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# Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*

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ABSTRACT: The effect of low water temperatures on the development and viability of larval and adult *Anguillicola crassus* (Nematoda) in the final host *Anguilla anguilla* was studied. European eels were experimentally infected with *A. crassus* and then maintained for 4 mo at 4, 9, 10 and 19°C. Larval development showed a temperature-dependent pattern and was significantly retarded at low temperatures. Third-stage larvae survived a 4 mo period at 4°C without being affected, although they were not able to invade the swimbladder wall at this temperature. In contrast, adult worms were severely harmed during a 4 mo period at 4°C, as reflected by increased mortality and decreased growth and reproductivity compared to the worms maintained for the same period at 18°C. Starvation of the eels for 4 mo at 19°C did not affect the development and growth of the nematode. The experimentally obtained results support the hypothesis that the spread of *A. crassus* in boreal regions, e.g. Northern Europe, is restricted by the natural ambient temperature regimes.

KEY WORDS: Eel · *Anguillicola crassus* · Development · Temperature · Starvation

## INTRODUCTION

After *Anguillicola crassus* (Nematoda, Dracunculoidea) was imported from the Far East to Europe in the early 1980s, it spread rapidly across the continent. Ten years after the first report of its presence in 1982 in eels from the Weser-Ems region, Germany (Neumann 1985), this parasite had become established over almost the whole of Europe (Moravec 1992). However, in southern Sweden Höglund et al. (1992) reported *A. crassus* populations being restricted to areas affected by thermal discharges from power stations on the Baltic coast, whereas eels from inland lakes were uninfected. These results implied that the water temperature required for a complete life-cycle of *A. crassus* is not naturally available in the areas examined.

The effect of water temperature on the hatching of *Anguillicola crassus* eggs and on the free-living second-stage larvae ($L_2$) was extensively examined by Thomas & Ollevier (1993). In accordance with the findings of Kim et al. (1989, cited in Nagasawa et al. 1994) they showed that the optimum temperature for hatching lies in the range between 15 and 30°C. At these temperatures the eggs hatch within a few days. At 10°C hatching is severely delayed, taking almost 1 mo, whereas at 5°C the $L_2$ in the egg sheath survive but rarely emerge.

The survival of free-living $L_2$ is also dependent on temperature. Although the published data do not correspond perfectly with each other (De Charleroy et al. 1989, Petter et al. 1990, Thomas & Ollevier 1993), it is evident that at lower temperatures the survival time is noticeably prolonged. However, the infectivity of the $L_2$ to the intermediate host declines with larval age (Kennedy & Fitch 1990, Thomas & Ollevier 1993). Kennedy & Fitch (1990) reported $L_2$ survival of up to 160 d at 10°C. In contrast, they survive only up to 45 d at 23°C (Thomas & Ollevier 1993).

Subsequent development in the copepod intermediate host is also affected by the water temperature. At approximately 21°C moulting to the third larval stage ($L_3$)

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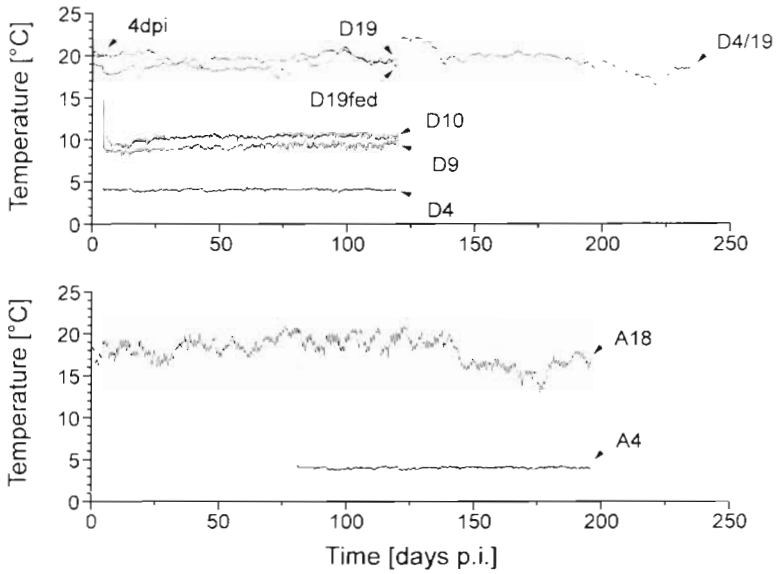


Fig. 1. Temperature regime to which eels were exposed during the experiment. Arrows indicate the times when eels of the different experimental groups (for abbreviations see Table 1) were examined. p.i.: post infection

occurs between Days 10 and 13 post infection (p.i.) of the copepods (Petter et al. 1989, De Charleroy et al. 1990, Thomas & Ollevier 1993). In contrast, the development is very slow at 12°C and moulting to $L_3$ does not occur until Day 62, whereas at higher temperatures ranging from 18 to 29°C the process is accelerated and first $L_3$ appear on Day 6 (Petter et al. 1990). Furthermore, Petter et al. (1989) demonstrated that in the (unfortunately not further specified) range from 1 to 13°C the $L_2$ did not develop and even died within about 1 mo. However, the larvae were able to migrate into the haemocoel of the copepods. In consequence, Petter et al. (1989) concluded that the life-cycle of *Anguillicola crassus* is interrupted during the cold season and Kim et al. (1989, cited in Nagasawa et al. 1994) suggested that the nematode requires at least 15°C for a complete life-cycle.

However, there is a lack of information concerning the impact of temperature on *Anguillicola crassus* in the final host. Thus, in the present study the importance of water temperature for the invasion of the swimbladder wall by infective $L_3$ and their further development to mature adults via a fourth larval stage ($L_4$) was investigated under controlled laboratory conditions. Furthermore, the influence of low water temperatures on the viability and reproductivity of the adult worms dwelling in the swimbladder lumen of eels was studied.

## MATERIAL AND METHODS

**Source and maintenance of eels.** European eels *Anguilla anguilla* weighing 60 to 80 g were obtained from a commercial fish farm (Limnotherm, Bergheim, Germany) known to be free of *Anguillicola crassus*. The eels were placed in 100 l tanks and maintained in aerated tap water at different temperatures ranging from 4 to 20°C (Table 1 & Fig. 1). The water temperature was measured and recorded hourly with a temperature logger (EBI-85, Ebro Electronics, Ingolstadt, Germany) during the whole experimental period (Fig. 1).

**Infective larvae and infection.** Infective third-stage larvae ($L_3$) of *Anguillicola crassus* were obtained according to Haenen et al. (1994). Second-stage larvae were collected from the swimbladder lumen of naturally infected eels from the river Rhine. These larvae were fed to wild-caught planktonic copepods, mainly comprising *Thermocyclops* cf. *crassus* and *Mesocyclops leuckarti*, kept at 20°C. The infective $L_3$ were isolated 20 d p.i. from the intermediate hosts by the potter method described by Haenen et al. (1994) and stored in RMPI-1640 medium (Sigma, Deisenhofen, Germany) containing 0.2% kanamycin at 4°C until application.

Prior to infection the larvae were counted in a round bottom 98-well plate and suspended in approximately 100 µl RMPI-1640 medium. This suspension was administered to the eels perorally with a stomach tube (1.5 mm diameter). The wells were checked for remaining $L_3$ afterwards.

**Experimental design.** A summary of the experimental design and the number of eels of each experimental

Table 1. Experimental design showing the mean ± SD temperatures at which the different experimental groups were maintained for the times given at the top and the number of eels in each group (n). p.i.: post infection

| Group | n | Day 1 to 4 p.i. | Day 5 to 119 p.i. | Day 120 to 234 p.i. |
|---|---|---|---|---|
| 4dpi | 20 | 20.2 ± 0.2°C | – | – |
| D4 | 20 | 19.9 ± 0.1°C | 4.0 ± 0.1°C | – |
| D9 | 21 | 20.2 ± 0.2°C | 9.0 ± 0.4°C | – |
| D10 | 21 | 20.2 ± 0.2°C | 10.2 ± 0.5°C | – |
| D19 | 20 | 18.8 ± 0.1°C | 18.8 ± 0.7°C | – |
| D19fed | 19 | 19.9 ± 0.1°C | 19.7 ± 0.5°C | – |
| D4/19 | 28 | 19.9 ± 0.1°C | 4.0 ± 0.1°C | 19.4 ± 1.3°C |

| | | Day 1 to 80 p.i. | Day 81 to 115 p.i. |
|---|---|---|---|
| A18 | 36 | 18.4 ± 1.0°C | 17.7 ± 1.8°C |
| A4 | 46 | 18.4 ± 1.0°C | 4.0 ± 0.1°C |

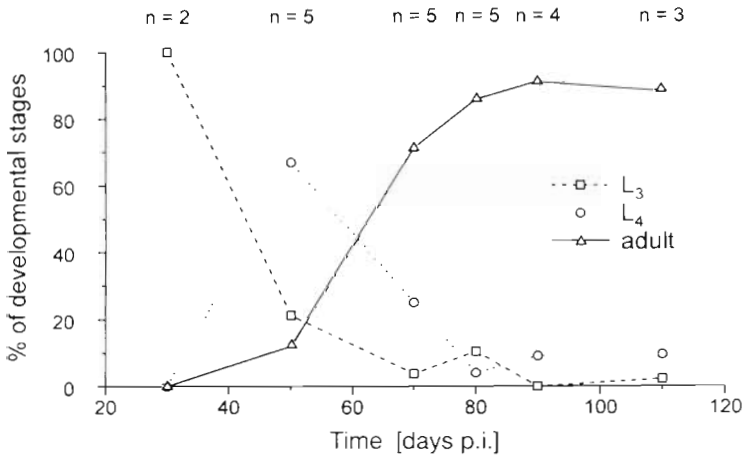


Fig. 2. *Anguillicola crassus*. Relative percentages of different developmental stages of the nematode out of total number of specimens recovered from the swimbladder of spot-checked *Anguilla anguilla* kept at 18°C. The number of eels examined (n) is given for each spot-check

group is given in Table 1. The impact of temperature on the larval development of *Anguillicola crassus* was examined by keeping eels infected with 20 $L_3$ each at 4, 9, 10 or 19°C for 115 d (groups D4, D9, D10 and D19). The intermediate temperatures of 9 and 10°C resulted from difficulties regulating the temperature; initially, 8 and 12°C were intended. To determine whether the maintenance at 4°C (group D4) was irreversibly harmful to the larvae, another group of eels (group D4/19) was first kept at 4°C for 115 d and subsequently at 19°C for additional 115 d. To allow the $L_3$ to migrate into the swimbladder wall, eels of all groups were kept initially at approximately 20°C for 4 d, following which the status of infection was determined (group 4dpi).

As eels are known to feed little at low temperatures (Tesch 1983) and to maintain uniform experimental conditions the eels of group D4, D9, D10 and D19 were starved in this experiment. To elucidate whether *Anguillicola crassus* were harmed by starving of the host, the results obtained from the eels kept at 19°C (group D19) were compared with those from identically treated, but fed, eels (group D19fed). These eels were fed twice a week with pellet food at a rate of 0.7 g $eel^{-1}$. Additionally, the eels of group D4/19 were also fed while being kept at 19°C.

To study the impact of cold water conditions on adult *Anguillicola crassus* eels were infected with 25 $L_3$ each. To allow the development of the nematodes from the larval to the adult stage all infected eels were first kept at 18°C. The developmental status of the worms was spot-checked (for number of eels see Fig. 2) from Day 30 p.i. onwards. Thus, it could be concluded that most of the $L_3$ administered to the eels became adult within 80 d p.i. Subsequently the remaining eels were divided into 2 groups and kept for a further 115 d at 18°C (group A18) and 4°C (group A4), respectively. During the maintenance at 18°C, the eels were fed in this experiment as described above.

At the end of the experiments the eels were killed and the swimbladder was examined for living and dead larvae and adults of *Anguillicola crassus*. As $L_3$ and $L_4$ cannot be distinguished from each other perfectly by means of light microscopy (Blanc et al. 1992), all larvae with a body length exceeding 1.5 mm were counted as $L_4$, in accordance with the results of Blanc et al. (1992). After determining the sex of all adult worms from each eel, the male and female *A. crassus* were counted separately. Living specimens were pooled from each eel, weighed and the mean wet weight for an individual male or female was then calculated. Nematodes showing no reaction to mechanical stimulation were considered dead and grouped with the decomposed specimens. The presence of $L_2$ (in the egg sheath or emerged) in the swimbladder lumen of the eels was used as proof of the successful reproduction of the nematodes.

**Statistical analysis.** Data are presented as mean values ± standard deviation (SD). The data sets of the recovery rate, the percentage of different developmental stages, the percentage of dead *Anguillicola crassus* and the mean wet weight of the adult worms obtained for the different groups (Table 1) were analysed using the *H*-test (Kruskal-Wallis) and/or the *U*-test (Mann-Whitney) for significant differences ($p \leq 0.05$).

## RESULTS

### Impact of temperature on larval *Anguillicola crassus*

The larval development of *Anguillicola crassus* in the final host showed a clear temperature-dependent pattern. The mean number of *A. crassus* recovered from eel swimbladders at the end of the experiment increased with the water temperature at which the eels were maintained (Fig. 3). The lowest recovery rate, determined for the eels kept 4 d at 20°C and subsequently 115 d at 4°C (group D4, 10.0 ± 10.1%), was nearly the same as the rate obtained from eels kept only 4 d at 20°C (group 4dpi, 10.3 ± 8.8%). At all the other temperatures examined significantly higher recovery rates were obtained. Statistical analysis revealed no differences in the recovery rate between group D9 (31.2 ± 13.5%), group D10 (41.9 ± 23.9%) and group D19 (34.5 ± 15.8%).

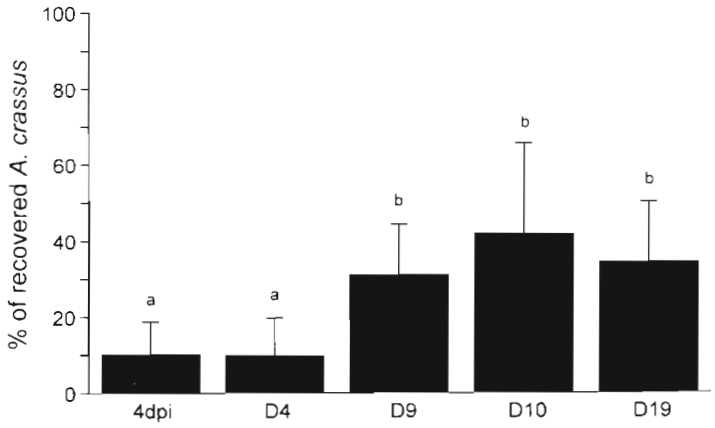


Fig. 3. *Anguillicola crassus*. Mean percentage recovery of nematodes from the swimbladder of *Anguilla anguilla* kept for 4 d at 20°C (4dpi), 4 d at 20°C and 115 d at 4°C (D4), at 9°C (D9), and at 10°C (D10) and continuously at 19°C (D19). a,b: Groups with a letter in common do not differ significantly at $p \leq 0.05$

Fig. 4 shows the percentage of the different developmental stages of *Anguillicola crassus* recovered at the end of the experiment. After 4 d at 20°C and 115 d at 4°C (group D4) as well as 4 d p.i. at 20°C (group 4dpi) only $L_3$, but no $L_4$ or adult worms were found. At 9°C (group D9) only a small proportion of worms (5.8 ± 14.3%) had developed to $L_4$. At 10°C (group D10) a considerably higher number of the nematodes reached $L_4$ (41.6 ± 32.3%) and even a few adult worms (0.4 ± 1.7%) could be found in the swimbladder lumen. However, 58.0 ± 31.9% of *A. crassus* still remained at $L_3$. Finally, at 19°C (group D19) the $L_3$ contributed only 1.9 ± 5.1%, and the $L_4$ 9.2 ± 12.2%, to all recovered specimens, whereas 88.9 ± 14.0% of the worms had reached the adult stage. In all swimbladders of eels from this group containing mature male and female *A. crassus* (85.0% of the examined eels), the successful reproduction of the parasite was manifested by a large number of $L_2$ in the swimbladder lumen. The results of the statistical analysis comparing the abundance of the developmental stages $L_3$ and $L_4$ and adults, respectively, with the eels maintained at the different temperatures are shown in Table 2.

Table 2. *Anguillicola crassus*. Results of the *U*-test ($p \leq 0.05$) comparing the abundance of the developmental stages of the nematode with *Anguilla anguilla* kept for 4 d at 20°C and 115 d at 4°C (D4), at 9°C (D9) and at 10°C (D10) and continuously at 19°C (D19). s: significantly different; ns: not sig-nificantly different; nd: not determined

| Groups | $L_3$ | $L_4$ | Adult |
|---|---|---|---|
| D4-D9 | s | s | nd |
| D9-D10 | s | s | ns |
| D10-D19 | s | s | s |

If experimentally infected eels which were kept first 4 d at 20°C and subsequently 115 d at 4°C were transferred to 19°C (group D4/19), *Anguillicola crassus* larvae continued their development without being negatively affected (Table 3). After 115 d at 19°C the recovery rate was not significantly different from the results obtained from the eels kept continuously for 119 d at 19°C (group D19). In eels that were maintained previously at 4°C the percentage of adult worms and their mean wet weight was significantly higher than in eels solely kept at 19°C

### Impact of eel starvation on *Anguillicola crassus*

The results obtained from the eels that were fed (group D19fed) compared to those which were starved and kept at nearly the same temperature (group D19) are presented in Table 3. Data analysis revealed no significant difference between the 2 groups with respect to the recovery rate, the percentage of the developmental stages, the wet weight of the adult worms or their reproductivity.

### Impact of temperature on adult *Anguillicola crassus*

The results from the spot-checked eels kept at 18°C are shown in Fig. 2. After 50 d p.i. the first adults of

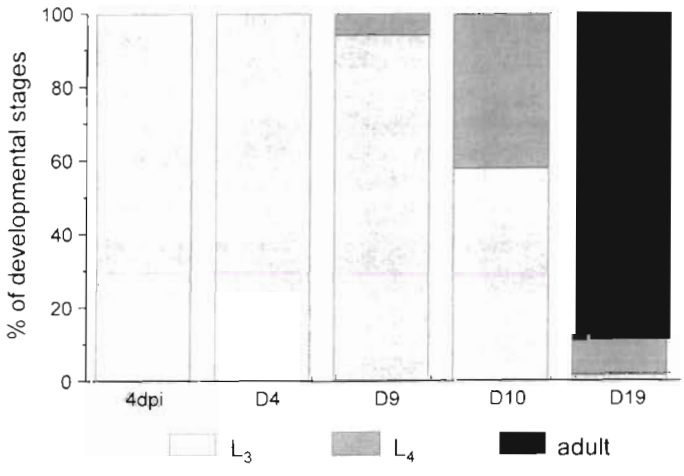


Fig. 4. *Anguillicola crassus*. Relative percentages of different developmental stages of the nematode out of total number of specimens recovered from the swimbladder of *Anguilla anguilla* kept for 4 d at 20°C (4dpi), 4 d at 20°C and 115 d at 4°C (D4), at 9°C (D9) and at 10°C (D10) and continuously at 19°C (D19)

Table 3. Influence of a cold water period and starvation on the development of *Anguillicola crassus*. The following are given: mean percentage of *A. crassus* recovered from total number of $L_3$ administered, percentages of developmental stages from the recovered specimens, mean wet weight of male and female *A. crassus* and percentage of *Anguilla anguilla* containing $L_2$ in the swimbladder lumen. Sample sizes (n) are given in parentheses

| | Group D19fed[a] Mean ± SD (n) | Group D19[a] Mean ± SD (n) | Group D4/19[b] Mean ± SD (n) |
|---|---|---|---|
| % recovery | 48.2 ± 24.3 (19) | 34.5 ± 15.8 (20) | 37.9 ± 26.4 (28) |
| % of $L_3$ | 13.1 ± 31.2 (19) | 1.9 ± 5.1 (20) | 0.8 ± 3.9 (26) |
| % of $L_4$ | 2.9 ± 5.7 (19) | 9.2 ± 12.2 (20) | 1.4 ± 4.5 (26) |
| % of adults | 84.0 ± 30.8 (19) | 88.9 ± 14.0 (20) | 97.8 ± 5.8 (26) |
| Wet weight of males (mg) | 9.8 ± 6.5 (16) | 14.2 ± 7.6 (18) | 17.4 ± 10.1 (24) |
| Wet weight of females (mg) | 100.4 ± 46.7 (16) | 124.3 ± 42.3 (20) | 169.0 ± 43.7 (22) |
| % of eels containing $L_2$ | 78.9 (19) | 85.0 (20) | 78.6 (28) |

[a]119 d at approximately 19°C. [b]4 d at 20°C, 115 d at 4°C and 115 d at 19°C

*Anguillicola crassus* appeared in the swimbladder lumen and 67% of the nematodes had reached $L_4$. At Day 80 p.i. more than 85% of the worms were adult and from this time on the percentage of adult worms changed only by a small amount. Thus the period of time during which moulting from $L_4$ to adults occurred ranged from 50 to 80 d at a water temperature of 18°C.

The impact of the low water temperature on adult *Anguillicola crassus* became obvious when comparing the data obtained from eels which were kept from Day 81 to Day 185 at 4°C (group A4) and 18°C (group A18), respectively (Table 4). Although the recovery rates showed no significant differences between the 2 groups, all the other data revealed that adult worms were deleteriously affected by the low temperature. The percentage of dead adult worms and the percentage of larval stages were significantly higher for the eels maintained at 4°C. Additionally, the mean wet weight of adult *A. crassus* and the number of swimbladders containing $L_2$, indicating reproductive activity of adult worms, in eels kept at 4°C was approximately 10 times less than the values determined for eels kept at 18°C.

Table 4. Impact of water temperature on adult *Anguillicola crassus*. The following are given: mean percentage of *A. crassus* recovered from total number of $L_3$ administered, percentages of developmental stages from the recovered specimens, percentage that were dead, mean wet weight of male and female *A. crassus* and percentage of *Anguilla anguilla* containing $L_2$ in the swimbladder lumen. Sample sizes (n) are given in parentheses

| | Group A18[a] Mean ± SD (n) | Group A4[b] Mean ± SD (n) |
|---|---|---|
| % recovery | 29.8 ± 26.8 (36) | 36.4 ± 25.9 (46) |
| % of $L_3$ | 7.7 ± 19.3 (30) | 16.2 ± 16.0 (41) |
| % of $L_4$ | 7.3 ± 18.6 (30) | 15.7 ± 11.9 (41) |
| % of adults | 85.0 ± 27.5 (30) | 68.1 ± 18.6 (41) |
| % of dead *A. crassus* | 10.1 ± 22.2 (30) | 39.1 ± 27.5 (41) |
| Wet weight of males (mg) | 37.2 ± 19.2 (24) | 4.7 ± 8.3 (33) |
| Wet weight of females (mg) | 187.2 ± 72.4 (24) | 16.4 ± 11.1 (31) |
| % of eels containing $L_2$ | 75.0 (36) | 8.7 (41) |

[a]195 d at 18°C. [b]80 d at 18°C and 115 d at 4°C

## DISCUSSION

Development and viability of *Anguillicola crassus* in the final host were shown to be temperature-dependent. The growth and development of worms did not differ significantly between the starved and fed eels kept at approximately 19°C. Considering the reduced metabolism of host and parasite at lower temperatures, one can therefore deduce that starvation of the eels over 4 mo at 4, 9 or 10°C does not affect the nematodes. Results of Polzer & Taraschewski (1993) revealed that adult worms are able to degrade haemoglobin, which might be sufficient as a nutritive source. Thus, the findings of the present investigation can be attributed exclusively to the impact of temperature.

The pattern in the relative proportions of developmental stages of *Anguillicola crassus* after 115 d at different temperatures can be explained by retarded larval development at lower temperatures. This would be expected, as temperature is known to be an important factor controlling the development of poikilothermic organisms. At about 10°C, a critical limit appears to be reached as the relatively small rise in temperature from 9 to 10°C caused a significant speeding up of larval development.

The results indicate a doubling of the time to the first appearance of

adult *Anguillicola crassus* in the swimbladder lumen at 10°C compared to 18°C. A developmental period of approximately 50 d at 20°C as revealed by the spot-checked eels is consistent with the results of Haenen et al. (1996), who observed the first *A. crassus* in the swimbladder lumen 48 d p.i. at 18 to 20°C. Nevertheless, at the end of the experiment the relative proportions of developmental stages were highly variable within experimental groups, reflecting individual differences in the specimens.

The increased recovery rate at higher temperatures leads to the assumption that not only the duration of development but also the mobility of invading $L_3$ is affected by the ambient temperature. This becomes obvious at 4°C, since after 4 d at 20°C and 115 d at 4°C the number of recovered *Anguillicola crassus* was as low as after 4 d at 20°C. This shows that the $L_3$ do not invade the swimbladder wall simultaneously and also that at 4°C they were unable to invade the swimbladder wall at all.

At 4°C no sign of larval development could be detected over a period of 4 mo. However, the percentage of adult *Anguillicola crassus* and their mean wet weight was significantly higher when eels were maintained after the cold spell for a further 4 mo at 19°C compared to eels maintained solely for 4 mo at 19°C. At least 2 hypotheses might explain these findings. It seems possible that the $L_3$ pass a diapause at 4°C and further development at increased temperatures is stimulated due to the preceding cold period. On the other hand, development from $L_3$ to $L_4$ might not be entirely stopped but only severely retarded. In this case, one also has to assume a correlation between temperature and larval development. Based on the results obtained from the eels kept at 9 and 18°C, the time required from infection to the third moult from $L_3$ to $L_4$ at 4°C clearly exceeds 115 d. This could explain why no development was observed until Day 115 at 4°C. Although it seems unclear whether larval development continues at 4°C or if it is interrupted until the temperature increases again, it can be concluded that a 4 mo period at 4°C does not influence the viability of $L_3$.

In contrast to the larvae, adult *Anguillicola crassus* were severely harmed during a 4 mo period at 4°C. Comparing the nematodes kept at 18°C to those kept at 4°C, the latter showed a significantly lower wet weight. This may reflect a drastically reduced or even arrested metabolism and consequently reduced feeding activity. The 4 times higher number of dead adult worms illustrated the irreversible harm caused by the low temperature. As a result, the reproduction of *A. crassus* was also clearly reduced. The few $L_2$ found in the eels kept at 4°C might even have been left over from the period spent at 18°C. This appears reasonable as free-living $L_2$ survive a considerable time at low temperatures (e.g. Kennedy & Fitch 1990). The small number of dead adult worms found in the eels kept at 18°C is considered to reflect common natural conditions, as shown in the field studies of Kennedy & Fitch (1990) throughout 1 yr.

In summary, the life-cycle of *Anguillicola crassus* comprises 2 phases in which a sufficiently long period at 4°C causes irreversible damage: firstly, $L_2$ in the intermediate host (Petter et al. 1989) and, secondly, the adult nematodes in the swimbladder lumen of the final hosts. Generally, a decrease in temperature leads to an increase in time required for a complete life-cycle, probably due to a reduced metabolism of the nematode.

The results presented here together with the facts already known concerning the influence of low temperatures on development in the intermediate host (Petter et al. 1989, 1990) are suitable to explain why *Anguillicola crassus* in Scandinavia only occurs in anthropogenically heated waters, but not under natural temperature conditions as assumed by Höglund et al. (1992). Our results clearly contradict the hypothesis of Kennedy & Fitch (1990) that the adults of *A. crassus* 'appear to be able to survive and reproduce in eels under any conditions that the host can withstand'. Thus, the description of *A. crassus* as a successful coloniser presented by these authors does not apply under cold water conditions. Whilst the temperature regime prevailing in the freshwaters of Central Europe obviously still enables the parasite to build up a stable population, in Scandinavia this might be prevented by an increased mortality of adult worms during the longer winter (with a temperature minimum of 4°C in freshwater and even less in the brackish Baltic) and retarded development because of lower temperatures in summer Due to the expected high mortality of adults during long winters, prior to extensive reproduction in early summer gravid specimens must be recruited from the hibernated larvae. A comparison of the water temperatures in southern Swedish lakes (Raab & Vedin 1995) and Lake Constance, southern Germany (Müller 1983–1994) illustrates the different temperature conditions. In southern Sweden the water temperature exceeds 5°C for approximately 7 mo and the 10°C threshold for approximately 4 mo per year whereas in South Germany the periods are 10 and 6 mo, respectively. Furthermore, the mean highest summer temperature in the southern Swedish lakes is about 2°C lower than that of Lake Constance. However, looking at the data from all areas of Europe where *A. crassus* occurs (e.g. Canestri-Trotti 1987, Taraschewski et al. 1987, Køie 1991), no influence of latitude on the prevalence or abundance of *A. crassus* is apparent. Such a gradient from northern to southern Europe might be masked by many other factors such as

the time the parasite has already been in the region, the availability of intermediate and paratenic hosts or the immunological status of the respective eel population. Interestingly, no clear seasonal pattern in infection of eels as a result of the seasonal variation in temperature has been shown so far (Kennedy & Fitch 1990, Möller et al. 1991, Thomas & Ollevier 1992, Würtz et al. 1998), although in Japan and Korea a decrease in prevalence during the cold season has been suggested (Egusa et al. 1969, Kim et al. 1989, both cited in Nagasawa 1994). In any case, under field conditions the impact of low winter temperatures on the life-cycle of *A. crassus* can easily be hidden by the retarded larval development, the longevity of larval stages and delayed mortality of adult worms, as could be concluded from our results.

Concerning North America, where *Anguillicola crassus* was recently introduced into the southern USA (Johnson et al. 1995, Fries & Williams 1996), the large Canadian eel populations of the St. Lawrence River or of Newfoundland (Tesch 1983) will in all probability not become infected by the parasite during its northward expansion, due to the low winter temperatures.

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