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10-2019

Temporal, spatial, and biological variation of nematode epidemiology in American eels

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Warshafsky, ZT; Tuckey, Troy D.; Vogelbein, WK; Latour, RJ; and Wargo, AR, "Temporal, spatial, and biological variation of nematode epidemiology in American eels" (2019). *VIMS Articles*. 1780.
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Canadian Journal of Fisheries and Aquatic Sciences

TEMPORAL, SPATIAL, AND BIOLOGICAL VARIATION OF NEMATODE EPIDEMIOLOGY IN AMERICAN EELS

Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2018-0136.R2
Manuscript Type:	Article
Date Submitted by the Author:	17-Nov-2018
Complete List of Authors:	Warshafsky, Zoemma; Virginia Institute of Marine Science, Aquatic Health Sciences Tuckey, Troy; Virginia Institute of Marine Science Vogelbein, Wolfgang; Virginia Institute of Marine Science Latour, Robert; Virginia Institute of Marine Science, Fisheries Science Wargo, Andrew; Virginia Institute of Marine Science, Aquatic Health Sciences
Keyword:	EPIDEMIOLOGY < General, PARASITES < Organisms, ESTUARIES < Environment/Habitat, CATADROMOUS SPECIES < Organisms
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

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Manuscripts

1 TEMPORAL, SPATIAL, AND BIOLOGICAL VARIATION OF NEMATODE
2 EPIDEMIOLOGY IN AMERICAN EELS

3
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11 **ABSTRACT**

12
13 American eels (*Anguilla rostrata*) are infected by the non-native parasitic nematode
14 *Anguillicoloides crassus*, which can cause severe swimbladder damage. We investigated
15 epidemiology of *A. crassus* to better understand its population-level effects on American eels.
16 Nematode prevalence, abundance, and intensity, and swimbladder damage were quantified in
17 glass eels, elvers, and yellow eels from the lower Chesapeake Bay and related to season of
18 capture, river system, and total length. Age-variant force-of-infection and disease-associated
19 mortality were estimated using a three-state irreversible disease model, which assumes recovery
20 is not possible. Results showed glass eels have very low infection prevalence and severity
21 compared to elvers and yellow eels. Nematode abundance varied by season, river, and eel length,
22 whereas swimbladder damage varied by season and eel length. Nematode abundance and
23 swimbladder damage were weakly positively correlated. Force-of-infection, based on
24 swimbladder damage, peaked at age 2 and disease positive eels had an estimated lower annual
25 survival probability of 0.76 compared to disease negative eels. Full understanding of American
26 eel population dynamics will require broader knowledge of cryptic disease-associated mortality
27 throughout North America.

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40 INTRODUCTION

41

42 The American eel (*Anguilla rostrata*) is an economically and ecologically important, yet
43 relatively data-poor, species distributed along the Atlantic coast of North America and
44 throughout the Gulf of Mexico (ASMFC 2012). The American eel population has been declining
45 for the past several decades and is currently considered depleted and at a historically low level of
46 abundance according to the most recent stock assessment update by the Atlantic States Marine
47 Fisheries Commission (ASMFC 2017). Several hypotheses have been proposed to explain the
48 species' decline such as overfishing, pollution, changing climate, altered habitats and food webs,
49 parasites, and emergent disease (Castonguay et al. 1994; Haro et al. 2000). One such proposed
50 hypothesis is the impact of the introduced parasitic nematode, *Anguillicoloides crassus*, which
51 can cause severe damage to the swimbladders of American eels.

52 Infection by *Anguillicoloides crassus* is endemic in the Japanese eel (*Anguilla japonica*)
53 in Asia, but significant pathology or notable negative population level impacts have not been
54 observed (Sokolowski and Dove 2006). In contrast, the emergence, rapid spread, high
55 prevalence, and pathogenicity of *A. crassus* have been linked to declines in wild European eel
56 (*Anguilla anguilla*) populations and in European eel aquaculture facilities in Asia (Barse et al.
57 2001; Ooi et al. 1996). Within the American eel population, the parasite was first discovered in
58 1995 in a Texas aquaculture facility and was first observed in wild animals in South Carolina
59 that same year (Fries et al. 1996). Since its discovery, the distribution of *A. crassus* has expanded
60 rapidly and now occurs in eels in the Gulf of Mexico northward to Nova Scotia (Rockwell et al.
61 2009). In the Chesapeake Bay, where approximately 60% of the annual U.S. catch of eels is
62 landed (ASMFC 2017), *A. crassus* was first detected in 1997 (Barse and Secor 1999) and

63 currently can be found in all major tributaries with prevalences as high as 80% (Barse et al.
64 2001; Fenske et al. 2010).

65 Eels become infected by consuming intermediate hosts such as copepods and ostrocods
66 or by ingesting paratenic hosts (i.e., intermediate hosts in which parasite development does not
67 occur) such as fishes, amphibians, snails, or insect larvae (Thomas and Ollevier 1992; Moravec
68 1996; Moravec and Skorikova 1998). Once inside the eel, the parasite moves from the alimentary
69 tract through the body cavity, eventually taking up residence in the swimbladder where it
70 matures, sexually reproduces, and then dies and decays or is forced out through the pneumatic
71 duct (Haenen et al. 1989; De Charleroy et al. 1990). Damage to the eel occurs as a result of
72 larval nematode migration through the swimbladder wall, feeding on blood by adults, and
73 inflammation and degradation of dead adults within the swimbladder lumen (Sokolowski and
74 Dove 2006). Damage clinically manifests as increased opacity, thickening, and pigmentation of
75 the swimbladder wall (Lefevbre et al. 2011). Tissue damage by *A. crassus* can be so severe that it
76 results in complete degradation and loss of swimbladder function (Molnar et al. 1995; Wurtz et
77 al. 1996; Kobayashi et al. 1990). However, the adverse health impacts of this infection and its
78 impacts on eel population dynamics are not well understood, in part due to a complex
79 relationship between parasite abundance and degree of swimbladder damage, suggesting that
80 severe damage may prevent subsequent infections (Lefevbre et al. 2013).

81 Previous studies addressing the impacts of *A. crassus* have mainly focused on infection
82 prevalence and mean intensity (Fenske et al. 2010; Aieta and Oliveria 2009; Hein et al. 2014),
83 but these metrics may provide an inaccurate or incomplete characterization of the multifaceted
84 epidemiology. Potential discrepancies in the relationship between parasite abundance and
85 swimbladder damage may be caused by intermediate and paratenic host dynamics, *A. crassus*

86 dying within the swimbladder and decaying or being cleared, and the possibility that a highly
87 damaged swimbladder may not serve as a suitable habitat for *A. crassus* (De Charleroy et al.
88 1990; van Banning and Haenen 1990). Additionally, previous analyses have focused mainly on
89 yellow eels and have not included younger life stages, despite potential susceptibility of those
90 stages to infection (Hein et al. 2015; De Charelory et al. 1990). Finally, it is not known if *A.*
91 *crassus* causes infection-associated mortality (Lefebvre et al. 2013), which greatly limits
92 understanding of the population level impacts of this parasite in American eels.

93 The objectives of our study were to (1) quantify prevalence, abundance, and intensity of
94 *Anguillicoloides crassus* infections in glass, elver, and yellow stage American eels in the
95 Chesapeake Bay; (2) clarify the relationships between parasite abundance and swimbladder
96 damage and the impacts of capture location, eel size, and season on the infection; and (3) model
97 *A. crassus* force of infection in Chesapeake Bay, including exploration of spatiotemporal
98 covariate effects, and evaluate disease-associated mortality. Collectively, our results support
99 ongoing American eel management efforts by addressing key questions related to patterns in
100 disease prevalence and population impacts of *A. crassus*.

101

102 **METHODS**

103

104 **Field collections**

105 Glass and elver stage American eels were collected from six sites within the lower
106 Chesapeake Bay from March through June 2015 using Irish eel ramps (Figure 1). Traps were
107 placed in areas of freshwater runoff and a dam that impeded the eels' upstream movements. The
108 six sites were on the James River (Wareham's Pond), York River (Bracken's Pond and Wormley

109 Pond), Rappahannock River (Kamp's Millpond), and Potomac River (Clark's Millpond and
110 Gardy's Millpond). Sampling was conducted according to protocols established by the ASMFC
111 for monitoring young-of-year glass eels (ASMFC 2012). Traps were checked a minimum of two
112 days per week, with increasing frequency depending on the strength of glass eel ingress. On the
113 first sampling day of each week, a maximum of 30 glass eels and 20 elvers were collected if
114 possible, followed by up to 10 glass eels and 5 elvers each subsequent sampling day depending
115 on their availability. This sampling technique was designed to optimize collecting enough eels to
116 detect the nematode at low prevalence and reducing potential sampling biases such as
117 autocorrelation within a catch (i.e., pseudoreplication associated with cluster sampling).
118 Differentiation between glass eels and elvers was based on pigmentation stage (Haro and Kruger
119 1988), with fully pigmented eels considered elvers, and the incompletely pigmented eels
120 considered glass eels.

121 Yellow stage American eels were collected from 2013 to 2015 by the VIMS Seine
122 Survey and Juvenile Fish Trawl Survey (Tuckey and Fabrizio 2013) and opportunistically from
123 the Virginia Department of Game and Inland Fisheries Electrofishing Survey. The Trawl and
124 Seine Surveys sampled eels in primarily brackish and tidal fresh water, whereas the
125 Electrofishing Survey sampled eels in mainly freshwater locations. Yellow eels were collected
126 within the James, York, and Rappahannock River watersheds from all three surveys, whereas
127 yellow eels in the Potomac River watershed were collected exclusively by electrofishing.

128

129 **Laboratory processing**

130 Weights (nearest 0.001 g) and lengths (nearest 0.01 mm total length) were obtained from
131 glass eels and elvers that were euthanized using clove oil, measured, and then frozen (-18°C) for

132 storage. Yellow eels were euthanized using an ice slurry, frozen immediately, and weighed
133 (nearest 0.1 g) and measured (nearest 1 mm) following thawing just prior to dissection. For all
134 eels, the swimbladder was removed after thawing and opened to enumerate adult *A. crassus*.
135 Counts of larval *A. crassus* in the swimbladder wall were quantified for glass eels under a
136 dissecting microscope, after placing the swimbladder between two glass slides. Only adult *A.*
137 *crassus* parasites were recorded for elvers and yellow eels. Swimbladder condition was
138 quantified using the Swimbladder Degenerative Index (SDI; Lefebvre et al. 2002). This index
139 quantifies three swimbladder attributes—bladder wall opacity, thickness, and pigmentation and
140 exudate (e.g. dead worms, erythrocytes, decaying swimbladder tissue, eggs, and L2 stage of *A.*
141 *crassus*; Lefebvre et al. 2002) with each of these ranked from 0 (healthy, normal condition) to 2
142 (severe condition). The three attribute scores were added together to generate a total SDI ranging
143 from 0 to 6. Due to the difficulty of detecting eggs and L2 stage *A. crassus* in swimbladders,
144 these two types of exudate were not used to determine the presence of exudate in elvers and
145 yellow eels, but were used in glass eel analyses.

146 Sagittal otoliths were extracted from elvers and yellow eels and processed for age
147 determination (Michaud et al. 1988; Cieri and McCleave 2000; Morrison and Secor 2003).
148 Otoliths were mounted on a glass slide with CrystalBond™ and sanded down in the frontal plane
149 until the core was visible. The otolith was then flipped and the opposite end was sanded down
150 until the otolith was transparent and annuli were clearly visible. Annuli were quantified using a
151 compound microscope. Each otolith was read independently by two readers and those specimens
152 with annulus counts that differed were read again. Final age assignments were based on
153 consensus between readers. Because eels have been in the Atlantic Ocean for about one year
154 prior to metamorphosing into glass eels and the first annulus may not be laid down until after a

155 full year in coastal waters (ASMFC 2017), one year was added to all ages. Protocols for
156 sampling and euthanizing eels were approved by the College of William & Mary's Institutional
157 Animal Care and Use Committee.

158

159 **Statistical analyses**

160 Infection prevalence (proportion of eels infected with nematodes), mean abundance
161 (average number of nematodes across all eels surveyed, infected and uninfected), and mean
162 infection intensity (average number of nematodes per infected eel) were calculated from all eel
163 stages and river systems using adult *A. crassus* counts (Bush et al. 1997). Glass eel analysis
164 included both the adult and larval stages of the parasite. For all following analyses, a plausible
165 set of candidate model parameterizations was defined based on hypotheses regarding potential
166 effects of covariates (i.e., the covariates included in models have been shown to be important in
167 other studies or represent plausible hypotheses unique to this study). Final models were selected
168 using a combination of goodness of fit measures and Akaike's Information Criterion value (AIC;
169 Burnham and Anderson 2002).

170

171 *Parasite abundance*

172 For glass eels, the probability of infection by *A. crassus* larval and adult stages combined
173 in relation to glass eel total length (TL) was investigated using a binomial generalized linear
174 model with a logit link function. The inflection point, or the TL at which the probability of being
175 infected is 0.5, of the binomial model was calculated and its standard error was estimated using
176 the delta-method (Seber 1982). Glass eels were excluded from subsequent analyses due to low
177 prevalence and infection intensity.

178 For elvers and yellow eels, preliminary analyses comparing AIC values of the fully
 179 saturated model (all covariates in both count and zero model components) to the fully saturated
 180 count model that includes only the intercept in the zero component indicated that parasite count
 181 data were zero-inflated (i.e., an excess frequency of zeros in the dataset; $\Delta AIC = 74.6$).
 182 Therefore zero-inflated negative binomial models were used to explore the effects of covariates
 183 on parasite abundance and the probability of a false zero, or the absence of parasites due to
 184 design, survey, or observation error (Zuur et al. 2012). The covariates included river system
 185 (James, York, Rappahannock, Potomac), season (created by assigning the date of capture into the
 186 four seasons based on the solstices and equinoxes of that year), SDI, and TL. Multiple model
 187 parameterizations were considered that reflected different combinations of covariates for the
 188 count and false zero model components. The dispersion parameter ($d = \sum \frac{residual^2}{df(residual)}$), which
 189 indicates overdispersion when $d > 1$, and the Pearson residual versus fitted value plots were used
 190 to evaluate goodness of fit of the models. Partial predictions from the most empirically supported
 191 model were generated using marginal means (Searle et al. 1980).

192

193 *SDI*

194 The swim bladder degenerative index (SDI) is an ordered categorical response variable,
 195 requiring a specific regression framework to capture the sequential nature of the data. The
 196 ordinal logistic regression (or cumulative logit model) meets this requirement by modelling the
 197 probability of an eel having a certain level of swimbladder damage (i.e., SDI score) or lower
 198 against all higher levels (Agresti 2010; Hedeker 2003; McCullagh 1980) such that:

$$199 \quad \text{logit}(P(Y_{ij} \leq c)) = \theta_c - (\mathbf{x}_{ij}^t \boldsymbol{\beta} + \mathbf{u}_{ij}^t \boldsymbol{\alpha}_c + v_i) \quad (1)$$

200 The cumulative $\text{logit}(P(Y_{ij} \leq c))$ represents the cumulative probability of the j^{th} eel from the i^{th}
201 catch having a c^{th} level of swimbladder damage. The parameter θ_c represents strictly increasing
202 model thresholds (1, ..., $C - 1$). The covariate vector \mathbf{x}_{ij} , which includes the intercept, follows
203 the proportional odds assumption such that there is only one vector of regression parameters $\boldsymbol{\beta}$
204 for each covariate. Covariate vector \mathbf{u}_{ij} follows partial proportional odds such that there is a
205 different vector of regression parameters $\boldsymbol{\alpha}_c$ for every level of c for each covariate (Agresti 2010;
206 Hedeker 2003; McCullagh 1980). The parameter v_i is the random effect for catch i distributed
207 $N(0, \sigma_v^2)$. Odds ratios ($Y \geq j$) are obtained through $\exp(\boldsymbol{\beta})$, and indicate the odds ratio of a
208 swimbladder having a damage level c or higher for a given level of a covariate (Agresti 2010;
209 Hedeker 2003; McCullagh 1980).

210 The proportional odds assumption (or parallel regression assumption), states that the odds
211 ratios of the different levels of a covariate are equal across all thresholds of swimbladder damage
212 levels, necessitating only one $\boldsymbol{\beta}$ coefficient. For example, for a given covariate (e.g. season), the
213 ratio of the odds of having a certain swimbladder damage level in one season (e.g. spring) to the
214 odds of having the same swimbladder condition in another season (e.g. winter) are equal across
215 all thresholds of swimbladder damage levels. This does not mean that the odds of swimbladder
216 damage within one level of a covariate are equal, but instead that the odds ratios of two levels of
217 a covariate are equal (i.e. proportional odds). This assumption can be relaxed by utilizing partial
218 proportional odds, which allows coefficient $\boldsymbol{\alpha}_c$ to vary with thresholds of SDI scores and
219 therefore not have equal odds ratios between levels of a covariate.

220 This approach was used to explore the effects of covariates river system, TL, season,
221 parasite abundance, and catch ID (random effect) on the odds of having attained greater than or
222 equal to the c^{th} level of swimbladder damage. Due to low sample size of higher parasite

223 intensities, a plus group was defined such that intensities ranged from zero to 10+. To aid
224 convergence and model interpretation, SDI scores were condensed into three ordered levels (low:
225 0-1, moderate: 2-3, severe: 4-6, such that $c = 0, 1, 2$). The proportional odds assumption was
226 evaluated by fitting multiple models with and without covariates as partial proportional odds, and
227 AIC was used for model selection. Overall goodness of fit was evaluated using the condition
228 number of the Hessian, for which values larger than 10^6 indicate that the model may be ill
229 defined (Christensen 2015). Partial predictions from the model with the most empirical support
230 were generated using marginal means (Searle et al. 1980).

231

232 *Epidemiology*

233 To estimate the probability that an uninfected eel becomes infected, termed force-of-
234 infection (FOI), and evaluate the potential presence of infection-associated mortality, we applied
235 a three-state irreversible disease model (see Heisey et al. 2006 for full details). The model is
236 designed to provide estimates of key epidemiological parameters from cross-sectional (i.e. data
237 representing multiples ages or cohorts) from binary prevalence-at-age data (i.e. either disease
238 positive or disease negative at a given age). The model assumes no vertical transmission (i.e.,
239 transmission from mother to offspring) or recovery to a state of full health. Force-of-infection
240 can be either age-invariant or age-dependent, and the Weibull, Pareto, Gompertz, and log-logistic
241 hazard functions were evaluated to identify the appropriate functional shape of the FOI curve
242 (Heisey et al. 2006). The model also allows for estimation of an additive disease-associated
243 mortality parameter that represents the additional mortality rate experienced by disease-positive
244 individuals relative to the background mortality rate of disease-negative individuals.

245 The effects of covariates month, season, and river system (all categorical) on force-of-
246 infection were investigated using log-linear models such that:

$$247 \lambda(t) = \lambda_0(t)e^{X\beta}. \quad (2)$$

248 The age-dependent FOI, denoted $\lambda(t)$, is modeled as the baseline FOI, λ_0 adjusted by the effects
249 of covariates included in the design matrix X and the associated vector of parameters β (Heisey et
250 al. 2006). The covariate month was redefined to represent two-month time periods (six levels)
251 starting with January/February. Due to low sample size of older eels, a plus group was defined so
252 that ages ranged from 1 to 12+. Disease-positive eels were those that had a swimbladder with an
253 SDI score ≥ 3 . Because *A. crassus* can die within the swimbladder and degrade or be expelled
254 through the pneumatic duct, prevalence does not meet the no-recovery assumption of the force of
255 infection model (i.e., damage to the swimbladder has occurred, but an absence of parasites does
256 not accurately capture the damage). As such, force-of-infection modelling was not conducted on
257 infection prevalence data.

258 All statistical analyses were performed using the R software package (R Core Team
259 2014). The 'pscl' package was used for fitting zero-inflated GLMs (Zeileis et al. 2008) and the
260 'ordinal' package (Christensen 2015) was used for fitting ordinal logistic regression models.
261 Results are presented as the mean or estimate \pm standard error.

262

263

264 **RESULTS**

265 ***Anguillicoloides crassus* infection and disease in glass eels**

266 A total of 1480 glass eels were collected from all six sites ranging in total length from 47.3 to
267 77.5 mm (mean: 57.6 mm \pm 0.103). Adult and larval nematode counts in glass eels were

268 combined and yielded an overall infection prevalence of 3.2%, mean nematode abundance per
269 eel of 0.047 ± 0.009 (range: 0-10), and mean infection intensity of 1.46 ± 0.195 . Only glass eels
270 collected in the Potomac and Rappahannock rivers were infected (Table 1). Glass eels had higher
271 infection levels of the larval stage of *A. crassus* (prevalence: 2.5%, mean abundance: $0.039 \pm$
272 0.009 , mean intensity: 1.57 ± 0.25 , range: 0-10) compared to the adult stage (prevalence: 0.8%,
273 mean abundance: 0.008 ± 0.002 , mean intensity: 1 ± 0 , range: 0-1). The probability of infection
274 by larval and adult nematodes combined increased with the length of glass eels (Figure 2). The
275 TL with a 0.5 probability of being infected was 78.3 ± 2.8 mm. Furthermore, only the more
276 advanced pigment stages of glass eels were found to be infected with larval and adult *A. crassus*,
277 and only the highest pigment stages of 5 and 6 showed any swimbladder damage. Overall,
278 minimal swimbladder damage was found with only seven glass eels having scores greater than 0
279 (mean SDI: 1.14 ± 0.143).

280

281 ***A. crassus* infection and disease in elver and yellow eels**

282 Across all six sampling sites, a total of 814 elvers were collected and total length ranged
283 from 49.0 to 238.0 mm (mean: 113.8 mm ± 1.02). Adult nematode prevalence was 59.2%, mean
284 abundance per eel was 1.51 ± 0.061 , mean intensity per infected eel was 2.44 ± 0.072 , and
285 average SDI was 1.62 ± 0.055 . When summarized by river system, James River elvers showed
286 the highest prevalence (66.7%), average abundance (1.83 ± 0.13), mean intensity (2.73 ± 0.13),
287 and average SDI (1.82 ± 0.11); however the difference in mean infection and disease levels
288 between sites was small (Table 1). There was substantial variation between individual elvers
289 such that infection intensity ranged from 0 to 10 nematodes and the full range of SDI scores (0-
290 6) was observed.

291 A total of 973 yellow eels were collected across all four river systems and all three
292 surveys. Total length of these individuals ranged from 60.0 to 700.0 mm (mean: 285.9 mm \pm
293 3.71). Adult nematode prevalence was 46.2%, mean abundance per eel was 1.35 ± 0.079 , mean
294 intensity per infected eel was 2.92 ± 0.136 , and average SDI was 2.44 ± 0.055 . Yellow eels from
295 the Potomac River showed the highest prevalence (55.2%), whereas those from the
296 Rappahannock River exhibited the highest mean abundance (1.53 ± 0.13) and mean intensity
297 (3.09 ± 0.22). Those from the James River displayed the highest average SDI (2.3 ± 0.08),
298 although mean differences between sites was again small (Table 1). As with elvers, there was
299 substantial variation among individuals in infection intensity and disease, such that infection
300 intensity in yellow eels ranged from 0 to 28 nematodes and the full range of SDI scores (0-6) was
301 observed.

303 **Parasite abundance**

304 The full zero-inflated negative binomial with river system, season, TL, and SDI as
305 covariates for both the zero-inflated and count components of the model received the most
306 empirical support (i.e., lowest AIC score; Table S1), had a dispersion parameter value close to
307 one (1.16), and the Pearson residual versus fitted value plot showed this model fit the data well.
308 This model was closely followed ($\Delta AIC < 2$, dispersion = 1.15) by a model without SDI in the
309 zero-inflated component. Due to the importance of SDI in the analysis and the lower AIC, the
310 full model was selected for inference.

311 When comparing parasite abundance (Table 2) using the count model, the estimated
312 effect for the Potomac River indicated lower mean parasite abundance relative to the James
313 River (-0.452 ± 0.112), whereas the estimated effects of the Rappahannock and York Rivers also

314 indicated lower mean parasite, though much smaller in magnitude (-0.135 ± 0.103 and $-0.015 \pm$
315 0.103 respectively, Figure 3). Regarding season, the estimated effects of spring and summer
316 indicated similar higher mean parasite abundances relative to fall (0.294 ± 0.130 and $0.224 \pm$
317 0.138 respectively), whereas winter showed a lower mean parasite abundance (-0.149 ± 0.316 ,
318 Figure 3). The estimated effect of TL indicated a positive relationship to parasite abundance
319 ($1.37 \times 10^{-3} \pm 4.42 \times 10^{-4}$). However, due to zero-inflation, the relationship between TL and
320 predicted parasite count was dome-shaped; the exception being both the Potomac River across
321 all seasons and winter across all river systems where the relationship was continuously
322 increasing due to very low zero-inflation (Figure 3). For SDI, parasite abundance was higher for
323 all scores compared to the baseline (SDI 1: 0.279 ± 0.124 , SDI 2: 0.512 ± 0.116 , SDI 3: $0.295 \pm$
324 0.131 , SDI 4: 0.502 ± 0.156 , SDI 5: 0.387 ± 0.194 , SDI 6: 0.500 ± 0.238 , Figure 3).

325

326 **Swimbladder condition**

327 The ordinal logistic regression model with the most empirical support included TL as a
328 proportional odds covariate, season and parasite abundance as partial proportional odds
329 covariates, and catch ID as a random effect (Tables 3 and S2). Inclusion of the random factor
330 resulted in a significant drop in AIC ($\Delta\text{AIC} = 128.7$), despite only a few eel catches appearing to
331 drive the effect (Figure S1), and therefore it was selected for inference. The condition number of
332 the Hessian for the selected model was 1.3×10^7 , which is slightly above the recommended value
333 of 10^6 . Dropping season from the model resulted in a lower Hessian value of 6.5×10^6 , but raised
334 the AIC ($\Delta\text{AIC} = 8$). The results for the other covariates were unchanged between the two
335 models, so season was ultimately included in the final model.

336 This model indicated that increasing TL was associated with an increase in the odds of
337 having a more damaged swimbladder (0.004 ± 0.001 , Figure 4). As such, the longer an eel, the
338 more likely it has a more damaged swimbladder. Also, the overall trend between parasite
339 abundance and swimbladder damage was a positive relationship. The parasite abundance
340 coefficients were negative (low versus moderate/severe condition: -0.2 ± 0.04 and low/moderate
341 versus severe condition: -0.12 ± 0.04) yet, when added to the positive model intercepts (low
342 versus moderate/severe condition: 0.39 ± 0.29 and low/moderate versus severe condition: $3.15 \pm$
343 0.31), the sum of the log odds was still a positive value. Therefore, increasing parasite abundance
344 resulted in an increased probability of swimbladder damage, but this increased probability was
345 reduced by the underlying negative relationship (Figure 4). The effect of season was minimal,
346 with only the estimated effect of having a severely damaged swimbladder in the summer relative
347 to fall indicating a decreased odds (-0.8 ± 0.29 , Figure 4).. The odds among the three
348 swimbladder conditions were not found to differ between the other seasons and fall (i.e.
349 estimates \pm standard error overlapped zero, Figure 4). Overall, eels had a higher predicted
350 probability of having a moderately damaged swimbladder (0.566 ± 0.063) compared to a low
351 (0.310 ± 0.042) or severely (0.124 ± 0.051) damaged swimbladder.

352

353 **Force of infection and disease associated mortality**

354 Sixty-four elvers and 661 yellow eels were included in the force of infection analysis,
355 ranging in age from 1 to 16 years (age range of 1 to 12+ used in analysis due to small sample
356 size of older individuals). Observed prevalence of swimbladder damage increased steeply from
357 age 1 to 2 and then slowed to a slight increase with age, whereas observed prevalence of *A.*

358 *crassus* infection was highest in the younger and older eels but lower in the middle ages (Figure
359 5).

360 Age-dependent force-of-infection models received more support than age-invariant
361 models when fitted to prevalence of swimbladder damage data (Table 4). The model with the
362 most empirical support was the log-logistic with month pairs and the mortality term, though
363 several other models were within three AIC units (Table 4). Across hazard models parameterized
364 with the same set of covariates, inclusion of the mortality term lowered the AIC for all except for
365 the Gompertz models (Table 4). Additionally, the combination of month pairs and the mortality
366 term resulted in the lowest AIC value within each set of hazard models, excluding the Gompertz
367 hazard models (Table 4). The unit hazard ratios (i.e., proportional difference in force of
368 infection) for month pairs relative to the baseline of November/December were
369 January/February: 0.026, March/April: -0.793, May/June: -0.294, July/August: -0.430, and
370 September/October: -0.486. Force of infection peaked at age 2 and then decreased with
371 subsequent ages (Figure 6). The disease-associated mortality term was estimated as 0.277 (95%
372 CI: 0.0845-0.507) and the annual survival ratio of a diseased eel relative to a non-diseased eel is
373 $e^{-(0.277)}$ (Heisey et al. 2006) or 0.76 (95% CI: 0.602-0.919).

374 Other cutoffs of swimbladder damage level considered indicative of infection (i.e. $SDI \geq$
375 1, 2, 4, 5, or 6) were explored. Similar results were found in these analyses such that the
376 mortality term was included in either the model with the most empirical support or in models
377 within 2 AIC units of the selected model. Additionally, month was usually (4 out of 6 cases) a
378 covariate in the model with the most empirical support. Likewise, force of infection peaked at
379 age 2 in almost all cases (Figure S3).

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381

382 **DISCUSSION**

383 Our study indicates that *A. crassus* infection and resultant swimbladder pathology vary
384 across host developmental stages and are influenced by environmental factors experienced by
385 American eels, which may ultimately result in disease-associated mortality. Infection
386 prevalences of over 50% were found in elvers and yellow eels, compared to only 2.5% in glass
387 eels. In general, similar patterns were observed in several previous studies of *A. crassus* in the
388 Chesapeake Bay region (Barse and Secor 1999; Barse et al. 2001; Fenske et al. 2010). Low
389 infection prevalence in glass eels, also observed by Hein et al. (2015) in South Carolina, are
390 potentially due to less time in the estuary and therefore less exposure to *A. crassus*, given that
391 eels first come in contact with the parasite in coastal waters (Van Banning and Haenen 1990).
392 This conclusion is also supported by finding mostly larval stage nematodes in glass eels in our
393 study, although Hein et al. (2015) found only adult nematodes. This difference could be due to
394 sampling location—glass eels in our study were caught in more brackish downstream sites (with
395 the exception of our Potomac sites) where they were most likely first exposed to *A. crassus*, and
396 eels collected in the South Carolina study were caught further up-river allowing more time for
397 infection by larval *A. crassus* and develop into adults (Hein et al. 2015). Our analyses also
398 indicated that season, size, and age differences in susceptibility, transmission, and mortality
399 could be drivers of observed in variation of infection and disease for elver and yellow eels.

400 Season appeared to be a minor source of variation in parasite abundance and
401 swimbladder damage for elver and yellow eels in our study. Parasite abundance was higher in
402 spring and lower during winter, whereas severe swimbladder damage had the highest probability
403 in summer and lowest in winter, though this effect was weak. These seasonal dynamics may be

404 driven by eel behavior and the abundance of *A. crassus* in the environment. For example, eels are
405 believed to be dormant in the cooler winter months (Kennedy and Fitch 1990), but as the water
406 warms in spring, they become active and commence feeding, which likely increases their
407 exposure to *A. crassus* through consumption of intermediate and paratenic hosts. Additionally, at
408 lower temperatures, the reproductive cycle of the parasite slows (Kim et al. 1989; Nagasawa et
409 al. 1994; Knopf et al. 1998), which may reduce parasite abundance and swimbladder damage in
410 the colder months. Seasonal differences could therefore indicate the existence of a lag between
411 acquiring parasites in the spring and accumulating damage from these parasites in the summer,
412 although future research is needed to explore the timing of infection and how this relates to
413 swimbladder damage.

414 For both parasite abundance and swimbladder damage, TL had a positive relationship. By
415 including zero-inflation in our analysis of parasite abundance, we were able to reveal that the
416 highest parasite abundances occur around 300 mm TL relatively consistently across seasons,
417 river systems, and swimbladder conditions. The mechanisms behind this relationship are
418 unknown, but it is likely that as eels get larger, they consume more prey and are more exposed to
419 *A. crassus*. Larger eels also have bigger swimbladders, which may provide more habitat for the
420 parasite. It is then possible that after eels reach a certain size, various aspects of parasite and
421 swimbladder damage accumulation drive prevalence downwards, possibly through disease
422 associated mortality as shown in the force of infection model. Evidence of such confounding
423 effects was supported by our finding that swimbladder damage was positively correlated with eel
424 length, but weakly correlated with parasite abundance.

425 River system was also found to be a potential source of variation in parasite abundance,
426 but not swimbladder damage. In particular, the Potomac River had overall lower parasite

427 abundance compared to the other river systems. These variations in infection, transmission, and
428 disease by season, size, and site observed in our study could be explained by a variety of
429 environmental and ecological factors (Hein et al. 2014; Moser et al. 2001; Fenske et al. 2010;
430 Hein et al. 2015; Morrison and Secor 2003; Machut and Limburg 2008; Aieta and Oliveira
431 2009). For example, higher salinity has been shown to have a negative effect on *A. crassus*
432 infection (Kirk et al. 2000; Lefebvre and Crivelli 2012), but we could not investigate this
433 covariate because we did not measure salinity in this study. Additionally, different locations and
434 seasons could have different availabilities of *A. crassus* or intermediate hosts and could vary
435 with other factors such as temperature, which could impact *A. crassus* transmission and infection
436 levels in eels (De Charleroy et al. 1989; Kennedy and Fitch 1990; Molnar et al. 1991; Molnar
437 1993). These and other environmental parameters warrant further investigation.

438 Parasite abundance and swimbladder damage are two metrics useful in characterizing *A.*
439 *crassus* infection, although we found that they are weakly correlated; a higher parasite
440 abundance may not directly correlate to more swimbladder damage. Although the estimated
441 mean parasite abundance was highest for the highest SDI score of 6 and lowest for the lowest
442 SDI score of 0, intermediate values of the metrics did not increase directly linearly. Additionally,
443 at higher parasite abundances, the probabilities of having a severely damaged swimbladder
444 versus a mildly damaged swimbladder were almost indistinguishable. Yet moderate damage had
445 an overall higher probability across the range of parasite abundances. A nonlinear relationship
446 was also found by several studies on European eels (Lefebvre et al. 2002; Lefebvre et al. 2013),
447 but is not well documented for American eels.

448 The nonlinear relationship between parasite count and swimbladder damage may be
449 caused by various aspects of the complex relationship between *A. crassus* and American eels.

450 Nematodes can die within the swimbladder and degrade or be cleared out, but leave behind
451 damaged tissue. Also, there may be a lag between nematode presence and damage accumulation
452 such that multiple infections may occur before damage accrues (Van Banning and Haenen 1990;
453 Molnar et al 1993; Wurtz and Tarachewski 2000). Additionally, density dependence among *A.*
454 *crassus* exists such that more adult nematodes within the lumen can arrest further movement of
455 larval nematodes into the lumen (Ashworth and Kennedy 1999). Furthermore, as a swimbladder
456 becomes more damaged, it becomes a less suitable habitat for nematodes (Van Banning and
457 Haenen 1990; Molnar et al. 1993). Therefore, a swimbladder can be in poor condition but it may
458 have no nematodes within it or it can appear healthy and harbor many parasites. Lefevbre et al.
459 (2002, 2013) suggested that the health state of the swimbladder may be a better long-term
460 indicator of overall infection history than number of living nematodes present at a given time.
461 Nematode count represents parasite pressure at a single point in time, whereas swimbladder
462 damage shows past and present damage, thereby potentially giving a more comprehensive
463 indication of infection severity.

464 The complexities in the dynamics of disease also likely play a role in the transmission, or
465 force of infection, of the parasite. The peak of FOI at age-2 indicated that most eels first become
466 infected shortly after entering the estuary. However, it is important to note that in this study FOI
467 was modeled using swimbladder damage rather than prevalence of the nematode, and how long
468 it takes infection to result in detectable disease is unknown. If, as our previous analyses indicate,
469 there is a lag between infection and swimbladder damage, our FOI results could also suggest that
470 eels acquire the majority of nematodes in the spring, which then results in peak visible
471 swimbladder damage in the summer months. This is consistent with our findings that parasite
472 abundance is highest in the spring and severe swimbladder damage is highest in the summer.

473 Eels likely do not accumulate more damage in the spring because they are not becoming re-
474 infected in the winter due to dormancy and lack of feeding (Kennedy and Fitch 1990) as
475 indicated by the lower FOI in March/April.

476 Importantly, the FOI model indicated that there is lower annual survival of eels with
477 moderate to severe swimbladder damage compared to those with very low or no damage. This
478 finding is supported by the overall low probability of eels having a severely damaged
479 swimbladder; if severe damage increases the likelihood of mortality, then there would a lower
480 chance of catching eels with such badly damaged swimbladders. Previous studies have shown
481 that higher *A. crassus* infection prevalence and intensity affect the ability of eels to swim,
482 tolerate hypoxic conditions or high temperatures, avoid hydraulic dams, and avoid predators and
483 fishing pressure (Molnar et al. 1991; Molnar 1993; Gollock et al. 2005; Lefebvre and Crivelli
484 2007), creating potential sources of elevated mortality. Because the FOI model is not able to
485 differentiate between mortality and recovery, more research is needed to determine if recovery
486 could also be occurring. The ability of the swimbladder to recover from infection is speculated
487 but not definitely shown to occur (Molnar et al. 1994; Székely et al. 2005; Lefebvre et al. 2012).
488 It is possible that due to the widespread availability of *A. crassus* intermediate and paratenic
489 hosts and the lack of acquired immunity (Knopf 2006), eels may be constantly exposed to the
490 nematode and never have the opportunity or ability to fully recover, although partial healing of
491 the swimbladder could be possible. Clearance of individual nematodes from the swimbladder
492 through either decay or forced exit through the pneumatic duct would result in fewer nematodes
493 within the swimbladder and would represent recovery by the definition of the FOI model if
494 disease prevalence is defined as nematode prevalence. Yet, the relationship between parasite load
495 and swimbladder damage is complex and fewer parasites does not necessarily mean a less

496 damaged organ. For these reasons, *A. crassus* infection prevalence was determined to not be a
497 suitable parameter for the FOI model.

498 In conclusion, parasite load and swimbladder damage, although related, illustrate
499 different components of this complex host parasite relationship. Parasite abundance shows
500 parasite pressure at a given point in time, whereas swimbladder damage is integrative and likely
501 represents the accumulation of disease and its negative impacts over time. Additionally, we have
502 shown that *A. crassus* infection may contribute to American eel mortality and therefore may
503 require consideration in future American eel stock assessments. A better understanding of the
504 timeline of the lifecycle of *A. crassus* would make it possible to determine if fluctuations in
505 parasite abundance and swimbladder damage are due to parasite availability or mortality. These
506 fluctuations could also be better informed with information regarding the lag between nematode
507 infection and swimbladder damage, in addition to the ability for eels to recover from infection.

508

509 **ACKNOWLEDGEMENTS**

510 This work was funded by the Virginia Sea Grant Graduate Research Fellowship and a
511 Virginia Institute of Marine Science Foundation Fellowship. The research would not have been
512 possible without the generous help from the VIMS young-of-year American Eel survey, which
513 collected glass eels and elvers and was funded by the Virginia Marine Resources Commission
514 (VMRC; through the Recreational Fishing Advisory Board and the Commercial Fishing
515 Advisory Board), as well as the Potomac River Fisheries Commission. Additionally, the VIMS
516 Juvenile Fish Trawl Survey and Striped Bass Seine Survey (funded by VMRC and Federal Aid
517 to Sport Fish Restoration and the Commonwealth of Virginia) collected yellow eels for this
518 study. The map in Figure 1 was created by Debra Gauthier of the Multispecies Research Group
519 at VIMS. We would like to thank two anonymous reviewers for valuable feedback. We also
520 appreciate the generous cooperation of the Virginia Department of Game and Inland Fisheries, in
521 particular Aaron Bunch, who organized the collection of yellow eels from various electrofishing

522 surveys around the state and allowed us to use their data for this research. This paper is
523 Contribution No. 3791 of the Virginia Institute of Marine Science, College of William & Mary.

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840 **TABLES**

841

842 Table 1. Prevalence (%), mean abundance (SE; range), and mean intensity (SE) of
 843 *Anguillicoloides crassus*, and swimbladder degenerative index (SDI; SE) in American eels by
 844 river system and eel stage.

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Location	Stage	N	Prevalence (%)	Abundance	Intensity	SDI
Potomac	Glass	116	5.2	0.05 (0.02; 0-1)	1 (0)	0.02 (0.01)
	Elver	269	59.6	1.26 (0.09; 0-10)	2.11 (0.11)	1.81 (0.1)
	Yellow	29	55.2	1.21 (0.24; 0-4)	2.19 (0.23)	1.97 (0.38)
	Total	414	44	0.91 (0.07; 0-10)	2.08 (0.1)	1.28 (0.08)
Rappahannock	Glass	249	2.4	0.02 (0.01; 0-1)	1 (0)	0.01 (0.01)
	Elver	114	53.3	1.13 (0.15; 0-9)	2.13 (0.2)	1.1 (0.17)
	Yellow	379	49.7	1.53 (0.13; 0-21)	3.09 (0.22)	1.03 (0.15)
	Total	742	34.2	0.96 (0.08; 0-21)	2.82 (0.17)	1.43 (0.06)
York	Glass	744	0	0 (0)	0 (0)	0.002 (0.002)
	Elver	230	64.8	1.72 (0.13; 0-10)	2.66 (0.15)	1.52 (0.1)
	Yellow	153	45.1	1.23 (0.23; 0-28)	2.77 (0.45)	2.85 (0.15)
	Total	1127	18.6	0.5 (0.05; 0-28)	2.7 (0.18)	0.67 (0.04)
James	Glass	371	0	0 (0)	0 (0)	0.01 (0.003)
	Elver	201	66.7	1.83 (0.13; 0-7)	2.73 (0.13)	1.82 (0.11)
	Yellow	412	43	1.24 (0.11; 0-18)	2.88 (0.19)	2.3 (0.08)
	Total	984	50.6	1.43 (0.08; 0-18)	2.82 (0.12)	1.32 (0.05)

846
847 Table 2. Model components and estimates from the most empirically supported model describing
848 the effects of covariates on count of *Anguillicoloides crassus* in American eels.
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Model Component	Parameter	Level	Estimate	Standard Error	
Zero-inflated	River system	James	Baseline	N/A	
		Potomac	-16.8	0.002	
		Rappahannock	-0.423	0.295	
		York	-0.357	0.288	
	Season	Fall	Baseline	N/A	
		Winter	-12.5	225	
		Spring	0.724	0.358	
		Summer	0.746	0.375	
	Count (negative binomial)	Total length		0.0064	0.001
			SDI		
		River system	0	Baseline	N/A
			1	-1.36	0.470
			2	-0.656	0.620
			3	-0.758	0.359
			4	-1.19	0.503
			5	-0.402	0.464
			6	-1.48	1.11
		Season	James	Baseline	N/A
			Potomac	-0.452	0.112
Rappahannock	-0.135		0.103		
York	-0.015		0.103		
Season	Fall	Baseline	N/A		
	Winter	-0.149	0.316		
	Spring	0.294	0.130		
	Summer	0.224	0.138		
SDI	Total length		0.0014	4 x 10 ⁻⁴	
		SDI			
	0	Baseline	N/A		
	1	0.279	0.124		
	2	0.512	0.116		
	3	0.295	0.131		
	4	0.502	0.156		
	5	0.387	0.194		
6	0.500	0.238			

850
 851 Table 3. Model components and estimates from the most empirically supported model describing
 852 the effects of covariates on swimbladder damage (low = 0, moderate = 1, severe = 2) from
 853 *Anguillicoloides crassus* in American eels. The “|” symbol in SDI Threshold indicates the
 854 threshold between swimbladder damage levels of the swimbladder degenerative index (SDI) for
 855 which the estimate of partial proportional odds applies for a given parameter. For example, 0|1
 856 indicates the probability an eel has an SDI of 0 versus all higher scores. Random effect estimate
 857 is the variance \pm standard deviation.
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Parameter type	Parameter	SDI Threshold	Estimate	Standard Error
Intercepts		0 1	0.387	0.288
		1 2	3.15	0.310
Proportional odds	Total Length		0.0042	0.001
Partial proportional odds	Season	0 1 fall	Baseline	N/A
		1 2 fall	Baseline	N/A
		0 1 winter	-0.425	0.911
		1 2 winter	0.641	0.971
		0 1 spring	0.204	0.251
		1 2 spring	-0.049	0.267
		0 1 summer	0.091	0.282
		1 2 summer	-0.800	0.294
	Parasite count	0 1	-0.201	0.036
		1 2	-0.117	0.036
Random effect	Catch ID		1.423	1.197

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877 Table 4. Assessment of force-of-infection model fits for presence of swimbladder damage ($SDI \geq$
 878 2) in American eels based on Akaike's information criterion. Months were grouped in to pairs
 879 starting with January/February (i.e. month pairs). Catch sites were grouped by river system
 880 (Potomac, Rappahannock, York, James Rivers). μ is the disease associated mortality term.

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Infection hazard model	ΔAIC					
	Null	μ	Season	Season, μ	System	System, μ
constant	40.6	7	35.1	11	40.3	6.5
Weibull	13.8	9	18.6	12.8	12	8.4
Pareto	9.2	7.5	13.9	11.8	8.5	7.9
Gompertz	5.3	7	10.4	11.6	5.5	7.5
log-logistic	11.2	3	15.7	6.4	10.4	5.5

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Infection hazard model	ΔAIC					
	Season, System	Season, System, μ	Month pairs	Month pairs, μ	Month pairs, System	Month pairs, System, μ
constant	35.1	10.8	27.8	3.4	31.8	6.3
Weibull	17	12.5	10.2	5.3	11.9	8.2
Pareto	13.4	12.4	6.1	4.7	8.5	7.9
Gompertz	12.5	10.7	3.1	4.7	5.9	7.9
log-logistic	15.2	9.4	6.3	0	9.3	4.8

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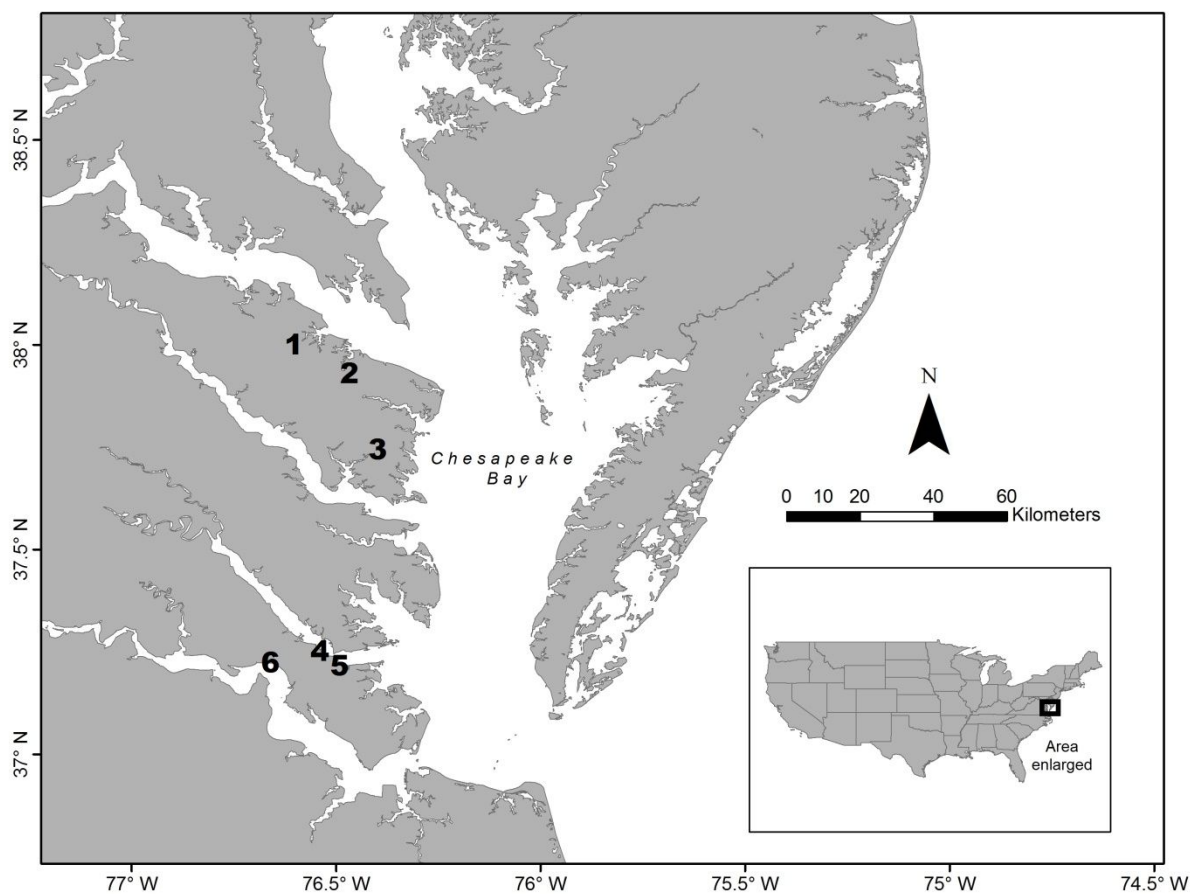
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899 **FIGURES**

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902 Figure 1. Map of collection sites for glass and elver American eels from lower Chesapeake Bay,

903 USA. Potomac River: (1) Gardy's Millpond, (2) Clark's Millpond; Rappahannock River: (3)

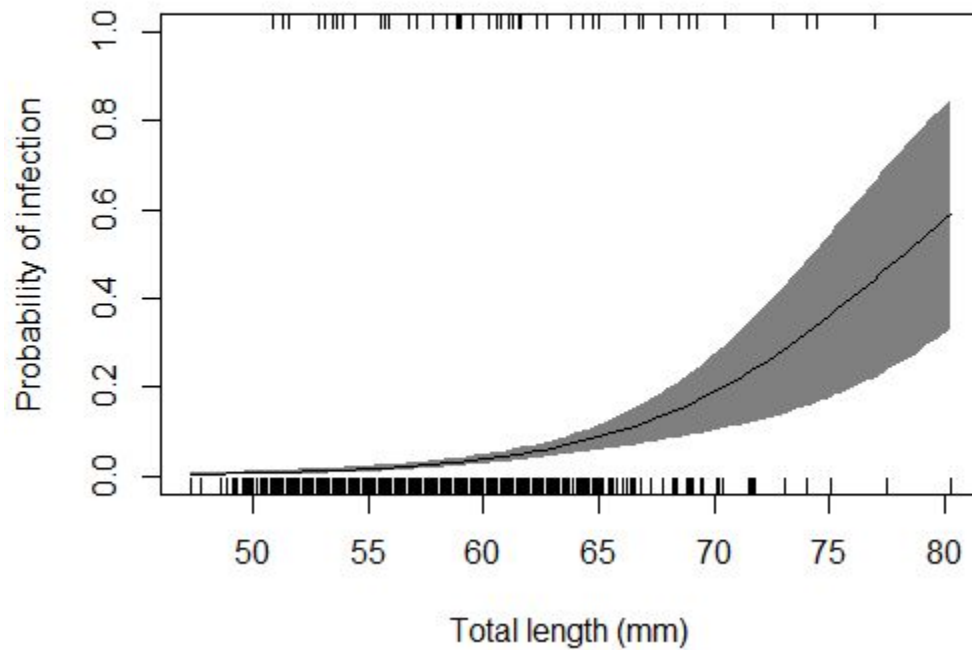
904 Kamp's Millpond; York River: (4) Bracken's Pond, (5) Wormley Pond; James River: (6)

905 Wareham's Pond. Map data from Arcinfo produced by Environmental Systems Research

906 Institute (ESRI, 1987).

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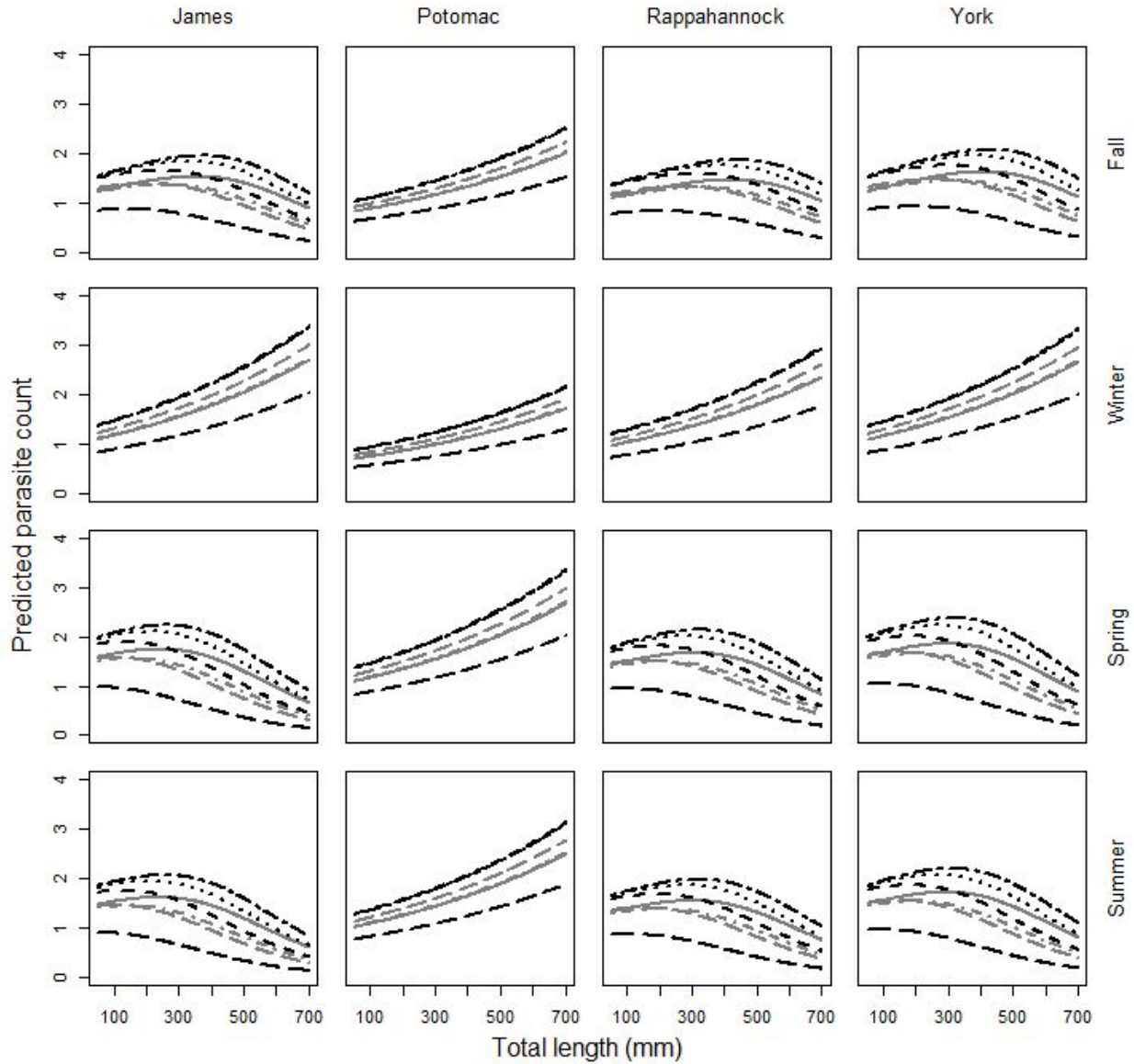
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910 Figure 2. Probability of infection with larval and adult *Anguillicoloides crassus* by total length
911 (mm) for glass eels. Black line represents binomial model results with 95% CI (grey shaded
912 area). Tick marks are individual eel observations of infected (top of plot) or uninfected (bottom
913 of plot).

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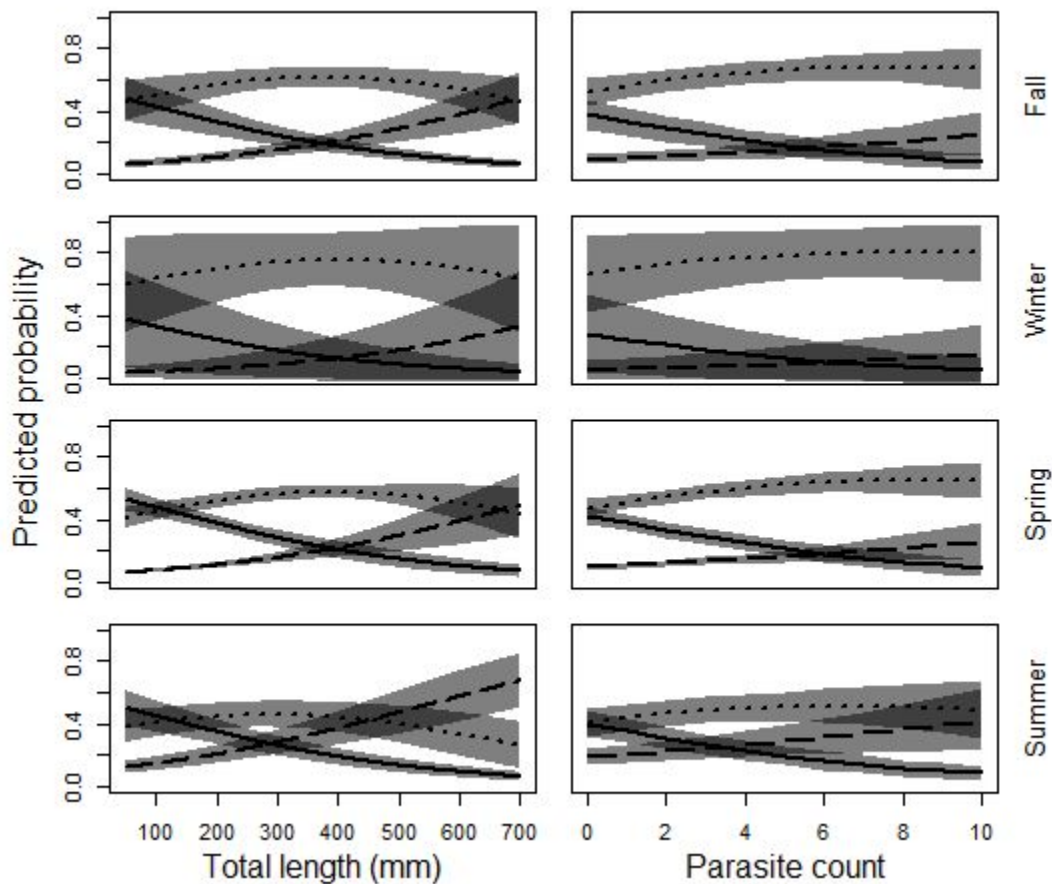
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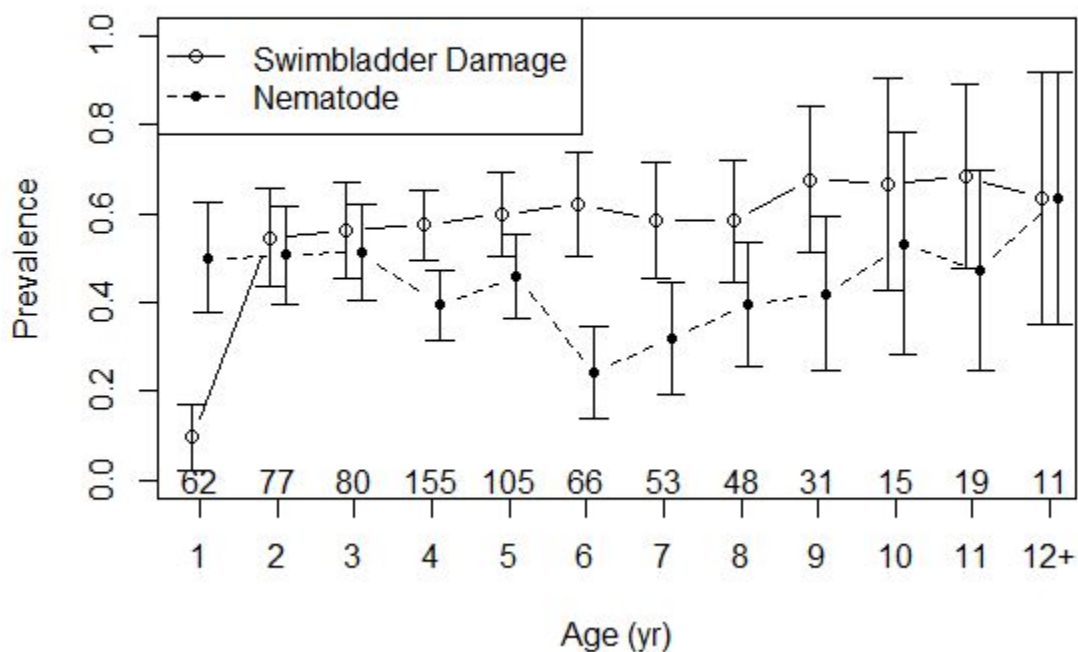
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 918 Figure 3. Predicted *Anguillicoloides crassus* parasite count for elver and yellow American eels
 919 for season of capture, system, total length (mm), and swimbladder degenerative index (SDI) total
 920 score. Results are from a zero-inflated negative binomial model. Individual lines represent SDI
 921 scores: 0 = ————, 1 = ————, 2 = ————, 3 = — · — ·, 4 = ·······, 5 =
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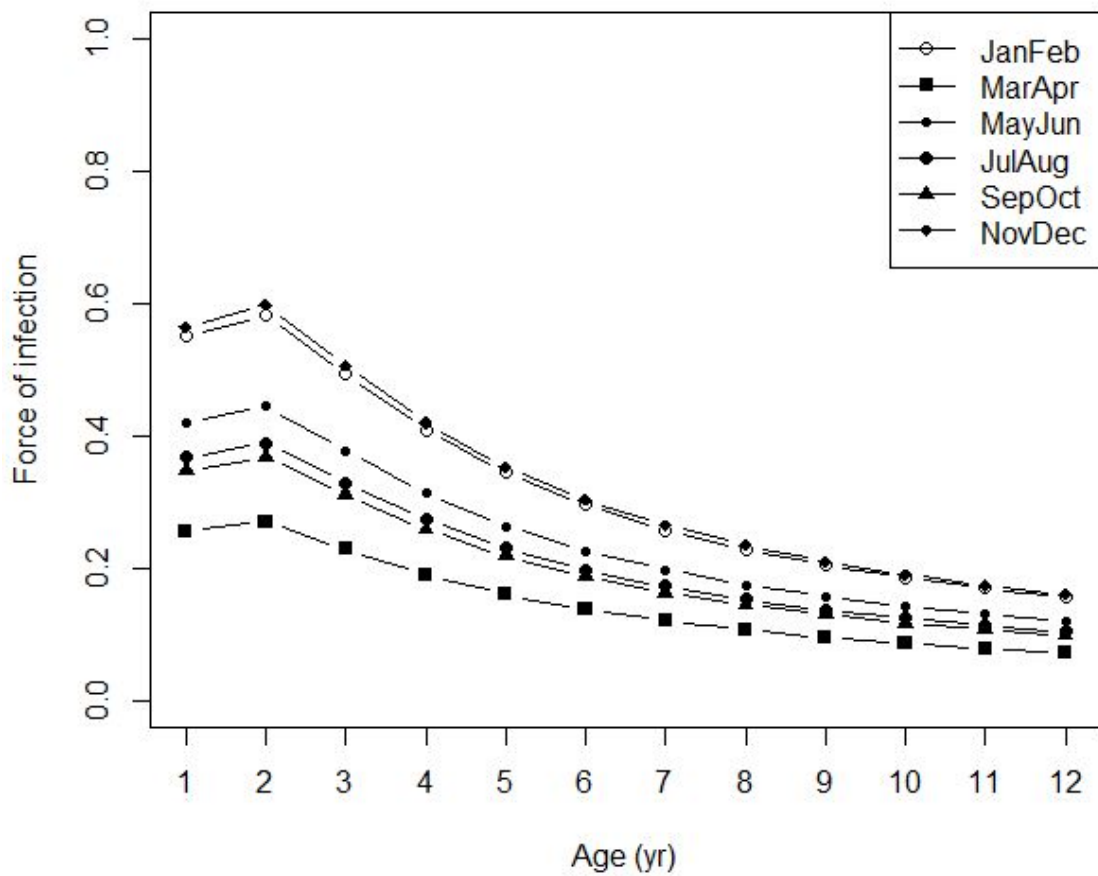
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 933 Figure 4. Predicted probability of elver and yellow eels being in a swimbladder condition
 934 category (low = SDI 0-1, ——— ; moderate = SDI 2-3, ; severe = SDI 4-6,
 935 - - -) by season of capture, total length (left panel), and *A. crassus* parasite count (right
 936 panel). Total length is held constant at its mean in right panel and parasite count is held
 937 constant at its mean in the left panel. Results are from the ordinal logistic regression with
 938 catch ID as a random effect.

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 948 Figure 5. Prevalence of swimbladder damage ($SDI \geq 3$; open circles with solid line) and *A.*
 949 *crassus* (closed circle with dashed line) prevalence by age of elver and yellow American eels
 950 with confidence intervals. Numbers above x-axis indicate sample size in each age group.
 951 Points are slightly offset for clarity.

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968 Figure 6. Force-of-infection of swimbladder damage by age for elver and yellow American eels
 969 by month pairs from best fitting force-of-infection model (log-logistic, month pair, mortality).

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