

Chapter 9

Impact of the Swim-Bladder Parasite on the Health and Performance of European Eels

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9.1 Parasitic Infections in Eels

A growing number of diseases including infections by parasites are thought to play an important role in the drastic reduction of eel in European rivers and lakes. Until now, the occurrence of about forty parasite species has been reported for the European eel. Bykhovskaya-Pavlovskaya et al. (1962) listed 30 species in this fish. Since then, several new species were recorded and among them highly pathogenic ones. Several of these species arrived by Pacific eels (*Anguilla japonica*, *A. australis*) that were introduced into Europe for experimental purposes. Parasitic infections leading to severe symptoms and eventually death of the fish are caused primarily by members of the latter group. The common occurrence of some helminthes like *Proteocephalus macrocephalus*, *Bothriocephalus claviceps*, *Acanthocephalus anguillae*, *Paraquimperia tenerrima* has been known for a long time (Murai 1971; Moravec 1994). There were also data on the pathogenic effect of some well known myxosporeans as *Myxidium giardi*, *Hoferellus gilsoni* (Copland 1983; Lom et al. 1986), but these parasites rarely caused any diseases. The first real pathogenic disease of parasitic origin in Europe was reported by Molnár (1983) who discovered *Pseudodactylogyrus anguillae* and *P. bini* in cultured eels. These monogeneans caused heavy losses in intensively cultured eel stocks (Buchmann et al. 1987; Buchmann 1993) but no losses were recorded in natural waters. From time to time reports appeared about severe infections caused by unknown or less known parasites affecting eels in natural water, but none of these parasites caused measurable losses in eel populations. Besides monogeneans, Moravec and Kjøie (1987) described *Daniconema anguillae*, a skrjabillanid nematode from the abdominal cavity of the European eel. Molnár and Moravec (1994) found a heavy infection by its larval stages in the fins of eels of Lake Balaton. *Myxobolus portucalensis* as

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described by Saraiva and Molnár (1990) seems also a common and frequent parasite but due to its specific location on the fins, losses of the hosts were not recorded. On the other hand an introduced nematode species, *Anguillicola crassus* proved to be highly pathogenic for eels and its role in the permanent decrease of eel populations in Europe should be seriously considered. A general review on the pathogenic helminth parasites of eels was presented by Kennedy (2007).

9.2 *Anguillicola* Infection in Eels

Five species of the genus *Anguillicola* are currently known. Four of these five species; *Anguillicola globiceps* Yamaguti 1935, *A. crassus* Kuwahara, Niimi et Itagaki 1974, *A. australiensis* Johnston et Mawston 1940 and *A. novaezelandiae* Moravec et Taraschewski 1988, are native and specific to Pacific eels, while the fifth one, *A. papernai* Moravec and Taraschewski 1988 is a parasite of the African species *Anguilla mossambica* (Taraschewski et al. 2005). In their original biotope, all of these species seem to be well adapted to hosts and they cause no apparent damage on them. Adults of all *Anguillicola* spp. live in the lumen of the swim-bladder and feed on blood (Fig. 9.1). As far as is known, none of the Pacific species can employ paratenic hosts in their life cycle (Puquin and Yuru 1980; Wang and Zhao 1980; Nagasawa et al. 1994). Introduced specimens of *A. crassus* in Europe however develop in several fishes as in paratenic hosts (Thomas and Ollevier 1992; Székely 1994; Moravec and Škoriková 1998), and cause heavy infections in the latter. This means that in the life cycle of the European eels, a more frequent indirect infection route through paratenic hosts and a less frequent direct route similar to the one of Far-East eels can be distinguished.

9.3 Life Cycle of *Anguillicola crassus*

The basic life cycle of *A. crassus* was studied by several researchers in the Far East and in Europe (Nagasawa et al. 1994; Kennedy 1994; Moravec et al. 1994a). In the Pacific Region adults (Fig. 9.2) are found in the swim-bladder of the Japanese eel where they feed on blood. Eggs are released into the swim-bladder and contain the second larval stage encased in the sheath of the first stage larva (Fig. 9.3). The larvae may hatch in the swim-bladder, or they may stay inside the eggs while they are passed out through the pneumatic duct of the eel and get into water via the intestine. The free-living larvae float for a while in the water and sink to the bottom, where they undulate in response to tactile or other similar stimuli. Larvae are captured and consumed by copepods either in water or on the bottom of the water basin. They are ingested by different species of cyclopoid copepods and pass into the hemocoel, where they molt to the third larval stage (Fig. 9.4).

Eels usually become infected in a direct way by eating infected copepods. Infected copepods can also enter the intestine of the eel through the gut content of small prey fishes. Larvae in the intestine of eel rapidly leave the copepods and migrate through



Fig. 9.1 Anguillicola-infected swim-bladders of Lake Balaton eels in 1991

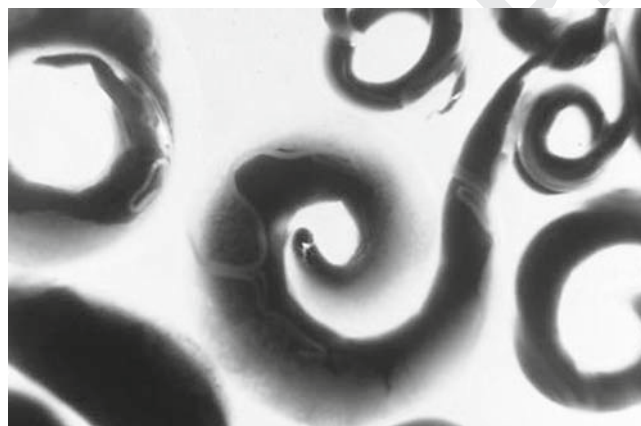


Fig. 9.2 Adult *Anguillicola* females containing L2 larvae in their uterus

the intestinal wall towards the swim-bladder (Wang and Zhao 1980). In the wall of the swim-bladder they molt to the fourth larval stage and then develop into adults. In the Pacific region *A. crassus* usually infects its host in low numbers and is not pathogenic to the natural host (Hine and Boustead 1974; Wang and Zhao 1980; Kennedy 1994).

The life cycle of *A. crassus* in Europe was studied by several authors (Haenen et al. 1989, 1994 1996; De Charleroy et al. 1990; Kennedy and Fitch 1990; Thomas and Ollevier 1992, 1993; Höglund and Thomas 1992; Moravec et al. 1993, 1994b). The above authors proved by their field and experimental studies that *A. crassus* in Europe develops using, in most cases, small prey fishes as paratenic hosts. At least 20 species of freshwater fishes as well as a snails, amphibians and insects have been identified as paratenic hosts for the third larval stage (Thomas and Ollevier 1992; Moravec 1996; Moravec and Konecny 1994; Székely 1994, 1995; Moravec and Škoriková 1998)

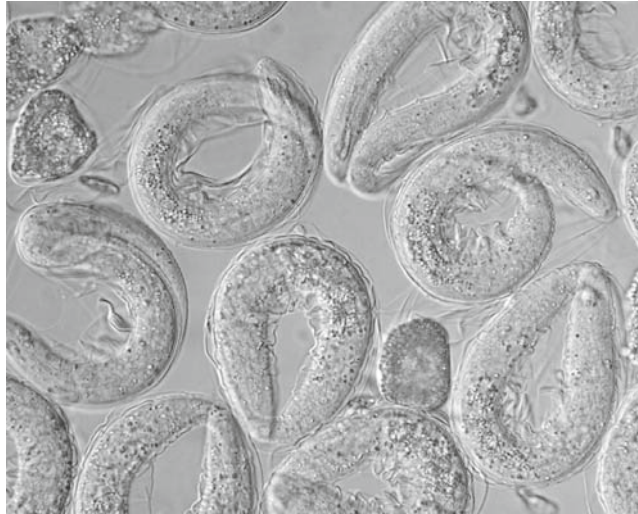


Fig. 9.3 Eggs of *Anguillicola crassus* containing second stage larvae



Fig. 9.4 Third stage *Anguillicola*-larva in a *Cyclops* intermediate host

(Fig. 9.5). Not all of them are of similar suitability: in some species only a few larvae survive as the host reacts against them, whereas in others, especially in species of Perciformes, larvae preserve their vitality for several months (Pazooki and Székely 1994; Székely et al. 1996). These authors concluded, that young eels may become



Fig. 9.5 Encapsulated third stage *Anguillicola*-larva from a paratenic fish host

infected by ingesting infected copepods but older and larger eels by ingesting infected fish. These studies also revealed that larvae in Pacific eels move rapidly through the intestinal and swim-bladder walls, but in the European eel they do not, and in case of host reaction they stay in the intestinal and swim-bladder wall before moving into the lumen of the swim-bladder and molting to the adult stage.

9.4 Transcontinental and Intracontinental Spread of *Anguillicola* Spp.

Contrary to the Pacific eels, which are infected with at least four *Anguillicola* spp., before intercontinental eel transmissions, no *Anguillicola* infection was reported in the European eel *A. anguilla* and the American eel *A. rostrata*. Little is known about the occurrence of parasites in the less studied Pacific eels (*A. celebesiensis* or *A. marmorata*). *Anguillicola globiceps* appears to occur only in wild *A. japonica* and may cause some thickening of the swim-bladder wall. It is more tolerant to cold temperatures than *A. crassus* (Nagasawa et al. 1994). In Japan, *A. crassus* is found in both wild and farmed specimens of *A. japonica*, but it does not appear to cause any damage to this host. On the other hand, Egusa (1979) and Egusa and Hirose (1983) reported that when *A. anguilla* was introduced into Japanese aquaculture ponds to increase stock production, it was found that both the prevalence and intensity of infection were higher in this introduced species than in *A. japonica*. Furthermore, *A. crassus* was pathogenic to, and caused mortality in, *A. anguilla*. Their observation was confirmed by experiments by Knopf and Manhke (2004) who described that recoveries of *A. crassus* are higher in *A. anguilla* (33.2%) than in *A. japonica* (13.8%). In the latter, development of the parasite is slower and

[Au1] a greater proportion of larvae is encapsulated in the swim-bladder wall and dies. Based on Japanese experiences, Egusa (1979) warned fish culturists that transmission of the Japanese eel to other continents might be a hazard. Despite Japanese experiences and Egusa's warning, two species of the Pacific eels were transported to Europe. *A. crassus* first appeared in Europe around 1980 (Peters and Hartmann 1986, 1987; Taraschewski et al. 1987; Moravec and Taraschewski 1988; Kennedy and Fitch 1990) and has spread through the eel trade throughout the continent and into North Africa (El-Hilali et al. 1996), and subsequently to North America (Barse and Secor 1999; Moser et al. 2001). The source of this *Anguillicola* infection was possibly Japanese eels brought to Europe for experimental purposes. Before this introduction to Western Europe, another shipment was transported for a comparative experiment to the Soviet Union and held in heated water. This shipment, however, transmitted only a monogenean (*Pseudodactylogyrus*) infection to the European eel (Golovin 1977), but no *Anguillicola crassus* infection was reported.

Other species have also been introduced into Europe when a stock of Australian eel (*A. australis*) was imported to Italy in 1975 and placed into Lake Bracciano. By these fishes, *Anguillicola novaezelandiae* (originally identified as *A. australiensis* by Paggi et al. (1982)) was introduced. It survived there for several years, reaching prevalence levels of 80% and intensities of up to 27 individuals per eel, but it caused no damage to the native *A. anguilla* and never spread from this isolated habitat to other lakes. Following the introduction of *A. crassus* into the lake in 1993, its numbers have declined (Moravec et al. 1994a). Though the chance for introduction of *A. globiceps* by Pacific eels is reasonable, there are no reports of its establishment. *A. crassus* spread very fast in Europe towards the East and North. It was found in Hungary in 1990 (Székely et al. 1991), in the Czech Republic (Baruš 1995), in Denmark (Køie 1988; Boetius 1990) and in Sweden (Höglund and Thomas 1992). The parasitosis spread to Africa; El-Hilali et al. (1996) and Maamouri et al. (1999) found it in Morocco and Tunisia, respectively. Barse and Secor (1999) discovered this parasite in the American eel (*Anguilla rostrata*). The spread of the parasite in each case could be attributed to human movements of infected eel stocks around the European continent and across the seas as a consequence of the unconsidered eel trade (Kennedy and Fitch 1990). Therefore, *A. crassus* appears to be an important pathogen for both species of Atlantic eels.

9.5 Differences of *Anguillicola* Infection Between Pacific Eels and Atlantic Eels

Anguillicola spp seem to be specific parasites for the genus *Anguilla* and they seem to easily infect other eel species than the original hosts. When European eels were first transmitted for experimental purpose to Japan (Egusa 1979; Egusa and Hirose 1983), this stock became quickly and heavily infected. Moreover, its infection rates surpassed the rate of their Japanese counterparts cultured in the same way and

density. At a similar way, infected Japanese and Australian eel stocks transported to Europe readily transmitted their *Anguillicola* infection to the European eel and an *Anguillicola novaezelandiae* (identified as *A. australiensis*) infection appeared in Italy (Paggi et al. 1982) and *A. crassus* infection in Western Europe (Hartmann 1987). This latter species became infective also for the American eel (*A. rostrata*) (Barse and Secor 1999). Of the two species, *A. novaezelandiae* seem to be less adaptable to the new biotope and host, and showed no ability for intensive spread. *A. crassus*, however, proved to be an aggressively disseminating species and rapidly colonized Europe, some African countries and Northern America (Peters and Hartmann 1986; El-Hilali et al. 1996; Barse et al. 2001; Kirk 2003). Besides colonization in the European eel, *A. crassus* demonstrated new qualities unknown in the original biotope, the Pacific region. Its much wider specificity for its first intermediate host, as it is able to infect several species of freshwater cyclopoid copepods as well as estuarine copepods, seems to be one of the most significant changes in its life cycle. This enables the parasite to complete its life cycle in freshwater and estuarine biotopes. The other major difference between the life cycle of *A. crassus* in Pacific eels and the Atlantic eels is the role of paratenic hosts. No paratenic hosts were found in the Pacific region and a direct way of infection route is supposed (Puquin and Yuru 1980; Wang and Zhao 1980; Nagasawa et al. 1994). On the other hand the heavy infection of the European eel with *A. crassus* is promoted by the large number of paratenic host, like prey fishes and their intensive infection with third stage *A. crassus* larvae (Thomas and Ollevier 1992; Moravec and Konecny 1994; Székely 1994, 1995). Differences in immune response to infection of the eels of the two regions may be a major factor to regulate the infection rate and balance between parasite and host. Nielsen (1999) proved that *A. japonica* mounts more effective immune responses to *A. crassus* than does *A. anguilla*.

9.6 Biology and Ecology of *Anguillicola crassus*

The life cycle of *A. crassus* is temperature related. It grows faster at higher temperatures and is retarded at low temperatures. Studies show that in most cases eggs containing second stage larvae and less frequently hatched larvae, leave the fish via the ductus pneumaticus of the swim-bladder and the gut. Eggs of *A. crassus* released from the worms do not hatch below 10°C and the rate of hatching increases with temperature up to 25–30°C. Hatching rate is also related to salinity, but the percentage of eggs hatched, and survival and infectivity of the second larval stage declines as salinity increases (Kirk et al. 2000a). A part of the eggs and larvae might be captured and eaten by copepods. The majority, however, sinks to the bottom. Second stage larvae attach to the substrate within 2 or 3 days. Kennedy and Fitch (1990) reported L₂ survival of 160 days at 10°C, up to 8 months at 7°C and 5 months at 24°C. In contrast, Thomas and Ollevier (1993) found that they survive only up to 45 days at 23°C. Survival and infectivity decrease exponentially with time (Kennedy and Fitch 1990) and with increasing salinity even though the parasite can survive in some saline

lagoons (Di Cave et al. 2001). Third stage larvae in some paratenic host fish species might survive for more than a year (Székely 1996). Third stage larvae infecting the swim-bladder of eels may survive in the wall of this organ for 4 months at 4°C. Over time they become unable to enter the lumen. Adult mortality increases over 4 months at the same temperature (Knopf et al. 1998). *A. crassus* prefers warmer temperatures and its life cycle is hindered at lower temperatures. It may explain why *A. crassus* is uncommon in, or absent from, the more northern boreal regions (Höglund and Thomas 1992; Thomas and Ollevier 1992, 1993; Knopf et al. 1998) where it may be restricted to thermal effluents (Höglund et al. 1992). The whole cycle can be completed in 90 days at suitable temperatures, but will normally take longer; at least 4 months (Haenen et al. 1989). Eels become vulnerable to infection as soon as they commence feeding in rivers or estuaries. Nimeth et al. (2004) found that even glass eels and elvers are susceptible to infection. Infection can continue throughout life, and in general infection levels tend to be higher in older and larger eels. Eels can recover from infection, but they are not immune to re-infection. Molnár et al. (1994) proved that in Lake Balaton eels there is a permanent reinfection, with recovery and intensification periods. There is no relationship between primary and secondary infections (Haenen et al. 1996) and higher doses of infection will normally produce more severe symptoms. It was initially thought that there was no antibody response to the parasites, but later studies by Sures and Knopf (2004) using ELISA have shown that the body wall of the parasite is a good antigen and significant levels of antibodies can be detected in the blood after 61 days. The infection rate might be affected by the antibody response. The response is suppressed by the initial rise in cortisol levels in all eels due to handling stress, which assists parasite establishment. Knopf et al. (2000a, b) has also reported a humoral response. Nielsen (1999) has shown that the antibody response of *A. japonica* to *A. crassus* is higher than that of *A. anguilla*, which suggests that an immune response may be involved in specificity and control of numbers.

Ashworth et al. (1996) and Ashworth and Kennedy (1999) have identified three density-dependent regulatory processes that may be responsible for this situation. Parasite induced copepod mortality is the first factor. Uninfected copepods might survive for 30 days post infection (pi), whereas the equivalent survival time for infected copepods is only 12 days pi. Mortality of infected copepods is also density-dependent, and heavily infected copepods may die before larvae reach the infective third stage. The second factor is the intensity of gravid females per infrapopulation, which remains relatively constant over time and is independent of the overall infrapopulation density. The third factor is the relation of gravid females to third and fourth stage larvae in the swim-bladder wall. The authors suggested that the presence of adult males and females in the swim-bladder could inhibit the movement of fourth stage larvae from the swim-bladder wall into the lumen and larvae were arrested in development in a density dependent manner. Density-dependent regulatory processes within the definitive host may be affected by environmental factors such as endocrine disruptors. Fazio et al. (2008a) showed that the steroid hormone 11-ketotestosterone induced a significant male-biased ratio in the nematode infrapopulations.

Kennedy and Fitch (1990) determined that adult parasites could survive in *A. anguilla* for up to 4 weeks when the eel was kept in 100% seawater. Survival declined in coastal lagoons of increasing salinity (Di Cave et al. 2001). Kirk et al. (2000a, b) showed that some adults could survive and continue to produce eggs in eels in 50% and 100% seawater for up to 6 months, but around 10% of the adult parasites were damaged following exposure to high salinity. Kirk et al. (2002) showed that the parasites are osmoconformers, achieving this by feeding on eel blood. Still, around 20% of the parasites could not tolerate the osmotic stress of living in eels in 100% seawater but died and disintegrated. It is thus possible that parasites of freshwater origin can survive in eels in coastal lagoons and estuaries as well as during the eel's migration to the Sargasso Sea. The life cycle could also be completed in waters of enhanced (up to 50%) salinity by using estuarine copepods such as *Eurytemora affinis* as an intermediate host, but it was considered unlikely that the parasites could transmit in sea water, as most marine copepods were of the wrong size to serve as intermediate hosts. The ability of the parasite to survive in eels in the Baltic Sea (Höglund and Thomas 1992; Reimer et al. 1994) could thus be due to transmission there or to the survival of freshwater infections.

9.7 Histopathological Changes Caused by *A. crassus* Infection

It is generally known for parasitic infections that during a low infection there is a kind of balance between parasites and hosts. At severe infection, however, parasitic diseases may have an important impact on the health of the hosts. As for *Anguillicola* infection, in the Pacific region this balance works well and heavy infections develop neither in Japanese eels (Egusa and Hirose 1983; Knopf and Mahnke 2004) nor in the Australian species (Kennedy 1994). On the other hand in the European eel, the newly colonized *A. crassus* caused heavy infections from the first years following its first detection (Hartmann 1987; Haenen et al. 1994). At a similar way in Hungary, heavy infection was recorded already at the time when the parasite was first found (Székely et al. 1991).

Severe pathological symptoms can develop in all sizes of eels. The effect of the parasite on its host will relate to the number of parasites present and the size of the eel. Changes are caused by matured worms and migrating larvae. The most evident visual effects can be observed on the swim-bladder but disease symptoms may develop in the intestinal wall as well. The major symptoms of infection include dilated blood vessels and congestion of blood vessels, haemorrhages, inflammation, thickening and fibrosis of the swim-bladder wall (Van Banning and Haenen 1990; Haenen et al. 1989, 1994; Molnár 1994; Molnár et al. 1991, 1993, 1994). There may be an increase in the spleen mass (Lefebvre et al. 2004), and finally the swim-bladder adheres totally to surrounding organs, such as kidney and intestine (van Banning and Haenen 1990).

The number of worms found in the eel is a vital, but not the only factor affecting the health of the fish. Van Banning and Haenen (1990), Haenen et al. (1996) and Csaba

et al. (1993) proved that changes in the swim-bladder wall have higher impact on the health of the host than the number of worms. Molnár et al. (1993) pointed out that migrating *Anguillicola* larvae showed up higher pathogenic impact on the changes in swim-bladder wall than blood sucking imago stages in the lumen. Van Banning and Haenen (1990), Csaba et al. (1993) and Molnár et al. (1993) supposed that pathological alterations in the swim-bladder and a general decrease in the host's resistance promote development of bacterial, fungal and other parasitic infections. Therefore heavy infection with *A. crassus* appears in most cases as a disease complex. Liewes and Schaminee-Main (1987) and Kamstra (1990) described seven stages of infection by the alterations in the swim-bladder:

- Stage 1** Swim-bladder is normal and without nematodes.
- Stage 2** Swim-bladder is normal but contains a few nematodes.
- Stage 3** Swim-bladder enlarged and partly filled with red-brown fluid. Swim-bladder wall can be inflamed.
- Stage 4** Swim-bladder much enlarged and filled with red-brown fluid. In this stage actively moving nematode larvae (L2) can be noticed.
- Stage 5** Rupture of the swim-bladder wall or the ductus pneumaticus is highly irritated. Secondary infections of surrounding tissues are externally visible as a swollen and inflamed abdomen.
- Stage 6** In this stage the swim-bladder wall (possible after rupture) is replaced by a thick layer of connective tissue. In the swim-bladder remainders of the nematodes can be found.
- Stage 7** The swim-bladder is replaced by a hard brown-black mass in which remainders of the nematodes can be found.

Csaba et al. (1993) examined eels during the massive eel mortality in Lake Balaton in 2001 and observed three major changes in the swim-bladder: (1) At the first stage of infection developing and mature worms were found in the lumen of the swim-bladder with a transparent wall, (2) In a progressed stage of infection, decayed, fragmented worms and a red-brown fluid were found in the lumen of the swim-bladder with an inflamed wall, and (3) With the most serious cases, the serosa and subserosa of the swim-bladder became as thick as 5 mm due to fibrous changes, but no worms inhabited the lumen. These authors found also a secondary bacterial infection at the final stage of anguillicolosis, but they recorded no increase of pesticides or heavy metals in the flesh of the eels. Molnár (1994) stated that changes in the swim-bladder wall were caused by the third and fourth stage larvae migrating in this organ. Larvae were detectable in the oedematous connective tissue of the subserosa and in the gas glands. Tissue proliferation consisting of epithelioid cells started to develop around migrating larvae blocking their route towards the lumen and finally most of the larvae became surrounded by a fibrous capsule. A similar nodule formation around invading larvae was observed in the wall of the intestine in which several hundreds of encapsulated or decayed larvae were found. Würtz and Taraschewski (2000) suggested that the histotrophic larvae did not create a severe cellular reaction. The leucocytes gathering around larvae seemed to be attracted rather to cellular debris resulting from the parasites' movements. Molnár (1994) however, observed that a granulation tissue consisting of epithelioid cells and

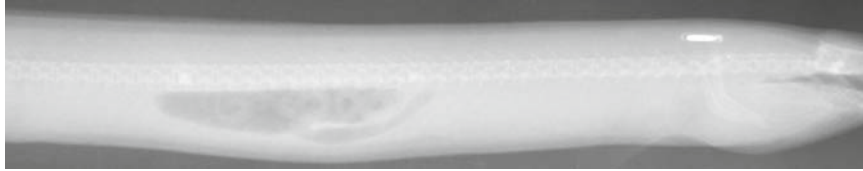


Fig. 9.6 X-ray of an *Anguillicola*-infected eel. The contours of the large *Anguillicola*-nematodes in the swim-bladder are well visible

macrophages was formed around larvae which restricted their movement of the larvae. Changes found in the epithelium are better attributed to the effect of blood sucking imago stages. Würtz and Taraschewski (2000) described that the epithelium of the heavily infected eel is characterized by large folds and cauliflower-like proliferation. Molnár (1994) found that during severe infection, serum filled cysts appeared in the tunica propria of the swim-bladder wall. These cysts were lined with epithelium and the serum filling their cavity contained pycnotic, rounded cells.

Beregi et al. (1998) and Székely et al. (2004) examined the anguillicolosis in the swim-bladder parallel with x-ray and CT-methods and with dissections (Fig. 9.6). They differentiated five major stages of changes in the pathological process:

- At grade 1** the swim-bladder gave a homogeneous radiographic shadow. When opening the lumen, no worms were found and the wall thickness did not exceeded 0.3 mm.
- At grade 2** radiographic shadow was not homogeneous, the contours of small worms were discernible. When opening the lumen of the mildly thickened wall, small worms were found in it.
- At grade 3** radiographic shadow of the bladder was deformed and contours of large worms were observed. By dissection, large worms and some fluid were found in the lumen of the dilated swim-bladder, which had a thickened portion at both ends.
- At grade 4** the swim-bladder gave a narrowed radiographic shadow. By opening the bladder the lumen was narrow, almost completely devoid of air but contained some exudates and dead worms. The wall of the swim-bladder in this stage of changes might reach 2 to 3 mm.
- At grade 5** no radiographic shadow of the bladder was found. By dissecting the abdominal cavity a degenerated small swim-bladder with thickened wall and atelectised lumen was found.

The long term change in the pathological status of a given *Anguillicola* infection was studied also by a radiodiagnostic method (Székely et al. 2005). Naturally infected eels caught in Lake Balaton were x-rayed at 0, 4, 8 and 12 weeks and changes in radiographic shadows of the different grades of infected swim-bladders were followed in 78 eels. Results obtained from individually tagged eels dissected after 3 months observations showed that the pathological status of the swim-bladder had deteriorated in 55% and remained the same in 37% of the cases. Tendency of improvement (one eel) and variable findings (7%) were recorded in a low percentage of cases only.

9.8 Effects of *A. crassus* Infection on the Physiology of Eel

There are several other, less visible, effects of *A. crassus* on *A. anguilla*. Boon et al. (1989) initially detected no significant difference in haematocrit and plasma proteins in infected eels but later (Boon et al. 1990a, b), using more sophisticated controls, these authors found a significant difference. Barus et al. (1998, 1999a) demonstrated lower level of methionine and aspartic acid in the muscles of infected eels, and significantly lower levels of muscle Ca, P, Fe and Mn. Scholz and Zerbst-Boroffka (1994) have determined that *A. crassus* is iso-osmotic with the eel body fluids, but that there are ionic differences in eel chloride levels in sea water composition. These ionic and osmotic changes impose stress on the parasites which are ionic and osmotic conformers.

Kelly et al. (2000) could not find significant differences in stress hormones, metabolic hormones or osmoregulatory status of infected eels and concluded that eels were able to adapt to chronic infection levels. However, Gollock et al. (2004) found that infected eels were more stressed under aquaculture conditions, when netted and exposed to air. They found that the cortisol response did not differ between infected and uninfected eels but that plasma glucose levels were higher in infected eels, and that glucose metabolism and utilization was increased i.e. stress of infection elevated glucose turnover. In a later study Gollock et al. (2005a) showed that acute temperature alone had little effect as an eel stressor. Under such conditions there was a lag in glucose metabolism in infected eels. There was no significant increase in hemoglobin levels when compared to the responses of uninfected eels as both groups showed a significant increase in hemoglobin. Gollock et al. (2005b) went on to demonstrate that infected eels exhibited a more pronounced stress response to hypoxia than uninfected individuals. Finally, Fazio et al. (2008b) found that the expression level of deep-sea rod opsin (DSO) gene in the eyes was significantly greater in infected wild eels. The authors suggested that the parasite may have an effect on the eel's silvering process.

9.9 Effect of *A. crassus* on the Survival of Eel

Despite intensive pathological changes and morphological degenerations in the swim-bladder, no outer symptoms are observable on infected eels. Kjøie (1991) could not find evidence of lack of appetite in infected European eels or difference in condition factor, but confirmed a greater mortality of infected eels during storage or transport due to stress and possibly to secondary bacterial infections. Other workers have found that the length/weight relationship does not differ between infected and uninfected European eels and that any difference in weight between infected and uninfected eels is not significant even if, paradoxically, the eels are suffering mortality due to the parasite (Barus and Prokeš 1996). Koops and Hartmann (1989) also found no difference in condition factor between infected and uninfected eels or a relationship between condition factor and parasite intensity. Möller et al. (1991) reported a higher condition

factor in infected eels but no change in the hepatosomatic index. The differences in estimation of the condition factor by the above authors might relate to the fact that in infected eels the enlarged swim-bladder filled by worms or fluid, as well as general fibrosis and serous infiltration of the abdominal serosa and inner organs, seemingly increase the body weight.

Contrary to the lack of outer symptoms, there are a series of observations on the effect of *A. crassus* infection on the physiological performance and survival of the eel. Thomas and Ollevier (1992) found that heavily infected eels were more easily sucked into power station intakes. Molnár and Székely (unpublished) experienced that the escape reaction of eels harvested by commercial fishermen differs significantly. They observed that less active eel specimens, first taken off by a landing-net from a densely filled fishing smack, were more infected than actively hiding specimens harvested from the smack at the end. Gollock et al. (2004) who examined the effect of netting and aerial exposure to the plasma glucose of eels infected with *A. crassus* concluded that these stressors potentially could result in decreased growth.

Molnár (1993) demonstrated that when uninfected and infected eels were deprived of oxygen, the severely infected eels died first. The impact of the oxygen shortage was temperature-dependent and the effect on individual eels related more closely to the thickening of the swim-bladder wall rather than to the number of parasites present. Infected eels had an increased demand for oxygen, but the presence of *A. crassus* impaired the functioning of the swim-bladder and this in turn could result in eel death.

The swim-bladder is an important but not a vital organ for the fish, at least in freshwater where they habitually live on (or in) the bottom. The fish can compensate most of its dysfunction relatively well. Nevertheless, Würtz et al. (1996) were able to demonstrate the importance of this organ. They proved, that there was in fact a significant correlation between the oxygen concentration in the swim-bladder and the level of *A. crassus* infection. The contribution of oxygen to the swim-bladder gas was reduced between 36% and 60% in naturally infected eels, and this related to the changes in the swim-bladder wall. Overall, the presence of parasites impeded the function of the swim-bladder as a buoyancy and hydrostatic organ by impairing the functioning of the gas gland.

9.10 Mortalities of Eel Caused by *A. crassus*

Since Egusa (1979) first observed the death of introduced European eels due to *Anguillicola* infection in Japan, data obtained in Europe proved that mortalities among intensively infected eel stocks can also occur. Eel mortalities in lakes in Central Europe, where eels are stocked to form the basis of fisheries, also suggest that *A. crassus* plays a role in these mortalities. The best documented of these mortalities occurred in Lake Balaton in Hungary (Molnár et al. 1991). The mass mortality of eels occurred during the summer of 1991, with an estimated loss of

250 t of eels in the western basin. In 1992 losses were lower, when 40 t were lost in the central basin as conditions improved in the western basin of the lake and the infection spread to the eastern basin. The last eel mortality was recorded in 2005 when the eastern part of the lake was affected but the losses in this region were more severe than in the other two. No other fish species was involved. Initially it was suggested that *A. crassus* alone might have been the cause. Subsequently, following a detailed examination of ichthyological and physico-chemical conditions in the lake, the opinion on the exclusive role of the parasite lessened. Effects of population density, the temperature and oxygen content in the water, co-infections with other pathogenic agents and a supposed intoxication with pesticides were considered. Infection levels were very high in the lake at the time of fish mortality and virtually all eels were infected, with 30–50 adults per eel and up to 200 larvae. Eel population densities were also very high. The effects of the parasites on eel swim-bladders were typical, with eels showing haemorrhagic swim-bladder walls that were thickened up to ten times in comparison with the normal condition, and with the swim-bladder filled with fluid, eggs and decaying and live adults. This, together with the known ability of the parasite to cause mortalities in eel farms, suggested the major role of anguillicolosis in fish mortality. It was clear that water temperature levels in the lake were unusually high during those summers while oxygen levels were correspondingly very low. Later on, it turned out that the lake was heavily overpopulated due to the repeated eel introductions and the extremely good survival of the glass eels. At the time when about 300 t of eel died, the mass of the eel population was calculated to be 1,000 to 1,200 t (Tátrai et al. 2002). Contradictory to the calculations, in the last 16 years (1991–2006) 2,584 t of eel was harvested and the yearly catch is still reasonable, although since 1991 no new introduction of eels happened. These conditions caused stress to the eels, and it now seems more likely that the combined effects of this stress together with heavy infection with adult and larval *A. crassus* invasion were the causes of the mortality (Molnár et al. 1991, 1994). On the other hand, the role of pesticides forwarded by Bálint et al. (1997) can be excluded as no other fish species was affected. In laboratory experiments the eel proved to be more resistant to piretroid-pesticides than cyprinid fishes (own experiences and Csaba G: personal communication).

[Au2]

[Au3]

Similar mass mortalities have been reported from other water bodies which have been stocked with eels, for example in the Vranov Reservoir in the Czech Republic (Barus et al. 1999). Here there was a loss of some 3–5 t in 1994, and the mortalities occurred also in summer when water temperatures were high, water oxygen levels low and eel densities were high. No other fish species were involved in losses. The conditions in Vranov Reservoir were similar to those of Lake Balaton. Barus and Prokeš (1996) stated that the conditions in both lakes were ideal for such epizootics as in these closed, shallow and overpopulated lakes the density of copepods and paratenic hosts facilitated the rapid increase of *Anguillicola* population levels. It is likely, therefore, that it was a combined effect of environmental and parasite induced stresses that caused the mortalities.

Conditions in the shallow, productive lakes of central Europe in which eels are stocked for commercial fisheries can clearly result in eel mortalities from time to

time, but these are not regular or inevitable occurrences. Schabuss et al. (2005) have noted a similarity in physical and chemical conditions between Neusiedler See in Austria and Lake Balaton. In both lakes a stocked eel fishery is going on. Infection levels with *A. crassus* were also high in Neusiedler See, but there was no occurrence of mass mortalities there over a period of many years. Despite similarities, however, there were great differences between the two lakes. Before there was a regular fisheries activity in Neusiedler See, in Lake Balaton eels were exclusively harvested by a trap at the outlet of the lake until the first eel mortality occurred and thus the density of the eel population was only moderately decreased. Ashworth and Kennedy (1999) suggested that parasite-induced mortalities might occur in all natural waters but that the eel density in these systems is never as high as in stocked lakes and that density dependent regulatory processes work against massive eel mortality in the former.

A. crassus can cause host mortalities in eel farms as well. Egusa and Hirose (1983) and Nagasawa et al. (1994) reported that *A. crassus* in *A. anguilla* in Japanese eel farms caused severe mortality of infected eels at their first experimental colonization to Japan and these were largely responsible for mortalities. Eventually, the mortalities in *A. anguilla* in Japanese eel farms due to the parasite were largely responsible for the abandonment of cultivation of *A. anguilla* in Japan (Taraschewski et al. 1987). In Europe, Liewes and Schaminee-Main (1987), Kamstra (1990), van Banning and Haenen (1990) and Mellergaard (1988) reported increased mortality of infected eels in Dutch and Danish eel farms. Contrary, however, to Japanese farms, eels in Europe were already infected in natural waters but disease symptoms developed later on during intensive culture.

9.11 Population Effects and Control

The experimental evidence that the functioning of the swim-bladder is impaired in infected eels and that they are more susceptible to stress and to human activities such as netting (Gollock et al. 2004) and transport (Køie 1991) suggests that infected eels in natural populations may respond differently to conditions than uninfected eels. It would seem very likely, for example, that infected eels would be more susceptible to natural avian predators. There is no direct evidence that this is the case, and such evidence would be very difficult to obtain, but even the reduction in swimming speed reported by Sprengel and Lüchtenberg (1991) must surely affect their escape response to predators.

Control measures are clearly impracticable in natural water bodies, and indeed may be unnecessary if *A. crassus* populations are regulated in a density-dependent manner at levels below those which cause mortality. If stocking densities can be reduced to a level at which the fishery remains commercial but the eel population does not rise to a density at which the eels are stressed, then mortalities are unlikely to occur. In the Lake Balaton where eel fisheries is depend on stocking with glass eels, the cessation of new stockings and intensive fisheries activity stopped the

chance of a massive eel kill and reduced the intensity of infection, although a relatively high level infection is still continues.

In eel ponds, control of the parasite levels may be difficult. A reduction of copepods' densities as the intermediate host can help. Egusa and Hirose (1983) suggested an intensive flow through the pond. Using chemicals to eliminate copepods might be effective but is not environmentally acceptable, as the effluent of the ponds would contaminate natural water bodies. In Europe, usually there are no problems in fish farms as the eel is cultured in intensive recirculation systems where no intermediate hosts exist. Eel farms are usually free from the parasite, or they harbor a low level infection due to the higher water salinity (Køie 1991). Taraschewski (2006) thinks that for farmed European eels, *A. crassus* is no more an economic threat, as reported from Japan in the 1970s (Egusa 1979) and Europe in the 1980s (Liewes and Schaminee-Main 1987; Van Banning and Haenen 1990; Kamstra 1990), due to progress in chemotherapy (Taraschewski et al. 1988; Hartmann 1987; Geets et al. 1992). The above authors reported about a number of nematocidal drugs that proved to be effective against *A. crassus*. Of the latter the most effective are levamisole and metrifonate in freshwater baths (Taraschewski et al. 1988). Levamisole appears to be the better of the two drugs as the cumulative lethal dose ratio is more favorable than with metrifonate (Taraschewski et al. 1988). Although these drugs have an excellent effectiveness against adult parasites in eels, the larvae in the swim-bladder wall are not affected. Ashworth and Blanc (1997) suggested applying levamisole to increase the success of treatment.

9.12 The Effects of Swim-Bladder Parasite Infection on Swimming Performance of Silver Eels

The reviewed effects of *A. crassus* infection on physiology and survival of the European eel mainly concerned the continental yellow eels stage. However, since the first appearance of *A. crassus* in Europe, concern has been expressed about possible effects of the parasite upon the migration of adult eels back to the Sargasso Sea to spawn. Yellow eels experience stress in their movements from freshwater to the sea and in their transition to silver eels. The silver eels themselves depend on their swim-bladder as a hydrostatic organ in the course of their migration to the Sargasso. Knowledge of the effects of *A. crassus* on the gas gland and oxygen concentration in the swim-bladder suggests very strongly that its ability to function as a hydrostatic organ will be impaired in infected eels and this must surely affect their vertical movements on migration as well as their swimming performance (see also Chapter 5).

Swim-bladder parasites drain energy due to their sanguivorous feeding and they cause mechanical damage on the swim-bladder wall. These two effects are hypothesized to impair the spawning migration of the European eel. Two earlier studies investigated the influence of *A. crassus* on the swimming of eel

(Sprengel and Luchtenberg 1991; Munderle et al. 2004). However, those studies were performed with small yellow eels (≤ 45 cm). Obviously large silver eels had to be tested over long distances and periods, as not only swimming speed but particularly a low cost of transport and a high endurance are crucial for long distance migration. Therefore, in a recent study (Palstra et al. 2007a), we have investigated both effects on swimming performance. We hypothesized that parasitic sanguivorous activities – related to parasite weight – reduce swimming endurance, while mechanical damage of the swim-bladder impairs buoyancy control. Recently, we have developed an experimental test to quantify swimming performance (Palstra et al. 2006) using 22 swim-tunnels suitable for exercising many large female silver eels at the same time. This test and set-up was used to investigate the relation between swimming endurance and the adverse effects of *A. crassus* infection. The relation between *A. crassus* infection and swimming efficiency was measured for 80 large female silver eels suffering various degrees of infection and swimming at various swimming speeds. It was found that:

- Oxygen consumption in rest and at critical aerobic swimming speeds were unaffected by infection (Fig. 9.7a, b).
- Critical aerobic swimming speed values tended to be negatively correlated with infection and damage levels (Fig. 9.7c).
- Eels with damaged swim-bladders dropped out early in comparison with the healthy eels; most of them could not swim faster than 0.7 m s^{-1} (Fig. 9.7d).
- Infected and damaged eels had at all speeds higher O_2 consumption levels and corresponding cost of transport (COT) levels.
- The optimum swimming speed of infected eels was significantly lower by 18% and that of eels with damaged swim-bladders by 21% in comparison with healthy eels (Fig. 9.7e).
- These eels also showed a higher (non significant) COT at their optimum swimming speeds by respectively 21% and 18% (Fig. 9.7f).

Hence, it could be concluded that both infected eels as well as eels without any parasites but with a damaged swim-bladder showed a considerable loss of swimming endurance. Simulated migration trials confirmed that eels with a high parasite level or with damaged swim-bladders show early migration failure ($< 1,000$ -km; Palstra et al. 2007b). Since swimming performance in both groups of eels was reduced, effects thus seem to be associated with swim-bladder dysfunction.

9.13 Implications for Eel Migration and Reproductive Success

The swim-bladder parasite has high impact during migration since (a) silver eels have much higher infection levels than yellow eels (Palstra et al. 2007a), (b) the infection level does not diminish during longer periods of up to 6 months (Székely et al. 2005; Palstra et al. 2007a), and (c) infection continues under salt water conditions (Kennedy and Fitch 1990; Kirk et al. 2000a, b, 2002; Palstra et al. 2007a).

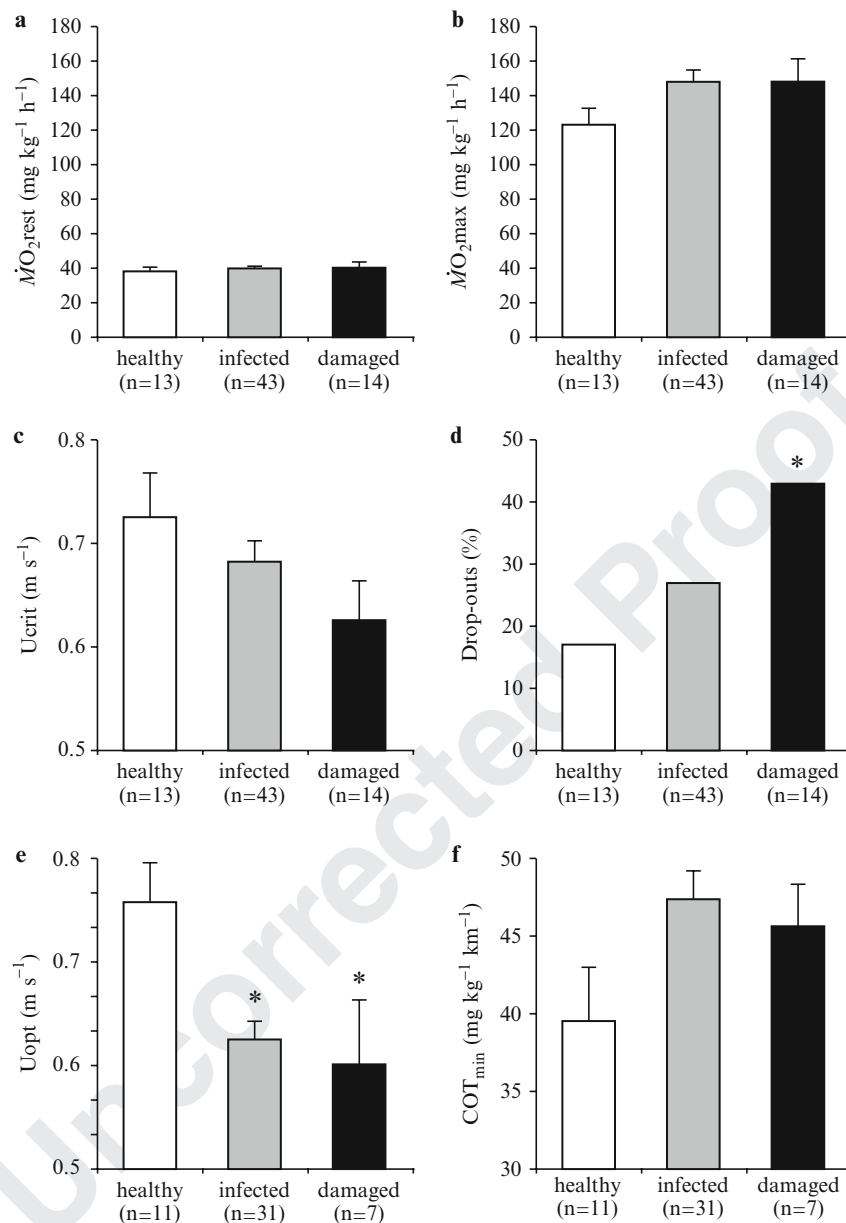


Fig. 9.7 Swimming parameters of healthy eels (white bars), infected eels (grey bars) and damaged eels (black bars). Healthy eels had large swim-bladders (SBI ≥ 10) without parasites. Infected eels had all-sized swim-bladders with parasites. Damaged eels had small swim-bladders (SBI < median 10.3%) without parasites. Significant differences ($P < 0.05$) are indicated by asterisk. No significant differences were found for (a) O_2 consumption in rest ($\dot{M}O_{2rest}$) (b) and maximal O_2 consumption ($\dot{M}O_{2max}$), (c) critical swimming speeds (U_{crit}) tended to decrease with increasing damage and (d) 43% of these eels dropped out before reaching U_{opt} (Mann-Whitney; $P = 0.03$), (e) eels with small swim-bladders had lower optimum swimming speeds U_{opt} (ANCOVA; $P = 0.01$) and (f) cost of transport (COT_{min}) tended to increase with increasing damage (Reproduced from Palstra et al. 2007a. With permission from Elsevier)

When we assume that eels continuously cruise at optimum swimming speeds, the trip would take only 3.5 months (Palstra et al. 2006). The 20% lower optimum swimming speed of infected and damaged eels may cause them to cruise slower, which extends the swimming period to about 4.2 months. They may arrive too late at the spawning grounds for final maturation and the spawning event itself.

[Au4] The cost of transport at optimum swimming speeds was about 20% higher in heavily infected and damaged eels than in healthy eels, making it likely that they will spend at least 20% more of their energy reserves on migration. This leaves less fat for egg production. In a study on the spawning characteristics of downstream migrating silver eels from the River Rhine (Palstra et al. unpublished data), a negative correlation was found between the number of parasites (up to $n = 46$ parasites per swim-bladder) and the relative gonad mass suggesting a direct effect of infection on maturation. Müller et al. (2001) concluded that *Anguillicola crassus* infection is not a barrier factor for the artificial induction of maturation. Egg quality may however be lower in these eels.

From the study of Palstra et al. (2007a), it can be concluded that fewer eels will be able to reach the spawning grounds. These eels may arrive too late and egg quality of these eels may be lower. With these effects on spawning migration and gamete quality, the swim-bladder parasite is a serious threat for the overall reproductive success of the European eel.

9.14 *Anguillicola crassus* Infection and Eel Decline

It was thought at one time that the decline in population levels of *A. anguilla* throughout Europe during the 1980s might be directly related to the spread and increase of *A. crassus* over the same period. A decline in eel populations and elver runs has been well documented throughout the continent (Moriarty and Dekker 1997), and a number of factors including overfishing of elvers and adults and global warming have been considered to be wholly or partially responsible. The correlation between the increase in *A. crassus* infection levels and decrease in host population levels might suggest a causal relationship, but doubt was thrown upon this suggestion when it was realized that a similar decline in magnitude of recruitment (98%) was taking place simultaneously in the *A. rostrata* population in North America at a time before *A. crassus* had spread to that continent. This decline was also blamed on overfishing and pollution. However, it has been suggested that the co-incidence in timing of the declines on both sides of the Atlantic implies an Atlantic-wide cause, e.g. changes in climate or Gulf Stream (Castonguay et al. 1994). Nevertheless, given the effects on basic physiology and survival during the yellow eel phase, and the impaired swimming performance of silver eels, it is very hard to believe that *A. crassus* is not at least partially responsible for, or does not contribute to the decline in eel populations and many workers believe that this is in fact the case (Køie 1991; Sures and Knopf 2004).

9.15 Conclusion

Anguillicola crassus is a pathogenic nematode of *Anguilla anguilla*. This parasite infecting originally pacific eels without obvious pathogenic symptoms was introduced into the Atlantic region in the years of 1980s, where it caused heavy infections and even massive fish dies in the European eel. Here the parasite has altered its life cycle by infecting a wide range of intermediate hosts, employing paratenic hosts and surviving as larvae for months in the swim-bladder wall. Pathogenic symptoms are partially caused by adult worms living in the lumen of the swim-bladder and feeding on blood, but major degeneration changes are caused by migrating larvae boring through the intestinal wall and causing proliferative, oedematous, fibrous and degenerative changes in the swim-bladder wall. Major pathogenic effects on eels result from haemorrhaging in, and thickening of the swim-bladder wall. At heavy infections no worms and air are formed in the swim-bladder thickened ten times compared to the original thickness. The process of development and changes in the swim-bladder wall were followed by x-ray and CT methods. The loss of oxygen concentration in the swim-bladder seems to reduce function as a hydrostatic organ, and increases the stress response of eels. In shallow lakes at warm temperatures this can result in mass mortalities. Experiments performed by the authors proved that swim-bladder infection had an adverse effect on swimming performance. It is, therefore feared that the parasite negatively affects the ability of eels to migrate to the Sargasso Sea and so contributes to the decline in eel populations.

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