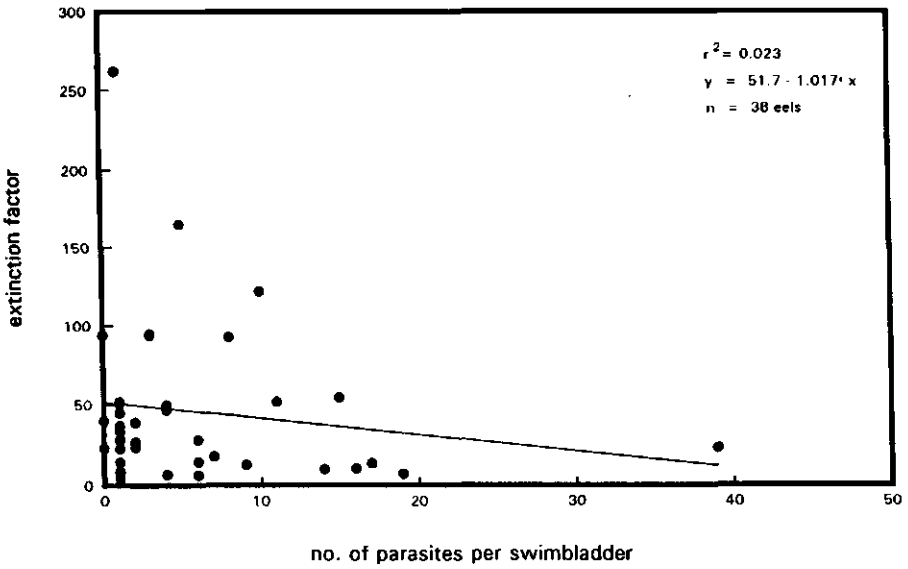


Fig. 4. Sera from naturally *A. crassus* infected Dutch eels tested in the ELISA. Extinction factor (optical density (OD) at 450 nm of serum from each naturally infected eel/OD 450 nm of IES (= positive control serum)) related to total no. of *A. crassus* found in the eels swimbladder. The regression line is shown, but the relation was not significant ($P>0.05$).

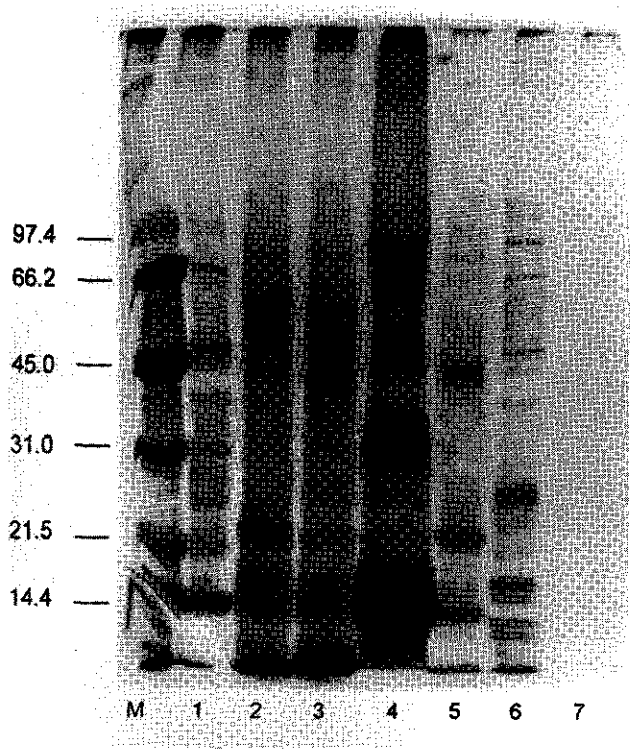


Fig. 5. SDS-PAGE electrophoresis of different antigens of *Anguillicola crassus*, silver stained. Molecular-weight markers are indicated in kD. Lane M = prestained low range standard (Bio-Rad); Lane 1 = cuticula (ELISA antigen of this study) of adults; Lane 2 = somatic female adults; Lane 3 = somatic male adults; Lane 4 = normal eel blood; Lane 5 = Höglund rotofor of adults; Lane 6 = Höglund cuticula of adults; Lane 7 = somatic antigen of L3-larvae.

The Western blots of the cuticula antigen of our ELISA with the positive control serum (IES) showed a protein band around 65 kD, which was not seen in the blot of the negative control serum (NES). However, this protein band was also seen in some of the sera from the infection experiment, which were negative in the ELISA, and in negative control serum from eels from the UK (provided by C.R. Kennedy). The control sera (NES and IES), the presamples of all eels at day 0, and other sera from the infection experiment showed a protein band of about 90-95 kD, which was also found in blots of sera from wild eels with a heavy *A. crassus* infection.

Discussion

In this study we examined the effects on European eels of various doses of L3-larvae of *Anguillicola crassus*, after oral administration. Only a few dose-effect experiments have ever been carried out with exact doses of *A. crassus* larvae. De Charleroy et al. (1990a) infected eels orally with eight L3-larvae of *A. crassus* each, isolated from common carps. They recovered a maximum of 38.2% of the larvae in the swimbladders at 28 days post infection (p.i.), whereas in some eels all eight larvae (100%) reached the swimbladder. Boon et al. (1990c) orally infected eels with a total of 5, 10, or 20 L3 larvae derived from glass eels; at 49 days p.i. they recovered a maximum of 30% of the larvae in the eels which received five larvae. Höglund and

Thomas (1992) recovered 22% from eels that had been infected with 40 L3 larvae of *A. crassus*.

In our study, we infected eels with 0 - 60 L3 larvae with low mean recoveries at day 112 p.i. of minimally 8.8% in group F-II (dose 20+20 L3-larvae) and maximally 26% in group B-II (dose 0+20 L3-larvae). The highest individual recovery was 100% in an eel infected with only one L3 larva. The recoveries in our experiment were clearly dose-related, but did not significantly differ between day 56 and day 112. We could not conclude therefore, that the eels developed specific resistance against reinfection after a primary infection. The reason for this absence of resistance may be, that the infection doses and/or the number of reinfections were too low.

The developmental stages of *A. crassus* detected at day 112 in the primary infected groups differed from those detected in eels dissected at day 56, since no adult parasites were found in the latter. We therefore concluded, that the quality of the infective L3 larvae at day 0 was less than at day 56. L3 larvae were no longer detected at day 112; apparently, these larvae developed into subsequent stages or they did not reach the swimbladder because of lack of viability or, less probable, because of aberrant migration (Haenen et al., 1989). At day 29, L4 larvae were present in eels of group G-II. This development is comparable to that found by De Charleroy et al. (1990b), who found L4 larvae 2.5 weeks p.i. at 20°C. Boon et al. (1990c) detected adult *A. crassus* 7 weeks p.i. at 25°C. Our eels were kept at 18-20°C, which explains why *A.crassus* in our eels developed more slowly to adults in 8 weeks.

Boon et al. (1990c) found a significant relation between infection dose and development of the parasite: at a higher dose the parasites were further developed than at a lower dose. These findings could partially be confirmed in this study; no relation between the various infection doses and the developmental stages was seen at day 56 (Fig. 2a). However, at days 85 (G-II) and 112 (other groups) (Fig. 2b) male adult *A. crassus* and L1/L2 larvae were found only in the swimbladders of the most heavily infected eels. This could have been a matter of chance, because in most wild infected eels in The Netherlands, there are far more female adult *A. crassus* than male (unpublished observations).

In our experiment, lesions were positively correlated with infection dose, but independent of reinfection of the eels. This supports the finding, that eels did not develop resistance against the parasite after a single infection.

Boon et al. (1990a) showed that Hct values decreased with higher infection dosages (5, 10, and 20 L3 larvae per eel). In wild eel, however, Boon et al. (1989) found no relation between parasite burden of the swimbladder and Hct. Höglund et al. (1992) found no correlation between infection with *A. crassus* and Hct, but detected slightly lower Hb concentrations in relatively heavy infected eels. In our experiment, Hct and Hb concentrations decreased dramatically because of the weekly blood sampling. Consequently, because sampling blood probably had more influence on these values than the infection, it was not possible to interprete these values related to experimental induced infection. Another problem was the high variance in these values within an eel population. This means, that bigger group sizes should be used in further experiments.

In this study all eels stopped eating entirely during the whole experiment, probably because of the stress of the weekly blood sampling. No dose-related changes in weight could be found in our experiments, a finding that is in contrast to findings of Boon et al. (1990c), who found dose-dependent loss of weight in the eels.

Fish show various nonspecific and immunological responses to parasitic infections (Van Muiswinkel and Jagt, 1984; Evans and Gratzek, 1989). Often, the nonspecific response is important (Hamers et al., 1992), sometimes the humoral response (McArthur, 1978; Whyte et al., 1987) or both (Whyte et al., 1989; Sharp et al., 1991). Also, a nonspecific tissue response, mediated by fibroblasts, sometimes resulting in capsule formation around the parasite (Van Muiswinkel and Jagt, 1984) occurs. A humoral response of the eel against *A. crassus* has already been demonstrated by Buchmann et al., (1991) and Höglund and Pilström (1994a in press, 1994b). This could be verified by ELISA for the IES and sera from naturally infected eels, but not for eel sera from the experiment.

Various explanations can be offered for these differences. First, we used adult somatic cuticula antigen of *A. crassus*, not whole adult antigen, because this antigen gave aspecific binding with the monoclonal anti eel immunoglobulin (Ig). However, Buchmann et al. (1991) found no aspecific binding of whole worm antigen with rabbit anti-eel serum. Perhaps our cuticula antigen was not the best choice, because L3 larvae in natural infections migrate through the tissues and are in direct contact with the animal's immune system, more than adult parasites, which just lie in the swimbladder lumen and suck eel blood. However, it is moretheless remarkable that many sera from infected wild eels were strongly positive in the ELISA. Moreover, Höglund and Pilström (1994a in press) proved the value of the cuticula antigen for their ELISA.

A second reason may be the fact, that we used infection dosages that were too low and/or the number of reinfections were too low to evoke a humoral response to the parasite. Wild eels probably have been reinfected many times and with probably a much higher total number of L3 larvae than we used in our experiment.

A third reason may be, that the production of humoral antibodies against *A. crassus* is suppressed by stress under experimental conditions. Stress may suppress the immune system of fish (Ellis, 1981). Naturally infected eels live under less stressful conditions than experimental eels and their humoral response is therefore less suppressed. No correlation between titer and number of *A. crassus* per eel was found in naturally infected eels. This was also found by Höglund (unpublished observations) for Swedish wild eels. An interpretation of these observations is difficult, because the history of naturally infected eels is not known.

The PAGE analysis of *A. crassus* antigen preparations showed protein bands with an approximate molecular mass of 43 kD in adult *A. crassus*, but also in normal eel blood. This means that the content of this band was probably not a specific epitope of *A. crassus*, in contradiction to findings of Buchmann et al. (1991). The 40 kD and 66 kD bands present in cuticula antigen and L3 somatic antigen are interesting, because they were not found in whole adults, nor in uninfected eel blood. However, Höglund and Pilström (1994a in press) found this 66 kD band also in whole worm homogenate of

A. crassus. The 90 kD protein band in female adult *A. crassus* could correspond with the 90 kD protein band found by Polzer and Taraschewski (1993) in adults (an aspartyl proteinase). More detailed research on *A. crassus* antigen preparations is needed to determine the important epitopes.

The protein bands found in our Western blots of 65 and 90-95 kD did not proof the presence of antibodies against *A. crassus*, because they were also detected in negative eel sera. They could possibly correspond with the protein bands found by Buchmann et al. (1991) of 67 and 94 kD respectively. These authors stated that at least the 94 kD band is not characteristic for antibodies against *A. crassus*. The 43 kD band found by them was also found in our blots. Unfortunately, this band was not specific for *A. crassus* infection, because it was also found in uninfected eel blood.

Because no specific antibodies against cuticula antigen of *A. crassus* were detected in our experiment, we do not support the hypothesis that eels develop a humoral response against *A. crassus* under experimental conditions. However, because the extinctions of sera from naturally infected eels in the ELISA were high, the hypothesis is still open for further research. Furthermore, we still do not know, to what extent the nonspecific response to migrating L3 larvae of *A. crassus* is important. The high number of inflammatory cells (mainly phagocytes) that gather around a migrating L3 larva 5-17 h post infection (Haenen et al., 1989, chapter 5 in this thesis) suggests, that this type of response might be important. This aspect should receive more attention in further studies.

Conclusion

Our experimentally induced infections of European eels with *A. crassus* revealed, that infection dosages up to 60 L3-larvae per eel, with or without a reinfection, caused lesions of the eel swimbladder, that were positively correlated with the size of infection dose. There was no evidence that the eels develop resistance against the parasite after infection, maybe as a result of the stressful experimental conditions. There was no effect of the infection dose on the weight of the eels, and the effect of the infection dose on Hct and Hb values was not detectable, due to the repeated blood sampling. The results of this study do not support the hypothesis that eels develop resistance and a humoral response against the parasite after a primary infection with *A. crassus*. However, naturally infected eels showed a high titer to cuticula antigen of *A. crassus*, and we cannot exclude the possibility that these eels do develop resistance against the parasite.

Acknowledgements

J.M. Broekhuijsen-Davies, H.P.A. Dorsman, C.P.H. Gaasenbeek, A.M. Ham-Hoffies, K. Hoebe, A. Hoogendoorn, S.H.M. Jeurissen, M.C.M. de Jong, N.S.C. Kuiper, J.N. van der Linden, J.W. Meijer and R. Spies are thanked for assisting in the experiments, testing, and preparation of the manuscript. J.H. Boon and J.H.W.M. Rombout are fully acknowledged for providing the monoclonals used in our ELISA and blots. We are also

thankful to K. Buchmann for providing us with rabbit-anti-eel Ig which was used in the pilot studies for our ELISA, and to C.R. Kennedy, who provided sera from *A. crassus*-negative eels from the UK.

References

Boon, J.H., Lokin, C.J.A., Ceusters, R. & Ollevier, F. (1989) Some properties of the blood of European eel (*Anguilla anguilla*) and the possible relationship with *Anguillicola crassus* infestations. *Aquaculture* **76** : 203-208.

Boon, J.H., Cannaerts, V.M., Augustijn, H., Machiels, M.A., De Charleroy, D. & Ollevier, F., 1990a. The effect of different infection levels with infective larvae of *Anguillicola crassus* on haematological parameters of European eel (*Anguilla anguilla*). *Aquaculture* **87** : 243-253.

Boon, J.H., Zuxu, Y. & Booms, G.H.R. (1990b) Effects of *Anguillicola crassus* and *Trypanosoma granulosum* infections on peripheral blood cells of European eel (*Anguilla anguilla* L.). *Bull. Eur. Ass. Fish Pathol.* **10(5)** : 143-145.

Boon, J.H., Augustijn, H., Cannaerts, V.M., Lokin, C.J., Machiels, M.A. & Ollevier, F. (1990c) 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.

Buchmann, K., Pedersen, L.O. and Glamann, J. (1991) Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Dis. aquat. Org.* **12** : 55-57.

De Charleroy, D., Cannaerts, V., Augustijn, H., Grisez, L., Boon, J.H. & Ollevier, F. (1990a) An improved method for artificial infection of the European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea). *J. Appl. Technol.* **6** : 182-188.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. & Ollevier, F. (1990b) The life cycle of *Anguillicola crassus*. *Dis. aquat. Org.* **8** : 77-84.

Ellis, A.E. (1981) Stress and the modulation of defence mechanisms in fish. In : Stress and Fish, A.D. Pickering (Editor), London, chapter 7 : 147-169.

Evans, D.L. & Gratzek, J.B. (1989) Immune defense mechanisms in fish to protozoan and helminth infections. *Amer. Zool.* **29** : 409-418.

Haenen, O.L.M. & Van Banning, P. (1991) Experimental transmission of *Anguillicola crassus* (Nematoda, Dracunculoidea) larvae from infected prey fish to the eel *Anguilla anguilla*. *Aquaculture* **92** : 115-119.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C. & Ollevier, F. (1989) Experimentally induced infections of the European eel, *Anguilla anguilla* L., with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. aquat. Org.* **7** : 97-101.

Haenen, O.L.M., Van Banning, P. & Dekker, W. (1994a) Infection of eel *Anguilla anguilla* (L.) and smelt *Osmerus eperlanus* (L.) with *Anguillicola crassus* (Nematoda, Dracunculoidea) in the Netherlands from 1986-1992. *Aquaculture* **126** : 219-229.

Haenen, O.L.M., Van Wijngaarden, T.A.M. & Borgsteede, F.H.M. (1994b) An improved method for the production of infective third-stage juveniles of *Anguillicola crassus*. Short communication, *Aquaculture* **123** : 163-165.

Hamers, R., Lehmann, J., Stürenberg, F.-J. & Taraschewski, H. (1992) In vitro study of the migratory and adherent responses of fish leucocytes to the eel-pathogenic acanthocephalan *Paratenuisentis ambiguus* (van Cleave, 1921) Bullack et Samuel, 1975 (Eoacanthocephala: Tenuisentidae). *Fish Shellfish Immunol.* **2** : 43-51.

Helleman, P.W., Sixma, J.J., van der Plas, P.M. & Dudok de Wit, C. (1975) Hematology. Agon Elsevier, Amsterdam, 287 pp.

Hirose, H., Sekino, T. & Egusa, S. (1976) Notes on the egg deposition, larval migration and intermediate host of the nematode *Anguillicola crassa* parasitic in the swimbladders of eels. *Fish Pathology* **11** : 27-31 (in Japanese with English summary).

Höglund, J., Andersson, J. & Hardig, J. (1992) Haematological responses in the European eel, *Anguilla anguilla* L., to sublethal infestation by *Anguillicola crassus* in a thermal effluent of the Swedish Baltic. *J. Fish Dis.* **15** : 507-514.

Höglund, J. & Pilström, L. (1994a, in press) Mechanical isolation and characterization of antigens for adult *Anguillicola crassus*. *Fish Shellfish Immunol.*, in press.

Höglund, J. & Pilström, L. (1994b) Purification of adult *Anguillicola crassus* whole-worm extract antigens for detection of specific antibodies in serum from the European eel (*Anguilla anguilla*) by ELISA. *Fish Shellfish Immunol.* **4** : 311-319.

Höglund, J. & Thomas, K. (1992) The black goby *Gobius niger* as a potential paratenic host for the parasitic nematode *Anguillicola crassus* in a thermal effluent of the Baltic. *Dis. aquat. Org.* **13** : 175-180.

Kuwahara, A., Niimi, A. & Itagaki, H. (1974) Studies on nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Phimetridae, Anguillicolidae). *Jap. J. Parasit.* **23 (5)** : 275-279.

Laemmli, E.K. (1970) Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature* **227** : 680-685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** : 265-275.

McArthur, C.P. (1978) Humoral antibody production by New Zealand eels, against the intestinal trematode *Telogaster opisthorchis* Macfarlane, 1945. *J. Fish Dis.* **1, 4** : 377-387.

Molnár, K., Baska, F., Csaba, Gy., Glávits, R. & Székely, Cs. (1993) Pathological and histopathological studies of the swimbladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Dis. aquat. Org.* **15** : 41-50.

Moravec, F., Di Cave, D., Orecchia, P. & Paggi, L. (1993) Studies on the development of *Anguillicola crassus* Kuwahara, Niimi, and Itagaki, 1974 (Nematoda: Dracunculoidea) in the intermediate host. *Folia Parasitol.* **40** : 39-48. Erratum in *Folia Parasitol.* (1993), **40** : 160.

Moravec, F. & Taraschewski, H. (1988) Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda, Anguillicolidae) of the swimbladder of eels, including descriptions of two new species *A. novazelandiae* sp.n. and *A. papernae* sp.n.. *Folia Parasitol.* **35** : 125-146.

Nagasawa, K., Kim, Y.-G. & Hirose, H. (1994, in press) *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.* 1994, in press.

Peters, G. & Hartmann, F. (1986) *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. *Dis. aquat. Org.* **1** : 229-230.

Polzer, M. & Taraschewski, H. (1993) Identification and characterization of the proteolytic enzymes in the developmental stages of the eel-pathogenic nematode *Anguillicola crassus*. *Parasitol. Res.* **79** : 24-27.

Sharp, G.J.E., Pike, A.W. & Secombes, C.J. (1991) Leucocyte migration in rainbow trout (*Oncorhynchus mykiss* [Waldbaum]): optimization of migration conditions and responses to host and pathogen (*Diphyllobothrium dendriticum* [Nitzsch]) derived chemoattractants. *Dev. Compar. Immunol.* **15** : 295-305.

Sokal, R.R. & Rohlf, F.J. (1981) Biometry. The principles and practice of statistics in biological research. W.H. Freeman and Company (Editors), New York, 2nd ed., 859 pp.

Thomas, K. (1993) The life cycle of the eel parasite *Anguillicola crassus* (Nematoda: Dracunculoidea). Thesis Katholieke Universiteit Leuven, België, Faculteit Wetenschappen, Departement Biologie, Laboratorium voor Ecologie en Aquacultuur, 157 pp.

Van Banning, P. & Haenen, O.L.M. (1990) Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In: F.O Perkins & T.C. Cheng (Editors), Pathology in marine science, Academic Press, inc., New York. Proc. 3rd Int. Coll. on Path. in Marine Aquaculture, Gloucester Point, Virginia, Oct 2-6, 1988: 317-330.

Van Banning, P., Heermans, W. & Van Willigen, J.A. (1985) *Anguillicola crassa*, een nieuwe aalparasiet in Nederlandse wateren. *Visserij* **38** (6-7) : 237-240.

Van der Heijden, M.H.T., Rooijakkers, J.B.M.A., Booms, G.H.R., Rombout, J.H.W.M. & Boon, J.H. (1994, in press) Production, characterization and applicability of monoclonal antibodies to European eel (*Anguilla anguilla* L., 1758) immunoglobulin. *Vet. Immunol. Immunopathol.*, in press.

Van Muiswinkel, W.B. & Jagt, L.P. (1984) Host-parasite relationships in fish and other ectothermic vertebrates. *Dev. Comp. Immunol.* Suppl. **3** : 205-208.

Whyte, S.K., Allan, J.C., Secombes, C.J. & Chappell, L.H. (1987) Cercariae and diplostomules of *Diplostomum spathaceum* (Digenea) elicit an immune response in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **31** Suppl. A: 185-190.

Whyte, S.K., Chappell, L.H. & Secombes, C.J. (1989) Cytotoxic reactions of rainbow trout, *Salmo gairdneri* Richardson, macrophages for larvae of the eye fluke *Diplostomum spathaceum* (Digenea). *J. Fish Biol.* **35** : 333-345.

Chapter 9

General Discussion

General Discussion

Before the early 1980s only papers from Southeast Asia were published about *Anguillicola crassus* (Kuwahara et al., 1974; Egusa, 1979), a common swimbladder parasite of Japanese eel *Anguilla japonica*. In Southeast Asia this nematode was regarded as an unimportant low-pathogenic parasite. However, after its introduction in the early 1980s into European eel *A. anguilla* populations, the parasite caused heavy infections in European eels and spread quickly throughout Europe. Only recently infections of this parasite have begun to decrease. However, for eel producing countries, such as The Netherlands, Belgium, Denmark, and Germany, the spread of this highly pathogenic parasite had serious economic impact, because it caused clearly visible severe internal lesions, which at eel farms became secondary infected with pathogenic bacteria, which killed the eels (Liewes and Schaminee-Main, 1987; Van Banning and Haenen, 1990).

This thesis described various aspects of *A. crassus* infection of wild fish in the years after introduction of the parasite into The Netherlands. Although Dutch eels already had a few parasite species (chapter 1) the swimbladder was obviously a not yet used niche. So, *A. crassus* became a successful parasite of eels in the freshwater lakes of The Netherlands. Bauer (1991) has shown, that parasites that are transported from one habitat to another can successfully acclimatize and establish themselves, if specific intermediate hosts, which are necessary in the life cyle of the parasite are available. In case of *A. crassus*, many different freshwater copepod species act as intermediate hosts, and in addition, eels prey on some paratenic hosts of *A. crassus*. In this respect, the fast establishment of *A. crassus* in Europe was not surprising.

Life cycle of *Anguillicola crassus*. The life cycle of *A. crassus* in European eel followed the standard path via copepods as intermediate hosts, as described for Japanese eels and which is common for Dracunculoid nematodes (Muller, 1971). In The Netherlands there are many copepod/cyclopoid species (De Nie et al., 1980), which may act as intermediate hosts. Moreover, many small freshwater fish species other than eel eat these copepods and thereby are infected with the third larval stage of *A. crassus* (chapter 3 and 4). These fish species act as facultative intermediate hosts, so called reservoir hosts or paratenic hosts (Baruš and Ryšavy, 1973), in which *A. crassus* larvae do not reach the pre-adult stage, for example in smelt and zander. Some fish species act as paradefinite hosts (C.R. Kennedy, pers. comm., 1993), in which the larvae become at least pre-adult, for example in ruffe, three-spined stickleback, and perch. It is not known whether the transmission of *A. crassus* to eels through other fish species is also important in Japan, as no literature was found on this phenomenon. Nonetheless, it enhanced the spreading of the parasite in Europe, since the third stage larvae of *A. crassus* can survive for up to one year in some paratenic host species (Kennedy, 1993). What factors determine the ultimate development of *A. crassus* in

paratenic hosts is not known, but physiological circumstances of the host may play an important role.

The impact of different abiotic (like temperature, weather, light intensity) and biotic factors (like population size of paratenic hosts and copepods) during the years studied is not known. However, the fact that the infection in small eels remained severe over the period 1986-1992 proves that the number of infective larvae of *A. crassus* in copepods of fresh waters was not a determining factor. Because of the intensive fishing activities in the Dutch freshwater lakes there are far more small eels than larger eels in these waters. Together with the decrease in intensity and prevalence of infection in larger eels the number of L2 larvae of *A. crassus* released by the small eels will be much higher than those released by larger eels. Therefore, it is obvious that the infection pressure of these *A. crassus* larvae via infected copepods to eels and other freshwater fish will not decrease very much on the short term.

The life cycle of *A. crassus* in Japanese eel was reported to last one year (Egusa, 1979; Puqin and Yuru, 1980). In The Netherlands under experimental conditions at 20°C the life cycle took about four months (chapter 5) and sometimes only two months (De Charleroy et al., 1990). The climate of Japan is in general colder and more diverse than that of The Netherlands: in January mean temperatures are in Japan -12 (north) to 10°C (south) and in The Netherlands -1 to 5°C; in July mean temperatures are in Japan 10 (north) to 27°C (south) and in The Netherlands 15 to 21°C. These differences could at least in the northern parts of Japan cause a longer duration of the life cycle of *A. crassus* in naturally infected eels compared to those in The Netherlands. It would be interesting, to test the duration of the life cycle of *A. crassus* in Japanese eels under laboratory circumstances at 20°C.

Pathological changes. The infection of eels of various sizes with *A. crassus* in The Netherlands was severe in the first four to five years after introduction of the parasite (chapter 2 and 6) to the naive eel population, reaching a maximum in prevalence and intensity between 1987 and 1989. After this time, prevalence and intensity decreased in larger eels (23-34 cm), but not in small eels (<18 cm). Molnár et al. (1994) also detected a decreasing prevalence and intensity of eels (>20 cm) with *A. crassus* in Lake Balaton (Hungary) since its introduction in 1990 (Székely et al., 1991) and suggests that this decrease is caused by increased resistance of the eels against the parasite. Egusa (1979) suggested that Japanese eels have acquired resistance to the parasite through a long history of contact with the parasite. A similar development could take place on the long term in European eel in Europe. Small eels would then continue to show a severe infection with *A. crassus* and build up resistance. Unfortunately, no data are present about how severe small eels from Japan are infected nowadays.

The European eels showed pathological changes (chapter 1 and 6), such as haemorrhages, congestion of blood vessels, and secondary inflammations. Moreover, swimbladders walls thickened as many layers of connective tissue, sometimes containing dead parasites, formed during infection. This severe fibrosis sometimes resulted in an airless swimbladder. Moreover, at eel farms, heavily infected

swimbladders often became infected additionally with bacteria, resulting in mortalities of eel.

During the course of the study, we noted that larger eels (23-34 cm, chapter 6) tended to a less severe infection with *A. crassus*. Therefore, the question arose, as to whether eels develop a mechanical and/or immunological resistance against *A. crassus*. European eels are known to build up some resistance against gill monogeneans (Slotved and Buchmann, 1993).

Lesions. The fibrosis that develops in the swimbladder, which is a tissue response to the parasite, and which is mediated by fibroblasts (Van Muiswinkel and Jagt, 1984), might be a mechanical barrier for migrating third stage (L3) *A. crassus* larvae (Hartmann, 1994). Very rarely, we detected what appeared to be a new swimbladder growing out of this severe fibrotic swimbladder complex (own findings, unpublished). If eels can generate a new swimbladder, this would explain why during the years of the study more larger eels had a rather normal swimbladder, containing only a few *A. crassus*, whereas the small eels (<18 cm) still were heavily infected and had fibrotic swimbladders. These observations indicate that the eels seemed to be able to restore their swimbladder and to remove fibrotic tissue containing dead encapsulated parasites. Haemorrages, dilation of blood vessels, and lesions, such as fibrosis, and pigment spots, were more severe at higher infection doses (chapter 8). However, the lesions were not dependent on whether the eels were primed or not. In other words, no increase of mechanical resistance after priming could be found. It is more likely that the total (primary and secondary) infection dose will determine the degree of fibrosis.

Immunological resistance. Several authors have suggested that eels develop immunological resistance against parasites (see also chapter 8). This type of resistance can result from a nonspecific response or a specific humoral response to the parasite, or both, in combination with complement activation (Woo, 1992).

Various studies describe nonspecific responses of fish against metazoan parasites (Davydov, 1978; Hoole and Arme, 1983). The role of the nonspecific response was not studied in this thesis. However, many mononuclear phagocytes were observed around migrating L3-larvae of *A. crassus* (Chapter 5, Fig. 3, and Chapter 6, Fig. 2) perhaps indicating a nonspecific acute response. Hartmann (1994) detected significant increased concentrations of superoxid anions in eel blood of infected eels, which would be a result of increased phagocytosis activity of the innate immune system as a reaction to the parasite. Molnár and Moravec (1994) detected macrophages adherent to the body surface of L3-larvae of *Daniconema anguillae* (Nematoda, Dracunculoidea) in the fin of an eel. We detected no white blood cells attached to *A. crassus*. This means that it is still unclear. However, it would be interesting to use the methods of Hamers et al. (1992), who studied the in vitro migratory and adherent responses of eel leucocytes to the eel-pathogenic acanthocephalan *Paratenuisentis ambiguus*, to study if L3-larvae of *A. crassus* can be inactivated by eel leucocytes.

Although fish are known to produce specific antibodies against metazoan parasites (Balakhnin and Davydov, 1988; Linnik et al., 1988; Evans and Gratzek, 1989), no clear

humoral response against *A. crassus* was detected in the eels that were experimentally infected (chapter 8). We orally infected eels with a maximum of 40 L3-larvae each, and challenged them with 20 L3-larvae each, but the sera, which were tested in our ELISA, were all negative during the 4-month sampling period. This indicated that the infection level was too low and/or the number of reinfections were too low to induce a specific immune response against the cuticula antigen of adult *A. crassus* that we used. Priming eels had no effect on the specific immune response. The maximum number of *A. crassus* recovered in the eel swimbladders that received 60 larvae each was only 13. Perhaps, much heavier and more frequent infections are needed with perhaps much longer periods of sampling before we can answer the question, if specific antibodies against *A. crassus* play a role. The Western blots showed no protein band indicating a specific immune response.

The fact that some sera from infected eels taken from the IJsselmeer showed high titers suggests that a humoral response may develop after reinfections. Similar findings were reported by Buchmann et al. (1991) and Höglund and Pilström (1994), who reported humoral responses in eel against *A. crassus*. However, whether the titers in our studies are truly specific for *A. crassus* is difficult to say. Only few eel parasite species were found in Dutch freshwater eels (see chapter 1). Therefore, cross-reactions seem unlikely or unimportant. Another explanation for the high titers in naturally infected eels might be that these eels are not immunologically suppressed by stress (Ellis, 1981). Handling stress may have played a role in the experimental eels, which may have caused an adverse effect on the immunological response.

It is known that maternal antibodies can be transferred, to fish eggs in some fish species of the genus *Tilapia* (Avtalion and Mor, 1992). The role of the possible transfer of maternal antibodies in the defence of eels against *A. crassus* is unlikely, however, since glass eels are already two years old when they arrive in the freshwaters of Europe.

Probably, eels have both the nonspecific and humoral response against *A. crassus*. Mackenzie (1987) gave a general description of immune responses against the human dracunculoid nematode *Dracunculus medinensis* in humans : activated macrophages engulfed larvae, suggesting that these cells may be involved in the killing of the larvae. Granulocytes infiltrated the area and in the beginning of the infection there were numerous eosinophils scattered throughout the lesions. Furthermore, neutrophils were detected next to the adult worm in loose areas. Immunoglobulin levels rose significantly, as did the levels of antibodies recognizing crude parasite antigen preparations. Evans and Gratzek (1989) mention that IgM-like antibodies were formed against metazoic parasites, like *Diplostomum spathaceum* (digenic trematode) in rainbow trout (*Oncorhynchus mykiss*). Szalai et al. (1988) suggested that the quillback (*Carpiodes cyprinus*), infected with *Neoechinorhynchus carpiodi* (Acanthocephala) has both a specific and a nonspecific response to the parasite.

Outlook for the future. Today, in the second decade after the introduction of *A. crassus* into The Netherlands, the infection intensity and lesions of *A. crassus* in bigger eels are declining, whereas the infection is still severe in small eels. It is difficult to

estimate, when the *A. crassus* infection in European eels in Europe will be stabilized like that in Japanese eels in Japan.

There are very many biotic and abiotic factors in the fresh waters which vary each year. Therefore, it is difficult to know whether the infection now affecting eels in Portugal and Greece will follow a similar pattern as the one in The Netherlands. For the eel farms at least, the problem could be solved : by buying uninfected glass eels or stocking eels, they protect themselves against *A. crassus.* This solution is also environmentally friendly, because no longer chemical treatments against the parasite are needed.

Conlusions

Epidemiology. Infection with the nematode *A. crassus* has developed very rapidly in European eels in The Netherlands with maximum prevalences in 1987-1988 and maximum infection intensities in 1988-1989 in the IJsselmeer and Markermeer. Larval stages of *A. crassus* were found in freshwater smelt *Osmerus eperlanus,* ruffe *Gymnocephalus cernuus,* perch *Perca fluviatilis,* zander *Stizostedion lucioperca,* three-spined stickleback *Gasterosteus aculeatus,* and pre-adult *A. crassus* in ruffe, perch, and three-spined stickleback.

Pathogenesis. The life cycle of *A. crassus* in European eel via copepods as intermediate hosts took more than 4 months at 20°C. At 17h post oral infection L3-larvae had reached the swimbladder; the L4-stadium was seen 3 month post infection (p.i.) and pre adults were seen 4 months p.i. in the same organ. Occasionally, aberrant migrating larvae were found. The indirect transmission of *A. crassus* to eels via oral administration of infected swimbladders of freshwater smelt and ruffe resulted in infected eels with adult *A. crassus* in their swimbladder. A method to produce vivid and countable larvae for the experimentally induced infections was developed.

Pathobiology and response. *A. crassus* caused acute pathological changes in the swimbladder of the European eel, like haemorrhages, dilation of blood vessels, fibrosis, and pigment spots, In the IJsselmeer and Markermeer the prevalence, intensity, and lesions related to the infection in larger eels (23-34 cm) decreased after 1988-1989; in small eels (<18 cm) these parameters were severe and did not change from 1986-1992. Larger eels (23-34 cm) from the Waddenzee showed a decrease of infection intensity and percentage of fibrotic swimbladders after 1987-1988, but the prevalence was high from 1987-1990. The pathological changes in eels could be generated with experimentally induced infections, and were dose dependent. However, they were not dependent on priming. No specific antibodies could be found in experimentally infected eels with up to 60 larvae/eel during a 4 month experiment. The experimentally infected eels showed no specific resistance against the parasite after priming. However, phagocytic cells were found around migrating L3-larvae, which suggests an innate defense of the host against the parasite. Dutch wild eels

showed a titer against the somatic cuticula antigen of adult *A. crassus*, which was not significantly related to number of parasites per swimbladder.

A. crassus infections in The Netherlands are still severe in small eels (<18 cm), but the related prevalences, intensities, and lesions are slightly decreasing in larger eels (23-34 cm). In the course of time, prevalences, intensities and lesions related to *A. crassus* infections have stabilized in larger eels and will probably continue to do so, because of some form of resistance to the parasite in these eels. Eel farms no longer have problems with this parasite because they use uninfected eels to stock their farms.

References

Avtalion, R.R. & Mor, A. (1992) Monomeric IgM is transferred from mother to egg in Tilapias. *The Israeli Journal of Aquaculture, Bamidgeh* **44(3)** : 93-98.

Balakhnin, I.A. & Davydov, D.N. (1988) Protective effect of natural antibodies against parasites in yearling carp. *Gidrobiologicheskii-Zhurnal* **24, 1** : 39-43 (in Russian).

Baruš, V. & Ryšavy, B. (1973) Forms and prevalence of reservoir habitationism in Nematoda. *Helminthologia* **14** : 401-419.

Bauer, O.N. (1991) Spread of parasites and diseass of aquatic organisms by acclimatization : a short review. *J. Fish Biol.* **39** : 679-686.

Buchmann, K., Pedersen, L.O. & Glamann, J. (1991) Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Dis. aquat. Org.* **12** : 55-57.

Davydov, V.G. (1978) Differences in the tissue reactions of fish in response to infection with *Diphyllobothrium latum* (L., 1758) plerocercoids. *Biologiya Vnutrennykh Vod, Informatsionnyi Byulleten* **39** : 68-71 (in Russian).

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. & Ollevier, F. (1990) The life cycle of *Anguillicola crassus*. *Dis. aquat. Org.* **8** : 77-84.

De Nie, H., Bromley, H.J. & Vijverberg, J. (1980) Distribution patterns of zooplankton in Tjeukemeer, The Netherlands. *J. Plankton Res.* **2** : 317-334.

Egusa, S. (1979) Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. In Thurow, F., 1979. Eel research and Management. *Rap. Proc. Verb. Reun. Cons. Int. Exp. Mer* **174** : 51-58.

Ellis, A.E. (1981) Stress and the modulation of defence mechanisms in fish. In : Stress and Fish, ed. A.D. Pickering, London, chapter 7 : 147-169.

Evans, D.L. & Gratzek, J.B. (1989) Immune defense mechanisms in fish to protozoan and helminth infections. *Amer. Zool.* **29** : 409-418.

Hamers, R., Lehmann, J., Stürenberg, F.-J. & Taraschewski, H. (1992) In vitro study of the migratory and adherent responses of fish leucocytes to the eel-pathogenic acanthocephalan *Paratenuisentis ambiguus* (van Cleave, 1921) Bullack et Samuel, 1975 (Eoacanthocephala : Tenuisentidae). *Fish Shellfish Immunol.* **2** : 43-51.

Hartmann, F. (1994) Untersuchungen zur Biologie, Epidemiologie und Schadwirkung von *Anguillicola crassus* Kuwahara, Niimi und Itagaki 1974 (Nematoda), einem blutsaugenden Parasiten in der Schwimmblase des europäischen Aals (*Anguilla anguilla* L.). Dissertation, Universität Hamburg, Verlag C. Shaker, Aachen, Deutschland, 139 p.

Höglund, J. & Pilström, L. (1994) Purification of adult *Anguillicola crassus* whole-worm extract antigens for detection of specific antibodies in serum from the European eel (*Anguilla anguilla*) by ELISA. *Fish Shellfish Immunol.* **4** : 311-319.

Hoole, D. & Arme, C. (1983) *Ligula intestinalis* (Cestoda : Pseudophyllidae): an ultra-structural study on the cellular response of roach fry, *Rutilus rutilus*. Int. J. Parasitol. **13, 4** : 359-363.

Kennedy, C.R. (1993) Distribution and ecology of *Anguillicola crassus*. Abstract of paper presented at Int. Workshop "*Anguillicola* and Anguillicolosis of Eels", Ceské Budejovice, Tsjechie, Oct 5-7, 1993.

Kuwahara, A., Niimi, A., & Itagaki, H. (1974) Studies on a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n.sp. (Phimetridae, Anguillicolidae). *Jap. J. Parasit.* **23, 5** : 275-279.

Liewes, E.W. & Schaminee-Main, S. (1987) Onderzoek naar de effecten van de parasiet *Anguillicola crassa* op de ontwikkeling van de paling (*Anguilla anguilla*) in een zoutwater palingmesterij. Intern report Texvis BV, Texel, The Netherlands.

Linnik, V.-Ya, Beznos, T.V. & Guzik, I.G. (1988) Immune reactions of carp to *Philometra lusiana* infestation. Veterinarnaya Nauka, Proizvodstvu **26** : 93-96 (in Russian).

Mackenzie, C.D. (1987) Immune responses in Onchocerciasis and Dracunculiasis. In : Immune responses in parasitic infections : immunology, immunopathology, and immunprophylaxis. Vol. I Nematodes. Ed. E.J.L. Soulsby. CRC Press, Boca Raton, Florida. Chapter 9 : 181-232.

Molnár, K. & Moravec, F. (1994) Third-stage larvae of *Daniconema anguillae* (Nematoda : Dracunculoidea) in the subcutaneous tissue of eel *Anguilla anguilla*. *Folia Parasitol.* **41** : 215-219.

Molnár, K., Székely, Cs. & Perényi, M. (1994) Dynamics of *Anguillicola crassus* (Nematoda : Dracunculoidea) infection in eels of Lake Balaton, Hungary. *Folia Parasitol.* **41** : 193-202.

Muller, R. (1971) *Dracunculus* and dracunculiasis. *Adv. Parasitol.* **9** : 73-151.

Puqin, W. & Yuru, Z. (1980) Observations on the life history of *Anguillicola globiceps* (Nematoda, Anguillicolidae). *Acta Zool. Sin.* **26** : 243-249.

Slotved, H.C. & Buchmann, K. (1993) Acquired resistance of the eel, *Anguilla anguilla* L., to challenge infections with gill monogeneans. *J. Fish Dis.* **16** : 585-591.

Szalai, A.J., Danell, G.V. & Dick, T.A. (1988) Intestinal leakage and precipitating antibodies in the serum of quillback, *Carpiodes cyprinus* (Lesueur), infected with *Neoechinorhynchus carpiodi* Dechtiar, 1968 (Acanthocephala : Neoechinorhynchidae). *J. Parasitol.* **73, 3** : 415-420.

Székely, Cs., Lang, M. & Csaba, Gy. (1991) First occurence of *Anguillicola crassus* in Hungary. *Bull. Eur. Ass. Fish Pathol.* **11 (4)** : 162-163.

Van Banning, P. & Haenen, O.L.M. (1990) Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In : Pathology in marine science, Ed. F.O. Perkins & T.C. Cheng, 1990. Academic Press, Inc., New York. Proc. 3rd Int. Coll. on Path. in Marine Aquaculture, Gloucester Point, Virginia : 317-330.

Van Muiswinkel, W.B. & Jagt, L.P. (1984) Host-parasite relationships in fish and other ectothermic vertebrates. *Devel. Comp. Immunol.* Suppl. **3** : 205-208.

Woo, P.T.K. (1992) Immunological responses of fish to parasitic organisms. *Ann. Review Fish Dis.* **2** : 339-367.

Summary

Samenvatting

Summary

In the 1980s an eel parasitic nematode, *Anguillicola crassus* (Nematoda, Dracunculoidea), which infects the swimbladder of European eels (*Anguilla anguilla*) and other freshwater fish species, was introduced into The Netherlands. This thesis describes the epidemiology, pathogenesis, and pathobiology of the parasitic infection.

Originating from Southeast Asia, the parasite caused severe swimbladder lesions in European eels soon after its introduction : dilation of blood vessels, inflammation and rupture of the swimbladder in both wild and farmed eels, often resulting in severe fibrosis of the swimbladder (chapter 2). High prevalences of infection were recorded (80 to 100% in 1987 in Dutch inland waters). Elvers became infected directly after entering the freshwater. Furthermore, the parasite was able to reach adulthood in the swimbladder lumen of these small eels. Infected farmed eels were particularly susceptible to secondary bacterial infections, which caused additional mortalities.

When fish from Dutch lakes were subsequently investigated for *A. crassus* (chapter 3), it was found that freshwater smelt (*Osmerus eperlanus*), ruffe (*Gymnocephalus cernuus*), perch (*Perca fluviatilis*), zander (*Stizostedion lucioperca*), and three-spined stickleback (*Gasterosteus aculeatus*) contained third stage (L3) larvae of *A. crassus* in their swimbladders. Roach (*Rutilus rutilus*) and bream (*Abramis brama*), however, did not contain the nematode. Pre-adult *A. crassus* were also found in ruffe, perch, and three-spined stickleback, but adult specimens were missing. It was suggested, that some of the fish species containing L3 larvae are preyed upon by eels and may act as paratenic hosts for the transmission of the parasite to eels.

To test whether L3 larvae of *A. crassus* could be transmitted from infected smelt and ruffe to uninfected eels, eels were force-fed with infected smelt or ruffe swimbladders (chapter 4). The L3 larvae migrated actively to the eel swimbladders, where they developed into adult *A. crassus*. It was concluded that eels can indeed become infected by eating infected prey fish.

In experimentally induced infections using oral inoculation of L3 larvae, the pathogenesis of *A. crassus* infection was studied (chapter 5). L3 larvae migrated directly through the intestinal wall and body cavity of the eels to the swimbladder within only 17 h. Fourth-stage *A. crassus* larvae were detected 3 months after infection, and pre-adults within 4 months after infection. The L3 larvae occasionally showed aberrant migration paths. The lesions of the swimbladders were less severe than those of naturally infected eels. *A. crassus* developed much faster in the European eels than in the Japanese eel, *Anguilla japonica*, as reported in the literature.

The pathobiology of *A. crassus* in The Netherlands was investigated from 1986 to 1992 in freshwater eels and smelts (chapter 6). Throughout the 6-year sampling period, young eels (up to 17 cm) showed severe lesions due to the parasite. Larger eels (23-34 cm) showed the highest prevalence of infection (96% from 1987 to 1988), and the highest intensity of infection, defined as the number of parasites per infected fish (about 16 per fish from 1988 to 1989). After 1989 the prevalence and the severity of the swimbladder lesions decreased. Although larger eels (23-34 cm) from the Waddenzee (salt water) showed high prevalences of infection (85-90%) from 1987 to

1990, the intensities of infection decreased (7.7 to 4.8 per eel) from 1987 onwards, and the percentage of fibrotic swimbladders decreased from 1988 (maximum 24.5%). Smelts showed a sharp decrease in prevalence (88% to 48%) of the parasite shortly after 1988. Thereafter the prevalence stayed rather constant, at about 40% of the smelt population. No pathological changes were observed in the smelt.

By improving our method for producing infective L3 larvae of *A. crassus,* we were able to isolate distinct L3 larvae from copepods (intermediate host) and to count exactly the infective L3 larvae for inoculating eels (chapter 7). This method was used in subsequent experiments.

To investigate why the *A. crassus* infection in naturally infected eels began to decrease, we conducted a dose-effect experiment in which some eels were primed and others not (chapter 8). At day 0 uninfected eels were orally infected with various doses up to 40 L3 larvae of *A. crassus* per fish. At day 56 eels were either killed and examined, or were reinfected with 20 larvae each. At day 112 all remaining eels were killed and examined. The numbers of *A. crassus* recovered from the eels ranged between 14-20% at day 56 and 9-26% at day 112. These percentages were positively related to the total infection dose. There was no difference in percentages between primary and secondary infection. The swimbladder lesions were also positively related to the total dose, but were again not related to reinfection.

An enzyme linked immuno sorbent assay (ELISA) was developed to test blood samples for antibodies against adult cuticula antigen of *A. crassus* (chapter 8). None of the sera from the experimental eels showed a titer in the ELISA, whereas sera from naturally infected eels showed high titers. However, when these sera were tested in Western blots, no protein band indicating specific antibodies against *A. crassus* cuticula antigen was detected. It was concluded that under our experimental conditions, the eels do not develop an antibody response or resistance against the parasite. Future research should focus on examining the possible roles of specific and nonspecific immune responses in the decrease in the *A. crassus* infection in naturally infected eels.

Samenvatting

Begin jaren tachtig werd de nematode *Anguillicola crassus* (Nematoda, Dracunculoidea), een zwemblaasparasiet van de Europese paling (*Anguilla anguilla*) en andere zoetwatervissoorten in Nederland geïntroduceerd. In dit proefschrift worden met name de epidemiologie, pathogenese en de pathobiologie van deze parasitaire infectie beschreven.

De parasiet is uit Zuid-Oost Azië afkomstig. Bij de eerste vondsten in de zwemblaas van Nederlandse paling bleek, dat de parasiet allerlei afwijkingen veroorzaakte, zoals verwijding van bloedvaten, ontstekingen en zelfs het openbarsten van de zwemblaas, vaak gevolgd door een sterke bindweefselvorming (fibrose). Hoge infectiepercentages werden gemeten bij paling uit de belangrijkste binnenwateren (80-100% in 1987). Uit het onderzoek bleek, dat glasalen geïnfecteerd kunnen worden zodra ze vanuit zee het zoete water hebben bereikt. Zelfs in deze kleine palingen bleek de parasiet volwassen te kunnen worden, waarbij de parasiet haar grootte aan die van de zwemblaas aanpast. Tevens werd duidelijk, dat palingmesterijen vooral last hadden van de parasiet door het optreden van secundaire bacteriële infecties van de zwemblaas.

Nadat het schadelijk effect van de parasiet voor de paling was vastgesteld (hoofdstuk 2), werd de rol van de parasiet bij andere zoetwatervissoorten onder de loep genomen (hoofdstuk 3). Hierbij bleek dat bij zoetwater-spiering (*Osmerus eperlanus*), pos (*Gymnocephalus cernuus*), baars (*Perca fluviatilis*), snoekbaars (*Stizostedion lucioperca*) en driedoornige stekelbaars (*Gasterosteus aculeatus*) derde-stadium (L3) larven van *A. crassus* in de zwemblazen kon worden gevonden, maar niet bij blankvoorn (*Rutilus rutilus*) en brasem (*Abramis brama*). Bij pos, baars en driedoornige stekelbaars werden bovendien pre-adulte *A. crassus* gevonden, maar geen volwassen wormen. Op basis van deze resultaten werd de hypothese geformuleerd, dat verscheidene zoetwatervissoorten, die als prooi dienen voor de paling tevens als paratenische gastheren voor *A. crassus* kunnen optreden.

De transmissie van de L3-larven van *A. crassus* vanuit geïnfecteerde spiering en pos naar ongeïnfecteerde paling werd onderzocht door palingen te voederen met de zwemblazen van beide vissoorten (hoofdstuk 4). Aan het eind van deze proef werden volwassen wormen gevonden in de zwemblazen van de paling.

De pathogenese van de infectie werd bestudeerd door jonge paling oraal te infecteren met L3-larven van *A. crassus* (hoofdstuk 5). De larven bleken de directe route dwars door de wand van het maagdarmkanaal en de buikholte richting zwemblaas te nemen en arriveerden op z'n vroegst na 17 uur in de zwemblaaswand. De eerste vierde-stadium larven werden 3 maanden na infectie aangetroffen en de eerste pre-adulte wormen na 4 maanden. Een enkele keer werd waargenomen dat de L3-larven afweken van de route en in het spierweefsel van de paling belandden. De weefselschade, die door de kunstmatige infectie werd veroorzaakt was veel minder ernstig dan die bij de wilde paling werd gezien. De periode waarin de cyclus van *A. crassus* werd voltooid bleek een stuk korter (circa 5 maanden) in vergelijking met de oorspronkelijke beschrijving van de cyclus (1 jaar) in de Japanse paling (*Anguilla japonica*).

De pathobiologie van *A. crassus* infecties in paling en spiering werden bestudeerd in het IJsselmeer, Markermeer en de Waddenzee in 1986-1992 (hoofdstuk 6). De kleinste palingen (tot een lengte van 17 cm) bleken gedurende die 6 jaren zeer ernstige zwemblaasafwijkingen te vertonen. Bij grotere palingen (23-34 cm) werden de hoogste prevalenties (infectiepercentages) tot 96% gevonden in 1987-1988 en de hoogste parasiet-intensiteit (aantal *A. crassus* per geïnfecteerde vis) van ongeveer 16 in 1988-1989. Daarna gingen zowel de prevalenties als de ernst van de zwemblaasbeschadigingen omlaag. Grotere paling (23-34 cm) uit de Waddenzee (zout water) vertoonden hoge prevalenties in 1987-1990 (85-90%), met een licht dalende parasiet-intensiteit (van 7,7 naar 4,8 per paling) en tevens dalende aantallen verdikte zwemblazen, uitgaande van 24,5% in 1988. Spiering vertoonde een scherpe daling van prevalentie na 1988 (van 88% naar 48%), waarna deze vrijwel constant bleef op een niveau van ongeveer 40%. Bij de spiering werden totaal geen pathologische reacties op de worminfectie gevonden.

De methode voor de produktie van infectieuze L3-larven van *A. crassus* werd verbeterd (hoofdstuk 7). Via een pottermethode bleek het mogelijk, levende L3-larven te isoleren uit copepoden (kreeftachtige tussengastheren voor de parasiet), en ze daarna exact te tellen voor het gebruik bij experimentele infecties.

Larven met behulp van bovenstaande methode gewonnen, werden gebruikt bij de dosis-effect proef (hoofdstuk 8). Ongenfecteerde palingen van een *A. crassus*-vrij bedrijf werden oraal geïnfecteerd met een dosis L3-larven variërend van 0-40 per vis. Na 8 weken werd de helft van de palingen opnieuw geïnfecteerd met 20 L3-larven per vis. De overige dieren werden gedood en onderzocht. Na nog eens 8 weken werden de overgebleven palingen onderzocht. Het percentage in de zwemblaas teruggevonden *A. crassus* varieerde van 14-20% na 8 weken en van 9-26% na 16 weken. Deze percentages waren recht-evenredig met de totale infectiedosis. Herinfectie als zodanig had daarop geen invloed. De zwemblaasbeschadigingen waren ernstiger bij hogere doses, maar waren onafhankelijk van herinfectie.

Een "enzyme linked immuno sorbent assay" (ELISA) werd opgezet om de bloedmonsters van de palingen te testen op aanwezigheid van antilichamen tegen cuticula antigeen van volwassen *A. crassus* (hoofdstuk 8). Geen van de experimentele sera vertoonde een titer in de ELISA, terwijl sera van zwaar geïnfecteerde wilde paling hoge titers in deze test vertoonden. Bij eiwitanalyse door middel van Western blotting werd echter geen karakteristiek eiwit gevonden dat overeenkwam met specifieke antilichamen tegen *A. crassus*. Er werd geconcludeerd, dat palingen onder de gegeven experimentele omstandigheden wel pathologische verschijnselen ontwikkelen na primaire en secundaire infectie, maar geen antilichaamrespons of resistentie ontwikkelen tegen *A. crassus*. Meer onderzoek is nodig om de mogelijke rol van de specifieke en de aspecifieke immunologische afweer te verduidelijken. Op dit moment kunnen we nog niet bewijzen, dat deze afweerprocessen de afname van de infectie in wilde paling kunnen verklaren.

Dankwoord/Acknowledgements

Dit proefschrift is tot stand gekomen door goede samenwerking met veel mensen van verschillende instellingen. Graag wil ik hierbij dan ook deze mensen hartelijk bedanken voor hun bijdrage.

Allereerst wil ik Paul van Banning (RIVO-DLO) bedanken voor zijn kameraadschap in deze: pas na enkele gezamenlijke publikaties kwam bij mij het idee op er een proefschrift van te maken. Gezien het frequente mede-auteurschap van Paul is het ook een beetje zijn proefschrift geworden. Gefeliciteerd dus, Paul!

Wim van Muiswinkel (LUW), mijn promotor wil ik bedanken voor zijn promotorschap bij deze studie, ondanks het feit, dat het immunologische deel van het proefschrift uiteindelijk qua resultaten enigszins tegenviel. Bedankt voor je positieve, vlotte en efficiënte begeleiding.

Fred Borgsteede (ID-DLO), bedankt voor je steeds aanwezige belangstelling voor het onderwerp en je kritische houding, die uiteindelijk uitmondden in copromotorschap. Je vormde een duidelijk klankbord voor mijn vragen rond het onderzoek voor dit proefschrift. Daarnaast ben ik je zeer dankbaar voor je praktische hulp bij de experimenten en het parasitologisch onderzoek van wilde paling.

De assistentie bij het bemonsteren voor de studies van de palingen uit IJsselmeer en Markermeer werd verricht door Jan van Willigen (RIVO-DLO) en de bemanning van MS 'Stern', o.a. Kees, Ad, Joop, Sjaak en Hendrik. Allemaal hartelijk bedankt voor jullie positieve houding, die de monsterdagen tot een feest maakten.

De experimenten met paling werden door Henk Dorsman, Johan Meijer en collegae van Dierverzorging en Biotechniek van ons instituut op vakkundige wijze uitgevoerd. Veel dank daarvoor.

Bij het seceren van grote partijen wilde paling en het tellen van de larvensuspensies voor de infectieproeven stonden Cor Gaasenbeek en Niek van der Linden (ID-DLO) steeds klaar om te helpen, waarvoor bij deze hartelijk dank.

Thomas van Wijngaarden, jouw inzet en doorzettingsvermogen als stagiaire van de LU Wageningen wierpen duidelijk vrucht af door de short communication (hoofdstuk 7) en de basis voor de ELISA voor hoofdstuk 8. Hartelijk bedankt hiervoor. Jan Cornelissen (ID-DLO) wil ik hierbij tevens bedanken voor het dagelijks begeleiden van Thomas en zijn kritische inbreng bij het opzetten van de ELISA.

Nora Kuiper, Herma Daus-Ooms en Ria Spies hebben geweldig geholpen bij het verwerken van de tekst van dit boekje. Victoria Thatcher heeft de verschillende hoofdstukken zeer grondig gescreened op Engels. Fred van Welie en Joop Iedema hebben de vele illustraties gereed gemaakt voor publikatie. Mart de Jong en Herman Huipen hebben geholpen met de statistische verwerking van de gegevens. Allemaal hartelijk bedankt.

Mijn medewerkers Aart Davidse en Françoise Keuzenkamp wil ik erg bedanken voor hun inzet en zelfstandigheid, waardoor ik in de gelegenheid was, me af en toe geheel aan mijn proefschrift te wijden, doordat zij volledig het overige werk overnamen. Met de werkdruk, inclusief het opzetten van de protocols voor sterlab ben ik jullie hiervoor zeer erkentelijk.

De discussie rond het laatste deel van het proefschrift vond plaats in een discussiegroep, waarin o.a. Ben Bokhout, Suzan Jeurissen, Leo van Leengoed van het ID-DLO en Lidy Verburg van de LUW vertegenwoordigd waren. Ik wil jullie bij deze bedanken voor jullie kritische, positieve bijdrage.

Phreddy Broekhuizen, Caspar Hoebe, Marjolein Ham-Hoffies en Arie Hogendoorn hebben geweldig geholpen bij de gelelectroforese, Western blots en het analyseren van de palingbloedmonsters op haematocriet- en haemoglobinegehalten. Rob Buijs en Hans van Lieshout hebben de verwerking van het formalinemateriaal tot histologische coupes voor hun rekening genomen. Allemaal hartelijk bedankt.

De Organisatie ter Verbetering van de Binnenvisserij te Nieuwegein wil ik bedanken voor het beschikbaar stellen van de wilde paling uit de Waddenzee voor onderzoek ten behoeve van dit proefschrift.

Thom van der Heijden, Jan Rombout en Jan Boon van de LUW hebben hun monoclonalen anti paling Ig beschikbaar gesteld voor de ELISA, waarvoor mijn hartelijke dank. Johan Höglund, thank you very much for the control sera and your help with the set up of the ELISA. Dr. K. Buchmann, thank you so much for sending me the polyclonal anti eel Ig. Dr. C. Kennedy, dr. K. Molnár, and dr. Cs. Székely are thanked very much for sending me the sera from *Anguillicola crassus*-negative and -positive eels. Dr. C.J. Secombes is acknowledged for giving me the opportunity to learn laboratory tests related to nonspecific immune responses of fish.

Verder zijn er vele mensen op de achtergrond actief geweest voor het welslagen van het onderzoek, zoals de projectgroep visziekten met daarin Ed ter Laak en Jan van Oirschot, daarnaast Gethin Thomas, Arno Gielkens, Jaap van Leeuwen, Jaap van Dijk, Jan Haagsma, Diny Heuckeroth, en vertegenwoordigers van de ondersteunende afdelingen, waaronder technische dienst, expeditie, voedingsmedia, bibliotheek. Bedankt allemaal.

De directie van het ID-DLO wil ik bedanken voor het scheppen van de mogelijkheden voor het doen ontstaan van dit proefschrift.

Tineke van der Ploeg en Paul van Banning, bedankt voor jullie bereidheid tot het ondersteunen van mijn promotie als paranymfen.

Mijn echtgenoot, Frank Hepkema ben ik zeer dankbaar voor zijn support op alle fronten. Last but not least wil ik mijn ouders bedanken voor het aanmoedigen van het wetenschappelijk denken van jongs af aan en het vertrouwen, dat zij hadden in deze 'laatbloeier'.

Curriculum vitae

Olga L.M. Haenen werd op 11 augustus 1959 te Breda geboren. In 1977 behaalde zij haar Atheneum-B diploma aan het Thomas à Kempis College te Arnhem en begon de studie 'biologie', oriëntatie 'organisme' aan de Landbouwuniversiteit te Wageningen. Nadat in de kandidaatsfase een brede biologische basis was gelegd, werden in de doctoraalfase meer op visteelt en visgezondheid gerichte vakken gekozen, zoals respiratieproeven met Afrikaanse meerval, farmacokinetisch onderzoek van met antibiotica behandelde vissen, culturele antropologie van tropische vissers in India, en een praktijktijd van 6 maanden op een viskwekerij in Sri Lanka. De doctoraalstudie werd in 1985 voltooid met het behalen van het diploma. Direct hierna werd Olga Haenen als hoofd Sectie Visziekten aangesteld bij het DLO-Centraal Diergeneeskundig Instituut (thans DLO-Instituut voor Veehouderij en Diergezondheid) te Lelystad, waar zij de taak kreeg, een visziektelaboratorium op te bouwen voor 2e lijns visziektediagnostiek en exportonderzoek visziekten. Na enkele stages in het buitenland werd dit laboratorium in enkele jaren opgebouwd, waar zij samen met twee medewerkers full time werkt aan diagnostiek en onderzoek van visziekten. Het laboratorium is in 1993 uitgeroepen tot nationaal referentielab visziekten voor de Europese Unie. Olga Haenen heeft haar promotie-onderzoek binnen het ID-DLO uitgevoerd.

Scientific publications

Banning, P. van & Haenen, O.L.M. (1990) Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel *Anguilla anguilla*. In : Pathology in Marine Science. Ed. F.O. Perkins & T.C. Cheng. Academic Press inc., New York. Proc. 3rd Int. Coll. on Pathol. in Marine Aquaculture, Gloucester Point, Virginia, Oct. 2-6 1988 : 317-330.

Banning, P. van & Haenen, O.L.M. (1990) Aspecten van de aalzwemblaasworm *Anguillicola crassus*. *Dier-en-arts* **Juni/juli 1990** : 147-152.

Grondel, J.L., Nouws, J.F.M. & Haenen, O.L.M. (1986) Fish and antibiotics : Pharmacokinetics of sulphadimidine in carp (*Cyprinus carpio*). *Vet. Immunol. & Immunopathol.* **12** : 281-286.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C. & Ollevier, F. (1989) Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. aquat. Org.* **7** : 97-101.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire C. & Ollevier, F. (1991) Artificial infection of the European eel with third stage larvae of the nematode *Anguillicola crassus*. *J. Aquatic Animal Health* **3(4)** : 263-265.

Haenen, O.L.M. & van Banning, P. (1990) Detection of larvae of *Anguillicola crassus* (an eel swimbladder nemtode) in freshwater fish species. *Aquaculture* **87** : 103-109.

Haenen, O.L.M. & van Banning, P. (1991) Experimental transmission of *Anguillicola crassus* (Nematoda, Dracunculoidea) larvae from infected prey fish to the eel *Anguilla anguilla*. *Aquaculture* **92** : 115-119.

Haenen, O.L.M. & Davidse, A. (1993) Comparative pathogenicity of two strains of pike fry rhabdo virus and spring viremia of carp virus for young roach, common carp, grass carp and rainbow trout. *Dis. aquat. Org.* **15** : 87-92.

Haenen, O.L.M., 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 1986-1992. *Aquaculture* **126** : 219-229.

Haenen, O.L.M., van Banning, P., Dekker, W. & van Wijngaarden, T.A.M. (1993) Pathobiology and immunology of *Anguillicola crassus* related to European eel. *Folia Parasitol.* **4, 40** : 323-324 (Int. Workshop "*Anguillicola* and Anguillicolosis of eels", Ceské Budejovice, Tsjechië, Oct 5-7, 1993).

Haenen, O.L.M., van Wijngaarden, T.A.M. & Borgsteede, F.H.M. (1994) An improved method for the production of infective third stage juveniles of *Anguillicola crassus*. Short communication. *Aquaculture* **123** : 163-165.

Liewes, E. & Haenen, O.L.M. (1993) The Veterinary approach to eels. In : Aquaculture for Veterinarians : Fish husbandry and medicine. Ed. L.Brown, Pergamon Press Ltd. Oxford, England, chapter 18, 311-326.

Veenstra, J., Rietra, P.J.G.M., Coster, J.M., Stoutenbeek, C.P., ter Laak, E.A., Haenen, O.L.M., de Gier, H.H.W. & Dirks-Go, S. (1993) Human *Vibrio vulnificus* infections and environmental isolates in the Netherlands. *Aquaculture and Fisheries Management* **24** : 119-122.