

Invasive swimbladder parasite *Anguillicoloides crassus*: infection status 15 years after discovery in wild populations of American eel *Anguilla rostrata*

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ABSTRACT: A year-round survey of American eels *Anguilla rostrata* was performed at 5 localities in South Carolina (SC), USA, 15 yr after the first infection by the nematode *Anguillicoloides crassus* was reported from Winyah Bay, SC. Infections by adult stages of *A. crassus* in the swimbladder lumen occurred with a prevalence of 45 % (n = 479), a mean intensity (\pm SE) of 2.3 ± 0.2 worms per infected eel (range = 1–22), and a mean abundance of 2.0 ± 0.1 among all eels. Infections by larval stages of *A. crassus* in the swimbladder wall occurred with a prevalence, intensity, and abundance of 29 %, 2.4 ± 0.3 (range = 1–15), and 0.7 ± 0.1 , respectively (n = 471). Overall prevalence of the parasite (any stage) was 58 %, with a mean intensity \pm SE of 3.0 ± 0.2 and a mean abundance of 1.8 ± 0.2 . Biomass of the adult parasite stage varied significantly with eel body length, but the direction of the effect depended on salinity. Prevalence and intensity of infection by adult nematodes varied by locality but not by eel total length, salinity, or season. Larval prevalence was significantly greater in the winter and spring and also differed among localities. The lack of seasonal effects on infection by the adult worm stage contrasts with studies from higher latitudes in North America and Europe and may be due to the warmer winter temperatures at southern latitudes. Significant variation in infection among localities reflects possible differences in abundance of intermediate and/or paratenic hosts. Overall, infection levels were higher than previous reports for eels in SC but comparable to more recent reports from other areas in North America.

KEY WORDS: *Anguillicoloides crassus* · Parasitic nematode · Parasite distribution · Swimbladder · American eel · Population dynamics · Seasonality · Latitudes

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INTRODUCTION

The nematode *Anguillicoloides crassus* (Kuwahara, Niimi and Itagaki, 1974) infects the swimbladder of eels belonging to the genus *Anguilla* Schrank, 1798. Although some doubt exists over its precise origin (Lefebvre et al. 2012), it is thought to be a natural parasite of the Japanese eel, *A. japonica* Temminck and Schlegel, 1846. Over the past 30 yr, *A. crassus* has been invasively spreading around the globe infecting other eel species in Europe (see reviews by

Moravec 2006, Székely et al. 2009), North Africa (El Hilali et al. 1996), South Africa (Sasal et al. 2008), western Asia (Genç et al. 2005), and North America (Fries et al. 1996, Barse et al. 2001, Machut & Limburg 2008, Fenske et al. 2010), probably due to commercial movement of live eels. These invasions are of growing concern because the parasite is pathogenic to newly acquired host species (Knopf & Mahnke 2004, Taraschewski 2006). Also, *A. crassus* is thought to spread rapidly within new host populations (Székely et al. 2009) due to its non-specificity for

intermediate and paratenic hosts, which in Europe include a wide range of copepods, at least 37 fish species, as well as amphibians, mollusks, and insect larvae (Haenen & van Banning 1991, Thomas & Olivier 1993, Székely 1994). Eels become infected by consuming these hosts, which carry the larval stages of *A. crassus*. Once ingested, the larvae migrate to the swimbladder wall where they grow. Larvae may be arrested here (Ashworth & Kennedy 1999) or they may migrate to the swimbladder lumen, where they develop into adults before copulating and ovipositing. Since *A. crassus* damages the swimbladder of the eel host (Molnár et al. 1993, Würtz et al. 1996, Lefebvre et al. 2002a), infection leads to reduced swimming performance of both yellow and silver eel stages, and compromises the ability of silver eels to migrate to the ocean to spawn (Münderle et al. 2004, Palstra et al. 2007, Sjöberg et al. 2009).

Anguillicoloides crassus may have contributed to global declines in eel populations in recent years. The presence of the parasite and its effects on eel populations have been studied extensively in the European eel *Anguilla anguilla* (L., 1758) (Székely et al. 2009). Fewer studies have been performed on its occurrence in the American eel *A. rostrata* (Lesueur, 1817), despite its presence in the wild population since at least 1995 (Fries et al. 1996). Determining the status of this parasite in North America is of importance due to worrisome widespread declines in the panmictic *A. rostrata* population (ASMFC 2012), which led to petitions to include the American eel under the US Endangered Species Act.

In this study, we investigated the status of *Anguillicoloides crassus* infection in the coastal South Carolina (SC), USA, *Anguilla rostrata* population, where the parasite was first reported in wild American eels (Fries et al. 1996) and where local eel populations have shown signs of decline since at least 2001 (ASMFC 2012). We examined the effects of seasonality, salinity, and locality on *A. crassus* population dynamics over a 1 yr period. Current prevalence was compared to 2 earlier studies by Fries et al. (1996) and Moser et al. (2001) to assess the status of colonization of *A. crassus* in SC.

MATERIALS AND METHODS

Study sites and sampling

American eels were collected every month from 4 estuaries along the coast and 1 freshwater site in SC from January 2011 through January 2012. The 4



Fig. 1. Five sampling sites in South Carolina, USA: ACE Basin, Cooper River, North Inlet, Winyah Bay, and Little Pee Dee River. Areas sampled are outlined in dark gray

estuaries sampled were the Ashepoo, Combahee, and Edisto (ACE) Basin (22 sites between 32.89° and 32.59° N), North Inlet (17 sites between 33.35° and 33.33° N), Winyah Bay (34 sites between 33.43° and 33.30° N), and the Cooper River (30 sites between 33.02° and 32.93° N; Fig. 1). The freshwater site was the Little Pee Dee (LPD) River (9 sites between 34.18° and 33.81° N), which is located farther inland in north-central SC and flows into Winyah Bay (Fig. 1). The LPD River was sampled during May, June, October, and November 2011 only. Eels were captured from a wide range of salinities (0–36; Table 1) using both electrofishing (at salinities ≤ 8) and trapping (at salinities > 8) with standard minnow traps baited with fish scraps. Abiotic data (water temperature, salinity, and dissolved oxygen) were collected at the time of sampling. Once collected, eels were placed on ice and returned to the laboratory to be processed within 24 h, or they were stored at –20°C until dissection.

Necropsy

The total length (TL, to the nearest mm) and eviscerated mass (to the nearest g) of each eel were recorded. The swimbladder was resected and all adult *Anguillicoloides crassus* were removed from the lumen, counted, and weighed to obtain the total parasite wet biomass (to the nearest mg) before being stored in 70% ethanol at room temperature. Each swimbladder was pressed between 2 glass slides, and the total number of *A. crassus* larvae in

Table 1. *Anguillicoloides crassus* infecting *Anguilla rostrata*. Parasite stage (total = adult and larval stages combined), number of eels examined (N), prevalence, mean (SE) intensity, and mean (SE) abundance of *A. crassus* from each locality sampled

Locality Salinity	Stage	N	Prevalence (%)	Intensity	Abundance
ACE Basin 0.0–9.1	Adult	68	38	1.4 (0.1)	0.5 (0.1)
	Larvae	67	22	2.7 (0.7)	0.6 (0.3)
	Total	67	48	2.4 (0.5)	1.1 (0.3)
North Inlet 3.7–36	Adult	54	48	3.3 (0.7)	1.6 (0.4)
	Larvae	54	44	3.7 (0.8)	1.6 (0.5)
	Total	54	67	4.9 (1.0)	3.2 (0.7)
Cooper River 0.5–9.9	Adult	114	51	3.0 (0.4)	1.5 (0.3)
	Larvae	109	40	2.4 (0.5)	1.0 (0.2)
	Total	109	64	4.0 (0.6)	2.6 (0.4)
Winyah Bay 0.3–12	Adult	142	46	2.2 (0.2)	1.0 (0.1)
	Larvae	140	31	2.1 (0.3)	0.7 (0.1)
	Total	140	63	2.7 (0.3)	1.7 (0.2)
Little Pee Dee River ^a 0.0	Adult	101	40	1.2 (0.1)	0.5 (0.1)
	Larvae	101	12	1.2 (0.1)	0.1 (0.04)
	Total	101	44	1.3 (0.1)	0.6 (0.1)

^aEels were sampled in May, June, October, and November only

the swimbladder wall was recorded. A subsample (across all eels examined) of adult worms (n = 52) was analyzed for gender identification, and females were determined to be gravid (presence of eggs with larvae in uterus) or non gravid (absence of eggs) according to Moravec & Taraschewski (1988).

Analysis

Prevalence (percent infected), mean intensity (number of parasites per infected individual), and mean abundance (number of parasites per individual observed) of adult and larval nematodes were calculated according to Bush et al. (1997). Mean values are expressed \pm SE. Samples were divided into seasons by temperature as follows: winter, January to March 2011; spring, April to June 2011; summer, July to September 2011; and fall, October 2011 to January 2012. The small number of January 2012 samples (n = 16) were included with the fall 2011 samples because there was an insufficient number of samples to create a second winter category, and because the water temperature was similar to fall due to an unusually warm winter that year. For completeness, all analyses were repeated excluding the January

2012 data, but no differences in results were found.

Logistic regressions (logit-link) were used to test the effects of locality and season (fixed factors), and eel TL and salinity (covariates) on parasite prevalence (separate analyses for adult, larvae, and both stages combined) and presence or absence of gravid female *Anguillicoloides crassus*. Generalized linear models (GLM) were used to test the same effects on parasite intensity (adult and larvae separately, gamma distribution, log-link) and log-transformed parasite biomass (total weight of adult nematodes per eel, Gaussian distribution, identity-link). All possible 2-way interactions were initially included in each of the models tested, with any non-significant terms ($p > 0.05$) then removed by backwards elimination. Since sampling in the LPD River only took place in May, June, October, and November, eels from there were only included in the final analyses if season was not a significant factor at the other localities.

Prevalence of *Anguillicoloides crassus* in the LPD River, a tributary of the Great Pee Dee River, was calculated for comparison (2-tailed Fisher's exact test) with a historical report from the Great Pee Dee River (Moser et al. 2001). Prevalence of adult *A. crassus* in Winyah Bay was compared (2-tailed Fisher's exact test) to similar data from Fries et al. (1996), which was the first report of *A. crassus* in wild American eels.

Ashworth & Kennedy (1999) found a positive relationship between larval abundance and adult abundance of *Anguillicoloides crassus* and suggested that this may occur due to a high density of adults arresting larval development within an eel host. We examined whether a similar relationship existed in our samples using a linear regression.

RESULTS

Environmental conditions and occurrences of eels

Minimum and maximum water temperatures of 6.5 and 33.2°C were recorded in January 2011 and August 2011, respectively (Fig. 2a). The lowest and highest salinities sampled were 0 and 36 (Fig. 2b). The ACE Basin, Winyah Bay, LPD River, and the Cooper

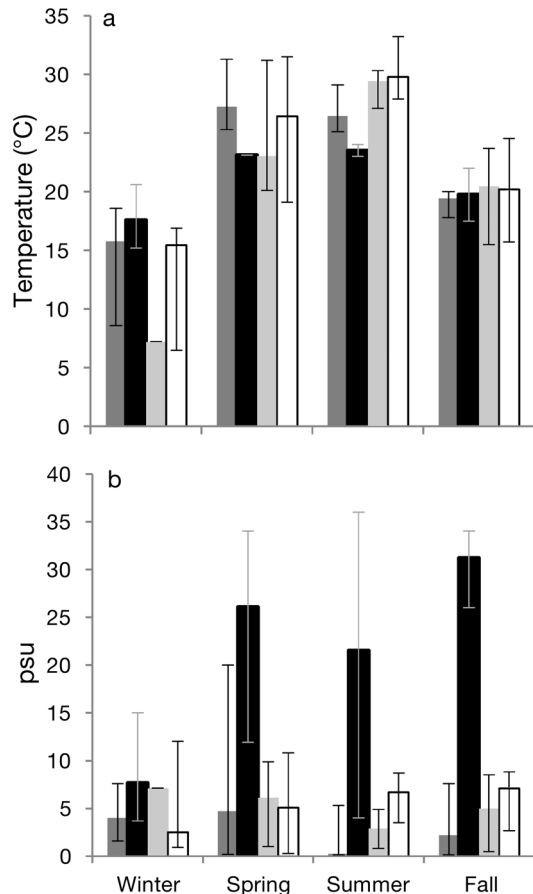


Fig. 2. Abiotic data from sampling sites. (a) Mean water temperature (°C), (b) mean salinity (psu). Water temperature was not measured during sampling in the Little Pee Dee River, and salinity there was always 0. Bars indicate the minimum and maximum values recorded. ACE Basin (dark gray), North Inlet (black), Cooper River (light gray), Winyah Bay (white). See Fig. 1 for site locations

River collection sites were all within main river channels. In contrast, North Inlet is a small ocean-dominated estuary with high salinity (maximum salinity = 36), and all collection sites were in narrow, shallow creeks and ditches. Salinity was significantly higher (1-way ANOVA, $p < 0.001$) in the North Inlet (mean = 18.5 ± 1.8) compared to all other sites (mean = 3.5 ± 0.2 , Fig. 2b). Of the 53 eels collected in North Inlet, only 16 came from salinities < 12 .

Overall infection status

A total of 479 eels ranging from 88 to 701 mm TL were collected. Across all seasons and locations combined, the prevalence of adult *Anguillicoloides crassus* was 45%, the mean intensity was 2.3 ± 0.2 (range 1–22), and the mean abundance was 2.0 ± 0.1

(Fig. 3a). Prevalence of larval *A. crassus* ($n = 471$ eels; 8 eels not screened) was 29%, with mean intensity of 2.4 ± 0.3 (range 1–15) and a mean abundance of 0.7 ± 0.1 (Fig. 3b). Prevalence of infection by any stage was 58% ($n = 471$) with a mean intensity of 3.0 ± 0.2 (range 1–36) and a mean abundance of 1.8 ± 0.2 (Fig. 3c). Prevalence of infection by adult and larval stages simultaneously was 17% ($n = 471$) with a

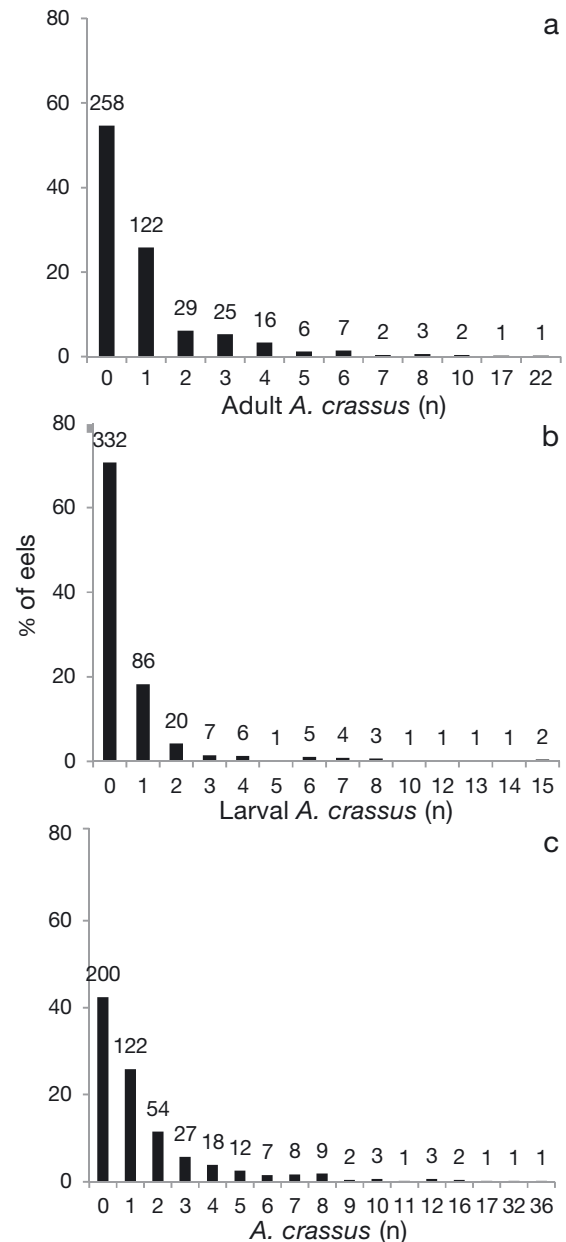


Fig. 3. *Anguillicoloides crassus* infecting *Anguilla rostrata*. Frequency distribution of *A. crassus* abundance per eel for (a) adult parasite stage (maximum = 22), (b) larval parasite stages (maximum = 15), (c) both stages combined (maximum = 36). Numbers above bars indicate the number of eels. Note that x-axes are non-linear

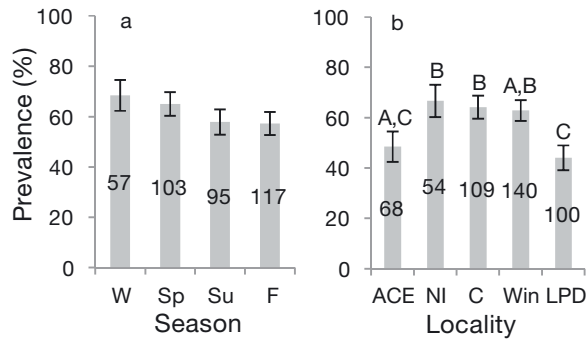


Fig. 4. *Anguillicoloides crassus* infecting *Anguilla rostrata*. Total prevalence (i.e. adult and larval stages combined) in relation to (a) season: winter (W), spring (Sp), summer (Su), and fall (F); and (b) locality: ACE Basin (ACE), North Inlet (NI), Cooper River (C), Winyah Bay (Win), and Little Pee Dee River (LPD). Error bars indicate SE. Numbers within bars indicate number of eels sampled. Groups that do not share a letter in (b) are statistically different from one another

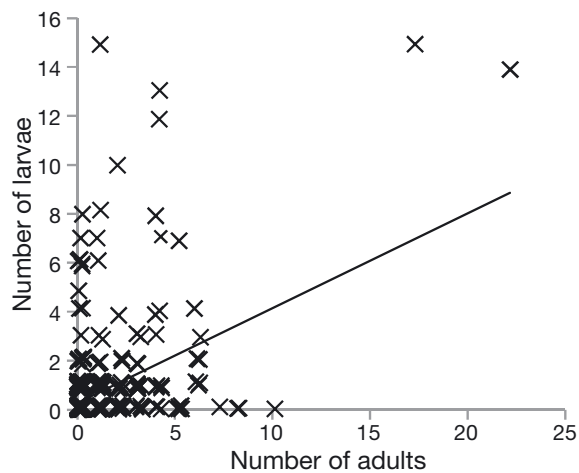


Fig. 5. *Anguillicoloides crassus* infecting *Anguilla rostrata*. Relationship between the number of adults in the swimbladder lumen and number of larvae in the swimbladder wall ($n = 471$). Note that data are jittered around values to avoid data overlap. Line equation: $y = 0.391x + 0.335$

mean intensity of 5.5 ± 0.6 . Prevalence, mean intensity, and mean abundance of adult and larval *A. crassus* for each locality sampled are provided in Table 1.

Prevalence of adult and larval stages combined

Total parasite prevalence (i.e. presence of adult, larval, or both parasite stages) varied significantly by locality (logistic regression, $p = 0.04$), but was not significantly affected by any of the other parameters tested ($p > 0.40$). Since season was not a significant factor (logistic regression, $p = 0.43$; Fig. 4a), samples

from the LPD River were added to the analysis. With this addition, locality remained significant ($p = 0.01$, Fig. 4b), whereas the other factors tested remained non-significant ($p > 0.57$). Post hoc tests indicated that prevalences in the ACE Basin and LPD River were significantly lower than in other localities (Fig. 4b, Table 1).

The number of larval parasites in the swimbladder wall increased significantly with number of adult parasites in the swimbladder lumen ($n = 471$, $p < 0.005$); however, adult number was generally a poor predictor of larval number ($r^2 = 0.16$, Fig. 5).

Infection by adult *Anguillicoloides crassus*

Initial analyses indicated that prevalence of adult parasites was not significantly affected by season ($p = 0.84$, Fig. 6b), and therefore the LPD River data were included for full analysis. This revealed a significant interaction between locality and TL ($p = 0.03$), with eels from the ACE Basin, Cooper River, North Inlet, and Winyah Bay having a flat or negative slope relating to adult parasite prevalence and TL, compared with eels from the LPD River, which had a positive slope (Fig. 7). Prevalence of adult *A. crassus* also varied significantly by locality ($p = 0.02$, Fig. 6a), with the lowest prevalence occurring in the ACE Basin. Prevalence of adult *A. crassus* did not vary significantly by salinity ($p = 0.57$).

Intensity of adult *Anguillicoloides crassus* varied significantly among localities (GLM, $p < 0.001$, Fig. 6c), but was not affected by any of the other parameters tested ($p > 0.49$; for season, see Fig. 6d). ACE Basin and LPD had the lowest mean intensities (1.4 ± 0.2 and 1.2 ± 0.1 , respectively), and North Inlet had the highest mean intensity (3.3 ± 0.7 ; Table 1, Fig. 6c).

Biomass of adult *Anguillicoloides crassus* was significantly affected by the interaction between salinity and TL ($p = 0.01$), but did not vary according to any other parameters tested ($p > 0.09$; Fig. 6e,f; LPD samples were included in analysis). Further investigation revealed that TL had a positive relationship with biomass at low salinities but a negative relationship at high salinities. However, when we repeated the analysis for each location separately, no significant interactions between salinity and TL were found.

Prevalence of gravid female *Anguillicoloides crassus* did not vary significantly with eel TL ($p = 0.46$), locality ($p = 0.71$), or season ($p = 0.86$). Prevalence of gravid females had a significant positive correlation with salinity ($p = 0.01$). Although the relationship was strengthened by the occurrence of a small number of

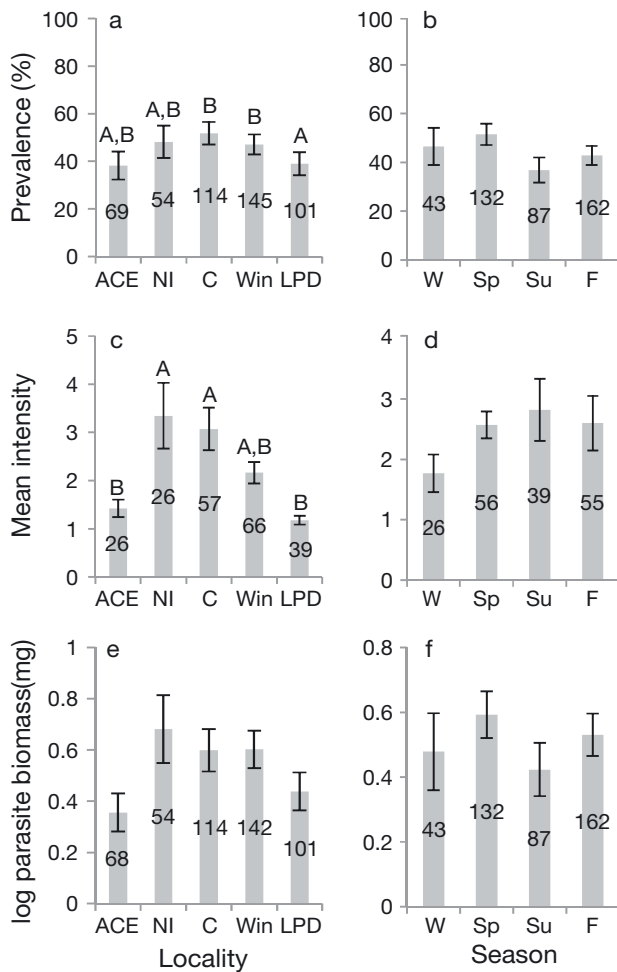


Fig. 6. *Anguillicoloides crassus* infecting *Anguilla rostrata*. Prevalence of adult nematodes in eels in relation to (a) locality and (b) season, mean intensity of adults in relation to (c) locality and (d) season, and mean log biomass of adults in relation to (e) locality and (f) season. Localities are ACE Basin (ACE), North Inlet (NI), Cooper River (C), Winyah Bay (Win), and Little Pee Dee River (LPD). Seasons: winter (W), spring (Sp), summer (Su), and fall (F). Error bars indicate SE. Numbers within columns indicate the sample size. Groups that do not share a letter are statistically different

gravid females collected at the high-salinity North Inlet site ($n = 5$), the relationship was still significant and positive ($p = 0.05$) when the North Inlet data were removed.

Infection by larval *Anguillicoloides crassus*

Initial analysis revealed that prevalence of larval *Anguillicoloides crassus* was significantly affected by locality ($p = 0.01$) and the interaction between salinity and locality ($p = 0.001$). However, because all eels captured from the highest salinities (>12) were col-

lected from North Inlet (Fig. 2b), it was not possible to distinguish whether the effect was driven by salinity or by location. With North Inlet samples removed from the analysis, the interaction between salinity and locality was no longer significant ($p > 0.38$), but locality alone ($p = 0.02$; Fig. 8a) and season ($p = 0.02$; Fig. 8b) were significant factors. Of the localities included in the final analysis, the Cooper River had the highest larval prevalence (40%) and the ACE Basin had the lowest larval prevalence (22%; Fig. 8a, Table 1). Prevalence of larvae was highest in the winter and spring and lowest in the summer and fall (Fig. 8b). Larval intensity was not affected by any of the terms tested ($p > 0.09$, Fig. 8c,d).

Comparison with historical infection

A total of 101 eels ranging from 160 to 629 mm TL were collected from the LPD River. Prevalence of infection by adult *Anguillicoloides crassus* in this system was 40% and was significantly higher (2-tailed Fisher's exact test, $p = 0.02$) than a 1998–1999 report of 25% prevalence ($n = 100$) in the Great Pee Dee River (Moser et al. 2001).

Comparison of prevalence of adult *Anguillicoloides crassus* in Winyah Bay between 1995 (Fries et al. 1996) and 2011 suggested that prevalence in 2011 was greater (46%; $n = 142$) than it was in 1995 (13%; $n = 8$; $p = 0.06$). To better compare the 2011 data with the small sample size from the 1995 study, prevalences for 2011 were calculated for 10 000 randomly re-selected groups of 8 individuals from the Winyah Bay dataset, targeting the same month sampled by Fries et al. (1996). This indicated that prevalence in 2011 was significantly greater than the 13% reported in 1995 ($p = 0.02$).

DISCUSSION

The prevalences of infection by adult (45%) and larval (29%) *Anguillicoloides crassus* in eels from SC estuarine habitats were comparable to those reported by Barse et al. (2001) in the Chesapeake area (46 and 40%, respectively). However, total parasite prevalence (all stages) was higher (58%) than those reported at other North American sites in New York (39%) (Machut & Limburg 2008) and the Chesapeake Bay (40.9%) (Fenske et al. 2010). Although it is not possible to determine when exactly *A. crassus* was introduced to SC, historical eel surveys can give a general time frame for its introduc-

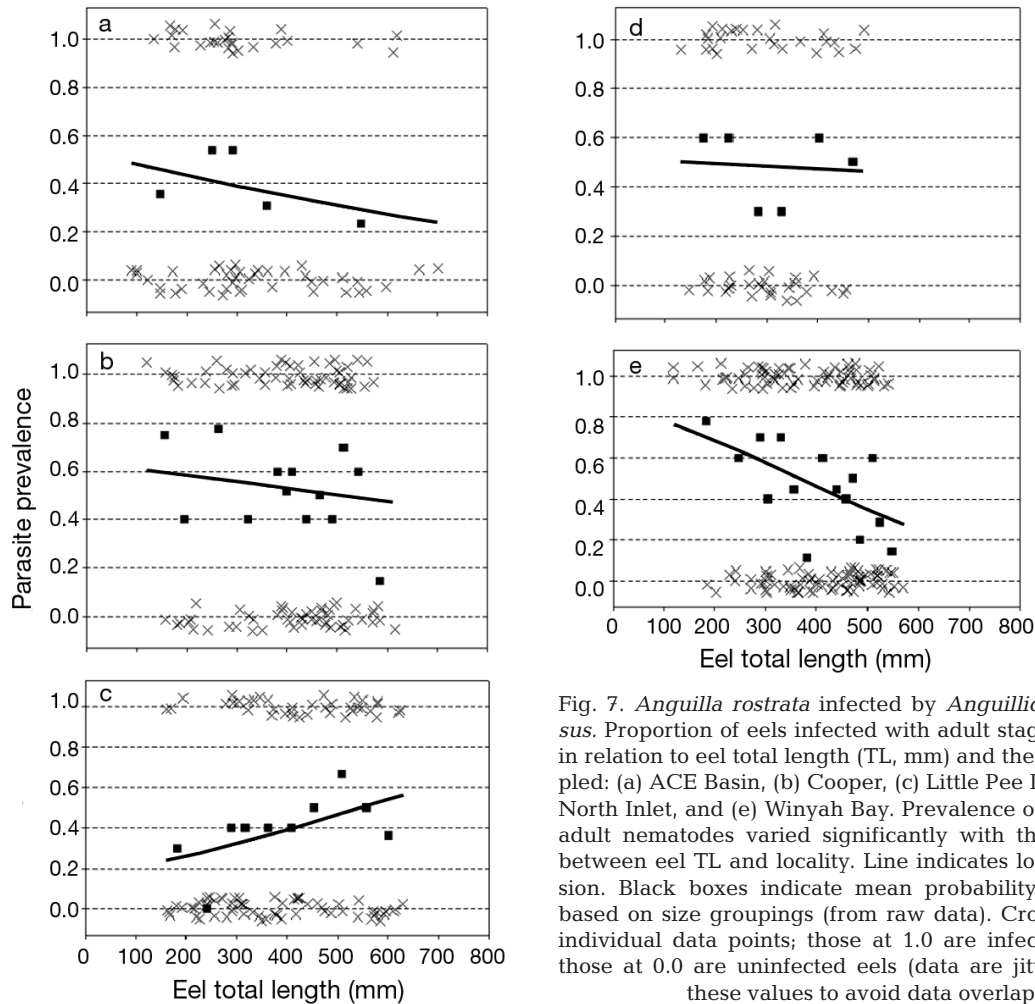


Fig. 7. *Anguilla rostrata* infected by *Anguillicoloides crassus*. Proportion of eels infected with adult stage nematodes in relation to eel total length (TL, mm) and the locality sampled: (a) ACE Basin, (b) Cooper, (c) Little Pee Dee River, (d) North Inlet, and (e) Winyah Bay. Prevalence of infection by adult nematodes varied significantly with the interaction between eel TL and locality. Line indicates logistic regression. Black boxes indicate mean probability of infection based on size groupings (from raw data). Crosses indicate individual data points; those at 1.0 are infected eels and those at 0.0 are uninfected eels (data are jittered around these values to avoid data overlap)

tion. No *A. crassus* were found during a 1977–1978 survey in the Cooper River (Hornberger et al. 1978), indicating that the parasite was introduced to this region between 1978 and 1995, when it was discovered in Winyah Bay (Fries et al. 1996). Comparisons of *A. crassus* prevalence between historical and current surveys showed a 15% increase in infection by adult *A. crassus* in the LPD River between 1999 (Moser et al. 2001) and 2011 (this study), although this small difference may be due to annual variability in infection; similarly, in Winyah Bay, prevalence of infection by adult *A. crassus* showed an apparent increase from 13% in 1995 (Fries et al. 1996) to 46% in 2011, although we acknowledge that the number of eels examined in the former study was very small ($n = 8$). These comparisons suggest a possible increasing trend in SC, but due to the small number of prevalence measurements (1995, 1999, and 2011), we can only speculate about what happened between 1995 and 2011 and cannot confidently address any patterns or trends.

Long-term studies in Europe on eels have shown rising trends in *Anguillicoloides crassus* prevalence (van Willingen & Dekker 1989, Kennedy & Fitch 1990, Lefebvre et al. 2002b, Audenaert et al. 2003), with the highest rate of increase occurring in the years soon after the introduction of the parasite. In some cases, this increase lasted over a decade and was followed by an eventual stabilization (van Willingen & Dekker 1989, Lefebvre et al. 2002b). In North America, the only multi-year comparison of *A. crassus* was carried out by Morrison & Secor (2003) in the Hudson River estuary, where prevalence increased from <20 to >60% from 1997 to 2000; it is not known whether prevalence of infection is still increasing or has stabilized there. In SC, our comparison between current and historic levels does not provide evidence of whether prevalence of infection by *A. crassus* has stabilized yet. Various mechanisms may contribute to the stabilization (or its delay), including host adaptation (Buchmann et al. 1991), density dependence (van Banning & Haenen 1990,

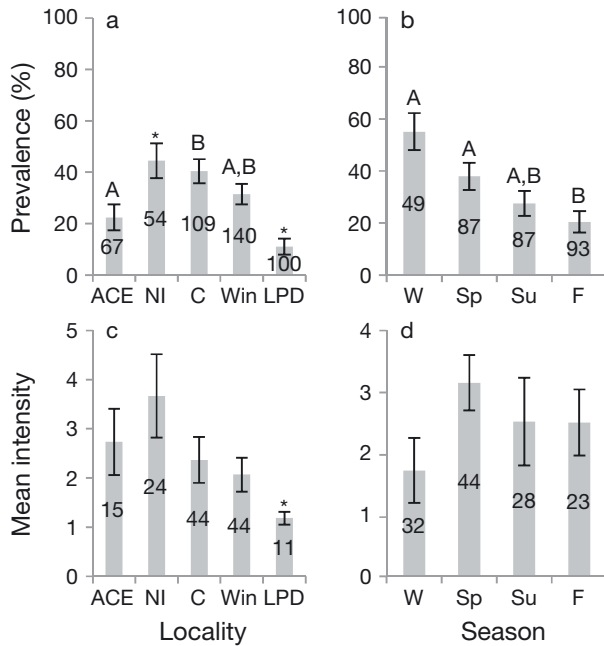


Fig. 8. *Anguillicoloides crassus* infecting *Anguilla rostrata*. Prevalence of nematode larvae in eels in relation to (a) locality and (b) season, and mean intensity of larvae (mean number of larvae per total number of infected eels) in relation to (c) locality and (d) season. Localities: ACE Basin (ACE), North Inlet (NI), Cooper River (C), Winyah Bay (Win), and Little Pee Dee River (LPD). Seasons: winter (W), spring (Sp), summer (Su), and fall (F). Error bars indicate SE. Numbers within columns indicate the sample size. Groups that do not share a letter are statistically different. Asterisks mark samples that were not included in the statistical analysis

Ashworth et al. 1996, Ashworth & Kennedy 1999, Lefebvre et al. 2002b), parasite-induced mortality of the host (Molnár et al. 1991, 1993, Baruš & Prokeš 1996), and presence and abundance of intermediate or paratenic hosts (Moravec 2006). To understand the role of such factors in these long-term trends, intermediate and paratenic hosts of *A. crassus* in North America need to be identified. Based on the history of European eel fate after *A. crassus* invasion, *Anguilla rostrata* could be at risk of mortality events, as documented by Molnár et al. (1991), or population declines (Van Banning & Haenen 1990, Molnár et al. 1993, 1994, Lefebvre et al. 2004) if the parasite's prevalence continues to rise.

Ashworth & Kennedy (1999) provided evidence that in European eels with high adult *Anguillicoloides crassus* subpopulations, the development of larvae appeared to be arrested and movement of larvae from the swimbladder wall to the lumen was inhibited in a density-dependent manner. We conducted a similar analysis to determine whether this trend also occurred in American eel and found a sig-

nificant positive relationship between the number of adult *A. crassus* in the swimbladder lumen and the number of larvae in the wall, indicative of density-dependent movement of larvae to the lumen. However, this correlation could simply be caused by a high density of *A. crassus* in the environment, resulting in high intensities of both stages of the parasite within the host. Without laboratory testing, we cannot confirm the density-dependent effects of *A. crassus* within American eel. If density-dependent effects do occur in American eel, this is one factor that supports eventual *A. crassus* stabilization in North America as seen in Europe (van Banning & Haenen 1990, Ashworth et al. 1996, Ashworth & Kennedy 1999, Lefebvre et al. 2002b).

Season had no significant effect on the prevalence or intensity of adult *Anguillicoloides crassus*, or on total (adult and/or larval) parasite prevalence. Larval parasite prevalence was, however, significantly higher in winter and declined through the rest of the year. The reproductive cycle of *A. crassus* slows down at colder temperatures (Kim et al. 1989, Nagasawa et al. 1994, Knopf et al. 1998, Székely et al. 2009), and seasonal patterns in infection have been reported at higher latitudes within North America (Fenske et al. 2010) and Europe (Lefebvre et al. 2002b). In contrast, both our study and that of Moser et al. (2001) in SC showed no seasonal patterns of infection by adult stages. Similarly, Neto et al. (2010) found dampened seasonality in *A. crassus* prevalence in low latitude (Portuguese) populations of European eel. In particular, the mild winter temperatures may have numerous consequences on the parasite life cycle, including continual development, increased survival of larvae, or increased reproductive success of adults (Knopf et al. 1998). The absence of seasonal fluctuation in the prevalence of gravid females and adult *A. crassus* biomass in our study support this latter idea. Also, the comparatively mild winter temperatures at lower latitudes may result in higher availability of intermediate hosts (likely small crustaceans: Kirk 2003, Székely et al. 2009) and higher consumption rates by eels (including potential paratenic hosts) during the winter, since eels cease feeding at extremely cold temperatures (Kennedy & Fitch 1990, Fukuda et al. 2009).

Unlike the prevalence of infection by adult *Anguillicoloides crassus*, prevalence of larvae varied significantly over the year, with the highest larval prevalence (55%) occurring in winter, and a decline occurring in summer (28%) and fall (26%). Lefebvre et al. (2002a) observed a similar summer decline in European eels with swimbladder damage, which is

known to be caused by *A. crassus* larvae (Molnár 1994, Würtz & Taraschewski 2000), and attributed it to eels not surviving harsh summer conditions. Molnár et al. (1991) also suggested that the combined stress of *A. crassus* infection and extreme environmental conditions, such as high water temperatures and low dissolved oxygen concentrations, explained a massive mortality event that occurred during summer months. Higher stress response and quicker mortality in infected eels compared to uninfected eels under hypoxic conditions was demonstrated experimentally (Molnár 1993, Gollock et al. 2005). Additionally, eels that had swimbladder damage were less tolerant of temperature and dissolved oxygen extremes than infected eels with no swimbladder damage (Lefebvre & Crivelli 2007). Thus, in SC, where water temperature during our sampling reached over 33°C (Fig. 2a) and dissolved oxygen levels fell as low as 2.9 mg l⁻¹, eels infected with *A. crassus* larvae may have been less likely to survive the stress during summers when conditions exceeded the lethal thresholds for infected eels of 27–28°C and dissolved oxygen values less than 3 mg l⁻¹ found by Molnár (1993). If mortality of larval-infected eels occurs during summer months, this would explain a concurrent decline in larval *A. crassus* prevalence among surviving eels. The seasonal variation in the prevalence of infection by larvae could also be a consequence of a seasonal variation in the presence of the yet unknown intermediate and paratenic hosts of *A. crassus* in our waters. Another explanation may be that larvae display seasonal movement from the swimbladder wall to the lumen as it is possible that they accumulate in the wall during the winter and migrate to the lumen as temperatures rise. However, we have no evidence for such behavior and if this were to happen, we would expect a seasonal increase in adult worms to occur, which our data do not show.

Neither prevalence nor mean intensity of the adult parasite varied significantly with salinity, matching the observation of Kirk et al. (2002) that adult *Anguillicoloides crassus* are able to survive a broad range of salinity conditions by osmoconforming with their host's blood. However, because we had restricted salinity ranges within localities (LPD salinity was 0; North Inlet had very high salinity), we were unable to determine whether the significant results for larval prevalence, adult parasite biomass, and prevalence of gravid females were actually caused by salinity itself or other habitat differences among localities. Other factors such as presence/absence of intermediate and paratenic hosts, eel density, anthropogenic

stressors, and water conditions that vary among localities may be more relevant factors. Martínez-Carrasco et al. (2011) explained a low prevalence of *A. crassus* in a hypersaline lagoon in Spain by the possible lack of intermediate hosts in the ecosystem, which had been identified as the copepod *Eurytemora affinis* Poppe, 1880 in surrounding areas. Additionally, Machut & Limburg (2008) saw higher *A. crassus* infection in urbanized watersheds and suggested that eels found in those areas were more susceptible to infection due to the anthropogenic stressors on their immune system. To better understand the effect of salinity on infection of *Anguilla rostrata* by *A. crassus*, future studies should cover sampling in freshwater and high salinities from multiple sites.

The effect of eel TL on the prevalence of adult *Anguillicoloides crassus* varied by locality. In eels from the coastal rivers and estuaries, there was a flat or negative effect of TL on prevalence of adult *A. crassus*, whereas eels from the inland LPD River showed a positive relationship between TL and prevalence of adult *A. crassus*. Infection may be expected to increase with eel TL because large eels are able to consume more infected intermediate and paratenic hosts, and because they provide a larger habitat for the parasites to establish (Möller et al. 1991, Thomas & Ollevier 1993, Molnár et al. 1994, Lefebvre et al. 2002b). The positive correlation between TL and infection that occurred in the LPD River, but not in the other systems, could be explained by differential diets of eels and availability of infected prey in these inland waters compared to estuarine environments or by the presence in freshwater, of healthier eels, which would be able to withstand the negative effects of the parasite. Identification and comparison of the intermediate and paratenic hosts in the 2 habitat types and assessment of swimbladder damage among freshwater and marine sites could help answer these questions.

Locality was a significant factor affecting the total parasite prevalence, adult parasite intensity, and larval parasite prevalence. In general, infection levels were lowest in the ACE Basin and the LPD River and were highest in the North Inlet. This may be related to biotope differences among the sampling sites. The North Inlet sampling sites consisted of shallow creeks and ditches and had high salinity levels (up to 36), whereas all other sampling localities were within main river channels and bays with relatively low salinity levels (0–12). The habitat at North Inlet is likely propitious to high densities of intermediate or paratenic hosts. Differences in biodiversity between the ACE Basin and the North Inlet have been re-

ported, with the ACE Basin having higher species richness (Smith 2012).

In conclusion, our data suggest that milder winters in the southern latitudes of the *Anguilla rostrata* range (including SC) may result in year-round infection by *Anguillicoloides crassus* and that more extreme summer conditions may induce host mortality. Hence, the effects of *A. crassus* infection on eel hosts probably differ across the species' range. This also suggests that infections by *A. crassus* may become a greater threat in more northern waters with global climate change and warming coastal water temperatures (NOAA 2012). Availability of intermediate and paratenic hosts likely plays a role in the establishment of infection hot spots and could explain variability among localities, which outlines the importance of identifying such hosts in North America.

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