LOCAL AND REGIONAL URBANIZATION: CORRELATES OF PARASITE COMMUNITY COMPOSITION AND RICHNESS IN THE SALT MARSH FISH, *FUNDULUS HETEROCLITUS*

James M. Alfieri[1,*] and Tavis K. Anderson[1,*]

[1]Department of Biology, Georgia Southern University, P.O. Box 8042-1, Statesboro, Georgia 30460.

Corresponding author:
James M. Alfieri, Graduate Program in Ecology & Evolutionary Biology, Texas A&M University, TAMU MS 2475, College Station, TX 77843. E-mail: jamesmalfieri@gmail.com

*Present address: James M. Alfieri, Graduate Program in Ecology & Evolutionary Biology, Texas A&M University, TAMU MS 2475, College Station, TX 77843; Tavis K. Anderson, Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, 1920 Dayton Ave, Ames, Iowa 50010.

ABSTRACT: This study examined the relationship between regional urbanization and the local transmission and establishment of parasites in the salt marsh fish, *Fundulus heteroclitus*. To determine the relative importance of abiotic and biotic factors across local and regional scales, we quantified the parasite community in *Fundulus heteroclitus* collected from 6 sites selected to reflect a gradient of urbanization (determined by variation in impervious surface). A total of 630 fish was studied, with 105 collected per site in 4 collection periods from 2015-16: concurrently, we measured local abiotic parameters and regional landscape descriptors. Trace metal concentrations differed between sites, but the relationship between contaminant concentration and urbanization was not consistent. Algal biomass, determined through measurement of chlorophyll *a*, was significantly higher in urbanized sites, but there was not a corresponding increase in total nitrogen or total phosphorus concentrations. Parasite component communities were different between sites, with the pattern primarily associated with the presence or absence of a complex life-cycle digenean, *Lasiotocus minutus*. The most consistent predictors of parasite infracommunity richness within *F. heteroclitus* were host size, % regional salt marsh, and regional patch density. Our data demonstrate that measures of regional urbanization do not have a consistent effect on the local abiotic environment and that parasite infracommunity richness is best explained through the consideration of regional abiotic and local biotic factors. This study demonstrates how anthropogenically driven landscape change influences fine-scale parasite transmission and parasite species richness.

INTRODUCTION

In areas associated with high levels of urbanization, there is significant alteration of estuarine habitat by fragmentation resulting in a mosaic of habitat sizes and shapes, and variation in the abiotic environment (Lowe and Peterson, 2014). This anthropogenic habitat modification has resulted in positive, negative, or neutral changes in the diversity and population dynamics of free-living communities (Lotze et al., 2006; Aronson et al., 2014). For parasites, transmission from one host to another involves life stages external to the host along with life stages forming the durable within-host association. Consequently, the effect of urbanization on parasite populations and communities may then be twofold; either directly through changes to, and variation in, the abiotic environment (e.g., availability of contaminants and altered physicochemical parameters, e.g., Anderson and Sukhdeo, 2010; Blanar et al., 2011; Pietrock and Marcogliese, 2003; Morley et al., 2003); or indirectly through modification of host population dynamics (e.g., Hechinger et al., 2007; Anderson and Sukhdeo, 2013a).

The mechanism by which regional urbanization affects local abiotic and biotic conditions, and potentially the transmission and establishment of parasites is likely mediated by the construction of impervious surfaces (Paul and Meyer, 2001). Impervious surfaces increase the volume and rate of terrestrial runoff following rain events, leading to more frequent introductions and higher concentrations of contaminants and freshwater into estuarine habitats, with a range of effects on free-living organisms (Sanger et al., 1999). For example, a regional landscape analysis by Lerberg et al. (2000) found that the watersheds that contained greater than 50% impervious surfaces were associated with higher concentrations of metals (Cu, Cr, Pb, Zn, Cd, and Hg) resulting in a decrease in the abundance and richness of crustaceans and bivalves. Hutchins et al. (2014) found a positive correlation between the amount of impervious surfaces in a regional

62 landscape and the concentration of nutrients in the water column, which can lead to declines in
63 fish abundance through habitat degradation, toxic algal blooms, and altered algal communities
64 (Johnson et al., 2009). In a study contrasting urban and undeveloped estuaries, Krebs et al. (2014)
65 demonstrated that urban estuaries had lower, variable salinities, resulting in altered fish community
66 structure. Additional studies have demonstrated how thermal effluent associated with industrial
67 discharge in estuaries alters fish growth rate and the fish community (Jones et al., 1996), and how
68 human development may alter pH, dissolved organic matter, and oxygen concentration affecting
69 foraminiferal faunas (Hayward et al., 2004). A common theme among these studies is that the
70 regional construction of impervious surfaces alters the local abiotic environment via increases in
71 contaminant amounts and variation in physicochemical parameters, generally resulting in
72 significant changes to the free-living community.

73 The free-living community, and changes in its diversity and dynamics due to urbanization,
74 is likely to affect the diversity and richness of parasites for two reasons. Firstly, the host is
75 exploited as an energy source and critical habitat, and over evolutionary time this has resulted in
76 parasite life strategies that are integrally coupled with host species dynamics. This essential
77 interaction has been used to demonstrate how parasites can be indicators of free-living diversity
78 (Hechinger et al., 2007), and it has been argued that a healthy ecosystem is one that is rich in
79 parasites (Hudson et al., 2006). Further, complex life cycle parasites are largely dependent upon
80 trophic interactions, which occur more frequently in locally stable communities (Anderson and
81 Sukhdeo, 2013a). In increasingly urbanized salt marshes, host population dynamics may be less
82 stable, resulting in reduced diversity or less predictable host dynamics, causing a concomitant
83 decrease in the richness of parasites (Anderson and Sukhdeo, 2013b). Concurrently, a feature of
84 increasingly urbanized salt marshes is habitat fragmentation, resulting in higher densities of hosts

85 in habitat patches (Wang and Moskovits, 2001). Higher host densities may lead to increased
86 abundance of directly transmitted parasites, as the probability of a parasite transmission stage
87 encountering a suitable host is increased (Arneberg et al., 1998).

88 Many of the studies that examine the interaction between host and parasite in urbanized
89 ecosystems have been conducted with human pathogens (Bradley and Altizer, 2007), on a local
90 spatial scale (Anderson and Sukhdeo, 2010; Blanar et al., 2011), or consider a single host and a
91 single parasite (Johnson et al., 2009). Further, linking the regional process of urbanization to local
92 transmission and diversity of parasite communities has yet to reveal consistent patterns
93 (Schotthoefer et al., 2011; Chapman et al., 2015; Blanar et al., 2016; Johnson et al., 2016). One
94 potential explanation for the absence of consistent results is that urbanization differentially affects
95 parasite species: the size and direction of effect is contingent upon parasite life history. For
96 example, Blanar et al. (2016) demonstrated a correlation between parasite community structure
97 and local contaminants and land use within 5 km. In this study, the relative abundance of the
98 complex life-cycle digenean was positively associated with an increase in crude oil contaminants,
99 whereas the opposite pattern was observed for the directly transmitted monogeneans. This
100 observation follows results of an earlier meta-analysis, where parasite life history (>1 obligatory
101 host in life cycle vs. 1 obligatory host in life cycle) and habitat (external vs. internal) informed
102 whether aquatic pollution had a significant effect on parasite population dynamics (Blanar et al.,
103 2009).

104 In this study, we assessed the effect of regional urbanization and the local abiotic
105 environment on the parasite community of the salt marsh fish, *Fundulus heteroclitus,* within 6 salt
106 marshes. We asked whether local factors, such as host size and the immediate abiotic environment,
107 or if regional landscape factors, such as land-use change and composition, were more important in

108 structuring the observed parasite community. We predicted that urban salt marshes would be
109 characterized by a high degree of regional habitat fragmentation and poor local abiotic conditions
110 (e.g., reduced water quality with nutrient enrichment and high contaminant concentrations).
111 Consequently, urbanized sites would have low diversity and prevalence of complex life cycle
112 parasites; but direct life cycle parasites would increase in diversity and abundance.

113 **MATERIALS AND METHODS**

114 **Study site description**

115 We examined three estuaries along the coastline of Georgia, USA: the Savannah River
116 Estuary, Sapelo Sound, and the St. Marys Estuary. The human population in the coastal counties
117 of Georgia increased by 62% between 1970 and 2000, and is expected to increase 51.2% over the
118 next 15 years (Ross and Barringer, 2006). The Savannah River Estuary experienced heavy
119 industrial growth during the first 60 years of the 1900s, resulting in high concentrations of
120 contaminants such as polychlorinated biphenyls, chlorinated pesticides, polycyclic aromatic
121 hydrocarbons (Alexander et al., 1999). In contrast, the landscape surrounding Sapelo Sound is
122 comprised mostly of agriculture and forested land, and there is relatively little urban development.
123 The landscape surrounding the St. Marys Estuary represents an intermediate between Savannah
124 River and Sapelo Sound; it is comprised of urban development, agriculture, and forest and there is
125 evidence of regular anthropogenic impact (Morrison et al., 2008).

126 From 2015-2016, we surveyed 6 salt marsh sites from within the three estuary systems that
127 reflected a gradient in urbanization, measured through % impervious surface within a 250 m radius
128 around each site (Figure 1). In the Savannah River Estuary, sites included Fort Pulaski (FP)
129 (32°1'9''N, 80°54'6''W), Cockspur Island (CI) (32°2'27''N, 80°51'1''W), Tybee (TB)
130 (32°0'23''N, 80°51'6''W), and Skidaway Island (SK) (31°58'32''N, 81°1'56''W). As a

representative site of the Sapelo Sound, Shellman Bluff (SB) (31°64'50''N, 81°22'3''W) was included. St. Marys (SM) (30°43'19''N, 81°32'34''W) was chosen as it is representative of the St. Marys Estuary.

Quantifying the regional and local environment

We defined the boundaries of the regional environment to the lowest level watershed provided by the United States Geological Survey (USGS) Watershed Boundary Dataset (WBD): the hydrological unit code (HUC) 12-digit boundary (Lotrich, 1975). We used the 2011 National Land Cover Dataset (NLCD) and National Oceanic and Atmospheric Administration's (NOAA) Coastal Change Analysis Program (C-CAP) for the regional land cover analysis. Regional landscape composition and change were measured using ArcGIS (ArcGIS Version 10.3, Environmental Systems Research Institute, Redlands, California). We also calculated a series of regional landscape metrics: patch density, largest patch index, edge density, mean salt marsh size, % salt marsh, % developed land, and total area using FRAGSTATS software (FRAGSTATS, version 4.2, University of Massachusetts, Amherst, Massachusetts). Patch density is an index that expresses the number of patches per unit area, and is a metric for landscape heterogeneity. Largest patch index is the size of the largest habitat patch relative to the size of the landscape, and is a measure of landscape dominance. Edge density is an index of the amount of habitat edges per unit area, and the contrast between developed land and salt marsh was weighted at 0.5 to evaluate salt marsh habitat fragmentation by urban structures or landscapes. Mean salt marsh size is an index of the size of a salt marsh habitat patch, with larger patches weighted in order to decrease the sensitivity of the mean to small habitat patch sizes. The % salt marsh and developed land is the percentage of the landscape comprised of those categories, and is a metric for habitat size and dominance. Total area refers to the area of the watershed. In addition, we used the National

154 Oceanic and Atmospheric Administration's (NOAA) Coastal Change Analysis Program (C-CAP)
155 to evaluate % landscape change within the HUC-12 digit watersheds for the period of time from
156 1996 – 2010.

157 We collected information on the local abiotic parameters from each site at flow and ebb
158 tide in March, June, September 2015 and February 2016. We measured pH, temperature, salinity,
159 and conductivity using a handheld multiparamenter instrument (Yellow Springs Instruments
160 Professional Plus; Yellow Springs Instruments, Yellow Springs, Ohio). In addition, we quantified
161 the trace metal composition, nutrient concentrations, and chlorophyll *a* concentrations during these
162 dates. To extract chlorophyll *a*, we followed a standard acidification method (EPA 445.0), whereby
163 a volume of 100 ml was filtered using Whatman GF/F filters (47 mm; 0.7 µm nominal pore size).
164 Phytoplankton pigments were then extracted from the filters in 8 ml of 90 % acetone at 0 °C for
165 24 hours. Fluorescence was measured using a Trilogy fluorometer (Turner Designs, San Jose,
166 California). For the nutrient and trace metal quantification, 2 samples from each collection time
167 and site were sent to the University of Georgia Laboratory for Environmental Analysis (Athens,
168 Georgia). These samples were filtered using a microwave-assisted nitric acid method (EPA 3015),
169 and total nitrogen (TN), total phosphorus (TP), and 72 metals were analyzed with inductively
170 coupled plasma mass spectrometry (ELAN 9000; PerkinElmer, Waltham, Massachusetts). In
171 addition to water analyses, we quantified the trace metal composition of sediment by collecting 3
172 - 8 cm core samples from randomized locations within each site in September 2015. These samples
173 were sent to the University of Georgia Laboratory for Environmental Analysis (Athens, Georgia),
174 and were prepared using a microwave-assisted nitric acid method (EPA 3052).

175 **Quantifying the parasite community in Fundulus heteroclitus**

176 We used the parasite community of a focal species, the common marsh killifish *Fundulus*
177 *heteroclitus*, to assess the impact of human modification of the environment on parasite
178 establishment and richness. The host community (benthic invertebrates, birds, fishes) transmits at
179 least 11 species of helminth parasites to the common killifish (Anderson and Sukhdeo, 2010; 2011;
180 2013b). In addition, the home range estimates of killifish are in concordance with our measure of
181 local urbanization (% impervious surface within a 250 m radius of each site) (Lotrich, 1975; Teo
182 and Able, 2003). At each site, killifish were collected using 5 minnow traps baited with dry dog
183 food. Traps were placed during the flood tide and collected during the ebb tide in March 2015,
184 with subsequent collections in June 2015, September 2015, and February 2016. We randomly
185 subsampled each collection to 30 killifish from each site on each collection date. The fish were
186 housed in the animal facility at Georgia Southern University and necropsied within 1 week
187 (IACUC #I14013).

188 Each fish was euthanized in a buffered 300 mg L^{-1} solution of tricaine methanesulfonate
189 (MS-222) until cessation of opercula movement followed by sex determination and measurements
190 of total length and weight. The MS-222 solution, all external surfaces, gills, and opercula were
191 examined for ectoparasites and helminths. The viscera, gonads, spleen, liver, heart, intestine, and
192 bladder were removed and examined for helminths. All helminths from the first collection were
193 heat fixed, stored in 70% ethanol, stained in acetocarmine, and mounted in Permount. Parasites
194 from all collections were identified with keys and primary literature (Khalil et al., 1994; Harris
195 and Vogelbein, 2006; Bray et al., 2008; Anderson et al., 2009).

196 **Statistical analyses**

197 To determine site-level differences in water pH, salinity, conductivity, water and sediment
198 trace metal concentration, we performed a series of one-way analysis of variance (ANOVA) tests,

199 using collection date as a blocking factor. All trace metal and nutrient concentration data were log
200 transformed to meet the assumptions of normality and homogeneity of variances. If a significant
201 difference was found ($P < 0.05$), a post-hoc multiple pairwise comparison using Tukey's honest
202 significance test was performed. To test for differences in parasite component community species
203 richness between sites, we conducted a Kruskal-Wallis one-way ANOVA (our data did not meet
204 the parametric assumptions of normality and equal variances). We followed this analysis with a
205 post-hoc multiple pairwise comparison using the Steel-Dwass test (JMP Pro 12.0.0, SAS Institute
206 Inc., Cary, NC).

207 To visualize the variation in parasite component community structure, we performed non-
208 metric multi-dimensional scaling (NMDS). In the NMDS, average abundances of parasite species
209 in each component community were calculated and square root transformed to control for the effect
210 of high abundance parasite taxa. A resemblance matrix was constructed using Bray-Curtis
211 dissimilarity distance, and stress was calculated using Kruskal's stress formula 1. To determine
212 whether there were significant differences in parasite component community structure, we
213 performed a permutational multivariate ANOVA (PerMANOVA). For this analysis, we used site
214 as a factor and performed 999 permutations. As an overall significant difference was found ($P <$
215 0.05), we performed post-hoc pairwise PerMANOVA tests to identify differences among the factor
216 levels, and used the Bonferroni correction to preserve the type I error rate. In addition to the post-
217 hoc pairwise PerMANOVA tests, we performed a two-way crossed similarity percentage
218 (SIMPER) analysis to identify the parasite species that contributed to 5% or greater of the variation
219 in component community structure between sites (PRIMER-E LTD., Plymouth, UK).

220 To determine the role of local and regional factors in determining parasite infracommunity
221 richness, we used fully cross-validated regression trees and random forests with 1000 trees using

222 R (R Core Development Team, 2016). Prior to this analysis, we conducted a variable reduction
223 process to simplify the 48 water and 65 sediment trace metal and nutrient concentration data (JMP
224 Pro 12.0.0, SAS Institute Inc., Cary, NC). This analysis generated a correlation matrix of the log
225 transformed concentration data, placed all variables in one cluster, and then conducted binary splits
226 based on the magnitude of the second eigenvalues. Variables were iteratively reassigned to
227 clusters, and cluster membership was finalized when the squared correlation to the first principal
228 component was highest. In each cluster, the variable that explained the most variation was used in
229 the subsequent regression tree and random forest analysis. In the regression tree and random forest
230 analyses the response variable was parasite species richness and the predictor variables were host
231 characteristics such as size and sex, local abiotic measures determined through the PCA variable
232 reduction process, and the complete set of regional abiotic metrics. To avoid overfitting, we used
233 a cost-complexity algorithm to prune the regression tree: this algorithm removed auxiliary nodes
234 that would result in no significant loss in the mean square error (De'ath and Fabricius, 2000). To
235 validate the structure of the generated regression tree, we used random forest methods to generate
236 predictions on the weighted average of response value based on 1000 regression trees (Liaw and
237 Wiener, 2014).

238 **RESULTS**

239 **Abiotic variation in regional and local environments**

240 The salt marsh sites were selected on a gradient of local urbanization measured by the
241 percent of impervious surface in a 250 m buffer (Figure 1: Table 1). This measure of local
242 urbanization was not linearly correlated with the regional metric of urbanization (% developed
243 land in the watershed: Table 1). Skidaway Island (SK), which was characterized by the least
244 amount of impervious surface in the 250 m buffer at 5%, contained 20.60% developed land in the

245 watershed. Tybee Island (TB), which was characterized by the greatest amount of impervious
246 surface in a 250 m buffer at 66%, contained 6.17% developed land in the watershed. Shellman
247 Bluff (SB), which was characterized by 7% impervious surface in a 250 m buffer, contained the
248 least percentage of developed land in the watershed at 3.77%. Fort Pulaski (FP) was characterized
249 by 35% impervious surface in a 250 m buffer, but contained the greatest percentage of developed
250 land in the watershed at 24.06%.

251 Water column trace metal concentration differed across sites for 10 metals, but there was
252 no pattern of increasing metal concentration corresponding with increasing impervious surface or
253 regional urbanization (Table 2). For all metals there was variation due to the collection date
254 blocking factor, however seasonal patterns could not be determined given the single year of
255 sampling. Similarly, sediment trace metal concentration differed across sites for 13 metals, but
256 there was no linear pattern associated with measures of impervious surface or regional urbanization
257 (Table 3). No statistically significant differences were detected for water column TN, TP, pH,
258 temperature, or salinity. Water column chlorophyll *a* concentration differed across sites, with
259 increases in concentration corresponding with increasing impervious surface ($P < 0.0001$, $F_{5,66} =$
260 9.6438: Table 4).

261 **The parasite community in Fundulus heteroclitus**

262 Eight taxa of metazoan parasites were identified from the 630 fish. The direct life cycle
263 parasites included the branchiuran *Argulus funduli*, the copepod *Ergasilus funduli*, the leech
264 *Myzobdella lugubris,* and the monogeneans *Swingleus ancistrus* and *Fundulotrema prolongis*. The
265 complex life cycle parasites included the digenean *Lasiotocus minutus*, the metacestode
266 *Glossocercus caribaensis*, and the nematode *Contracaecum* sp.. These 8 taxa of parasites infected
267 more than 70% of the killifish examined and the prevalence for: *A. funduli* was 15.08%; *E. funduli*

268 was 18.73%; *M. lugubris* was 1.90%; *S. ancistrus* was 25.40%; *F. prolongis* was 22.38%; *L.*
269 *minutus* was 13.17%; *G. caribaensis* was 47.62%; and *Contraececum* sp. was 6.34% (Tables 5 and
270 6).

271 There was a difference in mean infracommunity species richness between sites ($P < 0.0001$,
272 $H_5 = 50.73$: Figure 2). Post-hoc tests revealed no difference between sites TB and CI, and both of
273 these sites had lower infracommunity richness than sites SK, SB, FP, and SM. Assessed with
274 NMDS and PerMANOVA, the parasite component communities were separate, and there was a
275 global difference in parasite component communities between sites ($P < 0.001$, Pseudo $F_{5,12}$ =
276 6.11) (Figure 3). Pairwise PerMANOVA contrasts revealed no significant differences between
277 sites, however this was due to a conservative corrected alpha level of 0.003. Parasite communities
278 were generally similar between sites characterized by similar % impervious surface. Site SK
279 differed from sites CI ($P = 0.031$, Pseudo $F_{1,6} = 5.97$), SM ($P = 0.026$, Pseudo $F_{1,6} = 13.12$), and
280 TB ($P = 0.027$, Pseudo $F_{1,6} = 10.07$). Site SB differed from sites CI ($P = 0.032$, Pseudo $F_{1,6} = 5.99$),
281 SM ($P = 0.030$, Pseudo $F_{1,6} = 15.70$), and TB ($P = 0.032$, Pseudo $F_{1,6} = 13.59$). Site FP differed
282 from sites CI ($P = 0.027$, Pseudo $F_{1,6} = 4.21$), SM ($P = 0.04$, Pseudo $F_{1,6} = 7.53$), and TB (P =
283 0.031, Pseudo $F_{1,6} = 8.25$). SIMPER analysis indicated that the observed differences in parasite
284 community structure were driven by complex life-cycle parasites: the digenean *Lasciotocus*
285 *minutus* and the cestode *Glossocercus caribaensis* (Table 5). The sites containing higher local %
286 impervious surface were characterized by a lower prevalence and intensity of the digenean
287 *Lasciotocus minutus*. There was no difference in prevalence or intensity of the cestode
288 *Glossocercus caribaensis* prevalence or intensity among sites SK, SB, FP, CI, and TB – however,
289 site SM differed, with significantly higher prevalence and intensity of infection. Although the
290 SIMPER analysis did not identify any direct life cycle parasites that contributed to variation in

291 parasite communities, sites containing higher local % impervious surface generally had lower
292 prevalence and intensity for the monogeneans *Swingleus ancistrus* and *Fundulotrema prolongis*,
293 and the crustaceans *Argulus funduli* and *Ergasilus funduli* (Table 6).

294 **Correlates of parasite species richness in Fundulus heteroclitus**

295 The cluster analysis variable reduction process on the water and sediment trace metal and
296 nutrient concentrations resulted in 7 and 8 clusters, respectively. The 7 clusters in the water data
297 explained 77.1% of the variance, while the 8 clusters in the sediment concentration data explained
298 87% of the variance. Subsequently, we selected the variable that explained the highest variation
299 within each of the 15 total clusters for use in the regression tree and random forest analyses
300 (Supplemental Table 1).

301 We constructed regression trees that designated each of the 630 host observations of
302 infracommunity species richness to terminal leaf nodes: these terminal leaf nodes categorize the
303 local and regional drivers determining parasite species richness. We followed this regression tree
304 with a random forest analysis to assess the structure and validity of the generated regression tree.
305 For the tree using parasite species richness as the response variable, the optimum tree size
306 contained 5 leaves. In regression tree analysis, variable importance is determined as the reduction
307 in the loss function attributed to each variable at each split summed across the full tree. The
308 variables that were the most important in determining parasite species richness were host length,
309 host weight, regional % salt marsh, and patch density. Fish that had the highest infracommunity
310 richness were characterized by a total length less than 6.85 cm, weighed less than 11.25 g, and this
311 was independent of regional factors (mean richness of 3.158, N = 38). Fish that had the lowest
312 infracommunity richness were characterized by a total length greater than 6.85 cm and were
313 collected from a site with a regional % salt marsh less than 42.92 (mean parasite species richness

314 of 0.7333, N = 150). The most important variables for determining parasite species richness in the
315 random forest models were host length, host weight, regional % salt marsh, largest patch index,
316 patch density, and mean salt marsh patch size. The local parameters host length, host weight, and
317 the regional parameters % salt marsh, and patch density were detected as important predictors of
318 parasite species richness in both approaches.

319 **DISCUSSION**

320 The goal of this study was to determine the role of local and regional factors in structuring
321 parasite communities. The sites, selected on an urbanization gradient, had significantly different
322 parasite component community composition and richness. Variation in the prevalence and
323 intensity of infection by complex life cycle parasites, the digenean *Lasiotocus minutus* and the
324 cestode *Glossocercus caribaensis*, were the primary cause for the differences in the parasite
325 communities between sites. Although the prevalence of direct life cycle parasites differed among
326 sites, this difference did not contribute to the overall variation in community composition. The
327 best predictors of parasite infracommunity richness included local and regional measures: host
328 size, % regional salt marsh, and patch density. The data do not support the prediction that regional
329 urbanization is linearly associated with decreased parasite infracommunity richness; rather, our
330 results suggest that habitat size, both local and regional, and landscape heterogeneity have
331 differential effects on structuring the parasite community mediated by parasite life history.

332 Complex life cycle parasites can be broadly classified as autogenic, where the parasite
333 species completes its entire life cycle within the confines of an aquatic system, or allogenic where
334 the parasite uses hosts that are outside the aquatic environment (Esch et al., 1988). The extension
335 of this concept is that the local abiotic environment will have a greater effect on autogenic
336 parasites, as the parasites and hosts are continuously exposed to the aquatic environment. We

337 found that sites with the highest local impervious surface contained a lower prevalence and
338 intensity of the autogenic parasite *Lasiotocus minutus,* a digenean that uses the focal fish species,
339 *Fundulus heteroclitus,* as the definitive host, molluscs as the first intermediate host, and fish or
340 crustaceans as the second intermediate host (Stunkard, 1980). This pattern was expected, as this
341 parasite and associated hosts are continuously exposed to the external aquatic environment, and
342 would be exposed to any contaminants or deleterious physicochemical parameters. The second
343 complex life cycle parasite, the metacestode of *G. caribaensis*, is allogenic, and did not follow the
344 same pattern as *L. minutus*, and was prevalent in a site that had a high amount of impervious
345 surface. This allogenic cestode has fish-eating birds as definitive hosts and infects a number of fish
346 intermediate hosts (Scholz et al, 2004). This pattern was not expected, as there are still life stages
347 where the parasite and hosts are exposed to the external aquatic environment. However, it is
348 plausible to suggest that because the parasite spends less time in the aquatic environment the
349 parasite has a lesser risk of exposure to contaminants or deleterious physicochemical parameters.
350 Further, *G. caribaensis* has a vagile definitive host that may select habitat outside of urbanized salt
351 marshes, lessening the impact of local and regional urbanization. Consequently, the difference in
352 prevalence across sites may be due to the effect of the abiotic condition on parasite life stages
353 external to the host, or through effects on critical aquatic life cycle hosts, such as the mollusc
354 (Anderson and Sukhdeo, 2010).

355 Local abiotic factors have the potential to adversely impact the environmental stages of
356 parasites. For example, higher concentrations of nitrogen and phosphorus result in greater
357 mortality in *Fasciola hepatica* cercariae (Brglez and Wikerhauser, 1968), and experiments have
358 demonstrated that survival of cercarial *Philophtalmus* sp. is reduced at lower salinities compared
359 to survival at normal and high salinity (Lei and Poulin 2011). Similarly, Siddall and Des Clers

360 (1994) demonstrated that exposure to sewage sludge in seawater lowered the viability of cercariae
361 and miracidia of the digenean, *Zoogonoides viviparus*, due to trace concentrations of heavy metals
362 such as Hg, Cu, Cd, and Pb. However, our data show that local abiotic parameters (e.g., pH,
363 temperature, salinity, nitrogen, phosphorus) did not vary significantly across the sites, and the
364 regression tree and random forest analyses did not associate any local abiotic factor with parasite
365 species richness. Consequently, the dynamics we observe for the autogenic parasite, the digenean
366 *L. minutus*, are more likely to be determined by changes to the host population rather than affects
367 on life stages exposed to the environment.

368 Areas with higher levels of impervious surface are likely associated with stressors that
369 affect host populations. For example, King et al. (2004) demonstrated that the survival of the
370 marine bivalve *Tellina deltoidalis* could decrease to less than 10% in low salinity waters, which is
371 a common occurrence in urbanized watersheds following rain events. In a study germane to ours,
372 Hattenrath et al. (2010) manipulated nutrient concentrations in a marine system and found that a
373 toxin-producing dinoflagellate, *Alexandrium fundyense*, had a 4-fold increase in abundance and
374 toxin densities increased 8-fold. The nutrient concentrations in this experiment were parameterized
375 by field data collected during two harmful algal blooms in 2007 and 2008 that were associated
376 with large-scale bivalve population declines (Hattenrath et al., 2010). One striking pattern in our
377 data was an almost linear increase in chlorophyll *a* across the 6 sites, i.e., sites with more
378 impervious surfaces had a higher concentration of chlorophyll *a* (Table 4). Chlorophyll *a*
379 concentration is a proxy for phytoplankton abundance, and can be associated with eutrophication
380 and harmful algal blooms (Anderson et al., 2002). Further, chlorophyll *a* is often elevated in
381 estuaries nested in a developed landscape (Reed et al., 2016). The chlorophyll *a* values in our study
382 are not indicative of harmful algal blooms, but the linear increase in concentration suggests that

383 water quality is impacted. Though we did not measure mollusc abundance, it is plausible to suggest
384 that the elevated chlorophyll *a* levels and the consequent reduction in water quality affect the
385 molluscan first intermediate host and subsequently the observed parasite communities.

386 Through regression tree and random forest analyses, we demonstrated that host size was
387 the strongest determinant of parasite species richness in *F. heteroclitus*. Generally, larger hosts
388 contain more area for a parasite to colonize, allowing for a greater number of niches and concurrent
389 species (Guegan et al., 1992; Morand and Poulin, 1998). This species-area relationship is one of
390 the more consistent patterns in ecology (Arrhenius, 1921; Macarthur and Wilson, 1963), and
391 parasitologists have regularly found positive correlations between host size and infracommunity
392 richness (Rigby and Lawton, 1981; Morand et al., 2000; Behnke et al., 2001; Timi and Poulin,
393 2003; Kamiya et al., 2014). Our data demonstrate the opposite; that the strongest predictor for a
394 high infracommunity richness was a host with a smaller body size, which is in accordance with a
395 growing body of literature that finds no relationship between size and parasite richness (Feliu et
396 al., 1997; Krasnov et al., 2004; Korallo et al., 2007). Strona and Fattorini (2014) have argued that
397 ecological processes (e.g., competition and coextinction avoidance) are the more important
398 determinants of species richness, and the host size-species richness pattern is not a general
399 phenomenon (Guegan and Kennedy, 1993; Strona and Lafferty, 2013). Further, Anderson and
400 Sukhdeo (2011, 2013a) found that the predictors of parasite species richness in *F. heteroclitus*
401 were associated with ecological interactions and trophic generality. We suggest a similar
402 explanation for our data: smaller *F. heteroclitus* have a wider diet breadth facilitating infection by
403 complex life cycle parasites, and they also form higher density schools facilitating direct
404 transmission parasite establishment. This proposition is supported by Thompson (2015) who found
405 that *F. heteroclitus* displayed differential size-selective foraging where small individuals were

406 more likely to consume a diverse diet, consequently increasing the opportunity for infection by
407 multiple trophically transmitted parasite species. In addition, small *F. heteroclitus* individuals in
408 Georgia are generally restricted to surface pools in intertidal zone habitats resulting in increased
409 density which would likely increase direct parasite transmission (Kneib, 1987). Surface pools may
410 also act as focal points for complex life cycle transmission as small individuals in these pools are
411 vulnerable to predation by bird definitive hosts (Butner and Brattstrom, 1960; Kneib, 1982). Thus,
412 in this system, the negative relationship between body size and parasite richness is most likely due
413 to correlates with body size, such as trophic generality and other ecological interactions.

414 The secondary predictors for parasite infracommunity richness in *F. heteroclitus* were
415 associated with regional variables; the % regional salt marsh and patch density. Using a *Daphnia*
416 model system with 16 parasite taxa, Ebert et al. (2001) determined that time since colonization,
417 and pool volume - a proxy for host population size - were positively correlated with parasite
418 richness. To explain an increase of within-habitat transmission rates, they invoked principles from
419 general epidemiological models, arguing that the structure of the system allowed a lower
420 persistence threshold size for the parasite (Anderson and May, 1978; 1981; Gregory, 1990). A low
421 threshold allows for more parasite species to overcome that host density threshold and persist
422 (Dobson et al., 1992). The data from the present study similarly supports these models and the
423 observations of Ebert et al. (2001): larger contiguous areas of salt marsh patches, i.e. a greater %
424 salt marsh, likely contain a larger host population size, sustaining greater parasite species richness.
425 This proposition is further supported by Rudershausen et al. (2016) who determined that *F.*
426 *heteroclitus* population size was associated with creeks that had watersheds with high percentages
427 of contiguous marsh. Thus, the sites with a large % regional salt marsh likely contain larger

428 population sizes of *F. heteroclitus*, increasing the chances of a parasite encountering a suitable
429 host and establishing in the host population.

430 Coastal urbanization and anthropogenic disturbance are rapidly altering free-living and
431 parasite community dynamics. Our results suggest that regional urbanization may not always
432 negatively affect parasite species richness. The factors that influence the richness of the observed
433 parasite community are both local and regional, and the degree to which individual parasite species
434 respond to urbanization is determined by life-history strategy. Further, our data do not align with
435 the relatively general pattern relating species richness and host (habitat) size; instead, we suggest
436 that species richness in this system is determined by the interactions between free-living hosts and
437 their environment which is then detected as variation in the parasite community.

438 **ACKNOWLEDGEMENTS**

439 We acknowledge the advice and support provided by Dr. Clark Alexander and Mike
440 Robinson at the Applied Coastal Research Laboratory (Georgia Southern University), and Dr.
441 Sayed Hassad at the Laboratory of Environmental Analysis (University of Georgia). Field
442 assistance was provided by Emily Dodd, Sarah Dunn, Maria St. Jean, and Jackson Tomlinson.
443 Comments on an earlier draft by Drs. Risa Cohen, James Roberts, and Oscar Pung greatly
444 improved this manuscript. This research was funded in part by a Research Assistantship from the
445 Institute of Coastal Plain Science (Georgia Southern University) to JMA. TKA was supported by
446 the Office of Research Services and Sponsored Programs at Georgia Southern University (GSU)
447 and by an appointment to the USDA-ARS Research Participation Program administered by the
448 Oak Ridge Institute for Science and Education under contract number DE-AC05-06OR23100. The
449 funders had no role in study design, data collection and interpretation, or the decision to submit
450 the work for publication. Mention of trade names or commercial products in this article is solely

451 for the purpose of providing specific information and does not imply recommendation or
452 endorsement by the USDA. USDA is an equal opportunity provider and employer.

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665 FIGURE 1. Map of the 6 salt marsh study sites in coastal Georgia, USA, including 2011 land cover
666 data. The sites include Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island
667 (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound;
668 and St. Marys (SM) as part of the St. Marys Estuary. Red circles represent a 250 m buffer around
669 the study site. For the purpose of this work, land cover data was reclassified from the original 21
670 categories to 10 (Salt Marsh, Developed, Freshwater Wetland, Open Water, Forest, Agriculture,
671 Bare Land, and Grassland): 2 land cover categories were not present in the study area (Freshwater
672 Aquatic Bed and Unconsolidated Shore).
673
674 FIGURE 2. Mean infracommunity species richness (± SE) of complex and direct life cycle
675 parasites within the salt marsh fish, *Fundulus heteroclitus* (N = 630 fish), from 6 sites in coastal
676 Georgia, USA. The sites include Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and
677 Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of
678 the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The sites are presented
679 from left to right reflecting increasing levels of impervious surface.
680
681 FIGURE 3. Non-metric multidimensional scaling plot of complex and direct life cycle parasite
682 component communities of the salt marsh fish, *Fundulus heteroclitus*, from 6 sites in coastal
683 Georgia, USA. Distances are based on Bray-Curtis dissimilarities of the square root transformed
684 mean abundance of 8 parasite taxa of *Fundulus heteroclitus*. The sites include: Fort Pulaski (FP)
685 (closed triangles), Cockspur Island (CI) (open triangles), Tybee (TB) (open squares), and
686 Skidaway Island (SK) (open circles) in the Savannah River Estuary; Shellman Bluff (SB) (closed

687 circles) as a representative of the Sapelo Sound; and St. Marys (SM) (closed squares) as part of
688 the St. Marys Estuary.

Table I. Regional characteristics of six salt marsh sites on coastal Georgia, USA. The sites include: Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The variables measured reflect regional (HUC-12 digit watershed) characteristics of landscape heterogeneity and composition. These data were obtained from the National Landcover Dataset, and analyzed in FRAGSTATS and ArcGIS. The units of measure are acres for Total Area, # of land use class patches per watershed area for Patch Density, % of the landscape that the largest land use class patch comprises for Largest Patch Index, the number of edges between different land use classes per watershed area for Edge Density, and mean size of salt marsh patches for Mean Salt Marsh Size.

	SK	SB	FP	CI	SM	TB
% Impervious surface	5	7	35	45	60	66
Total Area	8482	10441	47497	18101	11179	18101
Patch Density	39.6	29.9	24.2	23.9	28.2	23.9
Largest Patch Index	11.7	7.9	19.5	26.9	17.2	26.9
Edge Density	119.8	115.5	93.1	99.1	104.8	99.1
Mean Marsh Size	2.5	3.5	4.1	4.2	3.6	4.2
% Salt Marsh	39.2	25.6	3.2	46.7	33.2	46.7
% Developed	20.6	3.8	24.1	6.2	20.4	6.2
% Area Change to Developed (1996 – 2010)	6.4	1.9	18.5	2.6	7.3	2.6

Table II. Water column trace metal concentrations at six salt marsh sites on coastal Georgia, USA. The sites include: Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The sites are presented from left to right reflecting increasing levels of impervious surface. The presented trace metals (μg L^{-1}) represent those that differed across sites.

	SK		SB		FP		CI		SM		TB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fe	994.39	351.45	909.00	356.76	1040.67	405.33	1083.11	424.73	1448.53	578.33	1287.66	414.82
Zn	66.09	25.75	75.23	29.76	59.57	23.49	50.73	19.5	62.22	24.6	91.26	37.25
Br	1.31	0.57	1.15	0.51	1.21	0.51	1.24	0.60	1.65	0.64	1.59	0.69
Sr	0.04	0.02	0.03	0.02	0.04	0.02	0.04	0.02	0.04	0.02	0.05	0.02
Cd	0.98	0.41	0.94	0.37	0.61	0.23	0.54	0.20	1.28	0.53	1.31	0.50
Sn	0.09	0.03	0.18	0.07	0.12	0.06	0.10	0.04	0.18	0.07	0.15	0.07
Ce	5.24	5.24	0.26	0.18	0	0	1.35	1.3	3.69	2.98	9.34	4.45
Pr	0.65	0.61	0.12	0.05	0.07	0.02	0.26	0.16	0.5	0.34	1.21	0.57
Sm	0.04	0.01	0.13	0.04	0.07	0.02	0.13	0.05	0.05	0.03	0.24	0.15
Au	0.96	0.61	9.83	8.18	2.38	1.62	4.84	2.85	3.02	1.49	0.93	0.53

Table III. Sediment trace metal concentrations at six salt marsh sites on coastal Georgia, USA. The sites include: Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The sites are presented from left to right reflecting increasing levels of impervious surface. The presented trace metals (mg Kg^{-1}) represent those that were determined to vary significantly between sites.

	SK		SB		FP		CI		SM		TB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Li	44.66	3.91	52.99	1.99	40.36	4.09	57.94	3.84	48.94	3.14	40.60	2.65
Na	520.78	29.69	520.01	43.93	404.42	26.07	390.18	11.78	379.78	24.12	294.79	47.17
V	42.27	5.13	54.21	4.52	49.19	3.94	52.20	0.96	40.73	3.49	29.45	4.25
Co	13.74	2.71	9.69	1.1191	6.82	0.55	8.15	0.26	8.05	0.67	6.16	0.42
Cu	32.18	0.83	48.88	18.85	20.15	2.38	21.72	1.96	24.68	2.81	15.24	2.15
Zn	151.82	33.22	146.31	46.33	49.35	6.12	104.05	32.03	69.10	3.16	36.26	6.53
Ga	2.09	0.32	2.54	0.13	2.11	0.14	2.88	0.12	2.48	0.11	1.89	0.15
I	0.01	0	0.01	0	0.02	0	0.01	0	0.01	0	0.01	0
Ba	25.70	2.30	30.60	1.34	23.77	1.30	31.98	1.02	28.05	2.30	21.91	0.75
Nd	20.51	1.07	23.81	1.83	22.79	0.93	21.98	0.18	21.19	0.72	18.51	0.77
Lu	0.04	0	0.07	0	0.05	0	0.06	0.01	0.06	0	0.04	0
Pb	2006.08	936.37	1681.16	1293.47	0	0	0	0	352.15	352.15	0	0
Th	14035.24	917.71	14414.14	314.00	16319.36	1005.79	16876.34	263.17	16883.67	596.68	13906.43	1107.74

Table IV. Mean chlorophyll *a* concentrations (µg L^{-1}) ± SE at six salt marsh sites on coastal Georgia, USA. The sites include: Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The sites are presented from left to right reflecting increasing levels of impervious surface. Collections, labeled A through D, occurred in March 2015 (A), June 2015 (B), September 2015 (C), and February 2016 (D).

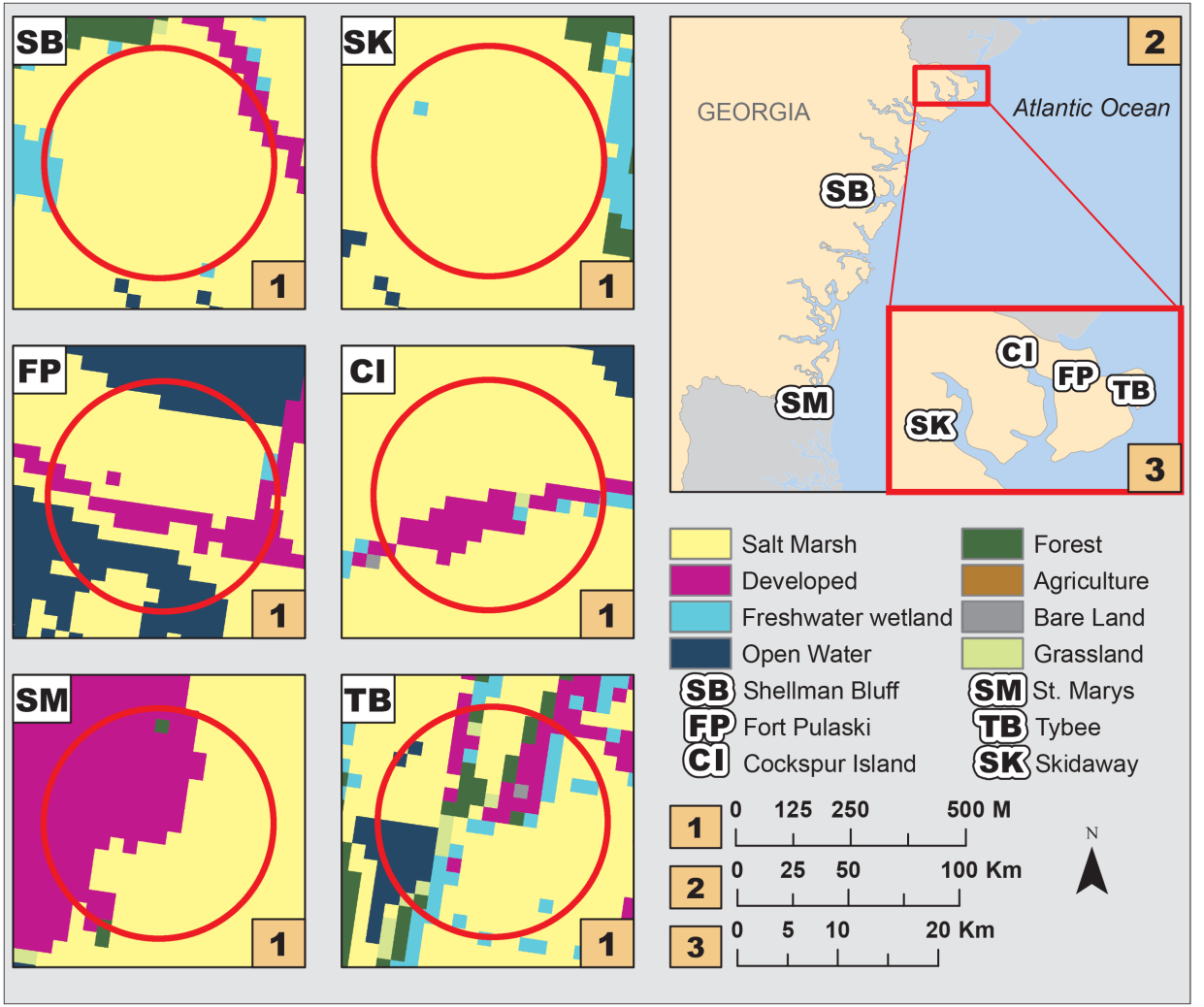
	SK		SB		FP		CI		SM		TB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	5.45	0.36	6.39	0.66	8.05	1.24	14.15	0.84	11.16	0.92	13.80	0.28
B	8.87	0.60	7.88	1.55	12.36	0.91	18.41	0.57	19.87	1.23	19.13	1.99
C	6.99	0.50	6.24	0.99	8.89	0.81	8.39	3.63	10.46	1.14	10.58	0.21
D	6.29	0.85	7.80	0.78	8.13	0.54	8.47	0.18	8.81	0.80	14.04	0.50

Table V. Prevalence (%) and mean intensity (±SE) of the three complex life cycle parasite taxa infecting the salt marsh fish, *Fundulus heteroclitus*. The sites include: Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The sites are presented from top to bottom reflecting increasing levels of impervious surface.

	Lasciotocus minutus		*Glossocercus caribaensis*		*Contracaecum* sp.	
	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity
SK	26.7	14.0 ± 2.9	44.8	4.0 ± 0.6	1.0	1.0 ± 0.0
SB	24.8	24.6 ± 4.4	39.0	3.9 ± 0.5	7.6	1.0 ± 0.0
FP	22.9	9.5 ± 1.7	448	4.2 ± 0.5	13.3	1.0 ± 0.0
CI	2.9	7.3 ± 2.4	35.2	3.4 ± 0.6	5.7	1.0 ± 0.0
SM	1.9	4.0 ± 3.0	91.4	6.0 ± 0.5	6.7	1.0 ± 0.0
TB	0	-	30.5	2.2 ± 0.3	3.8	1.0 ± 0.0

Table VI. Prevalence (%) and mean intensity (±SE) of the 5 direct life cycle parasite taxa infecting the salt marsh fish, *Fundulus heteroclitus*. The sites include: Fort Pulaski (FP), Cockspur Island (CI), Tybee (TB), and Skidaway Island (SK) in the Savannah River Estuary; Shellman Bluff (SB) as a representative of the Sapelo Sound; and St. Marys (SM) as part of the St. Marys Estuary. The sites are presented from top to bottom reflecting increasing levels of impervious surface.

	Myzobdella lugubris		*Swingleus ancistrus*		*Fundulotrema prolongis*		*Ergasilus funduli*		*Argulus funduli*	
	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity
SK	0	-	40.0	2.8 ± 0.4	29.5	4.6 ± 1.0	24.8	3.4 ± 0.7	15.2	1.4 ± 0.2
SB	1.9	1.0 ± 0	36.2	3.1 ± 0.3	20.0	4.2 ± 0.9	19.0	3.8 ± 0.7	20.0	1.4 ± 0.2
FP	9.5	1.2 ± 0.2	38.1	4.5 ± 0.7	29.5	6.0 ± 1.1	14.3	3.6 ± 0.7	24.8	1.4 ± 0.1
CI	0	-	8.6	2.0 ± 0.5	16.2	3.6 ± 0.8	20.0	3.9 ± 1.1	7.6	1.1 ± 0.2
SM	0	-	13.3	2.7 ± 0.5	20.0	3.6 ± 0.9	22.9	3.4 ± 0.9	12.4	1.2 ± 0.2
TB	0	-	16.2	1.4 ± 0.3	19.0	3.5 ± 0.5	11.4	3.1 ± 0.5	10.5	1.3 ± 0.1


SB
SK
FP
CI
SM
TB
1
2
3
GEORGIA
Atlantic Ocean
Salt Marsh
Developed
Freshwater wetland
Open Water
Forest
Agriculture
Bare Land
Grassland
SB Shellman Bluff
FP Fort Pulaski
CI Cockspur Island
SM St. Marys
TB Tybee
SK Skidaway
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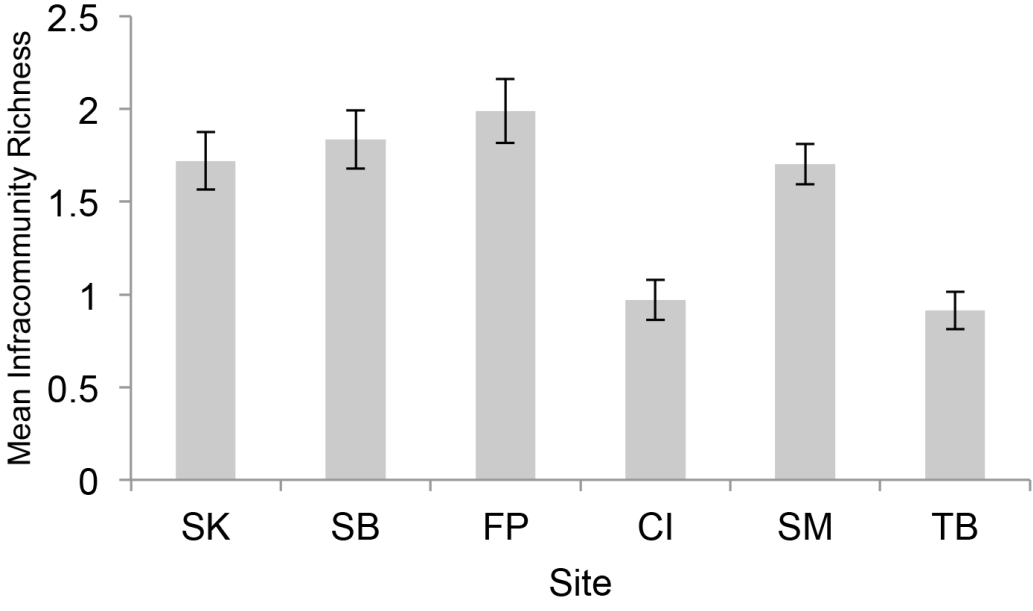
0 25 50 100 Km
0 5 10 20 Km
N


Mean Infracommunity Richness
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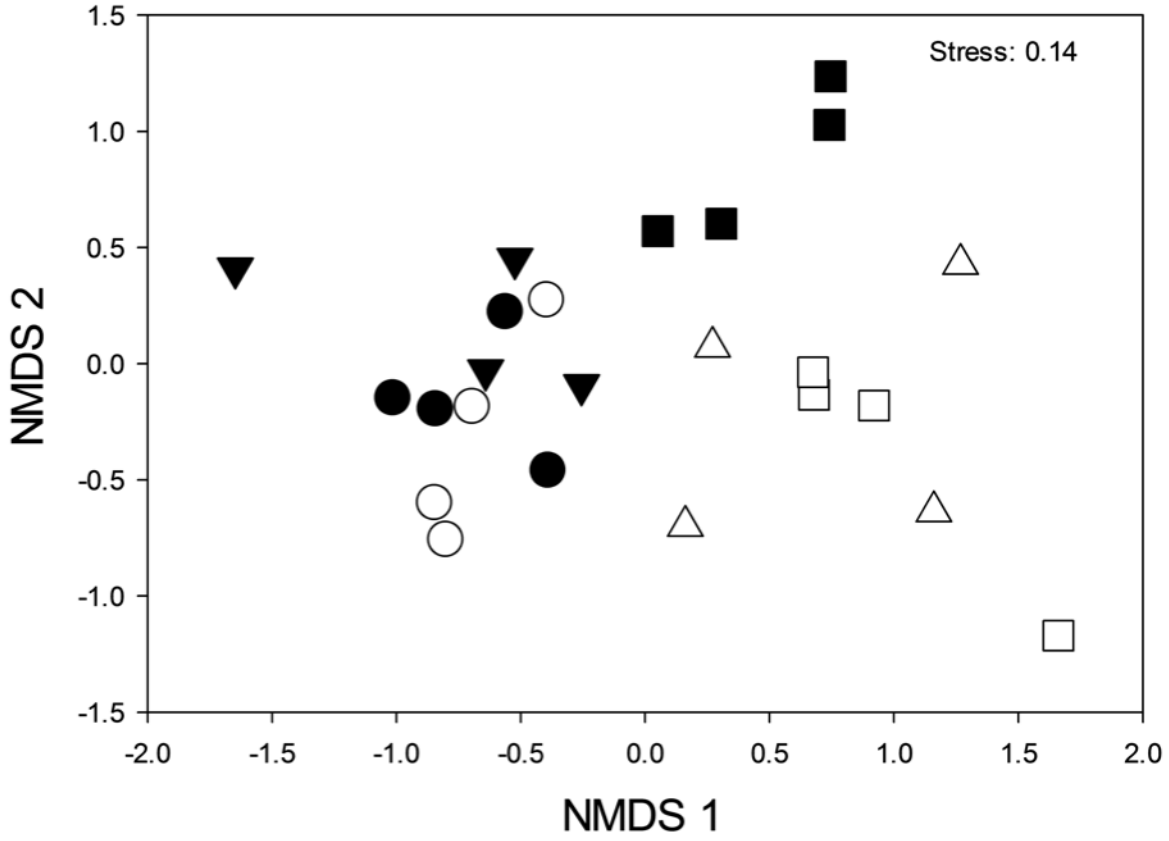
2
1.5
1
0.5
0
SK
SB
FP
CI
SM
TB
Site


Stress: 0.14
NMDS 2
NMDS 1
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0