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Prevalence and intensity of *Anguillicola crassus* infection of the European eel, *Anguilla anguilla* (L.), in Lake Võrtsjärv (Estonia)

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Abstract. Nonindigenous swimbladder nematode *Anguillicola crassus* was probably introduced into Lake Võrtsjärv via importation of live young eels (standard length of 20–30 cm) from Germany in autumn 1988. Since 1992, the parasite has been found in eels that inhabit this shallow eutrophic lake. The aim of the investigation was to determine the prevalence and intensity of *A. crassus* infection in the eel population of L. Võrtsjärv in different years, and to assess the possible effect of the parasite on eel fishery. Since 1992, 623 eels were examined for *Anguillicola.* The prevalence of infected eels ranged from 51% to 86% in different years. The average number of worms per infected eel (mean intensity) ranged from 4.0 ± 0.6 in 1999 to 12.6 ± 2.5 in 1993, the maximum number of the parasite was 92 per eel. The mean number of parasites in a swimbladder of eel was not related with the length of the host fish. No statistical difference was found for the condition factor of infected and noninfected fish. Although under normal environmental conditions *Anguillicola* has not caused serious problems to eels in L. Võrtsjärv, high intensity of parasite infection together with other unfavourable factors (e.g., high temperature, oxygen deficiency, intensive algal bloom, etc.) may lead to mass eel kills.

Key words: *Anguillicola crassus*, prevalence and intensity of infection, Lake Võrtsjärv.

INTRODUCTION

The increasing demand for eels for commercial and aquaculture purposes in Europe has led to world-wide importation of live eels into some countries (van Banning & Haenen, 1990). Such a situation involves the risk of introducing nonindigenous parasites and pathogens into fish farms and natural water bodies.

Anguillicola crassus (Kuwahara et al., 1974) is a nematode parasitizing in the swimbladder of eels (*Anguilla* spp.). It originates in Southeast Asia where it is a natural parasite of the Japanese eel, *Anguilla japonica* (Kuwahara et al, 1974; Egusa, 1979; Beregi et al., 1998).

Anguillicola was introduced into European inland waters probably during the mid-1970s with imported live eels from the Far East and New Zealand (Koops & Hartmann, 1989). In 1982 it was reported from Germany (Koops & Hartmann, 1989) and Italy (Molnár et al., 1993). The appearance of this parasite in Hungary was recorded in 1990 by Székely et al. (1991). The exotic parasite spread rapidly throughout the continent. Now this nematode is distributed over whole Central Europe, but also eel from Egypt is infected. In late 1987, *Anguillicola* was reported for the first time in Britain (Kennedy & Fitch, 1990) and in 1998 in Ireland (Ewans & Matthews, 1999). Lately this parasite has been found also in the eastern regions of North America (Barse, 1999). Glass eels seem to be uninfested because the parasite does not propagate in saline waters (Koops & Hartmann, 1989). But transport of young brown eels for stocking and the live transport of large eels for consumption are real factors in continuing the spread of *Anguillicola.* The parasite has established itself in many European eel populations and will spread further.

In eels of L. Võrtsjärv *A. crassus* was first found in 1992. Probably, this parasite was introduced into the lake with young eels (standard length of 20–30 cm) from Germany in autumn 1988 (Kangur, 1994).

Once introduced into a lake or river, *A. crassus* spreads rapidly among the eel population. Distribution within an aquatic system is generally through intermediate hosts and movement of other fish. Spread between localities is generally through transport of infected eels (Kennedy & Fitch, 1990). It was observed that eel can be infected very soon after immigration of elvers into fresh water. For example, wild young eels had already prevalence of infection of 80% after living only 6–8 weeks in freshwater L. IJsselmeer (The Netherlands) (van Banning & Haenen, 1990).

European eel is not a natural host for *A. crassus* (van Banning & Haenen, 1990). An absence of native swimbladder nematodes in Europe is a factor in the rapid distribution of *A. crassus* as there is a lack of competitors and resistance of the host.

According to Kennedy & Fitch (1990), *Anguillicola* is a successful colonizer because it has high reproductive potential and a relatively simple life cycle, with many intermediate and paratenic hosts. Eggs and free-living larvae are capable of surviving and remaining infective for long periods in fresh water. Experimental studies by Kirk et al. (2000) showed that *A. crassus* can survive and reproduce in eels in estuarine and marine simulated conditions and therefore could survive also during the spawning migration of eels to the Sargasso Sea.

The developmental cycle of *A. crassus* involves an obligate intermediate host, different species of copepods and ostracods, in which the nematode larvae reach the third stage, a stage that is infective to the definite host – eel (De Charleroy et al., 1990; Moravec & Škoríková, 1998). Many copepod and ostracod species have been reported to serve as intermediate hosts for *A. crassus* (De Charleroy et al., 1990; Haenen & van Banning, 1991; Moravec, 1996). The infected intermediate hosts, which are ingested by the definite host (eel), are currently the source of *A. crassus* infection. Various fish species were found to serve as paratenic hosts in which the nematode larvae remain alive and keep their capability to infect eels (Haenen & van Banning, 1991). Not only prey fishes, but also aquatic snails, amphibians, and larvae of aquatic insects can serve as paratenic hosts of *A. crassus* (Moravec & Škoríková, 1998).

According to Ashworth & Kennedy (1999), third larval stages of *A. crassus* entering an eel migrate first to the swimbladder wall where they moult to the fourth larval stage before moving into the lumen where they grow and mature. The third larval stage of *A. crassus* can survive for prolonged periods also within the swimbladder wall of paratenic hosts. Adult worms inhabit the lumen of eel swimbladders, where they feed on host blood (Békési et al., 1997). The parasite's full developmental cycle is completed in less than 2 months at 20 °C (Székely et al., 1991).

Since the introduction of *A. crassus* into Europe, anguillicolosis has been a considerable problem in several countries (Békési et al., 1997). Mortality due to the infection has been reported by Sarti et al. (1985), Hartmann (1987), Molnár et al. (1991). A species-specific mass mortality of eel (400 tonnes) occurred in L. Balaton in summer 1991. Most probably this eel-kill was caused by a massive infection by the nematode *A. crassus* (Biro, 1992).

Eel is the most important commercial fish in L. Võrtsjärv today as it accounted for up to 60–70% of the total value of annual fish catch in the 1990s (Kangur, 1998). Therefore the success of fishery in this lake depends to a great extent on the abundance and condition of the eel population. The nonindigenous parasite *A. crassus* may endanger the eel fishery in L. Võrtsjärv.

The aim of the investigation was to determine the prevalence and intensity of *A. crassus* infection in the eel population of L. Võrtsjärv in different years and to assess the possible effect of this parasite on the eel fishery in this lake. A correlation between the length of eel and the intensity of infection was established. The effect of *Anguillicola* infection on the condition factor (according Fulton) of eel was elucidated.

STUDY AREA

Lake Võrtsjärv (270 km^2) in central Estonia (Fig. 1) is the second largest lake in the Baltic countries. It is a very shallow turbid water body with a mean depth of 2.8 m and maximum depth of 6 m (Jaani, 1990). The lake is strongly eutrophic: mean total nitrogen concentration is 1600 mg m^{-3}, total phosphorus concentration 54 mg m^{-3}, and mean Secchi depth 1.1 m (Haberman et al., 1998). During the ice-free period, Secchi depth does not usually exceed 1 m. The water is slightly alkaline (pH ~ 8) with a high seston content. The ice cover lasts from November to April, on the average 135 days. In winter, oxygen deficit can occur under the ice cover. The lake is polymictic with some short (1–2 week) stratification periods during summer and weak inverse thermal stratification in winter (Nõges & Nõges, 1998). The mean annual range of water level fluctuations is 1.4 m (Huttula & Nõges, 1998).

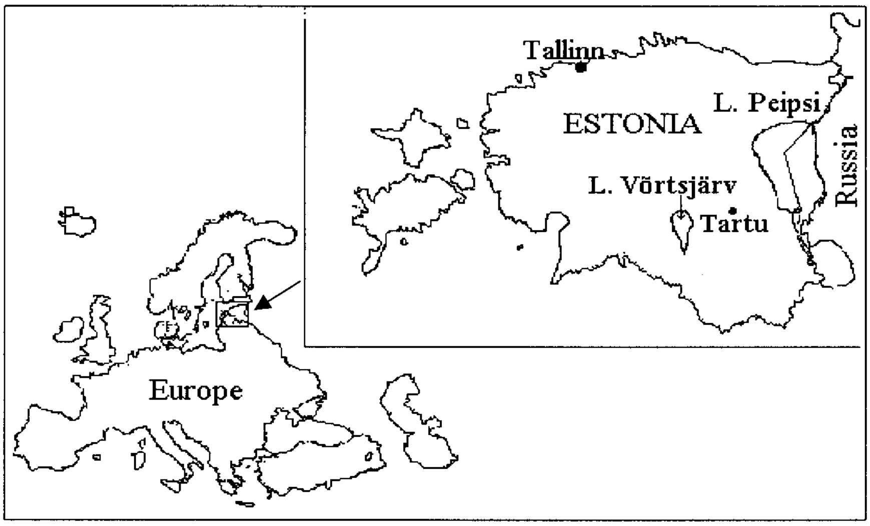

Fig. 1. Location of Lake Võrtsjärv.

According to present data, one lamprey and 34 fish species inhabit L. Võrtsjärv or lower reaches of its tributaries (Pihu, 1998). About 10 of them have commercial and recreational importance. As a result of the stocking of glass eels, *Anguilla anguilla* (L.) is the most important game fish in Lake Võrtsjärv (Kangur, 1998).

MATERIAL AND METHODS

A total of 623 eels were collected since 1992 from L. Võrtsjärv and examined for the presence of *A. crassus* in swimbladders. The eels were caught by experimental trawl in the open water period of 1992–2000 and by commercial fence traps during 1994–2000. The sampled fish were measured (standard length, Sl) with an accuracy of 1 cm and weighed with an accuracy of 5 g. After that the eels were dissected and all nematodes found in the lumen of the swimbladder were counted. The length of the examined eels ranged from 17 to 99 cm (Table 1).

The prevalence (% of eels infected) and mean intensity of *A. crassus* infection (average number of worms per infected eel) were determined in different years. The intensity of infection was elucidated by different length groups of host.

For statistical analysis STATISTICA for Windows (StatSoft, Inc, 1995) was applied. The Pearson correlation analysis was used to measure relationships between variables. Student's t-test was used to compare the mean length and condition factor of infected and uninfected eels. In the statistical tests the level of significance α was 0.05.

Table 1. Number and measurements of the studied eels from L. Võrtsjärv

Year	Number of eels	Sl, cm		Number of infected eels
		Mean	Range	
1992	8	65.5	56–72	5
1993	28	64.8	30–83	18
1994	48	64.4	53–75	29
1995	149	69.5	51–91	76
1996	14	68.4	45–84	9
1997	71	69.1	34–87	43
1998	77	61.3	24–96	49
1999	112	63.4	48–99	68
2000	115	69.4	51–90	99
2001	1	17	–	1
Total	623		24–99	397

RESULTS

Prevalence of *A. crassus* infection

From 623 eel swimbladders examined 397 contained *Anguillicola*. The parasite length ranged from 2 to 42 mm. The prevalence of the infected eels varied between 51% and 86% over the years, with no apparent trend (Fig. 2). The proportion of eels containing the parasitic nematode was commonly about 60–64%. However, in 1995 the prevalence of infection was markedly low (51%), whereas in 2000 a maximum of 86% was recorded.

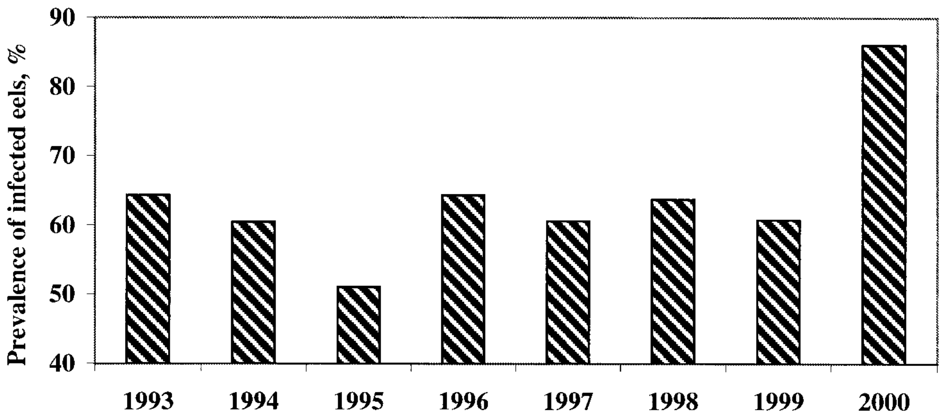

Fig. 2. The mean yearly prevalence (%) of *Anguillicola crassus* in the eels from L. Võrtsjärv.

Intensity of *A. crassus* infection and measurements of the host

The number of parasites found in a swimbladder of eel was highly variable. Infection intensity ranged from 0 to 92 worms per eel (Fig. 3). Distribution of infection intensity in eels showed that most swimbladders contained less than 10 parasite specimens.

The average number of worms per infected eel (mean intensity) ranged from 4.0±0.6 in 1999 to 12.6±2.5 in 1993 (Fig. 4). A significant decreasing tendency ($r = -0.8$; $p < 0.05$) was found in the mean intensity of *A. crassus* infection from 1992 to 2000.

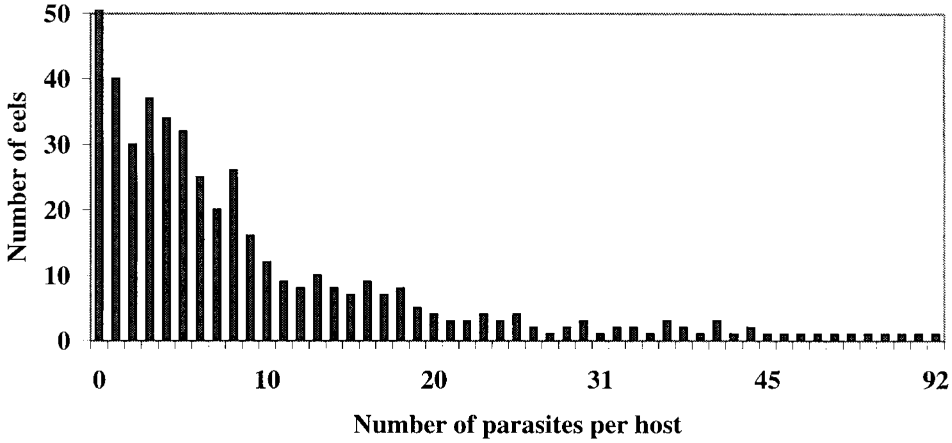

Fig. 3. Distribution of infection intensity in eels (n = 623) from L. Võrtsjärv.

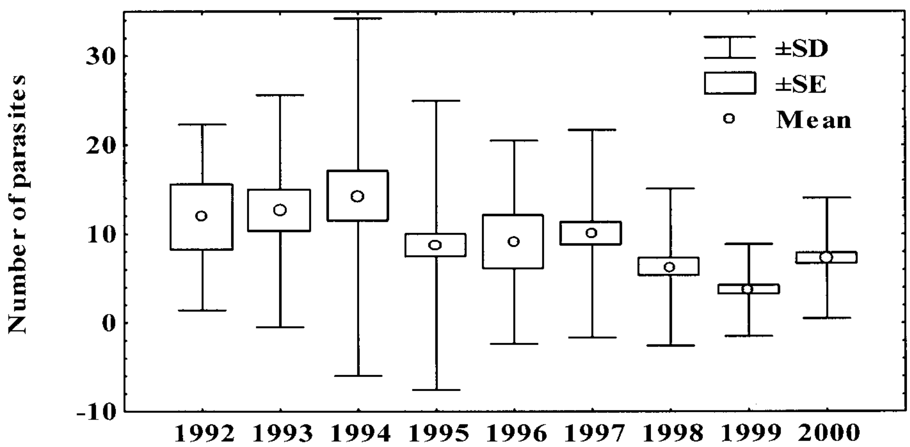

Fig. 4. Mean number of *Anguillicola crassus* in a swimbladder of eel from L. Võrtsjärv in 1992–2000.

The length frequency distribution of eels investigated shows that 60–79 cm long fish were the most numerous (Fig. 5). The eels smaller than 50 cm were represented with a few specimens only, and the number of larger eels (Sl > 80 cm) was considerably low as well. However, the proportion of infected eels was quite similar in all length groups, constituting about 60–65%.

According to our data, the mean number of parasites in a swimbladder of eel was not size-related with the host fish (Fig. 6). None of the samples analysed in this study gave a significant correlation between fish size (length) and infection intensity.

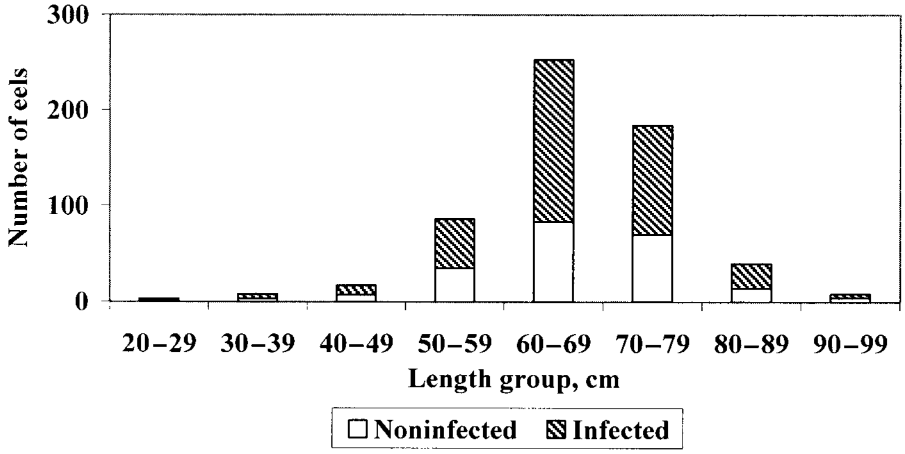

Fig. 5. Length distribution of the investigated eels.

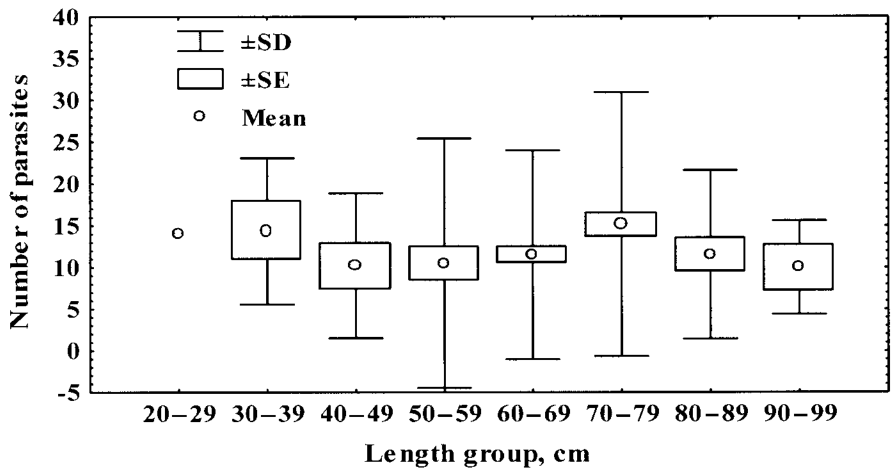

Fig. 6. Mean number of *Anguillicola crassus* in a swimbladder of eel of different length groups.

Also, no statistical difference was found for the condition factor of infected and uninfected fish. The correlation between the condition factor of eel and the number of parasites per fish was not significant either. Some infected eels, however, were slightly heavier than the uninfected fish.

DISCUSSION

Massive fish-kills caused by parasites in natural waters are rare (Molnár et al., 1991). In natural systems, parasites characteristically cause periodic and sporadic fish-kills. However, in the case of introduction of a parasite to a new biotope, mass mortality may sometimes occur. The introduction of *A. crassus* to susceptible populations of eel in Europe, including L. Võrtsjärv, occurred relatively recently. The pathological changes in European eel caused by *A. crassus* appear to be severer than in the Japanese eel who is a natural host for this parasite (van Banning & Haenen, 1990).

Anguillicola is infecting eels of all sizes. The nematode may even reach the adult phase in the young eels from 8.5 cm upwards, adapting their size to the restricted lumen of the small swimbladder (van Banning & Haenen, 1990). In L. Võrtsjärv, the parasite was found in eels of all length groups (17–99 cm).

There is a consensus among scientists that *Anguillicola* spp. may severely affect growth and survival of eels in rivers and lakes (Koops & Hartman, 1989). Growth and feed conversion in farmed eels are negatively influenced by the parasite. However, according to Molnár et al. (1991), *A. crassus* is a moderately pathogenic parasite in natural waters.

The parasite gives severe pathological effects in *A. anguilla* and heavy infection leads to host mortality (Egusa, 1979; Nagasawa et al., 1994). The pathological changes in eel have been thoroughly studied by van Banning & Haenen (1990), Molnár (1993), and Molnár et al. (1993). They established that the direct damage done by *A. crassus* arises from the blood-sucking of mature worms as well as from changes produced in the swimbladder wall by the migration of larvae. A considerable thickening of the swimbladder wall was found in *Anguillicola*-infected eels (van Banning & Haenen, 1990; Békési et al., 1997). It is not clear what may happen to infested eels during their spawning migration at sea where eels have to travel long distances in open oceanic waters with a known day–night change of swimming depth (Tesch, 1998). Probably the infested eels do not reach their spawning areas in the Sargasso Sea (Winfield et al., 1993).

Mortality of eels is mainly caused by destruction of the swimbladder wall (van Banning & Haenen, 1990), and it generally occurs at high water temperature (Molnár et al., 1993).

Eel kills due the *Anguillicola* infection have not been established in L. Võrtsjärv yet. However, there is a real risk for mortality of eels, because the prevalence of infected eels was high (86% in 2000). The latest mass kills of fishes, including eels, occurred in winter 1996 in L. Võrtsjärv. The role of the parasite in the eel kill was not detected then, although the parasite infection may have contributed to the mortality.

In L. Balaton a highly intensive infection caused by the new parasite coincided with a massive eel kill in summer 1991 (Molnár et al., 1991; Biro, 1992). Deceased fish were infected by 30 to 50 specimens of adult nematodes in the lumen of the swimbladder. No mortalities of other fish species in the lake were observed. Mass mortality of eels infected with *A. crassus* was concluded to have resulted from high infection rates combined with high temperatures (Molnár et al., 1991). Lake Balaton is a shallow lake, where the water temperature in summer may rise up to 28 °C. Although L. Võrtsjärv is also a very shallow lake, its water temperature during summer is usually lower than in L. Balaton. In L. Võrtsjärv, the mean water temperature in July reaches 20.1 °C (Haberman et al., 1998). However, in lasting calm and sunny periods the surface layer may warm up to 25 °C. Besides, in comparison with L. Balaton, the intensity of *A. crassus* infection was usually lower in L. Võrtsjärv: most eels contained less than 10 specimens of the parasite in their swimbladder lumen.

Haenen & van Banning (1991) found that the larvae of *A. crassus* are non-specific in choosing their hosts, and the parasite may be transferred from one host to another, when eaten. Larvae of *A. crassus* were detected in various small freshwater fishes (e.g. smelt, *Osmerus eperlanus* (L.); perch, *Perca fluviatilis* L.; and pikeperch, *Stizostedion lucioperca* (L.)) that are preyed by eels. Of the 13 fish species examined in large numbers in L. Balaton, ruffe, *Gymnocephalus cernuus* (L.), and European catfish, *Silurus glanis* L., showed as high as 100% prevalence of infection (Székely, 1994). According to Lehmann et al. (1996), ruffe is probably the most important infestation and re-infestation source with the swimbladder parasite *A. crassus* for older eels in Nordrhein-Westfalen (Germany). In L. Võrtsjärv, ruffe may be the most important infestation source with *Anguillicola* for eels as well. Ruffe is very abundant in the lake, and it is consumed by eel (Kangur et al., 1999).

Levels of infestation have been recorded to rise from 10% to 50% within a year (Belpaire et al., 1989; Koops & Hartmann, 1989). In L. Võrtsjärv, prevalence of *Anguillicola* infected eels increased 25% within a year (from 61% in 1999 to 86% in 2000).

Neither the prevalence nor the intensity of infestation show significant seasonal patterns (Thomas & Ollevier, 1992). Further, no correlation between the condition factor of the eels and intensity of infection with *A. crassus* could be found (Wuertz et al., 1998). No statistical difference was found for the condition factor between individuals for infected and uninfected fish (Koops & Hartmann, 1989). These findings are in accordance with our results in L. Võrtsjärv. However, Hartmann (1993) found that the condition factor as well as the swimming activity decreased with increasing infection rate. Thomas & Ollevier (1992) established a positive but weak correlation between eel length and number of adult nematodes in the swimbladder, while no correlation was found between eel length and total parasite burden.

According to Koops & Hartmann (1989), under normal environmental conditions and in relatively shallow inland waters, *Anguillicola* need not cause an immediate problem to eels; however, under situations of stress infested eels may react more sensitively. Although *A. crassus* has not been found to cause serious problems to eels in L. Võrtsjärv up to now, high prevalence and intensity of the parasite infestation together with other unfavourable factors (e.g., high temperature, oxygen deficiency, intensive algal bloom, etc.) may cause mass mortality of eels.

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Euroopa angerja (*Anguilla anguilla* (L.)) invadeeritus parasiidiga *Anguillicola crassus* Võrtsjärves

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1992. aastal avastati Võrtsjärves uus kalaparasiit – *Anguillicola crassus*. See angerja ujupõies elutsev nematood sattus järve ilmselt 1988. aasta sügisel koos Saksamaalt toodud 20–30 cm pikkuste asustusangerjatega. Et kindlaks teha *A. crassus*'e esinemissagedus Võrtsjärve angerjatel ja kalade invadeerituse intensiivsus ning hinnata parasiidi võimalikku mõju järve angerjamajandusele, uuriti aastatel 1992–2000 623 angerjat. Invadeeritud angerjate esinemissagedus varieerus aastati 51–86% vahel. Keskmine *Anguillicola* hulk ujupõies oli $4{,}0 \pm 0{,}6$ (1999. a) kuni $12{,}6 \pm 2{,}5$ (1993. a), kusjuures suurim ühest ujupõiest leitud parasiitide arv oli 92. Olulist seost ei märgatud ujupõiest leitud parasiitide arvu ja angerja pikkuse vahel, samuti ei erinenud invadeeritud ja invadeerimata angerjate tüsedus. Võrtsjärves ei ole seni kindlaks tehtud angerjate massilist hukkumist nimetatud parasiidi tõttu, nagu see on juhtunud mõnes teises Euroopa järves (näit Balatonis). Ilmselt ei tekita *Anguillicola* normaalsetes keskkonnatingimustes Võrtsjärve angerjapopulatsioonile olulist kahju. Kuid suur parasitaarne koormus koos teiste ebasoodsate teguritega (näit kõrge temperatuur, hapnikupuudus, intensiivne vetikate õitsemine ja muu) võivad mõjuda angerjatele hukutavalt.