

Comparison of infection success, development and swim bladder pathogenicity of two congeneric *Anguillicola* species in experimentally infected *Anguilla anguilla* and *A. japonica*

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Abstract Two closely related parasites, *Anguillicola crassus* and *Anguillicola novaezelandiae*, originally parasitizing swim bladders of the Japanese eel (*Anguilla japonica*) and the Short-finned eel (*Anguilla australis*), respectively, were used for analyzing the infection success of each parasite species on either long-known, recently acquired or new definitive host species and the associated effects on the eels' swim bladders. On that account, European eels (*Anguilla anguilla*) and Japanese eels were experimentally infected with both *Anguillicola* species in the laboratory. Susceptibility of the two eel species to both parasite species was determined by analyses of infection data. Subsequently, histopathological effects of the nematodes on the hosts' swim bladders were characterized according to already established indices.

The present study revealed significant differences between the four different host-parasite systems regarding recovery rates, infropopulations, and damage levels. Both nematode species achieved significantly lower recovery rates in Japanese eels than in European eels, since the examined swim bladders of Japanese eels contained a high amount of dead encapsulated larvae, whereas those of European eels contained only living nematodes. Encapsulation of larvae in Japanese eels was associated with a distinct thickening of the swim bladder walls. The swim bladders of uninfected Japanese eels turned out to be generally thicker than those of European eels. Infection with both *Anguillicola* species resulted in a further thickening process of the swim bladder walls in Japanese eels, whereas those of European eels showed only

minor changes. The two established classification systems turned out to be inapplicable, since the measurements and the macroscopic evaluations of the swim bladders of the two infected eel species did not entirely correspond to the underlying criteria.

Keywords *Anguillicola crassus* · *Anguillicola novaezelandiae* · Swim bladder · Damage level · European eel · Japanese eel

Introduction

Anguillicoloid swim bladder parasites that have accidentally been introduced into the population of European eels (*Anguilla anguilla*) belong to the best known examples of parasites that negatively affect newly acquired hosts. The nematode species *Anguillicola crassus* was introduced to Europe via import of infected eels from Taiwan in the 1980s (Neumann 1985), whereas *Anguillicola novaezelandiae*, the endemic parasite of the Short-finned eel (*Anguilla australis*) was transported from New Zealand to Lake Bracciano, Italy, in 1975 (Moravec and Taraschewski 1988). These two closely related species differ with regard to their natural hosts and thus to their infection potential and their pathogenicity towards other eel hosts. While various studies were focused on the susceptibility of the European or the Japanese eel towards *A. crassus* both in wild eel populations and in experimental studies (Knopf et al. 1998; Knopf and Mahnke 2004; Knopf 2006; Münderle et al. 2006; Han et al. 2008; Heitlinger et al. 2009; Weclawski et al. 2013), there is only one study dealing with experimental infections with *A. novaezelandiae* in intermediate hosts (Moravec et al. 1994) and two in European eels (Grabner et al. 2012; Dangel et al. 2013).

In order to comparatively analyze the infection success of the two nematode species *A. crassus* and *A. novaezelandiae* in

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the two eel species *A. anguilla* and *A. japonica*, experimental infections of all four possible host-parasite combinations were performed in a common garden experiment. Based on recent studies, it can be assumed that the infection success of *A. crassus* in the European eel will exceed the rate in the Japanese eel, as the immune system of the Japanese eel has already been adapted to *A. crassus*, while the European eel has only been exposed to *A. crassus* within the last three decades (Knopf 2006). With respect to *A. novaezelandiae*, the first hypothesis being tested is that its infection rate is higher than the rate of *A. crassus* in both eel species since the former parasite has never naturally encountered the Japanese host species and has only encountered the European host species in Lake Bracciano for a period assumed to be too short to allow for adaptation processes in the population of the European eel.

Infestation of swim bladders with *A. crassus* usually causes damage of the swim bladder wall including thickening, inflammation, fibrosis, and changes in the epithelial cells (Knopf et al. 2008; Würtz and Taraschewski 2000). In severe cases, pathologic alterations even lead to a complete loss of the swim bladder lumen, or the lumen becomes totally filled with worms. From these massive alterations of the swim bladder, one may expect a loss of its function which will be especially relevant when eels start their spawning migration through the Atlantic (Sures and Knopf 2004a), with diurnal vertical migrations ranging between 40 to 1,000 m (Aarestrup et al. 2009). In order to specify the degree of swim bladder damage, a number of analyses of histopathological effects on the swim bladder walls of the respective eel hosts have been performed (Hartmann 1994; Haenen et al. 1994; Molnár et al. 1993, 1995; Würtz and Taraschewski 2000; Abdelmonem et al. 2010; Neto et al. 2010), and the results were used in order to classify swim bladder damages into categories (Liewes and Schaminee-Main 1987; Csaba et al. 1993; Hartmann 1994; Molnár et al. 1994; Beregi et al. 1998; reviewed by Lefebvre et al. 2011). From these studies, it emerges that infections with *A. crassus* may cause severe swim bladder damages in European eels (Kirk 2003), whereas the impact on Japanese eels is usually rather low (Egusa 1992). Data on natural infections of the Short-finned eel with *A. novaezelandiae* (Lefebvre et al. 2004a, b) revealed only little—if any—damage. Accordingly, within the present study, degenerative changes of the swim bladder walls of two different eel species were directly compared following experimental infection with the two different nematode species and related to the already existing knowledge on histopathological changes and their related categorization. The underlying hypothesis is that a higher degree of swim bladder wall damage in European and Japanese eels is to be expected following infection with *A. novaezelandiae*, since both eel species are not adapted to this parasite.

Material and methods

Source and maintenance of final and intermediate hosts and parasites

This study includes two different eel species (*A. anguilla*, *A. japonica*) and two different nematode species (*A. crassus*, *A. novaezelandiae*). Uninfected European eels were purchased from a German eel farm known to be free of *A. crassus* infections (Albe Fischfarm, Haren/Ruetenbrock, Germany) and were kept at a constant temperature of 20 °C in aerated tap water. Eels were fed with pellet food (DAN-EX 2848, BioMar A/S, Brande, Denmark) twice a week. Japanese eels were imported from an eel farm in Japan (Omori-Tansui Co., Ltd., Miyazaki, Japan) and were kept in tanks separated from the other eel species but under the same conditions. In order to exclude already existing infections, a group of 10 eels of each species was dissected prior to experimental infections and their swim bladders were screened for the presence of nematodes. Cyclopid copepods, serving as intermediate hosts, were caught from an urban pond, kept in small tanks, and fed twice weekly. Second stage larvae (L2) of *A. crassus* were extracted from swim bladders of European eels that were previously infected in the laboratory (see Grabner et al. 2012). Second stage larvae of *A. novaezelandiae* were collected from swim bladders of naturally infected Short-finned eels in New Zealand (see Dangel and Sures 2013).

Experimental infections

In order to generate third stage larvae (L3) of both nematode species, copepods were individually infected by placing two L2 per copepod into 24-well plates filled with tap water. In the following 3 weeks, the copepods were fed twice a week, and subsequently, the L3 were separated from the copepods by means of a tissue potter (55 ml tissue grinder, Wheaton, Millville, New Jersey, USA) (see Haenen et al. 1994). The remaining suspension of larvae in 0.8 % saline was poured through a common paper tea filter into a centrifuge falcon tube, allowing the larvae to migrate through the membrane of the filter. After at least 2 hours rest time, the larvae were collected from the bottom of the tube by means of a Pasteur pipette and then stored in medium (Minimum Essential Medium Eagle, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 8 °C until use.

Infections of European eels were performed with 20 L3 of either parasite species. In order to achieve a comparable intensity, 40 larvae were used for infections of the Japanese eel, since previous studies suggest a lower susceptibility of Japanese eels towards *A. crassus* compared to European eels (Knopf and Mahnke 2004). The respective eels were selected according to comparable sizes of about 100–250 g per individual. Eels were gently wrapped in a well-soaked cloth, and

subsequently, a small-sized volume of the suspension containing the larvae was administered by means of a stomach tube (1.5 mm diameter; B. Braun Melsungen AG, Melsungen, Germany) as described by Sures and Knopf (2004b). Each infection group was kept separated from the other groups in large tanks at a constant temperature of 20 °C. In order to prevent unintended release of *A. novaezelandiae* individuals to the environment, all occurring waste water was heated to at least 80 °C in order to kill the L2 potentially discharged by the hosts. Uninfected control groups of both eel species were kept separated from the infected groups but under the same conditions.

Examination and analysis of host-parasite systems

All groups (see Table 1) were kept for 90 and 120 days post infection (dpi), respectively. Prior to examination, the eels were beheaded, measured, and weighed. Then, the swim bladders were removed and cut open. Adult nematodes were collected in physiologic saline, divided according to genders, counted, and immediately stored in –80 °C for further analyses.

The swim bladders were macroscopically examined for any alterations or damages, which were categorized into degenerative levels on the basis of classifications by Hartmann (1994), modified by Knopf (1999) (Schadensklasse (SK)) and Lefebvre et al. (2011) (Swimbladder Degenerative Index (SDI)). The SK classification is based on a macroscopic diagnosis regarding thickness and opacity of the swim bladder wall, whereas the SDI classification uses three criteria: transparency/opacity, pigmentation/exudate, and thickness. After removal and macroscopic examination, the swim bladders were compressed between two plexiglass plates and checked for the presence of dead or living L3 or L4 by means of a stereomicroscope with a magnification of $\times 8$ –40. After recording the number of larvae, a sample of each swim bladder was stored for histological analyses in a tissue embedding cassette (Histosette I, M498, VWR International GmbH, Darmstadt, Germany), immersed in a formalin solution containing 10 % formaldehyde.

Recovery rates were determined in percent by dividing the total number of recovered nematodes by the total number of administered larvae. In order to describe the composition of

infrapopulations, mean intensities were calculated by dividing the total number of nematodes found in one host by the total number of hosts that have been infected (see Bush et al. 1997). Statistical analyses of all data were performed by means of the *t* test (GraphPad Prism 5, GraphPad Software, Inc., USA).

Histological analyses

The fixed swim bladder samples were embedded in paraffin wax, sectioned into layers of 5 μ m by means of a microtome (Leica Microsystems), and applied to microscope slides. These sections were dried and subsequently stained with hematoxylin and eosin. The histological samples were examined and photographed using a light microscope (Olympus). The thickness of the swim bladder walls was measured by means of an image-processing program (Image J, National Institutes of Health, USA). Histopathological data were checked for significance using the *t* test (GraphPad Prism).

Results

Infection success and development of *Anguillicola* spp. in *Anguilla* spp.

Recovery rates of both *Anguillicola* species at 90 dpi are significantly higher in European eels compared to Japanese eels, with a slightly higher infection success for *A. novaezelandiae* compared to *A. crassus* (Fig. 1). In Japanese eels, recovery rates of both *Anguillicola* species ranged below 10 %. European eels infected with *A. crassus* contained 17 % L3, 6 % L4, and 76 % adults, whereas the infrapopulation of *A. novaezelandiae* consisted of 96 % adults and 4 % L4 (Fig. 2). Both nematodes in Japanese eels as hosts lack living larval stages but contain 46 % (*A. crassus*) and 59 % (*A. novaezelandiae*) dead larvae, located in the swim bladder walls.

At 120 dpi, recovery rates of adults (Fig. 3) of both nematode species in the European eel were similar with means of 41.4 % (*A. crassus*) and 39.5 % (*A. novaezelandiae*). In the Japanese eel, only a mean of 6.3 % (*A. crassus*) and 3.6 % (*A. novaezelandiae*) adults were found. When including larval stages, however, the highest recovery rate was found for European eels infected with *A. crassus* followed by European eels infected with *A. novaezelandiae*. In the Japanese eel, *A. novaezelandiae* showed a higher recovery rate compared to *A. crassus*. Values concerning adults as well as all stages of both *Anguillicola* spp. in the European and the Japanese eel featured a highly significant difference. Infrapopulations at 120 dpi (Fig. 4) showed a similar composition compared to the results at 90 dpi. The number of L3 found in European eels infected with *A. crassus* was slightly reduced, and at the same time, the number of L4 was increased. European eels infected

Table 1 Overview of experimental infections with eel and *Anguillicola* species

Host/parasite species	<i>Anguillicola crassus</i>	<i>Anguillicola novaezelandiae</i>
European eel	20 \times L3 (E20Ac)	20 \times L3 (E20An)
Japanese eel	40 \times L3 (J40Ac)	40 \times L3 (J40An)

Table shows host-parasite systems that were used for experimental infections, the amount of administered L3 stage larvae and the respective system labels

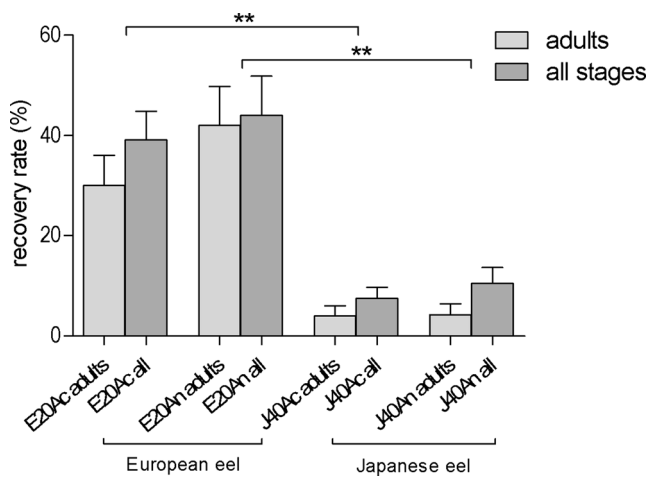


Fig. 1 Recovery rates of adults (light grey bars) and all stages (L3, L4, and adults; dark grey bars) of *Anguillicola* species in European and Japanese eels at 90 days post infection (dpi); with E20Ac European eel infected with 20× *A. crassus*, N=10; E20An European eel infected with 20× *A. novaezelandiae*, N=10; J40Ac Japanese eel infected with 40× *A. crassus*, N=10; J40An Japanese eel infected with 40× *A. novaezelandiae*, N=10

with *A. novaezelandiae* were not found to contain larval stages. In the Japanese eel infected with *A. novaezelandiae*, the share of dead larvae rose towards 71 %.

Histological analyses

Representative samples of the analyzed swim bladder walls of each group of eels are shown in Figs. 5 and 6. All layers of the swim bladders of Japanese eels—including the control group—were thicker than those of the European groups (Fig. 7). Both the epithelium and the muscularis mucosae showed higher thicknesses compared to all groups of the European eel. While the layers of the muscularis mucosae in Japanese eels were slightly thickened in consequence of infection with both *Anguillicola* species, the thickness of the epithelia partially increased threefold. Japanese eels infected with *A. crassus* featured the greatest thickening of all groups. Statistical tests showed a significant difference ($p=0.0308$)

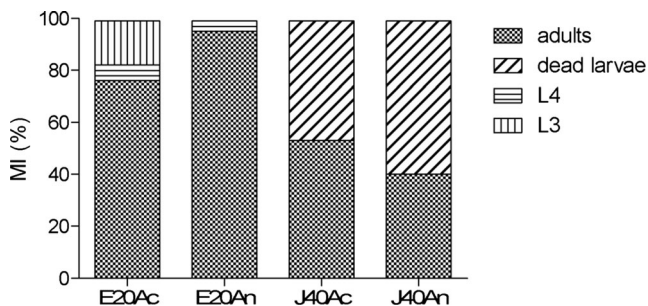


Fig. 2 Infrapopulations of *Anguillicola* sp. in European and Japanese eels at 90 dpi, with E20Ac European eel infected with 20× *A. crassus*, N=10; E20An European eel infected with 20× *A. novaezelandiae*, N=10; J40Ac Japanese eel infected with 40× *A. crassus*, N=10; J40An Japanese eel infected with 40× *A. novaezelandiae*, N=10

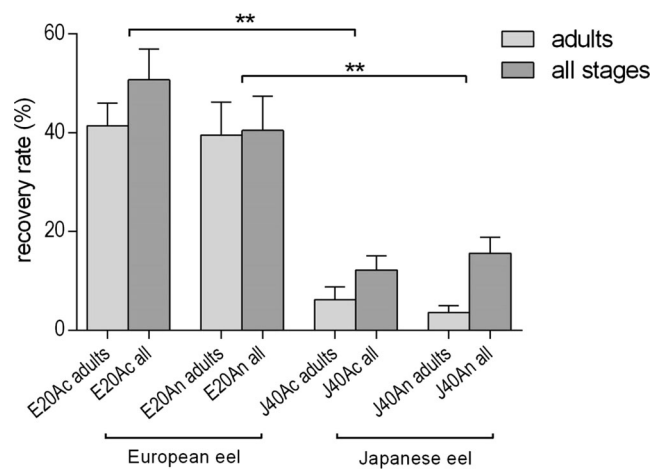


Fig. 3 Recovery rates of adults (light grey bars) and all stages (L3, L4, and adults; dark grey bars) of *Anguillicola* species in European and Japanese eels at 120 dpi, with E20Ac European eel infected with 20× *A. crassus*, N=10; E20An European eel infected with 20× *A. novaezelandiae*, N=10; J40Ac Japanese eel infected with 40× *A. crassus*, N=10; J40An Japanese eel infected with 40× *A. novaezelandiae*, N=10

between both uninfected control groups as well as between Japanese eels infected with *A. crassus* and *A. novaezelandiae*. Differences between European and Japanese eels infected with *A. crassus*, between European and Japanese eels infected with *A. novaezelandiae* as well as between uninfected Japanese eels and those infected with *A. crassus* were found to be highly significant ($p<0.008$) (see Fig. 7).

Damage levels of swim bladders

The percentages of the assessed swim bladder damage levels according to the SDI are presented in Fig. 8; those of the SK are shown in Fig. 9. While 25 % of the European eels infected with *A. novaezelandiae* had no visible damages and were thus ranked with an SDI of 0, all other groups showed certain swim bladder alterations. SDI 1 was found in approximately 92 % of European eels infected with *A. crassus*, in 75 % of European eels infected with *A. novaezelandiae*, in 50 % of Japanese eels

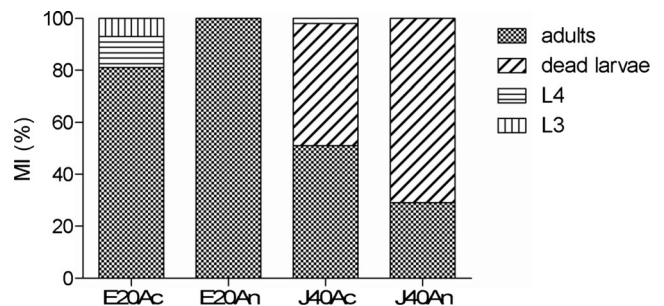


Fig. 4 Infrapopulations of *Anguillicola* sp. in European and Japanese eels at 120 dpi, with E20Ac European eel infected with 20× *A. crassus*, N=14; E20An European eel infected with 20× *A. novaezelandiae*, N=10; J40Ac Japanese eel infected with 40× *A. crassus*, N=10; J40An Japanese eel infected with 40× *A. novaezelandiae*, N=11

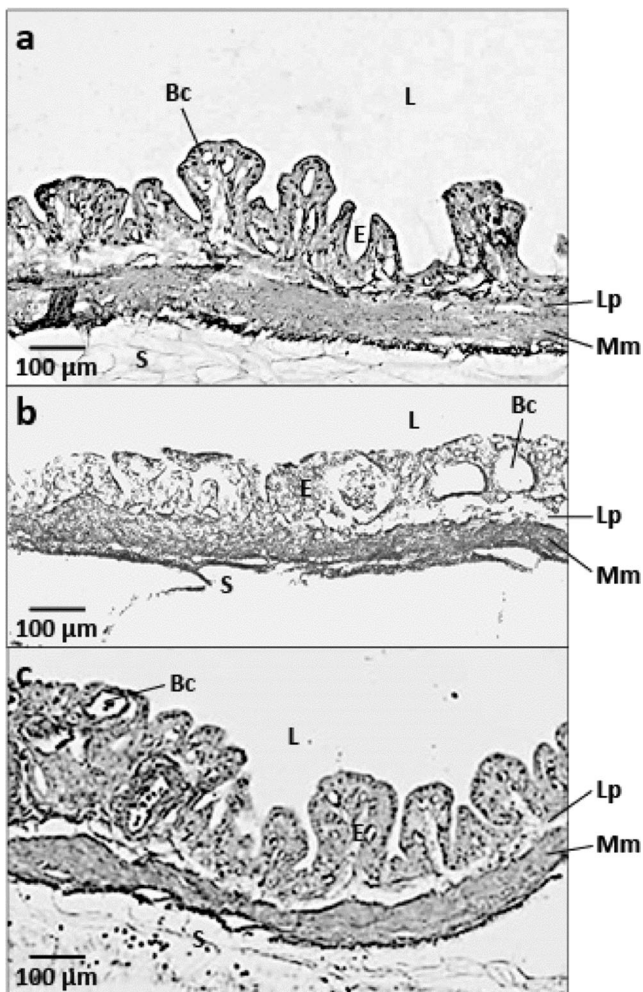


Fig. 5 Cross-sections of swim bladders of European eels at 120 dpi. **a** Cross-section of a swim bladder of an uninfected European eel. **b** Swim bladder infected with *A. crassus*. **c** Swim bladder infected with *A. novaezelandiae*. Bc blood capillary, E epithelium, L lumen, Lp lamina propria, Mm muscularis mucosae, S submucosa

infected with *A. crassus*, and in 10 % of Japanese eels infected with *A. novaezelandiae*. SDI 2 was only determined for European eels infected with *A. crassus* (~8 %) and Japanese eels infected with *A. crassus* (50 %) and with *A. novaezelandiae* (50 %). Solely swim bladders of Japanese eels infected with *A. novaezelandiae* correspond with SDI 3 (40 %).

Analyses of the swim bladders regarding the SK revealed a high percentage of SK 1 in European eels infected with *A. crassus* (~92 %) and with *A. novaezelandiae* (100 %). In Japanese eels infected with *A. novaezelandiae*, only 10 % of the swim bladders featured an SK 1. SK 2 was determined in approximately 8 % of the swim bladders of European eels infected with *A. crassus*; those infected with *A. novaezelandiae* showed no cases of SK 2. In Japanese eels infected with *A. novaezelandiae*, a share of 20 % was assigned to the SK 2 level. SK 3 was only identified in the Japanese eel infected with *A. novaezelandiae* with a share of 60 %. SKs for

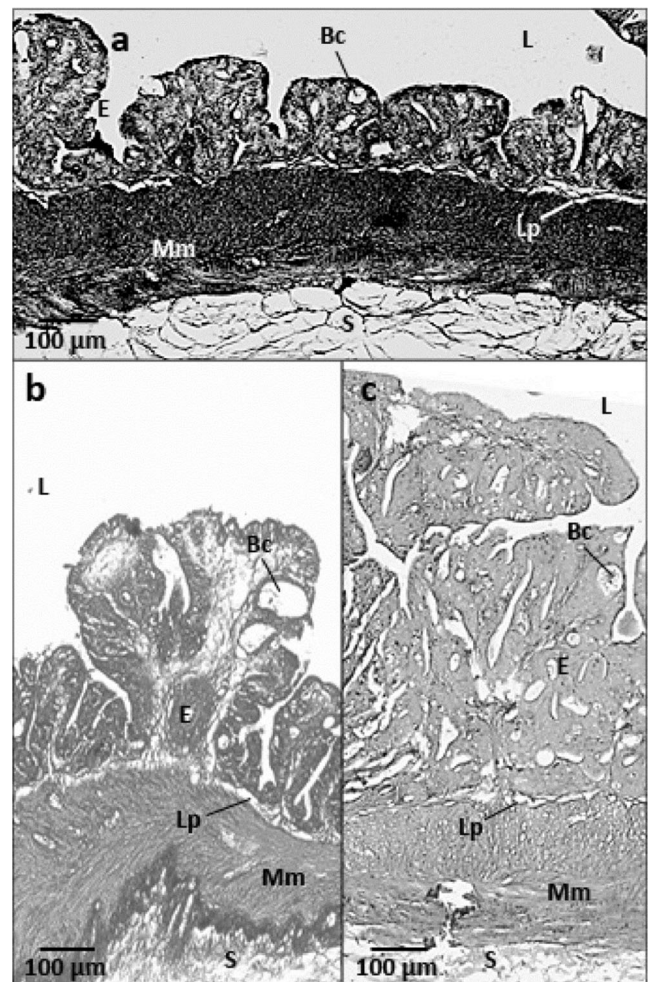


Fig. 6 Cross-sections of swim bladders of Japanese eels at 120 dpi. **a** Cross-section of a swim bladder of an uninfected Japanese eel. **b** Swim bladder infected with *A. crassus*. **c** Swim bladder infected with *A. novaezelandiae*. Bc blood capillary, E epithelium, L lumen, Lp lamina propria, Mm muscularis mucosae, S submucosa

Japanese eels infected with *A. crassus* could not be determined due to mismatch of features with the underlying study.

Swim bladders of European eels infected with both *Anguillicola* species featured no macroscopically visible thickening and no encapsulated larvae in their swim bladder walls. In contrast, 50 % of swim bladders in Japanese eels infected with *A. crassus* had a thickened wall and 40 % contained encapsulated larvae. When infected with *A. novaezelandiae*, 90 % of the swim bladder walls in Japanese eels were thickened and 100 % showed encapsulated larvae.

Discussion

The present study showed distinct differences in the recovery rate and the development of fully adapted, newly joined up,

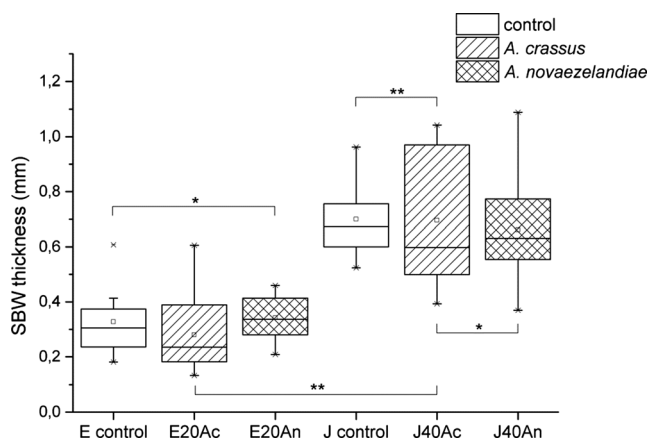


Fig. 7 Thickness of swim bladder walls of infected and uninfected European and Japanese eels at 120 dpi. Boxplots show ranges of thickness of swim bladder walls measured in mm. Data on *A. crassus* are shown in striped boxes, those on *A. novaezelandiae* in a check pattern, with E control European eel uninfected, $N=10$; E20Ac European eel infected with $20\times A. crassus$, $N=14$; E20An European eel infected with $20\times A. novaezelandiae$, $N=10$; J control Japanese eel uninfected, $N=10$; J40Ac Japanese eel infected with $40\times A. crassus$, $N=10$; J40An Japanese eel infected with $40\times A. novaezelandiae$, $N=11$

and completely unfamiliar host-parasite systems. Recovery rates were generally found to be higher in the European eel than in the Japanese eel irrespective of the particular nematode species they were infected with. Especially in the European eel, *A. novaezelandiae* featured a higher infection success and a faster development compared to *A. crassus*, which is in correspondence to the results of Dangel et al. (2013). These findings may lead to the conclusion that the development of *A. crassus* in the European eel is retarded due to the adapted immune response of the host, which has been living together with this parasite for more than 30 years now. On the contrary, *A. novaezelandiae* is able to develop more unopposedly in this virtually unadapted host. As already discussed by Dangel et al. (2013), *A. novaezelandiae* shows a much more synchronized development than *A. crassus*. While the former parasite

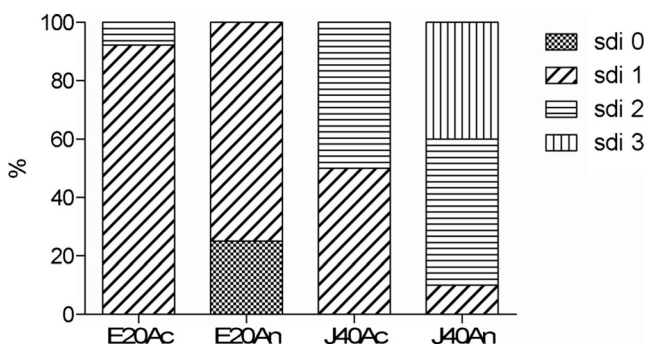


Fig. 8 Swimbladder Degenerative Index (SDI) at 120 dpi. Bars show distributions of SDI classifications according to Lefebvre et al. (2002), with E20Ac European eel infected with $20\times A. crassus$, $N=14$; E20An European eel infected with $20\times A. novaezelandiae$, $N=10$; J40Ac Japanese eel infected with $40\times A. crassus$, $N=10$; J40An Japanese eel infected with $40\times A. novaezelandiae$, $N=11$

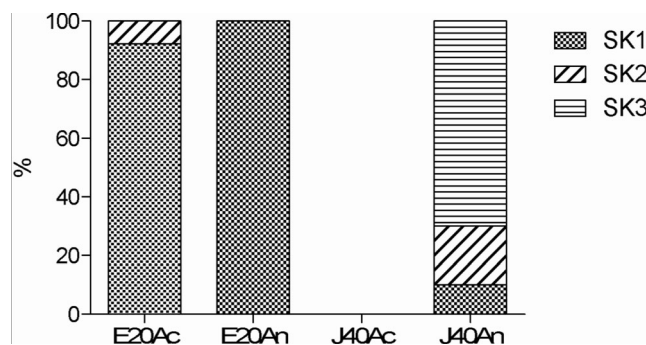


Fig. 9 Swim bladder damage levels (SK) at 120 dpi. Bars show distributions of swim bladder damage classifications according to Knopf (1999) with E20Ac European eel infected with $20\times A. crassus$, $N=14$; E20An European eel infected with $20\times A. novaezelandiae$, $N=10$; J40Ac Japanese eel infected with $40\times A. crassus$, $N=10$; J40An Japanese eel infected with $40\times A. novaezelandiae$, $N=11$

completed its development in the European eel at 120 dpi and no more larval stages could be detected, the latter parasite species featured an irregular development with both L3 and L4 stages at 90 and 120 dpi. The results on *A. crassus* in both eel species also correspond to a susceptibility study of Knopf and Mahnke (2004), in which similar recovery rates and infrapopulations for the Japanese and the European eel were determined, each infected with 30 larvae of *A. crassus* at 98 dpi. These findings imply that *A. crassus* in the European eel shows the least synchronized development of all four analyzed parasite-host systems. This might indicate that *A. crassus* in the European eel has an advantage over other parasite-host systems since it is able to produce more eggs over a longer period of time.

Interestingly, both parasite species performed quite similar in the Japanese eel. There were no L3 or L4 stages present, except for a single L4 finding of *A. crassus* at 120 dpi. The individuals found in the swim bladders of Japanese eels were either dead or fully developed. So the present study gives new insights into defence mechanisms of the Japanese eel towards a long-known parasite on the one hand and a new parasite species on the other hand. The results on the analyses of swim bladders in experimentally infected Japanese eels suggest that the revealed responses are not species-specific. Both systems involving Japanese eels featured thickening as well as encapsulation processes. From an evolutionary point of view, this reaction might be explained by the long-standing adaptation of the Japanese eel to *A. crassus*, which may have led to a similar response to *A. novaezelandiae*, although the host has never naturally encountered this parasite. The rather low recovery rates and the high amounts of dead encapsulated larvae in both Japanese eel-nematode systems point to the assumption that this eel species has developed a strong immune response, which is unspecific to the parasite species. Studies by Nielsen (1999) and Knopf and Lucius (2008) have proved that *A. anguilla* lacks a specific humoral immune response against antigens of *A. crassus*, which can yet be found in

A. japonica. This ability of generating a high immune competence against this long-known nematode may have led to a comparable reaction to the closely related species *A. novaezelandiae*. The effective response of Japanese eels to the particular antigens emerging from both parasite species is likely to contribute to a successful elimination of larvae penetrating the swim bladder wall. This reaction is also mirrored in the high percentage of encapsulated larvae found in Japanese eels which, in turn, corresponds to the findings on the thickening of their swim bladder walls. Although—indicated by the results of the control group—the swim bladders of Japanese eels inherently feature a higher thickness than those of European eels, the present study shows that the thickening of the swim bladder walls still correlates with the encapsulation of larvae. Since European eels had no encapsulated larvae at all, no thickening of their swim bladder walls occurred irrespectively of infection with either parasite species. As soon as encapsulated larvae were present in the swim bladder walls of Japanese eels, a further thickening occurred. While all layers of the swim bladder walls of Japanese eels were affected by thickening, those of European eels showed no sign of thickening. Except for the studies by Molnár (1994), Molnár et al. (1995), and Audenaert et al. (2003), in which an encapsulation of *A. crassus* larvae in European eels taken from Lake Balaton and from different sites in Belgium was observed, there has been no evidence for such a response of the European eel.

The present study generally revealed only minor damages of the swim bladders of European eels. A study by Würtz and Taraschewski (2000) pointed out that natural *A. crassus* infections of European eels caused a heavy folding of the epithelium, whereas laboratory infections were mainly characterized by inflammatory processes that were not associated with thickening. The described characteristic folding of the epithelium can yet be observed in Japanese eels experimentally infected with either parasite species (Fig. 5). It remains unclear if there are strong differences between naturally or experimentally infected eels. Studies by Würtz et al. (1998) and Abdelmonem et al. (2010) also disclosed a possible thickening of swim bladder walls in wild European eels. Würtz and Taraschewski (2000) concluded that a thickening of the swim bladder wall is much more likely in naturally infected European eels, whereas experimentally infected individuals normally show no signs of thickening unless other impairing factors are involved. This indicates that, apart from a non-recurring *Anguillicola* infection, other factors possibly occurring under natural conditions may lead to a thickening of the swim bladder wall. The present study shows those swim bladder damages that are solely associated with the respective *Anguillicola* infection and excludes all other factors that could have previously affected the swim bladders.

When regarding the results of the thickness measurements, there is a clear association between the respective thickening

process and the corresponding swim bladder damage level as one main criterion for both classification systems is the thickness of the wall or the volume of the lumen, respectively. In some cases, however, it was difficult to clearly define the particular damage class using the established indices. While the SDI by Lefebvre et al. (2002) takes all grades of damage into account by adding up all observed alterations, the SK by Hartmann (1994) and Knopf (1999), respectively, is limited to clearly defined grades, which cannot be combined or graded any further. This fixed classification made it impossible to conclusively assign the swim bladder damages of the Japanese eel to a particular SK (Fig. 8). In some cases, there was a variance of three grades within the SK classification, as there was an overlap of visible features belonging to three different grades. One might assume that this index is only applicable to the well-investigated host-parasite system of the European eel infected with *A. crassus*. It also seems to be questionable whether those damage classes may be equally employed on both wild and laboratory eels. The present study shows those swim bladder damages that are solely associated with the respective *Anguillicola* infection and excludes all other factors that could have previously affected the swim bladders. Other impairing factors or repeatedly occurring infections influencing the condition of swim bladders in natural eel populations might have led to more severe histopathological damages like extreme thickening, inflammation, and fibrosis that were previously described in other studies (Würtz et al. 1998; Würtz and Taraschewski 2000; Abdelmonem et al. 2010). Moreover, the results on the definite measurements of the swim bladder walls imply that the existing classification systems cannot be equally applied to both eel species. Hartmann has assigned a benchmark of up to 1.00 mm defining a normal swim bladder thickness; a value of up to 5.00 mm is classed with the most severe damage class of SK 5. The SDI by Lefebvre also takes a value of less than 1.00 mm as a basis for an unaffected swim bladder, whereas the highest damage degree (value 2) in this category is based upon a thickness value of more than 3.00 mm. Taking these specified values into account, the measurement results in this study are contradictory to the scales determined by the two mentioned classification systems. In this regard, almost all analyzed swim bladder walls would be regarded as unimpaired since most of them featured a thickness value lower than 1.00 mm.

A macroscopic evaluation of the thickness of swim bladders seems to be quite vague and arbitrary, so an objective classification based on visible alterations is difficult. Lefebvre et al. (2011) proposed involving two independent observers in order to ensure objectivity, but even with two observers, there was a high coefficient of variation with regard to the SDI. The present study suggests that it might be useful to include histological measurements into a damage classification, since thickness can be precisely measured. In this case, the criterion of easiness, which should generally pertain to an index, is

subordinated. A similar difficulty applies to the evaluation of opacity which is another underlying criterion of SDI and SK, since opacity cannot be precisely measured and a macroscopic evaluation might be biased as well.

The findings of this study suggest that the two considered damage classification systems proved to be unsuitable for comparing naturally and experimentally infected eels on the one hand and European and Japanese eels on the other hand. Since there has been no study focusing on swim bladder damages and a possible classification system for either naturally or experimentally infected Japanese eels so far, further research should be conducted on this host species.

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