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DENSITY DEPENDENCE WITHIN THE *ANGUILLICOLA CRASSUS* EEL AND COPEPOD SYSTEM

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The distribution of *Anguillicola crassus* and the role of density dependent factors operating within the host-parasite systems have been investigated. Since its introduction into the United Kingdom in 1987 *A. crassus* has spread rapidly to localities throughout the country. It is likely that its rapid progress has been aided by eel movements both natural and for imports and exports by the fishing industry, as its introduction was due to import and export of live fish. Post establishment colonization by the parasite within a river system is rapid, leading to high prevalence levels even after one year. Once this high level of prevalence is reached population levels seem to remain stable.

Laboratory experiments reveal that nematode larvae will remain in the eel swimbladder wall, if adults already exist in the lumen, for at least four months. Field data also show that the intensity of gravid females remains stable with varying total infrapopulation sizes. It is suggested that mechanisms of density dependent establishment and maturation inhibition operate within the definite host. Experiments continue.

Density dependent mortality has been studied in three species of copepod intermediate host. Mortality rate increases with the number of larvae introduced to each copepod in two species, *Cyclops viridis* and *Cyclops vicinus*. This relationship is unclear for the largest of the species, *Cyclops fuscus*. Mortality rate increases with decreasing copepod size. For *C. vicinus* mortality rate increases with temperature. Death rate of the intermediate host can be related to infection intensity, host size and temperature. It appears that *A. crassus* can exist at stable population levels and that it can exert density dependent effects on its own infrapopulations and intermediate hosts.

INFECTION RATES OF THE SILVER EEL *ANGUILLA ANGUILLA* POPULATION OF THE RIVER YSER BASIN (FLANDERS) WITH *ANGUILLICOLA CRASSUS* AND THE EFFECTS OF THE PARASITE ON THE MUSCLE COMPOSITION OF MIGRATING MALE SILVER EELS

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This paper describes the infection of the migrating silver eel population from the Blankaart Natural Reserve (River Yser basin) with *Anguillicola crassus* during autumn 1991. Possible effects of parasitic infection on body contents of male silver eel were analyzed by measuring lipid, protein and water content and energy value of muscle tissue from heavily and slightly or uninfected eels. Apart from differences in ash content, no evidence was found of significant variations in muscle composition between both groups.

SOME NOTES ON ANGUILLICOLOSIS IN ITALY

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During 1987–91 the authors have carried out research on *Anguillicola crassus* and particularly on: 1) the epidemiology of the parasite in eels (*Anguilla anguilla*) from several Italian regions (Veneto, Emilia-Romagna, Lazio, Calabria and Sicilia) and foreign countries (France, Spain, Morocco, Turkey and U.S.A.); 2) the therapy, testing several drugs and demonstrating the efficacy of levamisole at the dosage of 5 mg/l for 6 hours; 3) the life cycle, carrying out experimental infection of *Diacyclops* *bicuspidatus* (Copepoda, Cyclopidae) with second-stage larvae and then of glass-eels with infected copepods; and 4) the effects of *A. crassus* in farmed eels by statistical evaluation of some biometrical indexes.

AN AUTOMATIC IMAGE ANALYSIS SYSTEM FOR THE COUNTING, SIZING AND SEX-DETERMINATION OF *ANGUILLICOLA CRASSUS* (NEMATODA, DRACUNCULOIDEA), A PARASITE OF THE EUROPEAN EEL (*ANGUILLA ANGUILLA*)

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Image analysis has been used in the past for counting and sizing of bacteria, toxic algae, zooplankton and copepods in marine samples. Semi-automated methods involving image analysis were recently used for the determination of morphological parameters in parasites that are now measured using time-consuming manual methods. Because of a great need of data for the study of the distribution, ecology and population dynamics of *Anguillicola crassus* in France an automated imaging technique was performed.

Eels were obtained by fykenetting in the lake of Grand-Lieu, France. Parasites were collected by autopsy and fixed in Raillet's fixative.

The image analysis system consisted of a camera, a central processing unit, an output image monitor and a printer. The central unit was equipped with a set of cards for image acquisition, digitalization and processing. Samba (TITN-Alcatel) was used as the image processing software base together with the program MS-DOS windows (Microsoft).

With this easy-made preparation method and after optimisation of light conditions the dark-brown parasites were perfectly distinguishable from the medium. The parasites were then placed under a CCD monochrome camera connected to the image analysis system.

Image processing of the samples involved two main steps:

1) The first digitised image was displayed over 256 grey levels or in pseudo-colours, that is, colour-code based on their grey levels to improve contrast and better defined parasite size and shape. Within this first step a threshold band was determined and fixed for subsequent samples. This band allowed objects of interest to be automatically defined using up to 256 threshold bands based on grey levels. Moreover, an enhancement image technique was improved. Since we were interested in sizing the entire parasite we subjected the image to cycles of dilatation and erosion. Dilatation made the parasite boundary larger by one pixel, and made the cell boundary smaller by one pixel. The two processes do not entirely reverse each other, so by performing dilatation and erosion in a round of 3 the outer parasite boundaries remained much the same but the holes are filled and the boundaries become much more uniform.

2) Once the parasites are accurately defined by the image enhancement and threshold bands applied, the samples are ready for automatic counting and up to 15 size and shape parameters with sex determination are automatically calculated. When automatic count is performed the image turns into binary image and all objects in the image are located in colours. As the objects are located and analysed, they are outlined in white. If necessary, one can watch the progress of an automatic analysis to be sure that the program is identifying counting sizing and sexing correctly. This last step gives the ability of the user to watch the analysis while it proceeds and to intervene with semi-automatic analysis, thus ensuring that the system can be used even for difficult samples.

The relationships between the morphological parameters obtained through image analysis and morphological data directly measured using fixed or fresh samples were assessed by principal component analysis and multiple linear regression. A highly significant fit between the surface of the parasites and their length, width and weight was demonstrated.

Stepwise discriminant analysis was then used to set up a classification function for the sex prediction and a Jackknife validation procedure was performed. This classification function was implemented in the image analysis program and 88% of correct classification was achieved.

This method appears to be a valuable tool for collecting rapidly accurate data that will be used in the study of the epidemiology of anguillicolosis within eel populations.

DISTRIBUTION OF *ANGUILLICOLA CRASSUS*, POSSIBLE WAYS OF SPREADING AND CHANGES IN THE INFESTATION RATE IN SOME DANISH SEA AND FRESHWATER AREAS

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Anguillicola crassus was detected for the first time in Denmark in 1987 in Lake Arresø in northern Zealand and in 1988 a sampling program was established covering both inlets and lakes. In three of the places investigated in Jutland we found an infestation rate indicating that *A. crassus* had been introduced to these areas before 1987. Ringkøbing inlet in western Jutland had for many years delivered small eels for stocking purposes to the other two infected places. Ringkøbing inlet was the only locality on the west coast where *A. crassus* were found at that time and they were probably introduced to the inlet by the eel-trade with Holland or Germany. Lake Arresø was probably infested by contact with lorries from the Ringkøbing inlet area. We know of a few more cases where infected eels were transported from one lake to another by man.

As our knowledge became wider, the spreading of the parasite by man almost ceased but infestation of new places continued. In Denmark we have a stock of 50,000 cormorants, with a preference for feeding on eel, and the paratenic host, the black goby

(*Gobius niger*). If spreading by birds - especially the cormorant - is possible, they could be responsible for part of the unknown distribution. Yellow eel move to some extent in their local area and congregate during the winter-hibernation. Silver eels from the Baltic area, e.g. Poland and Germany, where infestation took place earlier than in Denmark, have to pass through the Belts and The Sound on their way to the Sargasso Sea. They could also have contributed to the spread of the parasite, specially when they were caught in Danish waters. In general, too little is still known about the part that animals play in the distribution of the parasite.

In samples collected specially for investigation of *A. crassus* the swimbladder was opened and the number of individuals were counted under weak magnification. The smallest specimens recorded were about 2 mm. In other eel samples we only recorded if *A. crassus* were present or not.

The development of the infestation rate is exponential during the first couple of years until approximately 100% has been reached. Thereafter a considerable decline occurs followed by a new increase. Just now it is impossible to predict the infestation rate. Will it continue to oscillate, and at what amplitude, or will it reach a steady state in connection with the possible development of an immune response to the *A. crassus*?

PHARMACOKINETICS OF LEVAMISOLE IN THE EUROPEAN EEL DEFINITIVE HOST OF THE NEMATODE *ANGUILLICOLA CRASSUS* (NEMATODA, DRACUNCULOIDEA)

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The aims of this study were to describe plasma disposition of levamisole in eels after single intravenous and single oral administrations. The investigation was designed to determine the most effective therapeutic regimen for the use of a product which had previously been shown to be clinically effective against *Anguillicola crassus* and to reduce risks of meat residues and adverse environmental effects.

The eels were held in freshwater at a mean temperature of 21°C and 20.8°C; the averages of pH and dissolved oxygen were 7.5 and 5.4 mg/l, respectively for both experiments. In the intravenous study the fishes were of 110 g mean weight and received a single intra cardiac injection of 8 mg levamisole per kg body weight.

In the oral study, the fishes of 116 g mean weight were divided in two groups. For one group (Group I), the drug was mixed with a commercial feed (AASS, TROUVIT) which was coated with 4% fish oil for the other group (Group II). The medicated feed was then administered by a gavage via a stomach tube.

In the intravenous experiment the plasma level of levamisole was quantified by gas chromatography with a thermoionic detector, the quantification limit being 0.5 µg/ml. In the other study the levamisole was determined by high-performance liquid chromatography, the quantification threshold being 0.035 µg/ml.

The principal pharmacokinetic parameters of levamisole after a single intravenous administration were as follows:

The extensive apparent volume of the central compartment indicates that the levamisole is initially rapidly distributed to tissues outside of the blood. The total clearance appears to be relatively high, indicating that the elimination of this liposoluble and weakly basic drug is rather fast.

K12 (h^{-1})	K21 (h^{-1})	Kel (h^{-1})	T1/2a (h)	T1/2Kel (h)	ACU0-∞ (µg.h.ml)	Vdss ($1.kg^{-1}$)	CLß (lh^{-1})	MRT (h)
0,9	0,25	2,1	0,22	3,99	17,04	1,67	0,77	2,16

The principal pharmacokinetic parameters of levamisole after a single oral administration were as follows:

	Ka (h^{-1})	Kel (h^{-1})	T1/2Ka (h)	T1/2Kel (h)	AUC ($\mu g.h.ml^{-1}$)	F %	MRT (h)	MAT (h)	T max (h)	C max (µg.h.ml)
Group I	0,18	0,10	3,9	6,7	4,9	34	15	14	8	0,34
Group II	0,22	0,05	3,1	13,3	6,3	44	16	15	8	0,41

As indicated by Tmax, the oral absorption of levamisole was slow in both groups. The Cmax, the AUC and thus the oral bioavailability were improved in Group II. For the purpose of designing therapeutic schemes this easily made formulation appears to be effective. Moreover, the pharmacokinetic parameters will allow multiple oral dosage simulations that will be discussed considering the life-cycle of *Anguillicola crassus* within its host.

We believe that the withdrawal period will be short,taking into account the MRI and the rapid elimination of the levamisole from the central compartment.

PATHOBIOLOGY AND IMMUNOLOGY OF *ANGUILLICOLA CRASSUS* RELATED TO EUROPEAN EEL

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Dutch wild eels *Anguilla anguilla* from the IJsselmeer and neighbouring waters were sampled several times during 1986–1992 to obtain an insight into the development of the infection with *Anguillicola crassus* in the years after its introduction. The eels were screened for presence and number of different developmental stages of *A. crassus*, and pathological changes.

Young eels (up to 17 cm) showed a heavy infection with severe pathological changes during the whole period, without any trend. In particular dilation of blood vessels, haemorrhages, inflammation, and ultimately severe fibrosis of the swimbladder with pigmentations were observed.

Larger eels (23–34 cm) from both the IJsselmeer and neighbouring Markermeer showed a trend of a decrease in infection percentage, with 1988 being significantly (statistical analysis by means of Generalized

Linear Model, $p<0.05$) the peak year (mean of the 2 waters 92.7%, n=302 eels). The intensity (mean no. of *A. crassus* per infected eel) showed a significant maximum in 1989 of 14.46 for both waters (n=246 eels). Not only did the number of parasites per eel decrease after 1989, but also their size, and the severeness of the lesions of the swimbladder. These trends were also seen in wild elvers from the Wadden Sea, close to the IJsselmeer, from 1988 onwards.

What is causing this development towards a less severe infection in the larger eels? One possibility would be the immune response of the eel. To monitor the eventual specific antibodies against the parasite an ELISA was set up. This test presented many problems, as the antigens were not purified enough and so many cross reactions occurred, and inexplicable extinctions were recorded. After the antigens were purified in a Western Blot, a 43kD band, like that described by Buchman et al. 1991, was recognized by *A. crassus*-positive eel sera, not by negative eel sera. This protein band will be focussed on further. The role of the aspecific immune response is still not known.

PATHOGENICITY OF *ANGUILLICOLA CRASSUS* INFECTING EELS IN LAKE BALATON

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After the introduction of *Anguillicola crassus* to Lake Balaton, in the late eighties, an extremely high infection was attained among the overpopulated eel stock of the lake and resulted in massive mortality in 1991 and 1992. In some large sized eels as many as 90 to 120 adult worms were found in the lumen and 200 to 300 third and fourth stage larvae in the wall of the swimbladder. In less chronic cases the swimbladder wall remained transparent and thin but in advanced cases the wall was thickened 10x. The mucosal epithelium covering the lumen was hyperplastic and the propria was filled by dilated blood vessels. Accumulation of serum in serosa and subserosal connective tissue fibres caused the latter to draw away from each other and form cisternae.

In less advanced cases third and fourth stage larvae were located in the loose connective tissue without signs of cellular host reaction. In advanced cases, however, larvae were surrounded by granulation tissue and mononuclear cell infiltrations. Larvae surrounded by firm connective tissue capsules occurred mostly in regenerated swimbladder walls.

It has been experimentally proved that at decreasing oxygen levels of the water the most heavily infected eels show the most severe signs and are the first to die.

We believe that losses are only rarely caused by the direct effect of the live worms (blood sucking by adults and continuous reinfection by larvae). Lethal damage by anguillicolosis should rather be attributed to indirect effects of infection such as thickening of the swimbladder wall and accumulation of exudate, semidigested bloodcells, second stage larvae and debris of decayed parasites in the lumen of the swimbladder. In the most severe cases, abnormal location of second stage larvae or adult worms in the wall of the swimbladder due to the rupture of the damaged tunica interna are regarded as lethal factors.

DISTRIBUTION AND ECOLOGY OF *ANGUILLICOLA CRASSUS*

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Given that is only ten years since *A. crassus* was first detected in Europe and little information is available on its ecology in Japan, it is remarkable how much is now known about it. This review will summarize what is known, but will also try to indicate significant gaps in our knowledge.

The link between parasite distribution and the eel trade is clearly established. It is equally clear that *A. crassus* has excellent natural dispersal abilities and all the attributes of a successful colonizer: good survivorship of free living larvae, wide specificity to its intermediate host and to paratenic hosts, use of the widespread and versatile eel as definitive host and high fecundity. Human activities facilitate dispersal, but it would do well unaided and will continue to expand its range.

Transmission dynamics appear normal, in that survival and infectivity of larvae are time dependent and mortality exponential, but the time scale is unusually long. Transmission dynamics to intermediate hosts appear normal, but to eels is not yet understood. Do plankton, where natural infection levels are low, transmit the infection to eels, or is it acquired via paratenic hosts? Are perch alternative but poor definitive hosts, or true paratenic hosts?

The population dynamics of *A. crassus* conform to expectation. It can breed and infect hosts all year round, and this and its high fecundity permits population size to increase rapidly after invasion. Epidemiological studies indicate that population size then levels off. A number of possible density-dependent, regulatory factors that might be responsible for such stability have been detected, including parasite overdispersion, parasite induced mortality in both hosts and a limit to the number of females per eel. However, we still know too little about the survivorship and dynamics of the L4, about preadults and about longevity and mortality of adults in eels.

When *A. crassus* infected European eels it invaded a vacant niche and so faced no competitors. We do not know, however, whether species of *Anguillicola* can and do compete amongst themselves, or what would happen if it invades e.g. Australia.

The biggest gaps in our ecological knowledge relate to the impact of *A. crassus* on natural eel populations and so on aquatic communities. Is it a significant cause of mortality in natural eel populations? Lake Balaton appears exceptional here, and also proves an opportunity to determine what happens to the existing fish community when eels decline. If *A. crassus* can affect migrating eels at sea and so reduce host fecundity, it is essential to determine what happens to natural fish communities when a key component declines or disappears. Studies on the ecology of *A. crassus* and other *Anguillicola* species in their natural eel hosts could be very informative in this context.

DYNAMICS OF *ANGUILLICOLA CRASSUS* INFECTION IN EELS OF LAKE BALATON

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Due to the introduction of *Anguillicola crassus* and subsequent extremely intense infections in eels, massive fish mortalities occurred in Lake Balaton in 1991 and 1992. During the summer months at least 200 tons of eels died in 1991 and about 40 tons in 1992. In order to investigate the spread and dynamics of infection a survey was initiated in September of 1991. In the course of this survey the incidence of mature and larval *A. crassus* infection, and changes caused by them in the swimbladder wall, were examined in three different regions of Lake Balaton. In the fall of 1991 the highest infection, lesion incidence and mortality were recorded mostly in the western basin of the lake. In the midregion (district of Tihany) only moderate losses were recorded. Changes in the swimbladder were less intensive and the number of worms was smaller than in the western region (Keszthely). Eels in the eastern part of the lake (Almádi, Kēnese) were also infected but the intensity of infection was low and no eels with thickened swimbladders were found.

In 1992 the intensity of infection decreased in the western part of the lake and only a moderate fish mortality was recorded. On the other hand the intensity of infection and pathological changes increased in the midregion, and mortalities were also concentrated in this district. In the eastern part of the lake symptoms became more severe and the number of eels with thickened swimbladders increased but no mortalities were recorded.

From this survey we have concluded that: 1) By 1991 practically all eels of Lake Balaton became infected by *Anguillicola crassus*. 2) Due to the high population of this fish in the lake the intensity of infection is higher than in other natural waters. 3) After a heavy infection with the worms severe pathological changes appear in the swimbladder and may result in mortality. 4) There was a tendency for the infection to spread from west to east.

THE COLONISATION AND DISTRIBUTION OF *ANGUILLICOLA CRASSUS* IN SWEDEN

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The distribution of *Anguillicola crassus* among migrating and yellow eels in Swedish coastal areas, lakes, rivers and farms has been monitored on a regular basis since 1987. So far, the parasite has only been established in two areas of the Swedish Baltic coast, both of which are affected by discharges of heated cooling water from nuclear power stations. Following the establishment of the parasite population in these areas, a marked seasonal pattern has been noted for prevalence and abundance which probably reflects the migration pattern of the eels. Only sporadic records of *Anguillicola* in yellow eels have been made elsewhere in coastal areas of Sweden, in samples from the south and west coast. However, reports made by local fishermen from these areas indicate that there is an increasing proportion of infected migrating silver eels in the late autumn catches. It is suggested that the majority of these eels are recruited from the southern and eastern Baltic and thus have been infected elsewhere. Only a single record has been made in inland waters, in 1993 in lake Hjälmaren in central Sweden. It has been demonstrated elsewhere, in Central Europe, that once *A. crassus* has been introduced it thereafter spreads rapidly and establishes itself. In general, this is not the case in Sweden. Our results indicate that primarily the climate but also the density of the eel population are important factors in determining the colonisation rate of *A. crassus*.

THE PROTEASES OF THE EEL-PATHOGENIC NEMATODE *ANGUILLICOLA CRASSUS*

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The proteolytic activities of homogenates prepared from the second-stage larva (L2) and the third-stage larva (L3) as well as the adult stage of the eel-pathogenic nematode *Anguillicola crassus* were examined using proteins and synthetic peptide substrates. Whole bodies of L2 larvae, the anterior third of the bodies of L3 larvae, and the anterior fifth of the bodies of adults were studied. Extract of L2 contained a trypsinlike proteinase exhibiting a molecular weight of 38,000 on gelatin-substrate gel electrophoresis. The proteinase showed azocollytic and slight keratinolytic activity at a optimal pH of 8.0. Also degradation of synthetic peptides for trypsinlike enzymes could be observed. An apparent molecular weight of 25,000 was determined for the trypsinlike proteinase of the L3. This enzyme showing a pH optimum at 8.0 possessed azocollytic, keratinolytic and slight elastinolytic activity. Synthetic peptides for trypsinlike enzymes were hydrolyzed too. In extracts of L3 also aminopeptidase activity with a pH optimum at 9.0 was present. Samples of adults contained an aspartyl proteinase with a molecular weight of 90,000. When hemoglobin was used as the substrate, the enzyme displayed optimal activity at pH 5.0. It was concluded that the trypsinlike proteinases of the larval stages are penetration enzymes, whereas that of the adult stage is a digestive enzyme. The leucine aminopeptidase found in extracts of the L3 larvae may play a role in the process of moulting.

SOME NEW DATA ON THE INTERMEDIATE AND PARATENIC HOSTS OF THE NEMATODE *ANGUILLICOLA CRASSUS* (DRACUNCULOIDEA)

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Three species of planktonic crustaceans, *Cyclops strenuus* and *Macrocyclops albidus* (Copepoda) and *Notodromas monacha* (Ostracoda), were experimentally infected with the eggs and second-stage larvae of the swimbladder nematode *Anguillicola crassus* originating from eels from Neusiedler Lake in Austria. At 20–22°C, third-stage larvae of the parasite developed in all these invertebrate hosts within 16–20 days p.i. Ostracods harbouring the nematode third-stage larvae (33 days p.i.) were fed to small eels (*Anguilla anguilla*), while infected copepods (20 days p.i.) were fed to seven other fish species. In these experiments, the larvae from ostracods proved to be infective for the definitive host and the ostracod was thus confirmed as a true intermediate host of *Anguillicola crassus*. *Notodromas monacha* represents a new experimental intermediate host of *A. crassus* and the second known invertebrate other than a copepod in which the larval development of this nematode up to the infective stage takes place. Five species of fish, cyprinids *Tinca tinca*, *Gobio gobio*, *Alburnus alburnus* and *Alburnoides bipunctatus* (the latter representing a new host record), and guppy, *Poecilia reticulata*, were found to serve as experimental paratenic hosts for *A. crassus*. In these hosts, the *A. crassus* third-stage larvae penetrate through the wall of the digestive tract into the body cavity of fish where they can survive unencapsulated for a period of a least two months. The larvae were frequently found near the host's swimbladder, but never in its walls or lumen. The larvae localized on the surface of the intestine were usually encapsulated, being covered by a thin fibrous layer; the encapsulated larvae were often found dead, sometimes with an already decomposing body. Considering literature data, distinct differences appear to exist in the forms of paratenic parasitism among the individual fish species serving as paratenic hosts of *A. crassus*. While some of them (e.g. cyprinids, guppy) may be assigned to the category of euparatenic hosts (for terminology see Odening 1976), others (e.g. percids and centrarchids) can be evaluated as metaparatenic hosts or even paradefinitive hosts. Paratenic hosts may undoubtedly play an important role in the transmission of *A. crassus* in ell populations.

ANGUILLICOLA CRASSUS AND *A. GLOBICEPS* (NEMATODA: DRACUNCULOIDEA) PARASITIC IN THE SWIMBLADDER OF EELS (*ANGUILLA JAPONICA* AND *A. ANGUILLA*) IN EAST ASIA: A REVIEW

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This review compiles information on the taxonomy, identification, geographical distribution, life cycles, host ranges, occurrence, development and growth in both intermediate and final hosts, pathogenicity, and control measures of *Anguillicola crassus* and *A. globiceps*, swimbladder nematodes of farmed and wild populations of two species of eels, *Anguilla japonica* and *A. anguilla*, in Japan, Korea, Taiwan, and China. *Anguillicola crassus* is distributed throughout these countries, while *A. globiceps* was previously reported only from Japan and China. These nematodes use cyclopoid copepods as intermediate hosts. Known intermediate hosts are *Eucyclops serrulatus* (Japan) and *Thermocyclops hyalinus* (Korea) for *A. crassus*, and *Mesocyclops leukarti*, *T. hyalinus*, *T. taihokuensis*, *E. serrulatus*, *Acanthocyclops viridis*, and *Cyclops strenuus* (China) for *A. globiceps*. *Anguillicola crassus* shows a seasonal occurrence in *T. hyalinus* with high prevalence in summer. Paratenic hosts are as yet unknown in East Asia. *Anguillicola crassus* is relatively common in farmed and wild populations of *Anguilla japonica* in East Asia, but *A. globiceps* is usually found in wild populations of *A. japonica* in Japan and China. In culture ponds, *A. crassus* is more prevalent and abundant in *A. anguilla* than in *A. japonica*. Although *A. globiceps* induces only the thickening of the host's swimbladder wall, *A. crassus* produces severe pathological effects in *A. anguilla* and heavy infection leads to host mortality. Prevalence of *A. crassus* in *A. japonica* cultured in Japan and Korea is relatively low in winter, while prevalence of *A. globiceps* in wild populations of *A. japonica* from Japan is high in winter.

EXPERIMENTAL OBSERVATIONS ON THE DEVELOPMENT OF *ANGUILLICOLA CRASSUS* (NEMATODA: DRACUNCULOIDEA) IN ITS DEFINITIVE HOST, *ANGUILLA ANGUILLA*

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The development of the swimbladder nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 in the definite host (eels) was studied under experimental conditions. Small eels, *Anguilla anguilla* (L.), with body length 8–16 cm were infected by feeding

them intermediate host copepods (*Cyclops strenuus* Fischer) harbouring third-stage larvae of this parasite. These experiments showed that, at 20–22°C, the development from the third- to the fourth-stage larvae lasted approximately 3 weeks, but some retarding third-stage larvae occurred in the wall of the host's swimbladder or hyperparasitizing in the cuticle of adult nematodes as late as 3 months p.i. Young adults developed in the lumen of the swimbladder within approximately 1 month and nonembryonated eggs first appeared in females 6–7 weeks p.i. The prepatent period was about 3 months and the patent period could be estimated to last no more than a month. Females degenerated soon after oviposition. The experiments confirmed that the size of mature *A. crassus* depended on the body size of its definitive host (eel). The size of the buccal capsule and the shape of the cephalic end of the smallest adults of *A. crassus* obtained were rather similar to those found in the larger specimens of *A. novaezelandiae*. Accordingly, while evaluating a species appurtenance in these two closely related species, the size of the buccal capsule and the shape of the cephalic end in relation to the size of body should always be taken into account.

OCCURRENCE OF *ANGUILLICOLA CRASSUS* IN EELS OF LAKE NEUSIEDL, AUSTRIA

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From March 1990 to October 1992 a fish ecological study was carried out at Neusiedler See. The main aim was gut analyses of the most abundant fish species, such as the eel (*Anguilla anguilla*). In Neusiedler See the diet of eels is dominated by aquatic insects, gastropods and *Asellus aquaticus*, whereas zooplankton and fishes are unimportant food items.

Anguillicola crassus was initially recorded for the first time from Neusiedler See in October 1990. Subsequent investigations of 10 formaldehyde preserved eels from September 1988 showed an infection with *A. crassus* in two eels. Over the period October 1991 to July 1992 90 eels with a total body length from 17 to 59 cm were examined for intestinal helminths with special attention to *A. crassus*. Prevalence of the nematode infection was 50%, with a range of intensity from 1 to 13 parasites per fish. One eel of 70 cm body length, caught in the Wulka, a tributary of Neusiedler See, harboured 32 specimens of *A. crassus*.

COMPARATIVE STUDY ON THE LEAD ACCUMULATION IN TWO DIFFERENT HELMINTHS OF THE EUROPEAN EEL *ANGUILLA ANGUILLA*

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The aim of the present study was to combine trace analytical and parasitological methods in order to investigate lead concentrations in different tissues (muscle, bile, liver and intestine) of the European eel *Anguilla anguilla* and in the two helminths *Anguillicola crassus* and *Paratenuisentis ambiguus*. The eels were caught by a fisherman from the river Weser near Petershagen, Germany.

The eoacanthocephalan *Paratenuisentis ambiguus* accumulated significantly more lead than the organs of the eel and the nematode *Anguillicola crassus*, with a mean lead value of 3.7 $\mu g\ g^{-1}$ wet weight.

The lead concentrations in the organs of eel were found to be 0.10 $\mu g\ g^{-1}$ wet weight for muscle, 0.03 $\mu g\ g^{-1}$ wet weight for bile, 0.18 $\mu g\ g^{-1}$ wet weight for liver and 0.09 $\mu g\ g^{-1}$ wet weight for intestine. Thus, the lead value of the bile was 100 times lower than that of the acanthocephalans.

The mean lead concentration of 0.02 $\mu g\ g^{-1}$ wet weight of *A. crassus* was significantly lower than that of *P. ambiguus* although the nematode feeds on blood. It is pointed out in many studies, that heavy metal transport in fish is via the blood. For this reason it could be expected that *A. crassus* shows relatively high lead levels. However, the lead accumulation of *A. crassus* was lower than that of the muscle of eel. Thus, it seems to be possible that *A. crassus*, which bore into the swimbladder wall for the ingestion of blood and use aspartyl proteinase to degrade hemoglobin is able to excrete heavy metals.

THE BIOLOGY OF *ANGUILLICOLA CRASSUS*: A REVIEW

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This Invited Introductory Review paper will attempt to summarize the current knowledge on the life cycle and biology of *Anguillicola crassus*.

Life cycle:

Adults live in the swimbladder lumen of the eel; eggs pass via the pneumatic duct and the digestive tract into the water, where they hatch, liberating second-stage larvae; when ingested by cyclopid copepods, these larvae grow in the haemocoele and moult into third-stage larvae; when these copepods are eaten by eels, the L3 larvae migrate to the swimbladder wall where they grow and moult twice before passing into the swimbladder cavity. Small fish may act as paratenic hosts in which the larvae remain alive and able to infect eels.

Viability of the free-living L2 larvae:

At 21°C, eggs hatched in the water within a few hours; hatching was delayed at low temperatures and was strongly inhibited

in sea-water. The lifespan of L2 larvae decreased when temperature and salt concentration increased; survival and infectivity were also reduced in alkaline water.
Development in the intermediate host:
Development up to the third-stage was obtained only in cyclopid copepods and in ostracods. The rate of development increased with temperature.
Paratenic hosts:
Various freshwater and coastal fish species belonging to several orders can be infected by the larvae. In Cyprinidae and Poeciliidae, only third-stage larvae were found and most of them remained in the body cavity; in Percidae, Gasterosteidae and Osmeridae, the larvae migrated to the swimbladder wall and some of them moulted into L4 and even pre-adult stages.
Development in the definitive host:
Third-stage larvae migrated directly through the intestinal wall and body cavity to the swimbladder wall within 17 h at 20°C. The prepatent period varied depending on the experiments: at 20°C, the entire life cycle was completed in less than 2 months; at 15–16°C, mature females were obtained at 21 weeks p.i.; in another experiment, only pre-adults were detected after 6 months p.i. In all experiments, there is considerable variation in the rate of development of individual worms.
Survival of adults in sea-water:
The survival of adult parasites and their eggs was not affected by acclimatization of eels to sea-water.

PARATENIC HOSTS FOR THE PARASITIC NEMATODE *ANGUILLICOLA CRASSUS* IN LAKE BALATON, HUNGARY

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A one-year study was conducted to determine which fish species may play a role in the life cycle of *Anguillicola crassus* in various habitats in Lake Balaton. The prevalence and intensity of the larval infection of fish species acting as paratenic hosts were studied, and observations were made on the types of paratenic host reactions against larvae. With the exception of one species, all the 20 fish species studied were infected by *Anguillicola* larvae; however, the prevalence and intensity of infection varied widely. Six new species (asp, white bream, Chinese rasbora, pike, river goby, European catfish), hitherto unreported as paratenic hosts, proved to be infected by larvae in Lake Balaton. Of the 13 fish species examined in large numbers, the ruffe and the European catfish showed the highest prevalence of infection (100%), followed by the river goby (83%), the white bream (79%) and the bleak (68%). Of these 13 fish species, the ruffe showed the highest intensity of infection by live larvae (mean intensity: 39.3 3rd stage larvae), followed by the European catfish (mean number of live larvae: 26.9) and the river goby (mean number of live larvae: 9.1). The mean number of live L3 in the bleak, a species regarded as the principal food source for eels, was 4.1. Specimens containing only dead or both dead and live larvae were much more common in cyprinid fishes than in species belonging to other taxonomical entities. In these fish, the process of encapsulation and subsequent necrosis of live larvae could also be observed. With knowledge of the feeding habits of eels, it appears that the bleak plays the most important role in the transmission of anguillicolosis. Other intensively infected fish species (e.g. the ruffe) may also contribute to massive infection of individual eels, even if they have a lower share in the eels' food structure.

ANGUILLICOLA CRASSUS: FINE STRUCTURE, PATHOGENICITY, TREATMENT

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Adults of *Anguillicola crassus* are invested by a three-layered cuticle. The thick outer layer has a gelatinous consistency and is surrounded by a multilaminated epicuticle. The gut filled with eel blood is lined by a thick intestinal wall containing numerous electron-dense and electron-lucent droplike inclusions.

Levamisole was found to be the best drug for treatment of *A. crassus*. The ratio between curative dose and lethal dose is about 1:100 and thus better than of other drugs like Metrifonate which also kills this parasite. Worms in eels treated by bathing with Levamisole show a vacuolization of their intestinal wall starting around the droplike inclusions and ending up in a total vacuolization of all tissues. The larvae L2 to L4 cannot be treated by drugs, probably because they do not feed on blood but on connective and other tissues. These larval stages do not have a gelatinous outer layer of the cuticle. The L2-larva contains a gut by the end of its development, but the gut has no lumen and thus does not contain any host tissues. In the L3-larva the gut is filled with collagen fibers and other remnants of host cells. The intestine of the L4 resembles that of the adult worm. However it does not contain blood but matter similar to that found in the L3. Several aspects of the pathogenicity of *A. crassus* have been investigated by different authors. In the European eel the pathological effects seem to be more pronounced than in *Anguilla japonica*.