The life cycle of *Anguillicola crassus*

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**ABSTRACT:** For some years now the parasitic swim bladder nematode *Anguillicola crassus* of the European eel *Anguilla anguilla* L., has been reported from several European countries. The entire life history of this parasite has recently been elucidated in our laboratory. Young larvae leave the swim bladder of the host via the pneumatic duct and reach the water through the digestive tract. They are ingested by small copepods (Cyclopoida), which act as intermediate hosts. Larvae remain in the hemocoel until the copepods are eaten by the final host, the European eel. Larvae penetrate through the intestinal wall and reach the swim bladder where they develop into adults. When infected copepods are eaten by other small fish, such as carp *Cyprinus carpio* L. or ide *Leuciscus idus* L., larvae do not reach the adult stage. However, when larger eels feed on such facultative reservoir hosts, they too become infected.

**INTRODUCTION**

*Anguillicola crassus*, a parasitic swim bladder nematode of the European eel *Anguilla anguilla*, was first observed in Belgium in December 1985 (De Charleroy 1986). This parasite, originating from South East Asia (Kuwahara et al. 1974), probably reached Western Europe in the early eighties via the import of infected eel for consumption or restocking (Peters & Hartmann 1986, Belpaire et al. 1989a, b). Very soon, it was apparent that this parasite had spread quickly through several countries, not only causing problems to eel growers, but also infecting a rapidly increasing percentage of the natural eel populations (Paggi et al. 1982, Neumann 1985, Peters & Hartmann 1986, Belpaire et al. 1989a, b). According to Chabaud (1965), nematodes from the suborder Camallanata always use a crustacean as intermediate host, often a copepod. Wang & Zhao (1980) noticed that several species of copepods are able to carry the infective stage of the parasite *Anguillicola globiceps* to the final host. De Charleroy et al. (1987) found that all of 10 Cyclopoida species tested were able to take up *A. crassus* larvae and carry them in their hemocoel. The species involved were: *Paracyclops fimбриatus*, *Macrocyclops albidus*, *M. fuscus*, *Eucyclops serrulatus*, *E. macruroides*, *Cyclops strenuus*, *C. vicinus*, *Acanthocyclops robustus*, *A. vernalis* and *Diacyclops bicuspidatus*.

Although copepods are not considered a main food item for eels, they are eaten by younger individuals (Lecomte-Finlger 1983, De Nie 1987) and as the eels grow, the size of their prey increases (Neveu 1981). According to Tesch (1977) the proportion of fish found in the stomach of the eels increases with length, and from a certain length on (40 to 50 cm) they show feeding patterns typical of piscivorous predators. The largest eels (>50 cm) feed almost exclusively on fish.

In nature, small eels as well as larger ones seem to become infected by *Anguillicola crassus*. According to Peters & Hartmann (1986) eels become already infested after they reach a length of about 20 cm and a weight of ca 10 g and as the eels grow, the frequency of infestation increases. Larval Stage 3 and 4 are also frequently found in the swim bladder of larger eels, which are believed not to feed regularly on such small prey. Referring to Barus & Rysavy (1973), who describe the different forms and prevalence of reservoir habituation in Nematoda, we tried to find out if the transfer of *A. crassus* from any other fish to eel was possible.
RESULTS AND DISCUSSION

Larva of Anguillicola crassus

In a few cases copulating nematodes were observed in swim bladders from eels infected with adult Anguillicola crassus. The liquid present in these swim bladders regularly contained tens of thousands of eggs, (mean length 90 μm, mean width 75 μm) containing L2 larvae. Already in the uteri of the fecundated females, L1 larvae moulted into L2 larvae. At oviposition, L2 larvae were present in the eggs of the parasite, still surrounded by the loose L1 cuticle. In the swim bladder most of the L2 larvae remained in the egg capsule, although a small percentage of larvae had already hatched (Figs. 1 and 2).

Intermediate host infection

Eggs containing L2 larvae brought into contact with freshwater hatched within a few hours at room temperature (ca 21 °C). Such larvae were used to infect Paracyclops fimbriatus. As expected P. fimbriatus fed with L2 carried A. crassus larvae in their hemocoel (mean 4.9 larvae copepod⁻¹). Compared with free living L2 larvae
Fig. 1. *Anguillicola crassus* L₂ as they can be found in the swim bladder of an infected eel. They are still surrounded by their egg sheath and the L₁ cuticle.

Fig. 2. *Anguillicola crassus*. Hatching of an L₂ from the egg. The larva (1), loose L₁ cuticle (2) and egg sheath (3) are clearly visible.
Fig. 3. *Anguillicola crassus*. *Paracyclops tibniatus* (intermediate host) infected with larvae (1) which are visible in the abdomen.

Fig. 4. *Anguillicola crassus*. L₀ larvae dissected out of the intermediate host *Paracyclops tibniatus* 10 d after infection with L₂ larvae. Mouling has already taken place.
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(mean length 250 μm, mean width 18 μm), no growth was observed during the first days post-infection, as a matter of fact, larvae became smaller during the first 3 d but from Day 4, larvae steadily started growing. Between Days 10 and 12 moulting to the L3 stage (Figs. 3 and 4) took place (mean length 716 μm, mean width 37 μm).

**Final host infection**

Eighteen of the 19 elvers infected with copepods carrying L3 larvae contained young *Anguillicola crassus* in their swim bladder (range 4 to 357, mean 99). Since we could not verify how many copepods each eel ate these numbers were of little quantitative value to demonstrate the infection efficiency, although it is worth mentioning for its qualitative value. Preliminary experiments using *Paracyclops fimbriatus* containing L2 larvae for 1 or 7 d gave poor infection percentages (5 to 9 %); we had expected completely negative results.

In the second experiment 26 of 30 glass eels contained young parasites (mean 3.3, max. 22). This indicates that even the very young glass eel stages can be infected. In those glass eels where the swim bladder was still very small, L3 larvae remained in the body cavity instead of penetrating into the swim bladder wall.

**Table 1. *Anguillicola crassus* number in swim bladder of 6 eels, each previously fed with one infected fish**

<table>
<thead>
<tr>
<th>Eel no.</th>
<th>Post-infection (d)</th>
<th>Fish</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>Ide</td>
<td>1 larva</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>Carp</td>
<td>72 larvae</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>Ide</td>
<td>11 preadults</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>Carp</td>
<td>2 adults</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>Ide</td>
<td>Not infected</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>Carp</td>
<td>6 adults, large no. of eggs</td>
</tr>
</tbody>
</table>

**Reservoir and final host infection**

The 10 control ide and carp examined, before the start of the experiment, all were negative. In the 10 infected fish no *Anguillicola crassus* were found in the swim bladder. Living L3 larvae of *A. crassus* were found free in the body cavity of all fish examined 15 and 60 d post-infection.

Five of the 6 eels fed with these L3 infected ide and carp, contained *Anguillicola crassus* (Table 1). We have no indications why these L3 larvae remained in the body cavity of ide and carp, although it could be linked to the developmental stage of their swim bladder.

![Fig. 5. *Anguillicola crassus*. Sections of L3 larvae (1) in the submucosa of the swim bladder of eel *Anguilla anguilla* where they pass during their migration to the swim bladder lumen (H & E staining)](image)
Fig. 6. *Anguiilloca crassus*. The difference between L₃ (1) and L₄ (2) larvae is clearly visible. The L₄ larvae are considerably larger and are stained darkly.

Fig. 7. *Anguiilloca crassus*. Nematode moulting from L₄ larva to preadult. The L₄ cuticle is still surrounding the parasite and is visible at the head end of the parasite (1).
carp samples taken from Belgian ponds and rivers. L₃ larvae were found in the body cavity as well as in the swim bladder. In perch Perca fluviatilis and pumpkinseed Lepomis gibbosus from field samples taken in Belgium, L₃, L₄ and even preadult stages were found in the swim bladder. However, these preadults exhibited an atypical morphology and would probably never have reached sexual maturity (Cannaerts 1989). Belpaire et al. (1989a) found 3-spined sticklebacks Gasterosteus aculeatus infected with A. crassus larvae in the swim bladder wall (River Yser, Belgium).

After infection of eels, the relatively mobile L₃ larvae did not immediately penetrate the lumen of the swim bladder but remained in the submucosa (Fig. 5). There they developed into the less mobile L₄ larvae (Fig. 6), which required just over 2 wk under our experimental conditions. These larvae were bigger and stained darkly, due to the presence of blood in their digestive system. After the last moult (Fig. 7) preadults (the gonadal system of which was not yet functioning) were found in the swim bladder cavity. Sexes of the parasites can easily be distinguished in the adult stages. The seminal vesicle is well defined in male parasites, whereas in female Anguillicola crassus the uteri and the vulva are clearly visible.

The 3 eels fed with infected glass eels 2 wk earlier contained 9 L₄, 12 L₃ and 7 L₄ Anguillicola crassus larvae in the wall of their swim bladder, respectively.

Release of eggs and larvae from the swim bladder

Shortly after an abrupt, artificial decrease in atmospheric pressure, large amounts of eggs were found in the upper region of the digestive tract of infected eel, indicating that the pneumatic duct probably serves as a route for Anguillicola crassus to passively leave the swim bladder. In the intestinal tract of infected eel, A. crassus eggs as well as free living L₂ stages were regularly observed; these are passed with the feces into the water.

LIFE HISTORY OF ANGUILLICOLA CRASSUS

The results presented here and other preliminary experiments allow us to give a more complete overview of the life cycle of Anguillicola crassus. Copulation between adult male and female parasites takes place in the swim bladder of the final host, the eel. The fertilized eggs develop in the female reproductive system and thus contain L₂ larvae at the moment of oviposition. Most of the larvae remain in the egg during their stay in the swim bladder, which they subsequently leave via the pneumatic duct, probably passively.

During or after passage through the digestive tract hatching occurs and L₂ larvae emerge from the eggs; the cuticula of the L₁ larvae forming a loose sheath surrounding the L₂ stage. These free-living L₂ larvae (ca 250 x 18 μm) fasten themselves by their tail-end to the substratum and wriggle their body intensively. This behaviour presumably stimulates predation by copepods. At this stage, larvae can stay alive up to 1 mo depending on external factors such as salinity and temperature (De Charleroy et al. 1989).

When eaten by a suitable intermediate host, larvae ensconce themselves in the hemocoel and start growing after a few days. The larvae moult in the copepod and reach the third and infective stage (L₃) after 10 to 12 d (at 21 °C) (Thomas & Ollevier 1989). Eels eating such infected copepods can become infected from the glass-eel stage onwards. When these infected copepods are eaten by small fish e.g. carp or ide, the L₃ larvae remain alive in the fish. These small reservoir hosts can be eaten by bigger eels, which thus become infected (Grisez 1988).

From the lumen of the eel intestine the L₃ larvae reach the swim bladder wall by passing through the intestinal wall and the body cavity (Haenen et al. 1989). Nematodes moult to L₄ after 2 to 3 wk in the swim bladder, suck blood and grow. They moult again, grow further, become sexually mature and commence reproducing in the swim bladder cavity. Under laboratory conditions (at 20 °C) the complete life cycle can take less than 2 mo.

It is easier to understand the exponential spread of Anguillicola crassus in Europe during recent years when taking into account the large quantities of A. crassus eggs found in the swim bladder of an infected eel (sometimes originating from a single pair of adults), the rather short period needed to complete the life cycle and the often careless import, export and restocking for commercial purposes.

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