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Parasite Distribution, Prevalence, and Assemblages of the Grass Shrimp, *Palaemonetes pugio*, in Southwestern Alabama, U.S.A.

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ABSTRACT: The grass shrimp, *Palaemonetes pugio*, harbors a number of symbionts within its North American range. Here, we document the distribution and seasonality of 4 taxonomic groups that use *P. pugio* as a host in coastal Alabama. We conducted a regional survey of 4 symbionts of *P. pugio* over 3 seasons and compared assemblages across space and time. The most common parasite was the metacercarial stage of the microphallid trematode *Microphallus turgidus*, which remained consistently prevalent over the 3 seasons surveyed. We also monitored the prevalence of *M. turgidus* at 2 sites monthly. Prevalence fluctuated significantly among seasons at these sites, but spatial heterogeneity appears to have a stronger influence on regional parasite prevalence. Distributions of 3 of 4 symbionts overlapped in a single geographic area (Mon Louis Island, Alabama, U.S.A.); however, multispecies infections of individual hosts were normally distributed within host populations. Sites surrounding Mon Louis Island had substantially higher parasite prevalence, particularly in the summer months. This area had a high quantity of *Spartina* marsh habitat, which we found influenced parasite prevalence, suggesting a role for wetland habitat in structuring parasite communities for this host.

KEY WORDS: *Palaemonetes*, parasite assemblage, distribution, wetland, ciliate, bopyrid, haplosporidian, microphallid trematode, Alabama gulf coast.

The grass shrimp, *Palaemonetes pugio*, serves as host for several parasites and symbionts in Alabama. We investigated how season, host demography, and habitat affected the considerable variation within parasite communities, with a particular focus on a common trematode. We were particularly interested in how variable environmental factors might affect parasite distributions.

The host organism

The grass shrimp functions as a critical trophic intermediate species. Its range extends from Texas to Florida and north to Massachusetts (Heard, 1982). *Palaemonetes pugio* is associated with the recycling of detrital biomass of estuarine and marine ecosystems (Welsh, 1975; McCall and Rakocinski, 2007; Robertson and Weis, 2007). This shrimp is an omnivore that consumes epiphytes, macroinvertebrates, and animal carcasses (Morgan, 1980), and in turn, is preyed upon by fishes, crabs, birds, and some mammals (Harrington and Harrington, 1972; Anderson, 1985). The combination of its extensive distribution, hardiness in the lab,

ease of capture, and tolerance for a wide range of salinities makes *P. pugio* a model organism for studies of estuarine and marine systems.

Parasites of *Palaemonetes pugio*

Palaemonetes pugio is host to a number of obligate, facultative, and transient symbionts (Overstreet, 1978). A common obligate parasite associated with *P. pugio* is the microphallid trematode, *Microphallus turgidus*, whose metacercariae encyst in the muscles of caridean shrimp (see descriptions in Heard and Overstreet, 1983, and Pung et al., 2002). The haplosporidian *Urosporidium crescens* is a hyperparasite commonly observed infecting *M. turgidus* metacercariae (Overstreet, 1978; Heard and Overstreet, 1983; Pung et al., 2002). The ectocommensal, *Lagenophrys eupagurus*, is a loricate ciliate of crustaceans (Clamp, 1973; Overstreet, 1978). Although these ciliates are not likely to disturb the development or metabolism of their hosts, they often cover most of the host's body, including walking legs, uropods, and the eyestalks (Clamp, 1973). The bopyrid isopod, *Probopyrus pandalicola*, is a parasitic castrator found in gill chambers of palaemonids. Because of the patchy and somewhat gregarious (distributed in large aggregate patches) behavior of *P.*

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pugio, all individuals within a shrimp population are equally likely to be exposed to the same parasites. Other researchers have clearly documented many of the relationships between *P. pugio* and its parasites (Clamp, 1973; Overstreet, 1978; Heard and Overstreet, 1983; Pung et al., 2002); however, parasitic community associations (those present within a given habitat) within grass shrimp populations have not been investigated.

Symbiont assemblages and community structure

The incorporation of parasites into community food web studies can document the flow of energy through trophic pathways overlooked in studies restricted to predator-prey interactions (Lafferty, 1997; Mackenzie, 1999; Marcogliese, 2005; Lafferty et al., 2006). Some parasites alter the behavior of *P. pugio*, which impacts predation success on these shrimp (Bass and Weis, 1999; Kunz and Pung, 2004). If parasites that influence behavior are limited to microhabitats within an ecosystem, predator feeding grounds would also be concentrated in those areas (Brown et al., 2002). Thus, analyses of data from field studies of parasite distribution can lead to a greater understanding of host distribution, environmental conditions, and energy transfer within communities. To determine the spatial and temporal distribution of the parasite community, we sampled *P. pugio* during 3 seasons for parasites across a spatial gradient and compared their distributions to environmental variables in southern Alabama.

MATERIALS AND METHODS

Study sites

Over 21 mo, we sampled populations of *P. pugio* from 22 locations in the Mobile Bay region (Fig. 1). We selected sites based on proximity to adequate shrimp habitat (emergent marsh, seagrass beds, or sufficiently complex substrate such as oyster beds or shallow sunken debris) and accessibility, with particular emphasis on western Mobile Bay and Dauphin Island, Alabama. Additional sites from the Mississippi Sound, eastern Mobile Bay, and local rivers were included in our surveys (Table 1). Sites ranged from 5 m² to 100 m². Each site was assigned 2 categorical characterizations: the first relating to the presence or absence of *Spartina* species (important marsh vegetation associated with the distribution of the primary host of *M. turgidus*, a hydrobiid snail), the second relating to the presence or absence of wetland habitat sufficient for supporting communities of additional obligate host populations (i.e., copepods, gastropods, and birds; Table 1). Although these designations are not comprehensive (considering they were assigned to each location during the preliminary survey) they are used to detect associations between parasite

abundance and distribution with habitat variability correlatively. The categorical distinctions for each site are intended to separate locations that serve as sources of parasite infections rather than ecological traps where infected hosts (infected elsewhere) are attracted. We visited the same 22 sites each season in order to document seasonal and regional patterns of prevalence and distribution.

Symbiont analyses

Shrimp were maintained alive in aerated plastic containers until processing, which occurred within 5 d after collection. All intact (i.e., the rostrum not broken) shrimp were measured for total length (rostrum to telson), sex, presence or absence of egg clutches, and presence or absence of parasites illuminated under a backlit dissecting scope. Voucher specimens of the parasites documented here were deposited in the USDA Agricultural Research Service U.S. National Parasite Collection under the following voucher numbers: *M. turgidus* 104644, *U. crescens* 104641, *Lagenophrys lunatus* 104645, and *P. pandalicola* 104643.

We counted metacercariae of the microphallid trematode *M. turgidus* individually under dissection scope and detected the presence of the hyperparasitic *U. crescens* infections by the appearance of inflammation and darkening of metacercarial cysts concurrent with a reddening of adjacent host tissue (Overstreet, 1978). Because enumerating hyperparasitic infections was imprecise, only their presence or absence was documented.

Lorica of *L. eupagurus* formed small, transparent protuberances not easily seen without a dissecting scope. We scanned shrimp hosts for infections of *L. eupagurus* by assessing the dorsal surfaces of the host's body. Because of their small size and the relatively large numbers occurring on infected hosts, we scanned only 1 side of each host, which allowed us to simplify records and reduce handling time of each shrimp. By observing only 1 side of each host, we have underestimated the prevalence of ciliates; however, infections, when present, were abundant and often covered multiple body parts. Thus, it is unlikely that many low-intensity infections were overlooked.

We always found the conspicuous bopyrid isopod, *P. pandalicola*, in mating pairs occupying the gill chambers of *P. pugio*. Adult females were relatively large and could be observed with the naked eye. Only established (fecund) bopyrids were documented in our surveys, although they were relatively rare at our sites.

The leech, *Myzobdella lugubris*, was also observed in relatively low numbers ($n = 2$). Because of their rarity and lack of host fidelity (i.e., they easily move among shrimp hosts), leeches were not included in our analyses.

Regional surveys

We conducted replicate surveys along the coast of Alabama, U.S.A. (Fig. 1), in the winter (January), spring (May), and fall (September) of 2007. We collected grass shrimp with a dip net. Sample sizes ranged from 8 to 217 (mean \pm SE = 52 \pm 2) shrimp per site. We recorded ambient water temperature and salinity (Table 1) for all sites in the May and September surveys with the use of a YSI model 85 conductivity and dissolved oxygen meter. Like the Wetland and *Spartina* designations, these values should not be considered comprehensive (they were taken once for each sampling for 2 seasons). Here temperature and salinity are

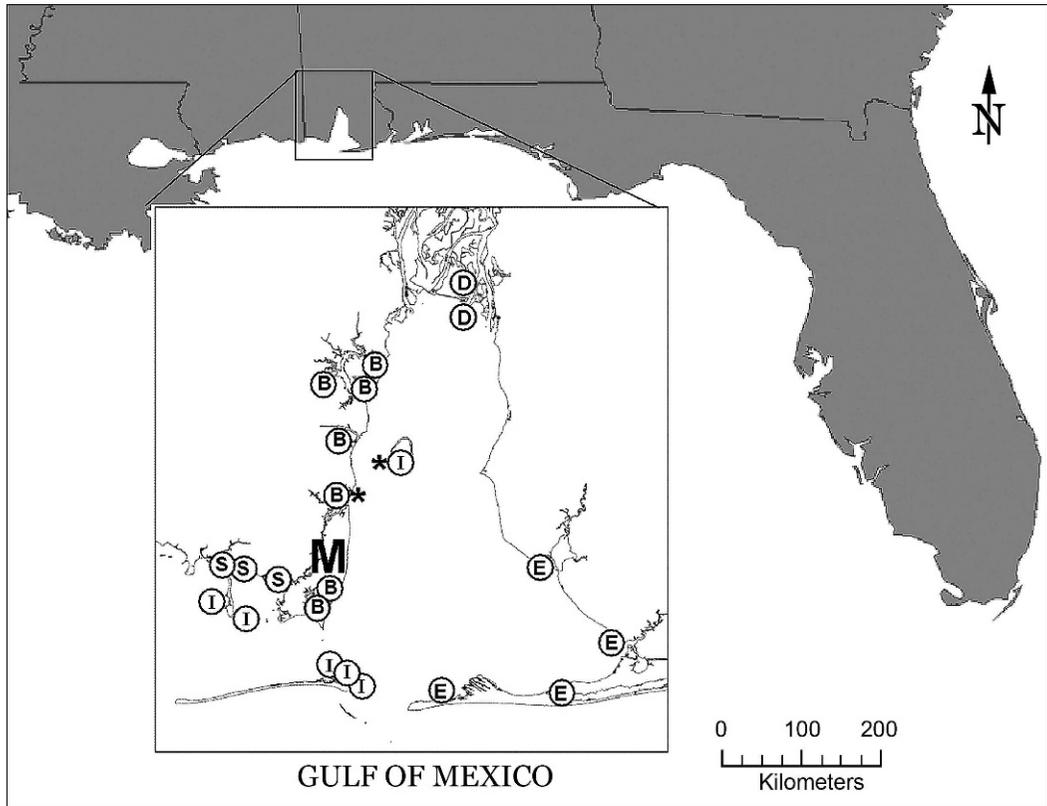


Figure 1. Map of the Gulf Coast of the U.S.A. highlighting the southwestern portion of Alabama where the Mobile Bay meets the Gulf of Mexico. Sampling sites are grouped into categories based on location and geographic properties; Mississippi Sound (S), western Mobile Bay (B), Mobile/Tensaw Delta (D), eastern Mobile Bay (E), and islands (I). Mon Louis Island (M) serves as the interface between the western Mobile Bay and the eastern Mississippi Sound. Sites with asterisks were sampled more frequently over a 21-mo period.

used to detect associations between parasite abundance and distribution with habitat variability correlatively.

Site-specific surveys

In addition to the seasonal surveys performed on a regional scale, we visited 2 adjacent sampling sites (Gaillard Island and Deer River, sites with asterisks in Fig. 1) more frequently for *M. turgidus*. At these sites, 18 sampling events (sampling monthly from spring until fall, and every 6 wk in winter) occurred over a 21-mo period beginning in March 2006. Gaillard Island is a dredge spoil depository that serves as a seasonal brown pelican rookery in the western Mobile Bay, Alabama, U.S.A. A preliminary survey indicated trematode infections were typically rare in *P. pugio* on Gaillard Island. As a reference site to Gaillard Island, we sampled the closest survey station on the mainland, Deer River, where preliminary surveys indicated high infection rates of *M. turgidus*.

Data analyses

To analyze the overall prevalence (pooling all sites) of the 4 symbionts through time, we used the Scheirer–Ray–Hare

(2-way extension of the nonparametric Kruskal–Wallis analysis of variance) test. Parasite assemblages (i.e., the combination of the site-specific prevalences of each symbiont within each *P. pugio* population) were compared among sites and seasons with an analysis of similarity (ANOSIM) with the use of Primer 6[®] software (designed for multivariate ecological research). ANOSIM tests the similarity of defined multivariate data sets as opposed to the variance around the mean used in a univariate analysis of variance tests. This multivariate approach allows for the analysis of community similarity with the use of all 4 symbionts as response variables, detecting geographic overlap in distribution of their assemblages. The independent variable used for population distinction was geographic area. Sampling sites where parasites were observed were assigned to 1 of 5 categories based on geography (see labels in Fig. 1: Mississippi Sound [S], western Mobile Bay [B], Mobile Delta [D], eastern Mobile Bay [E], and island sites [I], which included islands located in Mobile Bay and the Mississippi Sound).

We were also interested in whether the distributions of parasite species were correlated with one another and whether this was due to similar distributions across sites

Table 1. Site and host information for the 22 locations sampled for *Palaemonetes pugio* during 3 different seasons in 2007. Location coordinates expressed as degrees.hours.minutes.seconds. Categorical designation of a site with sufficient wetland habitat to support definitive host populations indicated by + (with wetland) or – (without wetland).

Site name	Location		Number of hosts	Average length (mm) ± SE	Average salinity (ppt) ± SE	Wetland designated
	Lat	Long				
Airport Marsh	30.15.25.15	–88.7.25.30	303	16.7 ± 2.9	22.5 ± 15.9	+
Appalachee River Marsh	30.40.40.51	–87.57.04.58	153	20.0 ± 3.4	5.4 ± 3.8	+
Bayfront Park	30.21.13.51	–88.07.02.78	195	26.1 ± 3.6	19.7 ± 14.0	+
Bayou Coden Mouth	30.22.15.22	–88.13.26.46	85	18.6 ± 2.2	24.3 ± 17.2	+
Billy Goat Hole	30.15.12.45	–88.05.01.11	83	24.8 ± 2.8	18.7 ± 13.2	+
Bon Secour River Mouth	30.14.21.18	–87.49.10.20	282	20.8 ± 2.5	22.3 ± 15.7	+
Callahan Road Marsh	30.22.09.53	–88.12.53.25	185	21.9 ± 2.7	27.7 ± 19.6	+
Deer River Marsh	30.31.11.47	–88.05.47.47	89	25.4 ± 3.3	15.8 ± 11.2	+
DI Causeway	30.20.09.38	–88.07.50.10	134	25.8 ± 2.3	21.4 ± 15.1	+
DISL Estuarium Marsh	30.15.02.51	–88.04.38.53	332	22.6 ± 2.8	19.3 ± 13.6	–
East Fowl River	30.26.47.57	–88.06.45.55	196	21.8 ± 2.4	15.9 ± 11.2	+
Fort Morgan Pools	30.14.04.02	–87.58.09.19	170	22.1 ± 3.1	22.5 ± 15.9	–
Gaillard Island Marsh	30.29.32.22	–88.01.46.18	165	17.4 ± 2.5	18.0 ± 12.7	–
Green Road Launch	30.22.05.53	–88.09.41.27	141	24.9 ± 2.3	20.7 ± 14.6	+
Helen Wood Park	30.34.19.54	–88.04.59.27	154	23.6 ± 3.4	13.4 ± 9.5	–
Isle aux Herbes	30.20.52.54	–88.15.41.00	94	21.4 ± 2.5	27.5 ± 19.4	–
Marsh Island	30.19.09.12	–88.13.21.56	99	20.9 ± 2.5	24.4 ± 17.3	–
Meaher Park Launch	30.39.59.45	–87.55.44.50	31	20.3 ± 3.7	6.1 ± 4.4	+
Rabbit Creek Marsh	30.35.07.42	–88.07.03.55	28	19.9 ± 2.3	8.7 ± 6.2	–
Weeks Bay Mouth	30.22.39.55	–87.50.01.07	137	23.4 ± 2.6	20.5 ± 14.5	+
West Little Lagoon Pool	30.17.34.08	–87.44.48.52	125	23.4 ± 3.2	26.3 ± 18.6	+
Yatch Club Road Marsh	30.34.55.54	–88.04.11.48	167	21.9 ± 2.6	12.9 ± 9.1	–

(spatial heterogeneity) or similar distributions within sites (host heterogeneity). To elucidate this, we calculated 3 values: (1) the observed frequency of double infections, (2) the expected frequency of double infections if all sites were pooled into 1 sample (i.e., if there was no spatial or temporal heterogeneity and parasites were distributed independently), and (3) the sum of the expected frequency of double infections among sites (if parasites were distributed independently). Comparing the sum of the expected to the expected of the pooled indicates whether heterogeneity among samples affects parasite distributions, while comparing the sum of the expected to the observed frequencies indicates whether parasites are independently distributed within a site (see Lafferty et al., 1994, and Kuris and Lafferty, 1994, for detailed descriptions of this analysis).

We used a repeated-measures multivariate generalized linear model (GLM) with JMP® statistical software to investigate environmental and demographic associations with parasitism. We used season as the repeated measure, and season was also tested as an independent factor. All first-order interactions were entered into the initial model and then (to preserve degrees of freedom) removed in sequence if nonsignificant. Any nonsignificant main effects were also removed. We tested the residuals of the final model for assumptions of normality. One analysis was conducted for the prevalence of each parasite species. The independent variables of the final model were Wetland (the presence or absence of adequate marsh habitat to support obligate hosts populations), Host Length, Temperature, Salinity (spring and fall), and Season (spring and fall). To avoid pseudoreplication, all variables were nested within season. For the haplosporidian, trematode prevalence was also entered as an independent effect.

In order to detect differences in parasite prevalence between the independent variables of site (Gaillard Island and Deer River) and season (winter = December, January, and February; spring = March, April, and May; summer = June, July, and August; and fall = September, October, and November), we used a Scheirer–Ray–Hare analysis to obtain adjusted *P* values for a 2-way analysis of variance. Relationships between host length and parasite intensity and prevalence were analyzed with the use of a linear regression for *P. pugio* populations sampled in both the regional and site-specific surveys.

RESULTS

Regional surveys

Overall regional parasite prevalence: We assessed 22 sites in the Mobile Bay region for seasonal and regional patterns of prevalence and distribution for parasites of *P. pugio* (Fig. 2). A total of 3,348 shrimp were processed during our surveys ($n = 1,594$ in January [winter], $n = 809$ in May [spring], and $n = 945$ in September [fall]). When analyzed with the Scheirer–Ray–Hare analysis, Season was not found to be a significant factor ($P = 0.95$) and the interaction between season and symbiotic Species was not significant ($P = 0.717$); however, Species was a significant factor ($P < 0.001$). The total prevalence for each parasite taxon was reanalyzed with the use of an ANOVA on ranks and a Dunn's

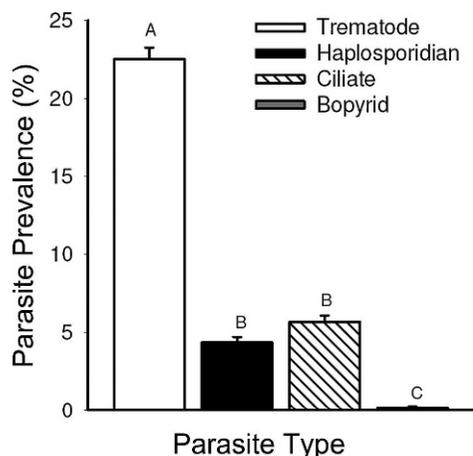


Figure 2. Regional prevalence of the 4 parasite types (trematodes, haplosporidians, loricate ciliates, and bopyrid isopods) pooled from all sites and seasons sampled. There is no significant difference in parasite prevalence for bars with the same letter.

test. Significant differences in prevalence among the 4 symbiont taxa were observed ($P < 0.001$) revealing microphallid trematodes as the most common parasites of *P. pugio* (Fig. 2). Prevalences of haplosporidians and loricate ciliates did not differ from one another, but were significantly greater than the prevalence of bopyrid isopods.

Parasite prevalence and distribution: Although overall prevalence did not change among seasons, the spatial distribution of each taxon fluctuated over time. Below we describe the spatial distribution of each symbiont in the 3 seasonal surveys.

Trematodes and haplosporidians: *Microphallus turgidus* was consistently found in high numbers along the western portion of the Mobile Bay and adjacent areas of the Mississippi Sound (Fig. 3). *Microphallus turgidus* was present on the eastern shore of Mobile Bay and Mobile Delta, with less seasonal consistency. Occurrence of the haplospor-

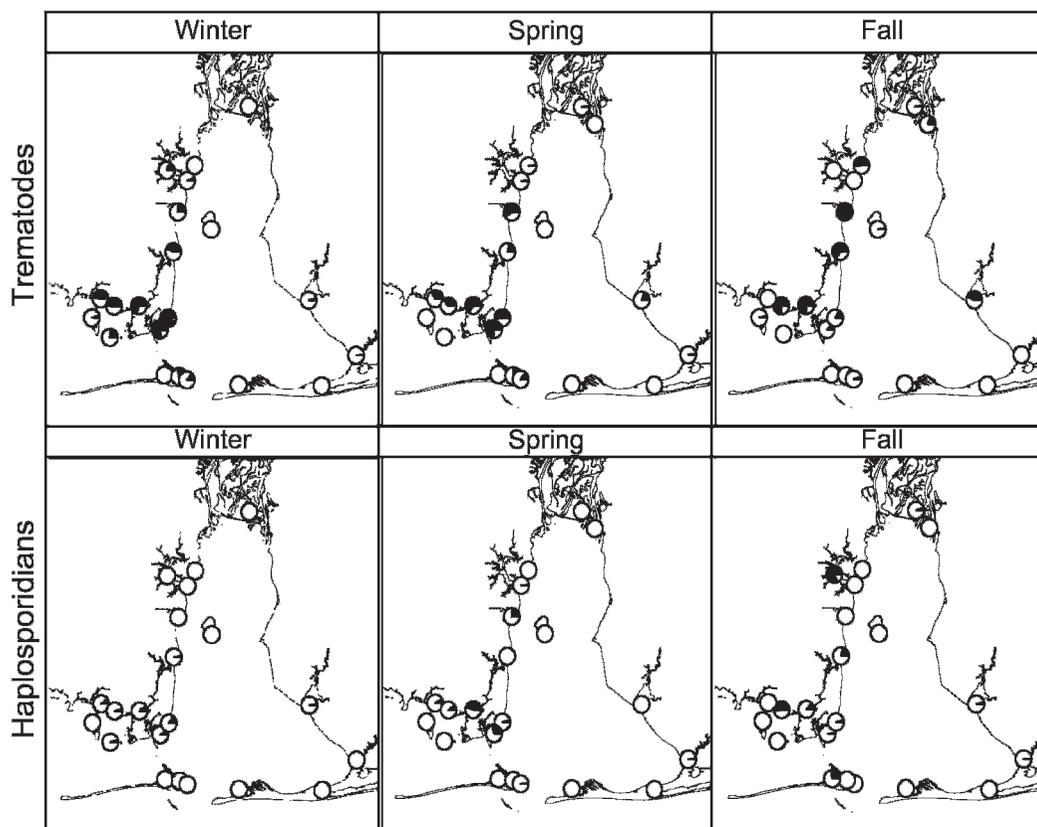


Figure 3. Local prevalences of trematodes and haplosporidians in the region of Mobile Bay, Alabama, U.S.A., in winter (January), spring (May), and fall (September) 2007. Pie charts reflect parasite abundance (dark wedge) for shrimp collected at each sampling site.

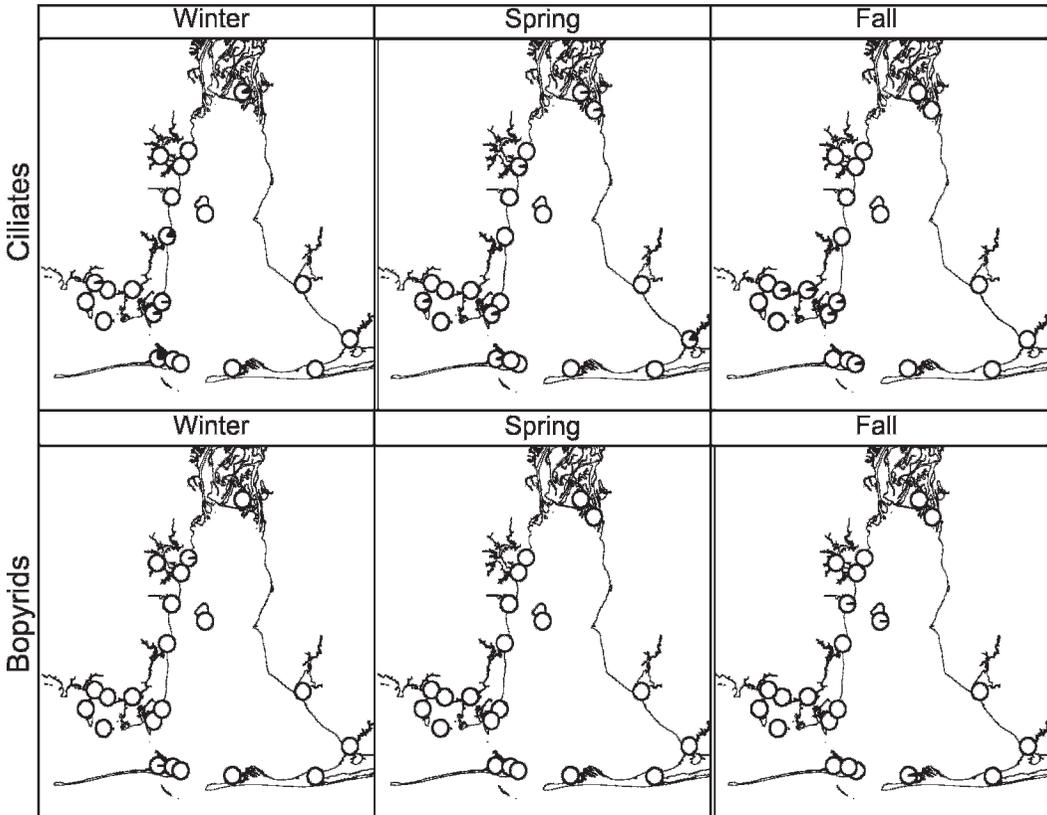


Figure 4. Local prevalences of ciliates and bopyrid isopods in the region of Mobile Bay, Alabama, U.S.A., in winter (January), spring (May), and fall (September) 2007. Pie charts reflect parasite abundance (dark wedge) for shrimp collected at each sampling site.

idian *U. crescens* was dependent on the presence of *M. turgidus*; however, not all sites with trematodes were found with hyperparasitic infections. Sampling sites where trematode infections were rare (prevalence $\leq 5\%$ of shrimp hosts) were consistently void of haplosporidian infection. The average intensity of trematode infections (6.4 ± 1.2 metacercariae host $^{-1}$) differed significantly from the trematode intensity of shrimp with concurrent haplosporidian infections (11.7 ± 1.8 metacercariae host $^{-1}$; $P = 0.006$). Furthermore, the length of shrimp with haplosporidian infections (27.65 ± 0.77 mm) differed from the mean size of all shrimp collected (25.18 ± 0.59 mm; $P = 0.009$). Nonetheless, the distribution of *U. crescens* was similar to that of their trematode hosts and highest prevalences occurred at the western Mobile Bay/Mississippi Sound interface (Fig. 3). Thus, locations where shrimp size accommodated higher trematode abundance were also the sites where haplosporidian infections were most common.

Loricate ciliates: The most restricted distribution of *L. eupagurus* occurred in winter when we collected ciliates at 6 sites with an average prevalence of 5.6%. As with the trematodes and haplosporidians, all of these sites were located along the western Mobile Bay and in the Mississippi Sound. In spring, the number of localities where ciliates were detected increased to 7, although the overall prevalence dropped to 2.2%. In fall, we documented regional ciliate prevalence of 6.5% in 10 shrimp populations located throughout the sampling region. Although overall prevalence of *L. eupagurus* fluctuated somewhat among seasons, we documented a west-to-east pattern of range expansion from winter through fall of 2007 (Fig. 4).

Bopyrid isopods: Of the over 3,300 shrimp observed in our surveys, only 6 were infected with the bopyrid isopod *P. pandalicola* (Fig. 4). Three infected hosts were collected in winter, no parasitic isopods were collected in spring, and 3 bopyrids were

Table 2. Association of trematode prevalence with environmental and demographic factors. $R^2 = 0.72$, $F_{16,24} = 3.85$, $P = 0.0015$.

Source	DF	SS	F ratio	Prob > F
Spartina[Season]	2	4,075.5606	5.5551	0.0104
Wetland[Season]	2	6,784.5990	9.2476	0.0011
Length[Season]	2	4.7534	0.0065	0.9935
Temperature[Season]	2	1,777.6763	2.4230	0.1100
Salinity[Season]	2	3,595.5009	4.9008	0.0164
Wetland * Length[Season]	2	7,652.5137	10.4306	0.0005
Wetland * Salinity[Season]	2	5,625.8257	7.6682	0.0027
Temperature * Salinity[Season]	2	3,439.3156	4.6879	0.0191

collected in the fall. Infections of bopyrids occurred at novel sites each season and not more than 1 mating pair of *P. pandalicola* was recovered at any site within the region.

Spatial analysis of parasite prevalence: With the use of the 5 predetermined geographic site categories in a 2-way analysis of (community) similarity (ANOSIM), we compared the independent factors of location (geographic site category) and season for each site's parasite community. This analysis revealed that symbiont assemblages did not vary significantly among seasons ($P = 0.21$), but varied significantly in space ($P = 0.018$; Fig. 1). Assemblages for sites grouped within S and B did not differ from one another ($P = 0.888$), but both differed significantly from D sites (S, $P = 0.002$; B, $P = 0.011$) and E sites (S, $P = 0.009$; B, $P = 0.053$). Island-site symbiont assemblages were not significantly different from any of the other site groups: B ($P = 0.117$), S ($P = 0.107$), E ($P = 0.481$), and D ($P = 0.425$).

Symbiont overlap within host populations: The distributions of the 4 symbionts showed nonrandom structure. The trematodes and haplosporidians were positively associated among samples (29 double infections expected among samples vs. 97 expected within samples) and within hosts (142 observed double infections vs. 97 expected within samples). Trematodes and ciliates were positively associated among sites (33 double infections expected among samples vs. 57 expected within samples) but not within sites (57 double infections expected within samples vs. 52 observed), indicating no interactions between ciliates and trematodes (significant based on nonoverlapping 95% confidence intervals). We found too few bopyrids to consider their inclusion in this statistical analysis.

Connecting environmental factors to prevalence patterns: A multivariate GLM was run for each

parasite taxon. Interactions among some variables suggested univariate analyses were not initially appropriate. For trematodes (Table 2), there was a strong wetland-by-host-length interaction. Prevalence increased with shrimp length at sites surrounded by wetland habitat, but this relationship was not significant at sites without wetland habitat (Fig. 5). Trematodes were considerably less prevalent at sites without wetland habitat, suggesting the main overall association between shrimp length and trematode prevalence was positive (a result confirmed by univariate associations between shrimp length and trematode prevalence; Fig. 6). There was also a strong wetland-by-salinity interaction for trematode prevalence. At sites with wetland habitat, trematode prevalence decreased with salinity, but this association disappeared in areas without wetlands. Trematode prevalence increased with temperature in areas with high salinity, but this effect also dissipated as salinity decreased. Trematode prevalence was significantly higher where *Spartina* was present. There was no significant effect of season on trematode prevalence.

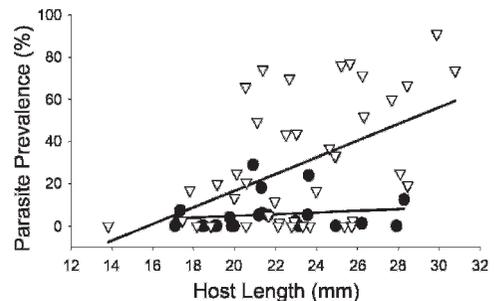


Figure 5. Linear regression plot of host length versus trematode prevalence for sites with (open triangles; regression line, $R^2 = 0.239$ and $P = 0.001$) and without (closed circles; regression line, $R^2 = 0.025$ and $P = 0.466$) marsh habitat present.

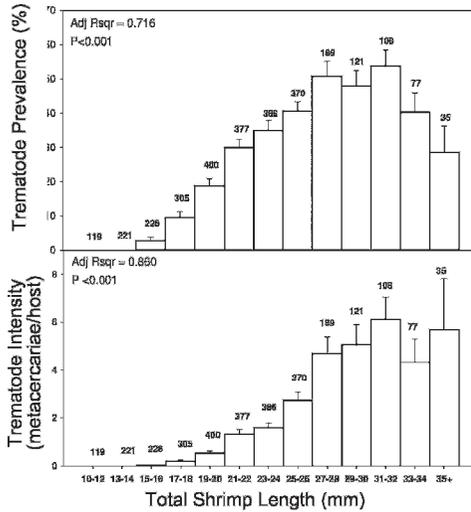


Figure 6. Frequency histograms of trematode prevalence (top) and intensity (bottom) for pooled regional survey data with error bars denoting standard error. Host size bin sample size is indicated above error bars and adjusted R^2 square values and P values are given for each analysis in the top left.

Consistent with our previous results, the prevalence of trematodes strongly influenced the prevalence of the hyperparasitic haplosporidian ($R^2 = 0.76, P < 0.001$). No other factors appeared to affect the distribution of this hyperparasite.

Lagenophrys eupagurus analyses suggested a significant interaction between temperature and salinity occurred as ciliate prevalence was high if temperature was high and salinity low or if temperature was low and salinity high (Table 3). Bopyrids were so rare that statistical explanation of their distribution was not possible. However, bopyrids are more prevalent outside the study area in locations with consistently low salinity (personal observation).

Site-specific survey

Trematode prevalence at Deer River ranged between 25 and 100%. In contrast, few shrimp on Gaillard Island contained metacercariae (0.0–13.5%).

A Scheirer–Ray–Hare adjusted 2-way ANOVA, with Season and Site as independent factors on arc-sine-square-root transformed data indicated significant differences in prevalence between the 2 sites ($P < 0.001$). However, these differences were influenced by seasonal effects, as a significant relationship was found for Season ($P < 0.001$) and the interaction between Site and Season ($P = 0.024$). Seasonal fluctuations in prevalence were observed at both sites (Fig. 7), but significant interactions did not occur uniformly between sites (Table 4). Host length was found to be positively associated with trematode prevalence ($R^2 = 0.138, P < 0.001$) and intensity ($R^2 = 0.170, P < 0.001$) at Deer River and Gaillard Island (prevalence $R^2 = 0.058, P < 0.001$; intensity $R^2 = 0.036, P < 0.001$).

DISCUSSION

Demography and habitat affected parasite distributions, particularly for the trematode metacercariae. We found that regional prevalence for all symbionts remained similar among seasons and the relative differences in prevalence within each symbiont community remained consistent throughout the year. The only exception to this was the loricate ciliate, which showed a trend for seasonal expansion throughout the study period. The community assemblage analysis (ANOSIM) suggested that site and geographic location were more important than season in determining parasite assemblages. This was confirmed in the site-specific analyses, where prevalence and intensity was always higher on the mainland than on the island site, with larger differences occurring in summer. Nevertheless, the more frequent temporal sampling at Deer River and Gaillard Island revealed substantial seasonal differences in the prevalence of *M. turgidus*. In all, results suggest that although seasonality may influence parasite prevalence, spatial heterogeneity has a greater influence on parasite abundance. Driving factors behind this trend appear to relate to host habitat (environmental variables such as marsh quantity and salinity) and parasite habitat (host size).

Table 3. Association of ciliate prevalence with environmental and demographic factors. $R^2 = 0.46, F_{6,34} = 4.89, P = 0.0011$.

Source	DF	SS	F ratio	Prob > F
Temp[Season]	2	8.5946	0.0279	0.9725
Salinity[Season]	2	1,356.8699	4.4087	0.0198
Temp * Salinity[Season]	2	3,801.7315	12.3524	<0.0001

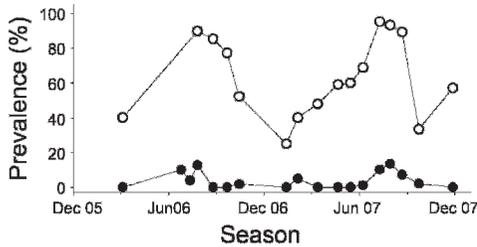


Figure 7. Trematode prevalence within *Palaemonetes pugio* populations on Gaillard Island (closed circles) and Deer River (open circles), Mobile County, Alabama, U.S.A.

Trematode intensities of *M. turgidus* tended to increase with host size; however, few of the largest shrimp observed in our surveys were parasitized. This may indicate increased mortality at elevated intensities or a survival benefit for shrimp that successfully avoid parasitic infection (as they live longer to reach a larger size). Our analyses suggest a positive association between prevalence and intensity of *M. turgidus* with host size, although trematode intensity in our samples was lower than reported by others (Pung et al., 2002). If heavily infected shrimp (≥ 25 cercariae host⁻¹) are 2.5 times more likely to be eaten

by a visual predator (Kunz and Pung, 2004), there may be increased mortality of highly parasitized shrimp in areas where birds and fish (trematode definitive hosts) are common. Although increased predation preference for shrimp having infection rates of 25 trematodes host⁻¹ was demonstrated by Kunz and Pung (2004), we rarely found trematode intensity to be that high (fewer than 5% of shrimp in our surveys). The average intensity found in Alabama was fewer than 5 trematodes host⁻¹. This study is in some ways complementary to those on the Georgia coast (Pung et al., 2002; Kunz and Pung, 2004); however, the environment along the north central Gulf of Mexico and the combinations of symbionts found on *P. pugio* in Alabama make this information novel and appropriate for future studies of *P. pugio* populations in our study region.

Urosporidium crescens was consistently absent from sites where *M. turgidus* prevalence was 5% or lower (Fig. 3), and the highest prevalence of the haplosporidian occurred in shrimp carrying many trematodes. If the haplosporidian–trematode interaction acts as an additional stressor for infected hosts, shrimp populations with high hyperparasite prevalence may suffer from increased mortality.

Table 4. Generalized linear model table of interactions for the 2-way analysis of site (Gaillard Island = GI, Deer River = DR) and season (winter = December–February, spring = March–May, summer = June–August, and fall = September–November).

Comparison	Difference of means	<i>t</i>	Unadjusted <i>P</i>	Critical level	Significant?
Within spring					
DR versus GI	0.803	8.839	0.000	0.050	Yes
Within summer					
DR versus GI	0.977	13.163	0.000	0.050	Yes
Within fall					
DR versus GI	0.768	8.454	0.000	0.050	Yes
Within winter					
DR versus GI	0.491	3.824	0.001	0.050	Yes
Within GI					
Summer versus spring	0.240	2.893	0.008	0.009	Yes
Summer versus fall	0.170	2.046	0.052	0.010	No
Summer versus winter	0.127	1.212	0.237	0.013	No
Winter versus spring	0.113	1.013	0.321	0.017	No
Fall versus spring	0.070	0.773	0.447	0.025	No
Winter versus fall	0.043	0.382	0.706	0.050	No
Within DR					
Summer versus winter	0.612	5.837	0.000	0.009	Yes
Summer versus spring	0.413	4.983	0.000	0.010	Yes
Summer versus fall	0.378	4.558	0.000	0.013	Yes
Fall versus winter	0.234	2.106	0.046	0.017	No
Spring versus winter	0.199	1.789	0.086	0.025	No
Fall versus spring	0.035	0.388	0.701	0.050	No

In addition to complications associated with predation and (hyper)parasitism, high infection rates may be fatal within *P. pugio* populations in the Gulf of Mexico due to differential mortality of hosts associated with increased environmental stress. Although increasing worldwide, the severity of hypoxic events in Mobile Bay tend to be greater than those along the mid-Atlantic coast (Diaz, 2001; Verity et al., 2006). Higher trematode intensities maintained in shrimp populations along the Atlantic (see Pung et al., 2002) may be facilitated by lower eutrophic and osmotic stress associated with more consistent salinities and regular tidal flushing (Verity et al., 2006). The elevated stress in estuarine areas of the northern Gulf of Mexico may also reduce the populations of gastropod hosts (associated with differential mortality conditions similar to those mentioned above), thus reducing overall trematode abundance. If this were the case, overall prevalence of *M. turgidus* infections in *P. pugio* populations would also be lower; however, prevalence rates in Alabama are similar to those reported elsewhere (Pung et al., 2004; Kunz and Pung, 2006).

The etiology of differential mortality of gastropod or crustacean hosts of *M. turgidus* may be elucidated by assessing the relationships between parasite intensity, prevalence, and host population dynamics with environmental variables such as temperature, salinity, dissolved oxygen concentrations, and pH of their aquatic environment, in addition to the biotic components of the habitat in which they live. Because some of the biotic variables included in our analyses (wetland and *Spartina*) were categorical rather than quantitative, and other environmental measurements (temperature and salinity) were taken only once at each site during 2 of the 3 sampling seasons, we are unable to offer a thorough analysis of the associations between trematode prevalence and salinity/habitat characteristics. Rather, we provide some potential approximations of what those associations could be. Here, we have demonstrated the correlative importance of the presence of marsh habitat, specifically *Spartina* species, and how these biological factors might interact with abiotic variables such as temperature and salinity.

In our region of study, temperature did not fluctuate greatly from one site to another within season, even though the sources and chemistry varied among sites. Therefore, we did not anticipate temperature to have a strong effect on the distribution of any of the parasites followed here; however, there were strong temperature and salinity interactions

regarding the abundance and distribution of trematodes and ciliates. These relationships agree with the observations of Poulin (2006), where trematode emergence from snail hosts increases nonlinearly as temperature increased, and Pung et al. (2002), where increasing salinity gradients were associated with elevated prevalence and intensity of *M. turgidus*. Although salinity may influence the rate of cercarial shedding, it is also suggested to influence the distribution of mollusk hosts that would ultimately control the distribution of parasites carried within gastropods (Skirnisson et al., 2004; Rogowski and Stockwell, 2006).

Publicly available aerial photographs and satellite images of the Alabama coastal region indicate a substantial expanse of marsh at the southeastern tip of Mon Louis Island, Alabama (M, Fig. 1) that separates the Mobile Bay from the Mississippi Sound. This is where we found the highest numbers of symbionts. Collection sites elsewhere had substantially smaller areas of marsh habitat compared to Mon Louis Island and were rarely used as foraging grounds for avian species (personal observation). Although we did not directly quantify wetland area, its qualitative categorization (presence or absence of marsh habitat suitable to support avian predators) had a significant relationship to the abundance of trematodes. Future quantitative analyses of this parameter are likely to support the theory that marshland (i.e., habitat quantity) may be more important than water quality in influencing symbiont diversity (Douglass and Pickel, 1999).

Marsh systems are critical nursery habitats for important commercial species (Lipcius et al., 2005; Nemerson and Able, 2005); they provide nutrients to primary producers (Lane and Brown, 2007) and function as transitional ecotones from terrestrial to marine environments (Lefeuvre et al., 2003). Fragmentation of marsh habitats decreases the diversity and abundance of species through loss of habitat and hydrological changes (Barendregt et al., 1995; Eggleston et al., 1998; Hovel and Lipcius, 2001; Madon et al., 2001; Reed and Hovel, 2006); however, researchers have shown that these effects can be reversed (Lipcius et al., 2005; Nemerson and Able, 2005).

Seasonal growth patterns of marsh vegetation tend to fluctuate in accordance with temperature and rainfall in the northern Gulf of Mexico (Rutledge and Fleeger, 1993). Although our classification system for marsh habitat did not change for each site among seasons, it is possible that areas with little marsh

habitat in winter could have sufficient marsh to support foraging bird populations as the growing season progresses through fall. Additionally, the amount of marsh we classified as suitable for host habitat may not reflect the quantity needed for all symbionts followed here. For example, the quantity of marsh habitat required to support gastropod and bird populations (obligate hosts for *M. turgidus*), may surpass the quantity suitable for *L. eupagurus*. Thus, growth of marsh vegetation from winter to fall of 2007 may have allowed for ciliate populations to proliferate in areas where they were previously rare or absent.

A common proxy for environmental health of an ecosystem is the abundance of native organisms within specific areas of interest. Although populations of small, cryptic, or rare species (such as the hydrobiid gastropods that serve as the primary host of *M. turgidus*) may be difficult to follow, *P. pugio* and its symbionts have the potential to serve as proxies for environmental stress in future impact studies of marshlands in Mobile Bay (Lafferty, 1997; Huspeni and Lafferty, 2004; Hechinger and Lafferty, 2005; Lafferty and Dunham, 2005; Whitney et al., 2007). Additionally, the analysis of the combinations of symbionts in an ecosystem-wide context would help to distinguish environmental factors (such as trophic interactions) influencing parasite prevalence.

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