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The effect of foraging and ontogeny on the prevalence and intensity of the invasive parasite *Anguillicola crassus* in the European eel *Anguilla anguilla*

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Abstract

Infection patterns of the invasive *Anguillicola crassus* nematode were investigated in a population of the European eel *Anguilla anguilla* where parasite invasion is very recent, Loch Lomond, Scotland. Intensity levels of the parasite were associated with differences in fish ontogeny and trophic ecology. Although eels foraged on both fish and invertebrates, individuals which were smaller and fed on invertebrates (>70% contribution to diet) were found to contain a greater number of swim bladder parasites compared to larger eel with a predominance of fish (>60% contribution) in their diet. Within affected fish, a significant negative relationship was found between fish length and parasite intensity, with smaller individuals having higher parasite intensity than larger individuals. This study indicates that food intake and infection risk are linked in this recently infected host–parasite system. From a management perspective increasing our understanding of how infection intensity and repeated exposure is linked to resource use in an ecosystem is important for the future management of this endangered species in Europe.

Keywords: Anguillicola crassus, eel, foraging, ontogeny, parasite prevalence.

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Introduction

The European eel (*Anguilla anguilla* L.) plays a pivotal role in a balanced aquatic ecosystem both as a predator and prey species. Since the 1970s, a continuous decline has been observed in recruitment of glass eels to continental waters (ICES 2013), leading to growing concerns over the long-term viability of the species (Dekker 2008). The factors underlying the collapse of this panmictic population (Als *et al.* 2011; Pujolar *et al.* 2013) are not fully understood. However, since the 1980s, a new threat to the European eel has emerged, the invasive parasitic nematode worm (*Anguillicola crassus* Kuwahara, Niimi & Itagaki 1974; Lefebvre, Fazio & Crivelli 2012). The parasite entered Europe through the transportation of its native host, the Japanese eel (*Anguilla japonica* Temminck & Schlegel 1846; Koops & Hartmann 1989). Since its arrival, it has spread rapidly across the continent (Kennedy 1993; Lefebvre *et al.* 2012) exploiting a naive host (Kirk 2003).

In areas where the parasite has established, it can reach a prevalence of over 90 per cent among eels within 4 years (Lefebvre *et al.* 2002), in part because of its ability to infect a range of intermediate and paratenic hosts (Kennedy 1993; Lefebvre *et al.* 2012; Becerra-Jurado *et al.* 2014). Its capacity to spread within a catchment is a direct consequence of the organism's adaptability, high reproductive output and short life cycle (Kirk 2003). There are four larval stages and a terminal adult stage in the life cycle of the *A. crassus* nematode (Lefebvre *et al.* 2012). Eggs are hatched in

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the eel's swim bladder and migrate out through the pneumatic duct and intestines before release into open water. Free living larvae can survive within the substratum in a dormant state for several days, particularly during periods of low water temperature (Kennedy & Fitch 1990). Copepod ingested larvae then burrow through the intestinal wall and spill out into the haemocoel (Thomas & Ollevier 1992). The parasite relies upon predator/prey interactions, with larval stages transmitted trophically through food-web interactions (Lefebvre *et al.* 2012). Eels have two routes of infection, either by direct consumption of copepods which act as the intermediate host or through ingestion of a paratenic host (Moravec & Skorikova 1998). It has been hypothesized that crustacean intermediate hosts serve as the source of infection for smaller eels (<20 cm), while larger eels mainly acquire infection by preying on paratenic hosts (Thomas & Ollevier 1992). Paratenic hosts are a very important part of the life cycle of *A. crassus* in Europe (Kirk 2003) and studies have shown that a wide range of eel prey organisms act as paratenic hosts (Moravec & Konecny 1994). These include at least 37 species of fish, including freshwater (Thomas & Ollevier 1992), estuarine and marine species (Hoglund & Thomas 1992). Frogs and newt tadpoles, snails and insect larvae have also been noted as paratenic hosts, but the importance of these hosts in nature is unknown (Moravec & Skorikova 1998).

The impact of *A. crassus* on European eels is well documented. In heavily infected eels, the swim bladder exhibits inflammation and thickening of the swim bladder wall (Molnar *et al.* 1993; Molnar 1994; Würtz, Taraschewski & Pelster 1996) reduced elasticity of the organ (Barry *et al.* 2014) and lowered resistance to environmental stressors (Gollock, Kennedy & Brown 2005). As a result of the physiological and physical changes to the structure of the swim bladder, reduced swimming speeds have also been recorded (Palstra *et al.* 2007) and it is most likely the silver eel stage, migrating to the open ocean that will be most impacted by these effects. *Anguillicola crassus* has been implicated as an important factor impeding stock recovery (VanBanning & Haenen 1991; Molnar *et al.* 1993). In the European context, efforts are being made to collate *A. crassus* infection data as part of a European Eel Quality Database (Belpaire *et al.* 2011) and to increase understanding of changing rates of infection in eels leaving continental waters.

Parasite infections have been noted to vary among size classes of their fish hosts and can reflect the trophic ecology of individuals (Bertrand, Marcogliese & Magnan 2008; Poulin & Leung 2011; Amundsen *et al.* 2013; Knudsen *et al.* 2014). Eel populations are known to display polymorphism, particularly in head shape, and at least in some populations the variation in morphology is strongly associated with foraging specialization (Lammens & Visser 1989; Proman & Reynolds 2000; Ide *et al.* 2011; Barry *et al.* 2015). Studies have shown that individuals exhibiting large robust and broad heads tend to be piscivorous and those with more delicate narrow heads feeding predominately on benthic invertebrates (Cucherousset *et al.* 2011). A recent study revealed that *A. crassus*-infected eels had a higher proportion of fish in their diet, suggesting piscivory increased chance of encounter with paratenic hosts in three riverine sites (Pegg *et al.* 2015). In contrast, a scenario posed by Lefebvre *et al.* (2013) suggests that individuals with high foraging rates also had the highest parasite burden, and the authors postulated that the most active foragers growing faster have a greater probability of becoming repeatedly infected via trophic transmission. Ultimately, the successful dispersal of *A. crassus* relies on predator/prey interactions within a host–parasite system; however, the trophic transmission of *A. crassus* remains poorly understood and studies of the early colonization and infection dynamics of this invasive nematode parasite are unknown.

Variation in foraging ecology between individuals may thus influence the encounter rate and parasite burden within the definitive host. This study aimed to (i) document the early stages of a recent invasion of the nematode parasite *A. crassus* in a large lake, Loch Lomond Scotland, and (ii) investigate the influence of trophic ecology on the intensity and prevalence of *A. crassus* in a previously naïve population of *A. anguilla*.

Methods

Sample collection

Eels were collected from two sites in Loch Lomond (56°05′N 4°34′W; Fig. 1) in June 2014.

Figure 1 The geographic location of Loch Lomond in Scotland and the location of the two sampling sites (★···♦).

All fish were collected using fyke traps ($n = 64$, 350–667 mm, north basin $n = 33$, south basin $n = 31$). Eels were killed (using a schedule 1 method; UK Home Office), weighed and measured, and the abdominal cavity dissected to enable a measure of parasite loading (or to confirm its absence). Swim bladders were removed and all *A. crassus* present in the swim bladder lumen were counted macroscopically. Body condition of each eel was calculated as relative condition factor according to Froese (2006), which measures the deviation of an individual from average weight for a given length in a sample population. Fat content was measured on live individuals using a Distell FM 692 Fat Meter. This meter has a microstrip sensor which measures the water content of a sample. The fat content of fish is correlated with the water content and thus the measurement of one can determine the other if the relationship between the two is known. The fat meter was calibrated (company calibration) to the fat/water relationship specific to European eel prior to taking measurements. Three measurements were taken along the body on both sides of the fish. The fat meter was then used to calculate the average per cent body fat for the individual based on the six readings.

For age analysis, one sagittal otolith from each pair was mounted onto a glass slide using 'Loctite' branded superglue. Otoliths were mounted concave side down and ground and polished on the sagittal plane using 1200 and 4000 grit silicon

carbide grinding paper until the origin and all growth rings could be observed under an optical microscope. Age was determined following WKAREA (2009) and otolith measurements were recorded using Image-Pro analysis software to allow back-calculation of size at age.

Stable isotope analysis

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide a longer-term signal of diet compared with the 'snapshot' information from stomach contents. Stable isotope ratios from fish muscle tissue typically reflect assimilated food during the summer growth period (Perga & Gerdeaux 2005). This information allows individual specialization in diet to be assessed (Bolnick *et al.* 2002). Samples of dorsal muscle from eels and potential fish prey and whole invertebrates were dried for 72 h in a drying oven. Dried tissue was ground in to a fine powder using a pestle and mortar. Invertebrates were analysed as bulk samples of whole individuals. High lipid concentration in muscle tissue can lead to particularly depleted $\delta^{13}C$ values (Post *et al.* 2007). Thus, a subset of eel tissue samples were lipid extracted to determine the effect of the lipid content on the stable isotope signature; 10–20 mg of ground tissue was soaked in a 2:1 chloroform: methanol (by volume) solvent mixture and the material suspended by stirring. After 15 min, the sample was centrifuged (835g for 5 min), the supernatant discarded (i.e. the analysis was not quantitative for lipids), and the sample resuspended in the solvent mixture. These steps were repeated at least three times or until the solvent ran clear. Finally, the sample was dried (60 °C). Given the large variation in individual lipid deposition and the confounding effect on $\delta^{13}C$ values, we undertook a correction for lipid level using a regression of the difference in $\delta^{13}C$ resulting from lipid extracted eels on their initial lipid concentration. Analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios was performed at the NERC Life Sciences Mass Spectrometry Facility, by continuous flow isotope ratio mass spectrometry (CF-IRMS), using an elemental analyser (Costech ECS 4010) coupled to a ThermoFisher Scientific Delta XP-Plus IRMS.

To estimate the long-term reliance of different prey to an individual's diet, a Bayesian stable isotope mixing model was implemented through SIAR (Stable Isotope Analysis in R, version 4.1.3; Parnell *et al.* 2010). Stomach content analysis (Figure S1) confirmed benthic invertebrates and fish as the main food source for eels in Loch Lomond. Baseline samples of potential eel diet were gathered from Loch Lomond. Potential fish prey was captured in fyke nets and potential invertebrate prey was collected from the foreshore via kick sampling. For SIAR, the fish and invertebrate prey signature was calculated as the mean ± SD $\delta^{13}C$ and $\delta^{15}N$ values of ruffe (*Gymnocephalus cernus* Linnaeus, 1758) for fish, whereas the invertebrate signature was calculated from the isotopic values of snails and *Asellus aquaticus* collected from the littoral zone. Following the approach of Cucherousset *et al.* (2011), averaged trophic fractionation factors with a large standard deviation were used in the mixing model (e.g. Post *et al.* 2007), fractionation of 1.0% (±1.0 SD) and 3.3% (±1.0 SD) for $\delta^{13}C$ and $\delta^{15}N$, respectively. The SIAR isotope mixing model uses a Bayesian approach to estimate relative dietary contributions and to consider uncertainties related to isotopic variation in the consumer and in the food sources as well as in the trophic fractionation factors (Parnell *et al.* 2010). Where resource use (benthic invertebrates or fish) of one group (infected) was outside the 95% credibility limits of another group (uninfected), groups were deemed to be using significantly different levels of the particular resource.

Data analysis

To identify the factors influencing the prevalence and intensity of *A. crassus* in the eel population, we used a generalized linear model approach. Parasite presence and absence (binomial response) among the eel population sampled and parasite intensity (parasite counts; Poisson response), the two primary response variables, were regressed with respect to five covariates (body length log10 transformed), body condition, proportion of invertebrates in diet (arcsine transformed), lipid content and one fixed effect (two-level factor; sampling sites × 2) in two separate GLMs for binomial and Poisson distributed data, respectively. Due to high collinearity between age and length (correlation factor; 0.9), age was dropped from the model. For parasite prevalence and intensity, maximal models including all covariates and fixed effects were fitted. A minimum adequate

model was generated by significance testing between models and the sequential removal of non-significant terms. The final model for parasite presence/absence and for parasite intensity contained only statistically significant terms. Inspection of diagnostic plots was used to ensure all model fit assumptions were satisfied. To test for differences between infected and uninfected fish, Welch *t*-tests were used to compare mean length, $\delta^{13}C$ and $\delta^{15}N$. All analyses were performed using R statistical software 3.1 (R Core Team 2014).

Results

Sixty-four eels were captured during the sampling period (size range: 350–661 mm). *Anguillicola crassus* was found in 43.8% of the eels collected in Loch Lomond. Mean intensity was 0.85 (SE ± 1.33) parasites per eel. The SIAR isotopic mixing model indicated that infected eels had a significantly higher reliance on invertebrates in their diet with only a small proportion of their diet being made up by fish (no overlap 95% Bayesian credibility interval; see Fig. 2), whereas uninfected eels had a significantly higher proportion of fish in their diet (no overlap in the 95% Bayesian credibility interval; see Fig. 2).

The minimum adequate model for prevalence among infected and uninfected fish revealed a significant effect of estimated proportion of invertebrates in diet (Table 1). The individuals with a higher estimated proportion of invertebrates in their diet exhibit higher probability of parasite infection ($Z = 1.61$, 3.728, $P < 0.001$; Fig 3). The minimum adequate model for the factors influencing intensity of parasites in an infected fish revealed a significant negative relationship between fish length and parasite intensity (Table 2), with smaller individuals having higher parasite intensity than larger individuals ($Z = 1.26$–2.192, $P < 0.05$).

Infected fish ($n = 28$) were significantly smaller than uninfected individuals ($n = 36$; $t = -3.14$, df = 60.88, $P < 0.001$). Infected fish were significantly more depleted in δC^{13} ($t = -4.2672$, df = 40.384, $P < 0.001$)) and $\delta^{15}N$ ($t = -7.4061$, df = 52.716, $P < 0.001$) than uninfected fish (see Table 3 and Fig. 2).

Discussion

The unpredictable nature of the invasion and establishment of non-native species means that information about mechanisms controlling the early stages of species invasions is only rarely studied. Given the difficulty in eradication of invasive species from aquatic environments (Clout & Veitch 2002), more information about the early

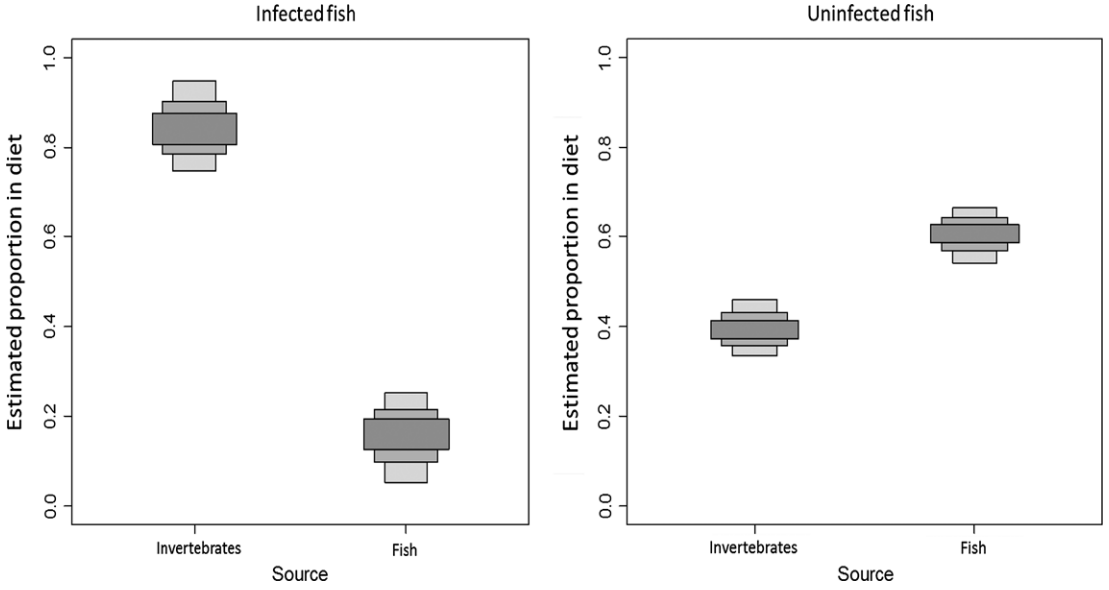

Figure 2 The relative proportion of each of the two food sources of infected and uninfected eels. Plots show 50%, 75% and 95% credibility intervals of the maximum-likelihood values estimated using SIAR.

stages of invasion has the potential to provide insights into the establishment process in a host–parasite system. This study uniquely addresses some of the ecological factors that impact on the transmission of an invasive parasite during the initial stages of parasite invasion.

The invasive nematode *A. crassus* has only recently invaded Loch Lomond. It was not detected on previous surveys (Barry *et al.* 2014). Introduction of *A. crassus* through an intermediate host in ballast water and introduction through the transportation of live bait are possible routes of entry to Loch Lomond. Levels of recreational boating activity are high on the loch and increased human activity has been shown to increase the probability of invasion by non-native species (Gallardo & Aldridge 2013) and Loch Lomond has been subject to invasion by a number of non-native species over the last few decades (Adams 1991; Adams & Maitland 1998). This invasive parasite is likely to have a negative impact on the currently abundant eel population within Loch Lomond (Adams *et al.* 2013). Studies have reported the effects of *A. crassus* on the condition of eels, and their ability to cope with environmental stressors infested with *A. crassus* (Gollock *et al.* 2005). Loss of appetite and vitality has been reported in cultured eels (VanBanning & Haenen 1991). However, the greatest effect may be the impact of the parasite on the swimming ability (Sjöberg *et al.* 2009) and swim bladder function (Barry *et al.* 2014). It is probably the silver eel stage, migrating to the open ocean that will be most impacted by these effects.

This study presented here shows patterning in the infection and transmission of the parasite in the eel population in the early stages of parasite establishment. Specifically, the ontogenetic stage and individual foraging strategy of eels have an influence on the intensity of infection by the invasive nematode parasite *A. crassus*. Although eels foraged on both fish and invertebrates in Loch Lomond, the incidence of infection with *A. crassus* was higher in eels that specialized on invertebrates as a food resource (>70% contribution to diet). In addition, the severity of infection (number of parasites in the swim bladder lumen) increased with the relative contribution of invertebrates in an individual's diet. The most likely explanation for observed patterns of parasite infection among eels sampled in Loch Lomond is trophic ecology, mediated through foraging behaviour and a potential 'encounter filter'. Eels are known to show individual specialization on available food resources (Lammens & Visser 1989; Proman & Reynolds 2000; Ide *et al.* 2011). The drivers behind ecological specialization and its consequences for individual fitness are poorly known. This study is among the first to demonstrate an effect of diet specialization on parasite loading by *A. crassus*.

Table 1 Parameter estimates for the minimum adequate model describing parasite intensity

Source	Estimate	SE	*Z* value	*P*
Intercept	2.7541	0.93593	2.943	–
Total length	−0.0474	0.02166	−2.192	<0.0001

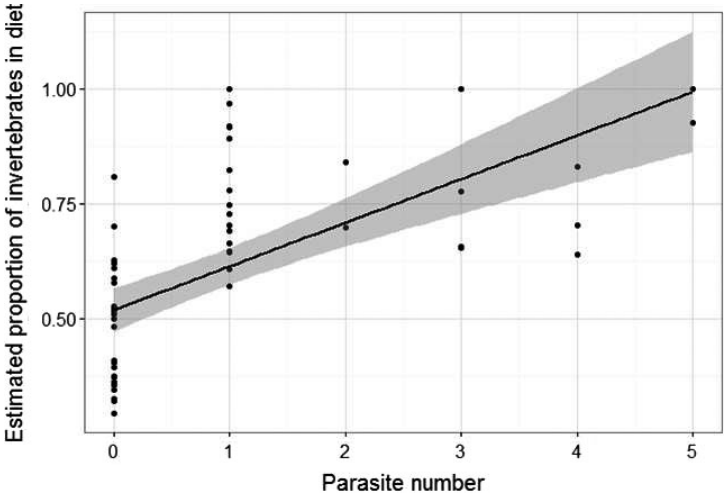

Figure 3 The relationship between estimated proportion of invertebrates in the diet and parasite number in swim bladder lumen. See text for significance.

Table 2 Parameter estimates for the minimum adequate model describing parasite prevalence

Source	Estimate	SE	*Z* value	*P*
Intercept	−9.814	2.621	−3.744	–
Proportion of invertebrates in diet	15.390	4.128	3.728	<0.0002

Table 3 Summary statistics of infected and uninfected eels

	Mean $\delta^{13}C \pm SD$	Mean $\delta^{13}N \pm SD$	Mean Length (mm) ± SD
Infected	−24.584 ± 1.03	11.53 ± 1.05	518 ± 95.9
Uninfected	−26.348 ± 1.58	9.25 ± 1.17	450.5 ± 75.3

The intraspecific variation in isotope ratios (variability in C and N) in eels from Loch Lomond suggests a level of dietary specialization on potential paratenic hosts as well as intermediate hosts. Exposure to parasites plays an important role in host vulnerability (Holmes 1990), with diet as the encounter filter and the main factor determining the number of parasites present in trophically transmitted species (Poulin 1995; Lafferty *et al.* 2008). In this study, smaller eels with a higher proportion of invertebrates in their diet had higher infection levels than larger eels with a greater proportion of fish in their diet. These findings contrast with those of Pegg *et al.* (2015) who reported an increased probability of infection in larger piscivorous eels. Parasite infections typically vary among size classes of their fish hosts, and differences in transmission rates often reflect ontogenic trophic niche shifts (Knudsen, Amundsen & Klemetsen 2003). In the literature, some authors have reported a decrease in the prevalence of *A. crassus* in the swim bladder lumen, with increasing eel length (Möller *et al.* 1991), while others have noted an increase (Thomas & Ollevier 1992). When *A. crassus* larvae are consumed by an intermediate host (i.e. a copepod), second-stage juveniles of *A. crassus* penetrate the digestive tract, enter the body cavity and moult into third-stage juveniles. Infected copepods move sluggishly and are more likely to occupy epibenthic zones in the water column than uninfected individuals (Kirk, Lewis & Kennedy 2000). Thus, we postulate that smaller and younger eels actively foraging in epibenthic zone on benthic invertebrates may have an increased chance of encounter, either directly through foraging on invertebrate paratenic hosts or feeding on infected copepods which may be occupying a similar epibenthic zone to invertebrate prey. Thus, the invertebrate prey of smaller eels may act as an important paratenic host for *A. crassus*. Larger eels in this study were found to feed predominately on ruffe which may also act as a paratenic host (Pietrock & Meinelt 2002); however, our infection rate results suggest that larger fish eaters may not be encountering infected intermediate and paratenic hosts as regularly as smaller invertebrate feeders.

The existing literature presents conflicting results on the relationship between trophic ecology and infection levels. For example, Morrissey & McCarthy (2007) reported lower *A. crassus* infection with increasing eel size in a marine environment, suggesting that larger eels typically do not feed directly on infected copepods and instead feed on larger crustaceans. In a naive host population of Lake Balaton, large eels (>100 g) acquired higher intensities of *A. crassus* than small eels (Barus & Prokes 1996). This relationship was interpreted as the result of greater consumption of infected intermediate and paratenic hosts by large eels. A recent study by Pegg *et al.* (2015) reported that eels from riverine sites, with a higher proportion of fish in their diet, had a higher probability of infection.

The disparity in observed field observations may be the result of trophic transmission dynamics which may be site specific. Thus, the trophic ecology of eels may act as a catalyst influencing encounter rate with *A. crassus* in certain systems and may also change as the parasite becomes more established in potential paratenic hosts within a system. A recent scenario posed by Lefebvre *et al.* (2013) is that the most active foragers (those consuming more intermediate/paratenic hosts) have an increased probability of infection. It may be the case that in Loch Lomond, the smaller eels feeding on invertebrates may be consuming more intermediate/paratenic hosts than larger fish eaters with invertebrate feeders consuming a higher diversity (feeding little and often) of prey and thus increasing their chance of infection. Our findings suggest that there is a link between infection and trophic parasite transmission in this host–parasite system. Seasonal variation in infection levels is an important factor to consider when examining prevalence and intensity in the definitive host. The majority of studies have failed to detect any seasonality in *A. crassus* prevalence (Kennedy &

Fitch 1990; Möller *et al.* 1991; Thomas & Ollevier 1992; Molnar 1994; Würtz *et al.* 1996). However, one long-term study noted a temporal pattern with maximum values of prevalence and mean intensity recorded each year in early summer and, to a lesser degree, in late winter (Lefebvre *et al.* 2002). The timing of this survey in early summer would coincide with peak prevalence noted in the study above. It is also important to note that prevalence of infected paratenic hosts (potential prey) of eels could change seasonally and thus the prevalence and infection rate found in this study could be higher or lower at other times of the year.

Conclusion

Given that this study is of a relatively early establishment of *A. crassus* into a system, it can be postulated that the observed patterns of prevalence and intensity may be temporally driven and will change as the parasite becomes more established at this site. Nonetheless, these results suggest that the individual hosts foraging behaviour can play an important role in the dispersal and establishment of *A. crassus* in this host–parasite system. Parasite infections have consequences on almost every aspect of fish behaviour. The behavioural consequences of *A. crassus* infection on eels are poorly understood during the continental stage (Lefebvre *et al.* 2012). Therefore, links in trophic transmission of *A. crassus* to host *A. anguilla* have significant importance. Factors relating to diet which may influence transmission of and repeat infections need to be investigated more robustly. The ability to identify potential mechanisms involved in the spread of *A. crassus* is imperative, given that conservation stocking has been identified as way of increasing numbers of eels (ICES, 2010). Trophic parasite transmission and the role of paratenic host warrant further research and should be considered when choosing appropriate stocking locations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Stomach contents of Uninfected (n = 8) and Infected (n = 12) individuals.

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