Factors Determining Distribution and Abundance of Delmarva Grass Shrimp (*Palaemonetes* spp.)

Robert E. Knowlton [1], **Ritindra N. Khan** [2], **Paul M. Arguin** [3], **Tayseer A. Aldaghlas**, and **Rabindra Sivapathasundram**, Department of Biological Sciences, George Washington University, Washington, DC 20052

ABSTRACT

Samples of grass shrimp were collected bimonthly at five stations, each with two sites: (A) wooden pilings (marina) and (B) mud/sand/shell flats. Populations, especially of *Palaemonetes vulgaris*, were least abundant during colder months, most numerous in September. Relative to *Palaemonetes pugio*, *P. vulgaris* abundance decreased with decreasing average salinity (30 to 15 ppt), to near zero at 12 ppt. At polyhaline stations *P. vulgaris* was significantly more abundant at "A," *P. pugio* at "B," sites. In an aquarium on a homogeneous substrate (sand), both species, alone and mixed together, usually exhibited a random distribution pattern. On four different substrates (arrayed in 32 cubicles), most animals settled on wood (37% of all observations) and mud (36%); few on shell (15%) and sand (12%). At 20 (vs. 40) ppt significantly more shrimp were observed on mud. More *P. pugio* selected mud, and more *P. vulgaris* wood, than any other substrate. The most significant differences in mixed (vs. single) species distributions occurred among *P. vulgaris* (increase on wood, decrease on mud). It is concluded that salinity and perhaps temperature influence spatial distribution and abundance of these species and, where sympatric, differential substrate selection and interference competition are important aspects of resource partitioning.

INTRODUCTION

Decapod Crustacea of the genus *Palaemonetes* are predominant members of shallow-water benthic communities in Atlantic and Gulf coast estuaries (Wood, 1967; Nixon and Oviatt, 1973; Heck and Orth, 1980; Anderson, 1985). "Grass shrimp" are of great ecological importance because of their position as opportunistic omnivores in estuarine food webs (Odum and Heald, 1972; Welsh, 1975; Morgan, 1980; Nelson, 1981; Chambers, 1981, 1982; Kneib, 1985; Posey and Hines, 1991; Uguccioni and Posey, 1992), and they are of some economic value in the fishing industry as bait. The most common species along ocean and bay shores of the "Delmarva" peninsula are *P. pugio* Holthuis and *P. vulgaris* (Say). Their distribution throughout this area, though, is not uniform. At some estuarine sites they coexist whereas at other places only one of the species occurs (Williams, 1984).

Salinity is thought to be a significant factor in controlling distributions of grass

Corresponding author.
Present Address: Dept. of Biology, Armstrong State College, Savannah, GA 31419.
Present Address: Dept. of Internal Medicine, Oregon Health Sciences University, Portland, OR 97201.

shrimp populations, inasmuch as *P. pugio* can be found from tidal fresh water to the ocean, while *P. vulgaris* exhibits a more restricted distribution (15-35 ppt) along the salinity gradient (Knowlton and Williams, 1970). Different salinity tolerances of the two species (Bowler and Seidenberg, 1971; Thorp and Hoss, 1975; Knowlton and Kirby, 1984; Knowlton and Schoen, 1984) could account, at least in part, for this distribution pattern. Thorp and Hoss (1975) compared temperature tolerances, concluding that winter temperatures do not affect habitat partitioning in these species. Since grass shrimp are demersal, characteristics of the substrate may also be an important factor. Chambers (1982) noted that *P. pugio* was abundant in muddy-bottomed creeks of high areas of Great Sippewisset (Massachusetts) marsh whereas *P. vulgaris* was predominant in "sandy" low-marsh creeks. Thorp (1976) studied relative abundances of the two species at sites of different substrate composition in the Newport River (North Carolina) estuary, and he discerned distribution patterns of each species, with or without the other species present, in an aquarium divided into mud and shell-mud substrate sections. In pilot studies conducted by Arguin et al. (1989), species differences in substrate selection patterns were exhibited when individuals of one or both species were introduced into an aquarium provided with four different substrates (mud, sand, shell, wood), but their results were based on only a few trials and one observation time per trial. Finally, Thorp (1976) provided evidence that interspecific competition may be important. Although he did not observe behavioral interactions, he found that *P. vulgaris* was able to displace *P. pugio* physically from a "preferred shell substratum," i.e., one that provided protection from predation. Chambers (1981, 1982) found that *P. vulgaris* seemed to dominate *P. pugio* in one-on-one same-sex interactions.

The main purpose of the present study, which had both field and experimental components, was to define the extent to which salinity and substrate, and presence/absence of the other species, affect the abundance and spatial distribution of these two species of grass shrimp. Populations of *Palaemonetes* spp. were sampled throughout the "Eastern Shore" region of Virginia, Maryland, and Delaware, at which time substrate observations, salinity readings, and other measurements were undertaken. In the laboratory, trials were run under controlled conditions to determine (1) dispersal patterns of each species (on a homogeneous substrate), (2) substrate preferences, (3) the influence of salinity on substrate selection, and (4) the extent to which the two species interact in relation to substrate choice.

MATERIALS AND METHODS

Field Studies

Bimonthly collections of *Palaemonetes* spp. were made during 1987 at five shoreline stations on the Delmarva peninsula selected mainly because they cover a wide range of salinity conditions (circa 12-30 ppt). On the ocean side stations were located at (1) Indian River Bay on Burton Island, Del.; and (2) Chincoteague Bay, Chincoteague, Va. In Chesapeake Bay stations were situated at (3) Kings Creek, Cape Charles, Va.; (4) Robin Hood Bay, near Saxis, Va.; and (5) Mezick Pond, Sandy Point State Park, near Annapolis, Md. Each station included two study sites:

(A) a marina consisting of wooden pilings, buttresses, docks, etc., with attendant algae and epifauna (e.g., barnacles, tunicates, sponges) and (B) nearby flats of sand, mud, oyster shells (or some combination) bordered by marsh vegetation (*Spartina alterniflora* at Stations 1-4, *Phragmites australis* at Station 5) and bare peat. At each station, collections were timed so that they would be accomplished at about the same time of day and stage of tide (about 2 hours prior to low tide). At each "A" and "B" site, salinity was measured with a refractometer, water and air temperatures with an ordinary pocket thermometer. The amount of oxygen dissolved in the water (sample collected in BOD bottle) at each site was determined by means of the "azide modification" (Hach Chemical Co.) of the Winkler method. Animals were collected using a long-handled Ward's "D-frame" (30 cm along straight edge, 30 cm deep) aquatic dip net dragged in a consistent manner (each time a linear haul of about 1 m) over a given area, so as to obtain crude estimates (average no./haul) of shrimp abundance. Collecting effort at each station was about 1 man-hour, but sampling was stopped earlier when approximately 300 animals were obtained. Captured animals making up each site sample were preserved in 95% ethanol. Later, the individuals in a sample were sorted and tallied according to species. The two species were differentiated on the basis of rostrum structure and other diagnostic features (as noted in Holthuis, 1952). Differences in numbers of each species between sites were analyzed using the Chi-square (X^2) test, while Pearson's product-moment correlation coefficient (rho) was calculated to test for the magnitude of correlation between percent *P. pugio* found at each site and each of the measured abiotic parameters (Glantz, 1987).

In October of 1989, additional samples of shrimp were collected from different areas of Station 3, where different substrates could be clearly demarcated. One such sample was taken from wooden pilings ("A" site), and one each from three areas (within site "B"): a flat composed of mud (13% sand, 41% silt, 46% clay), an oyster bar over sand, and bare sand. Again, the number of individuals of each species was determined for each sample.

Laboratory Experiments

Living shrimp of both species, sea water, and substrate samples were also collected from Station 1 (both sites), for utilization in the experimental part of the study, viz., tests designed to assess (1) distribution of individuals of one or the other species in an aquarium on a homogeneous substrate (sand), and (2) distribution of single-species populations, or the two species mixed together, at one of two levels of salinity, over an array of four different substrates (mud, sand, shell, and wood). Collections were made in the spring of 1990 and 1991, always around 1330 hr, under the following average conditions: 15 °C water temperature, 31 ppt salinity, 10.7 ppm dissolved oxygen. Prior to testing, collected animals were kept for about a week in "stock" aquaria containing a mixture of artificial (Instant Ocean® sea salts dissolved in deionized water) and collected sea water adjusted to 30 ppt salinity, and a small amount of gravel, in an attempt to reduce any field-derived acclimation that might have occurred and to allow the animals time to adjust to laboratory conditions. Stock tanks were exposed to natural daylight but all tests were carried out in a windowless room with a fluorescent ceiling light kept on throughout each test run.

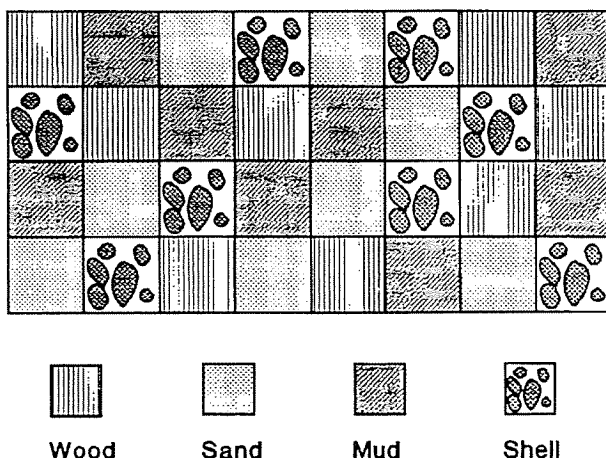In a preliminary experiment designed to determine whether or not individuals

FIGURE 1. Diagram of aquarium grid (top view) in "heterogeneous substrates" experiment, showing arrangement of cubicles, each containing the substrate indicated.

of each species distribute themselves randomly on a homogeneous substrate, sand was added to a 60 x 30 x 30 cm aquarium divided at the bottom into a "grid" of 32 cubicles (each one about 50 cm^2) by partitions made from clear acrylic plastic, with their upper edges situated about 1 cm above the substrate surface. Fifty individuals of a species were placed in the tank and allowed to distribute themselves until the following morning, at which time their location within the tank was noted. This procedure was done with each species separately and repeated once. Frequencies of occurrence of animals in the cubicles were then compared, using Chi-square, with expected frequencies in a Poisson distribution (Sokal and Rohlf, 1981). In a satellite study this analysis was extended to include additional runs using individuals of one or the other species, or both in equal numbers, and at a particular density (50, 100, 150, or 200 animals per tank). In each run, the number of animals in each cubicle was noted 15 times over 3 days.

The main experiment, consisting of three types of trials, was carried out using an aquarium in which each cubicle was "filled" with one of four substrates: sand, oyster shell (whole and broken), mud (1-cm layer over an understory of sand, to reduce turbidity), and wood (flat piece of driftwood lacking macroscopic epifauna, resoaked in sea water for one week, cut into squares to fit the cubicles and held underwater by a zinc weight attached to the underside by silicone caulking and coated with plastic marine varnish). The cubicles were arranged in such a way that the same substrates were never adjacent (Fig. 1), with each corner of the aquarium containing a different substrate (to equalize any "corner effects"). This heterogeneous substrate was not quite flat, with cubicle edges, shell, and the wooden blocks projecting 1-2 cm above the sand/mud surface. In each run of each trial, at noon animals were added to the aquarium, which was filled about halfway with an artificial-natural salt water mixture made up to the desired salinity. The number of animals in each of the 32 cubicles was noted at 3-hr intervals (± 30 min) until midnight, and at 0900 hr the next morning, to check for any diel differences in distribution patterns. At 0900 hr the tank was rotated 180°, to compensate for

any directional influences (e. g., ceiling light) within the room that might be attracting the animals, and six more observations of animal distribution were made during the second day as well (at 1200, 1500, 1800, 2100, 0000, 0900 hr). Only individuals clearly positioned on one of the substrates were tallied (i.e., swimming animals and those situated on a partition or along the aquarium side were not included). Two aquaria were used in each run, one containing water of 20 ppt salinity, the other with 40 ppt water. The tanks were placed side by side on a counter, separated by a distance of about 1 m; each run was repeated with the tanks in reversed positions. Two days prior to each test, animals to be tested at 20 ppt were brought out of the "stock" aquarium (where salinity = 30 ppt) and added to water of 25 ppt, then 20 ppt the next day. Another group of animals was acclimated to 40 ppt in the same two-step fashion. Salinity and temperature readings were taken every time the shrimp populations were observed; respective means ±standard deviations (n = 72) are 20.2 ± 1.1 ppt and 20.1 ± 1.7 °C, 39.7± 1.2 ppt and 20.3 ± 1.6 °C. The water in each tank was aerated by a single pump connected by tubing to an airstone positioned roughly in the middle, but the pump was turned off at least half an hour prior to an observation time because it was thought that the animals might be attracted to the bubbling air. Dissolved oxygen, checked prior to and during a run, was found to be above a level (4 ppm) that is well tolerated by both species (Welsh, 1975). The level of zinc in the test water did not exceed 45 ppb, comparable to levels found in many coastal waters (Bryan, 1976). The number of animals per tank to be used in each trial (60) was determined on the basis of results (see below) from the satellite study, i. e., a density that would be large enough to yield sufficient data but one that would not be likely to produce a non-random dispersion pattern and/or induce the animals to move around, thereby making counting difficult.

In the first trial type, 60 individual *P. pugio* were placed into each of the two aquaria and their locations noted, as described above. Then, a second trial was conducted in the same way, using *P. vulgaris* only. A third type of trial consisted of placing 30 members of each species simultaneously into the aquaria and subsequently noting the distributions of both species. In order to be able to differentiate the two species easily while they were both in the tank, individual *P. pugio* were dyed beforehand using a solution of Alcian blue, a process used by Coen et al. (1981) and determined by them not to affect the behavior and survivability of the shrimp. All three trials were repeated once; in the second run of the "mixed species" trial, individuals of *P. vulgaris* were dyed. In summary, the design of this experiment resulted in a total of 768 observations (i. e., number of shrimp on one of the four substrates) in the data set, or: 2 species x 2 "conditions" (i. e., single species, two species mixed together) x 2 salinities x 4 substrates x 2 days x 6 times (of each day) x 2 runs. Statistical analysis included a four-way analysis of variance (ANOVA) of the arcsine-transformed data, using a general linear models procedure (SAS Institute, 1985) with species, condition, salinity, and substrate as the main effects. Means of the factors were examined using Tukey's just significant difference (JSD) and graphical construction of confidence intervals (CI) about the means using the formula JSCI = mean ± JSD/2 (Sokal and Rohlf, 1981). In all analyses a probability (P) of less than 0.05 was required for significance.
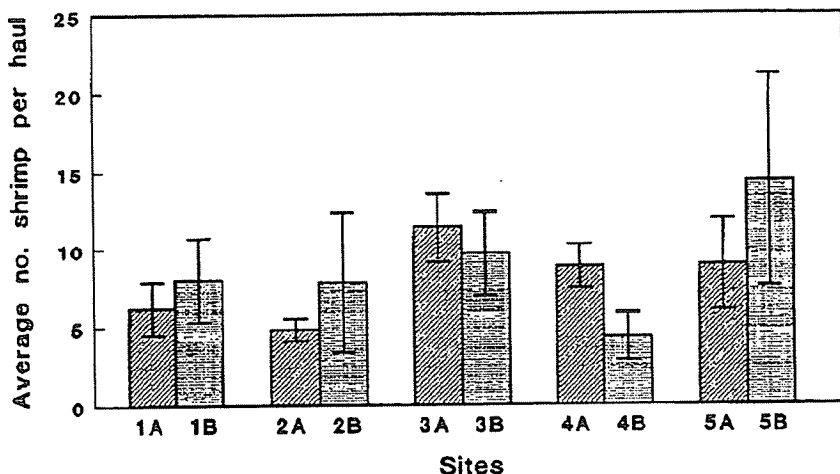
FIGURE 2. Mean abundance (approximate no. per haul of dip net) of *Palaemonetes* individuals (both species) at each collecting site (summed over the 6 months). Each vertical bar indicates standard error of the mean.

RESULTS

Abundance and Distribution in the Field

Of the 9,293 grass shrimp collected bimonthly from the five stations, nearly all were *Palaemonetes pugio* (68%) or *P. vulgaris* (32%). A third species, *P. intermedius*, was found in May (3 individuals at Sta. 2B) and July (1 animal, Sta. 5B); these animals, along with 7 juveniles (taken from Sta. 3A in September) of uncertain identity, were excluded from "species composition" calculations.

Figure 2 portrays average number of individual *Palaemonetes* (regardless of species) per haul (of the dip net) at each of the ten collecting sites (all months combined). A given haul yielded anywhere from zero to over 100 animals, but typically (i.e., 85% of the time) the yield in a haul ranged between 1 and 15. Maximum abundance was exhibited at Station 5B, where in May an average haul contained about 40 animals. Failure to collect any shrimp occurred on only one occasion — at Station 4B in January. Stations 4B and 2A were, on average, least productive, possibly because these sites are not as well protected from wave action and currents. The difference in means between all A and B sites (8.0 and 8.8 animals/haul, respectively) is not significant (t = 0.407 with df = 58; P = 0.686).

The same abundance data are presented by month (all stations combined), to illustrate seasonal variation (Fig. 3). During the winter months (January and March) animals were consistently less abundant, except at the A sites of the southern stations (3 and 4) of Chesapeake Bay. More animals appeared in May hauls, with 19% of *P. pugio* (but only a single *P. vulgaris* at Sta. 3) females bearing eggs. July samples contained numerous ovigerous females of both species and, at Stations 3 and 5, some juveniles. The highest numbers occurred in September, at which time populations contained year-old adults plus juveniles at all stations. (See Ganz and Knowlton, 1992, for an assessment of reproductive characteristics of these populations.)
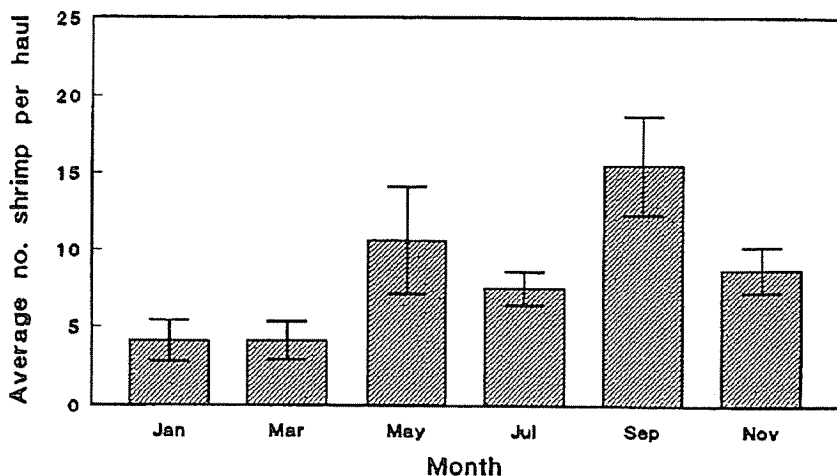
FIGURE 3. Mean number (per haul of dip net) of *Palaemonetes* individuals (both species) collected according to month (summed over the 10 sites). Each vertical bar indicates standard error of the mean.
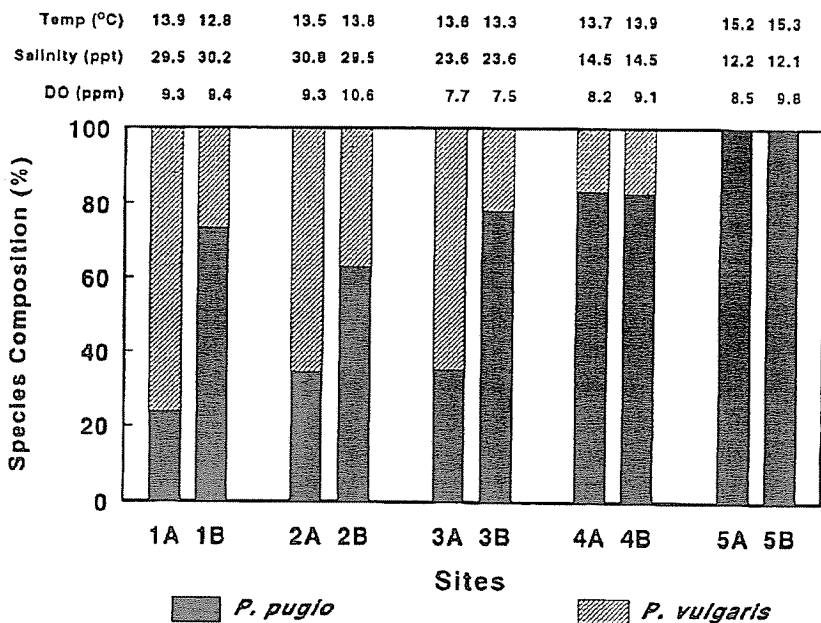

FIGURE 4. Species composition (mean % of *Palaemonetes pugio*) in samples collected at each site (summed over months), with average temperature, salinity, and dissolved oxygen (DO) levels measured. Percent *Palaemonetes vulgaris* = 100 - (% *P. pugio*), other and unknown species of *Palaemonetes* not being included in the total.

As shown in Fig. 4, which portrays average % of *P. pugio* collected over the year at the ten field sites, this species was more abundant than *P. vulgaris* at both A and B sites of Stations 4 and 5, where average salinities were less than 15 ppt; only one
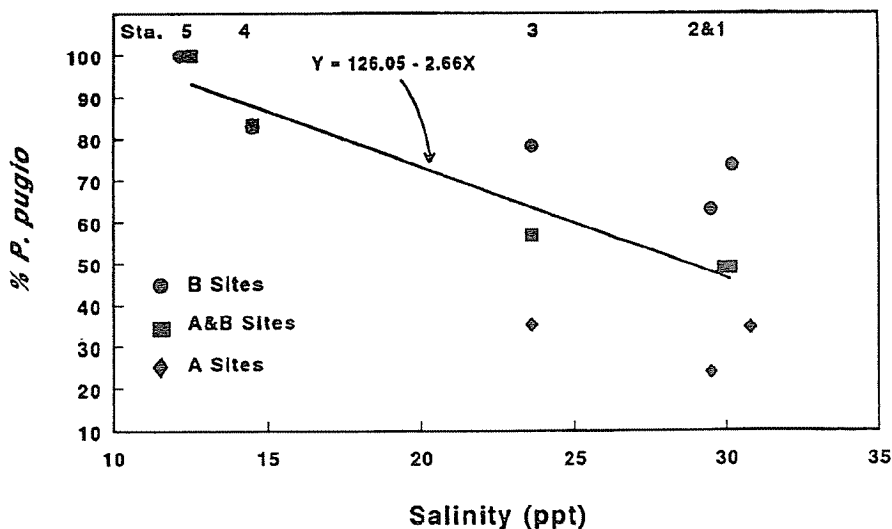
FIGURE 5. Mean % of *Palaemonetes pugio* in samples collected at each site (summed over months) in relation to mean salinity measured at each site and averaged over the year. Percent *Palaemonetes vulgaris* = 100 - (% *P. pugio*). Regression line is fitted to pooled (A & B sites) data.

P. vulgaris, a juvenile, was found at Station 5 (5B, September). At stations (1-3) where salinities were generally above 20 ppt, *P. vulgaris* dominated at A sites, *P. pugio* at B sites. The Chi-square test showed that differences in number of individuals of each species between the two sites were significant at Stations 1 (X^2 = 361.614 with df = 1; P < 0.001), 2 (X^2 = 92.845, P < 0.001), and 3 (X^2 = 321.108, P < 0.001), but not 4 (X^2 = 0.003, P = 0.959). Differences between only three sample pairs (Sta. 3, Jan.; Sta. 2, Mar.; Sta. 1, May) were not significant.

Figure 5 depicts average % *P. pugio* collected (all months) at the sites as a function of average salinity, to illustrate a linear relationship of species composition with this environmental parameter. As measured by Pearson's correlation coefficient (r) the negative correlation (r = -0.768) between % *P. pugio* and salinity is highly significant (t = 3.391 with df = 8; P = 0.009). When A and B sites are considered independently the relationship is even stronger (for A sites: r = -0.954, t = 5.502 with df = 3, P = 0.012; for B sites: r = -0.884, t = 3.270, P = 0.047). The slopes of the regression lines (-3.81 for A site data, -1.43 for B) are significantly different (calculated t = 2.908; critical value of t = 2.447 at df = 6, P = 0.05). The higher proportion of *P. pugio* (vs. *P. vulgaris*) at B (vs. A) sites of the polyhaline stations (1-3) is also seen in Fig. 5. A slight positive correlation (r = 0.488) between % *P. pugio* and temperature (A and B sites combined) was discerned but was found to be not significant (t = 1.582; P = 0.152). Virtually no correlation (r = -0.015) between *P. pugio* abundance and dissolved oxygen was exhibited (t = 0.043; P = 0.967).

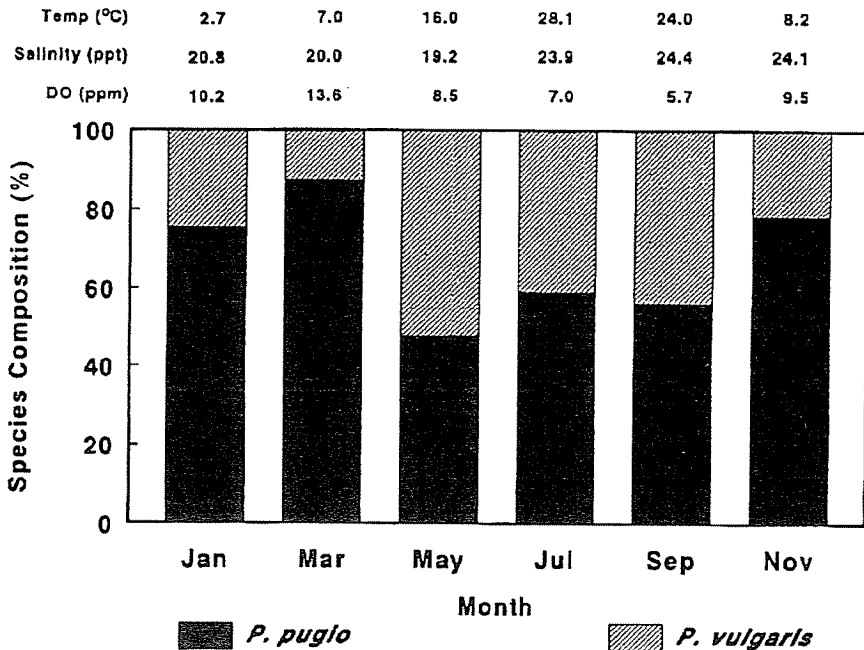| Temp (°C) | 2.7 | 7.0 | 16.0 | 28.1 | 24.0 | 8.2 |
| Salinity (ppt) | 20.8 | 20.0 | 19.2 | 23.9 | 24.4 | 24.1 |
| DO (ppm) | 10.2 | 13.6 | 8.5 | 7.0 | 5.7 | 9.5 |

FIGURE 6. Species composition (mean % of *Palaemonetes pugio*) in samples collected each month (summed over sites), with average temperature, salinity, and dissolved oxygen (DO) levels measured. Percent *Palaemonetes vulgaris* = 100 - (% *P. pugio*).

In Fig. 6 average % *P. pugio* collected at all sites during a particular month is indicated. It can be seen that relative abundance of each species was around 50% during the late spring and summer, and that *P. pugio* was proportionately more abundant (> 75%) during the colder months (Nov.-Mar.), when measured salinities were also lower (especially Jan.-Mar.) and oxygen readings higher. The correlation between % *P. pugio* and temperature is fairly strong ($r = -0.725$) but not statistically significant ($t = 2.107$ with $df = 4$; $P = 0.103$). The relationship with salinity is very weak ($r = -0.074$) and not significant ($t = 0.148$; $P = 0.890$). However, the correlation with dissolved oxygen is high ($r = 0.806$) and significant at $P = 0.053$ ($t = 2.724$).

Samples taken at four specific areas of Station 3, where substrate differences could be clearly discerned, differed with regard to relative abundance of the two species. On wood, *P. vulgaris* was more numerous (75.3% of individuals in sample) than *P. pugio* (24.7%), while *P. pugio* dominated in samples taken from shell (93.3%), sand (98.0%), and mud (100%).

Distributions Under Laboratory Conditions

Results from the preliminary "homogeneous substrate" experiment are shown in Table 1. These data (two runs combined) demonstrate a random distribution pattern of individuals of each species (separately, at a density of 50 per aquarium) because frequencies of occurrence (of animals in the cubicles) display little deviation from a Poisson series (that gives the frequency with which groups of 0, 1, 2,...

TABLE 1. Actual number of cubicles in aquarium grid (frequency) occupied by a given density of individuals* (no. individuals per cubicle) compared with theoretical number (expected frequency) reflecting a random distribution over a homogeneous substrate (sand).

No. individuals per cubicle	*Palaemonetes pugio*		*Palaemonetes vulgaris*	
	Frequency	Expected frequency	Frequency	Expected frequency
0	11	14.97	13	16.18
1	22	21.75	25	22.25
2	22	15.80	16	15.30
3	9	7.65	9	7.01
4	0	2.78	1	2.41

*In each run, 50 individuals of one or the other species were added to an aquarium with a 32-cubicle grid, but data were obtained from two runs for each species separately. Thus, the sum of (cubicle) frequencies = 64, as data from each run are pooled. The sum of the products (frequency x no. individuals per cubicle) is slightly less than 100 because a few animals were situated off the bottom when counted.

will be encountered if distribution is random). Calculated X^2 for *P. pugio* was 3.684, for *P. vulgaris* X^2 = 1.033 (critical value of X^2 = 5.991 at df = 2, P = 0.05). In the satellite study, it was found that distributions were generally random as long as shrimp densities did not exceed 100 per tank. Specifically, *P. pugio* distributions were random 80% of the time (n = 30), *P. vulgaris* 77%, under these conditions. At densities of 150 and 200 animals per tank, random distributions resulted only 10% and 7% of the time, respectively. Data from the mixed-species runs were similar, except that non-random patterns occurred about 50% of the time when 150 animals (75 of each species) or 200 animals were tested. Also, at the higher densities counts often exceeded 100% of the actual number of animals put into the tank, indicating that the animals were moving about more and that the same animal was occasionally being counted again. For these reasons, the main experiment was conducted using 60 animals per tank in each run.

In the "heterogeneous substrates" (main) experiment, the animals demonstrated decided substrate preferences. Considering all observations (i. e., in both runs regardless of conditions), 37 ± 3% (mean ± standard error) of all animals were seen on wood, 36 ± 4% on mud, 15 ± 2% on shell, and 12 ± 2% on sand. The data for the two runs are the same except for a 5% difference in animals on sand and a 4% difference in those on wood. Means analysis of data sorted according to time of day indicated that there was no significant diel variation in this pattern.

Results of the four-way ANOVA of the arcsine-transformed data (Table 2) show that neither interaction of the four factors (species x condition x salinity x substrate) nor any three-way interaction involving salinity was significant. However, highly significant differences in substrate selection were exhibited by the two species alone and both species mixed together (species x condition x substrate; Table 2). Also, a two-factor interaction between salinity and substrate was deter-

TABLE 2. Results of four-way analysis of variance performed on arcsine-transformed percentages of experimental populations of shrimp associated with one of four different substrates in an aquarium grid (Fig. 1).

| Source of variation	Degrees of freedom	Mean square	F value	P > F
Model				
Species	1	0.0000	0.00	0.9872
Condition	1	0.0012	0.14	0.7108
Salinity	1	0.0001	0.01	0.9303
Substrate	3	3.8306	437.38	0
Species x condition	1	0.0009	0.11	0.7425
Species x salinity	1	0.0001	0.02	0.8992
Species x substrate	3	1.1018	125.81	0.0001
Condition x salinity	1	0.0001	0.01	0.9363
Condition x substrate	3	0.2901	33.13	0.0001
Salinity x substrate	3	0.2342	14.09	0.0001
Species x condition x salinity	1	0.0000	0.00	0.9692
Species x condition x substrate	3	0.0606	6.92	0.0001
Species x salinity x substrate	3	0.0127	1.44	0.2285
Condition x salinity x substrate	3	0.0111	1.27	0.2825
Species x cond. x sal. x subst.	3	0.0155	1.77	0.1523
Error	736	0.0088	—	—
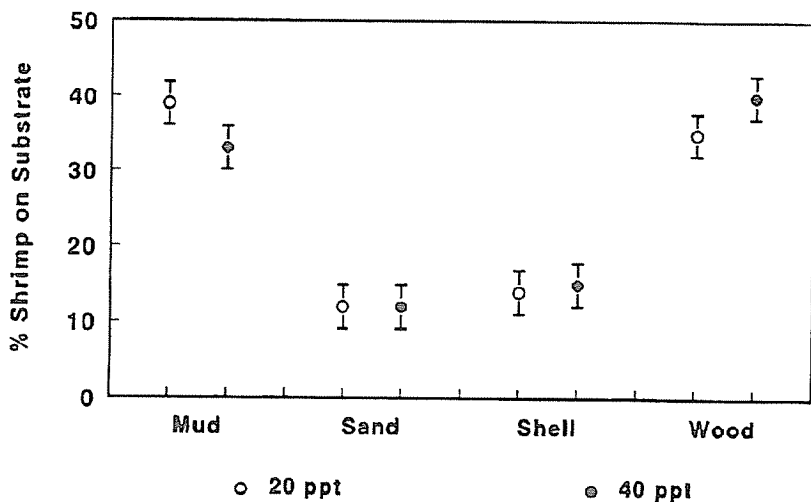
FIGURE 7. Mean % of individuals (n = 96) observed on each of four substrates grouped according to salinity level, regardless of species or "condition" (alone/mixed). Each vertical bar indicates confidence interval (JSCI) about the mean. Substrates were distributed in aquarium according to the pattern shown in Figure 1.
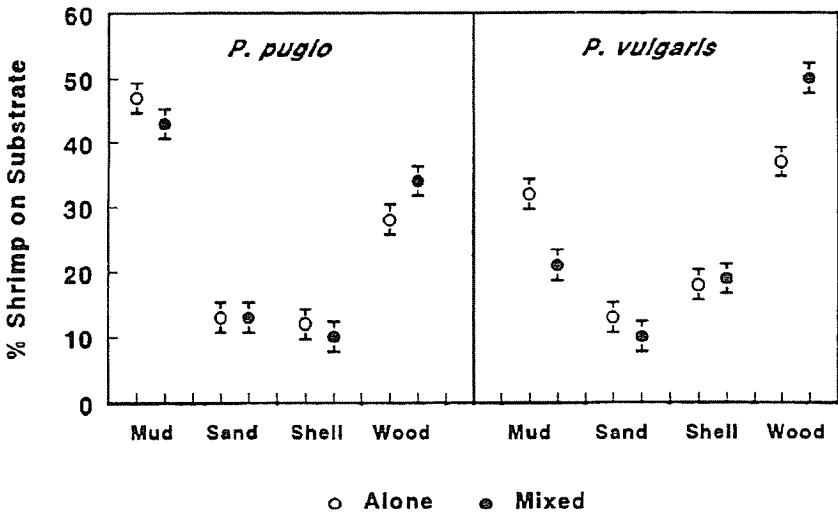
FIGURE 8. Mean % of individuals (n = 48) observed on each of four substrates grouped according to species and "condition" (the species "alone" or both species "mixed" in equal numbers), regardless of salinity level. Each vertical bar indicates confidence interval (JSCI) about the mean. See Fig. 1 for distribution pattern of substrates in aquarium.

mined to be highly significant, indicating that substrate selection of the two species (considered collectively) is influenced by salinity.

Salinity effects on substrate selection are depicted in Fig. 7. The number of animals (both species considered collectively) on sand and shell was seen to be about the same at both salinity levels, but at 20 ppt significantly more were observed on mud. At 40 ppt more animals occurred on wood than at 20 ppt, but the difference is not statistically significant.

The nature of the differences not related to salinity is illustrated in Fig. 8, which shows distributions (data from both salinity conditions pooled) of individuals grouped by species and condition. Proportionately more *P. pugio* selected mud, and more *P. vulgaris* selected wood, than any other substrate whether the other species was absent or present. Presence of *P. pugio* resulted in a highly significant increase in average number of *P. vulgaris* on wood and a highly significant decrease in the number on mud. A "shift" from mud to wood by *P. pugio* in the presence of *P. vulgaris* also occurred, but it was of lesser magnitude, the increase in number of *P. pugio* on wood being significant, the decrease in number on mud not so. Both species were considerably less numerous on shell and sand (vs. mud and wood). *Palaemonetes pugio* appeared on these substrates in about equal numbers, while *P. vulgaris* was significantly more common on shell. Numbers of one species on sand and shell did not differ radically in the presence of the other. Because effects of species, condition, and substrate are tied significantly together, neither the two-way effects involving any of these factors nor the independent effect of each of them can be assessed.

DISCUSSION

In the present study, abundance of both species was found to be greatest in September — the result of an influx of juveniles recruited from eggs hatched throughout the reproductive period (April-September). Similarly, peak densities of *P. pugio*, especially juveniles, in late summer-fall have been noted in populations inhabiting Bissel Cove, Rhode Island (Nixon and Oviatt, 1973; Welsh, 1975), North Inlet estuary, South Carolina (Sikora, 1977), Sapelo Island, Georgia (Kneib, 1987), and Galveston Bay, Texas (Wood, 1967). On the other hand, trawling in eelgrass (*Zostera*) meadows of 1-2 m depth in lower Chesapeake Bay, Heck and Orth (1980) collected maximum numbers of newly recruited *Palaemonetes*, mostly *P. vulgaris*, in June. With onset of winter, both species were seen to be less abundant (Fig. 3), *P. vulgaris* moreso than *P. pugio* (Fig. 6), especially at the northerly oceanside stations (1 and 2) and all the shallower marshy (B) sites. While this result may be due to greater difficulties inherent in winter sampling (ice, etc.), and while the observed negative correlations between % *P. pugio* and salinity/temperature do not necessarily indicate cause and effect, it might be inferred that, in response to low temperature and low salinity of late winter and early spring, *P. vulgaris* populations tend to migrate toward the south to deeper, warmer, more saline offshore waters, whereas *P. pugio* is more likely to remain inshore (perhaps even moving in to habitats vacated by *P. vulgaris*). Thorp and Hoss (1975) found that both species are quite tolerant of low temperature and rapid decreases (e. g., 10 to 2 °C); nevertheless Thorp (1976) observed a marked wintertime decrease in *P. vulgaris* abundance (relative to *P. pugio*) in two shelly areas at the mouth of the Newport River estuary. Chambers (1982) also noted a paucity of *P. vulgaris* during the winter months. In addition, Thorp's observation of greater numbers of *P. vulgaris* occupying cages in lower tidal zones, where temperature variations are less pronounced, indicates that this parameter may be a significant determinant of spatial distribution.

Results of the station and site analysis (Fig. 4) demonstrate that in the field *P. vulgaris* is more numerous at the marina (A) sites, in which wooden structures dominate, but only if the salinities are above 20 ppt. On the other hand, *P. pugio* is more evident on the "flat" marshy substrates (sand, mud, shell, peat, etc.) of the B sites and at low salinities (< 15 ppt) in general. Also, results from the October Station 3 census indicate that *P. vulgaris* is more likely to occupy wood and *P. pugio* the other substrates. Our field observations are in agreement with those of Thorp (1976), who found that *P. pugio* always occurred on muddy substrates and, with *P. vulgaris*, usually in shelly areas as well; the latter species was much less abundant on mud, especially in oligohaline creeks (wooden structures were not examined by him). The distribution along a salinity gradient (Fig. 5) may be accounted for, at least in part, by species differences in physiological tolerance of salinity and osmoregulatory capability. Knowlton and Schoen (1984) noted little difference in survivorship between the two species within the range 25-45 ppt, but much better survival of *P. pugio* below 20 ppt.

Data (e. g., Table 1) from pilot laboratory experiments established that grass shrimp (both species, whether alone or mixed together) generally distribute themselves randomly in the tank when all of their substrate choices are the same. If the animals exhibit clumping on a homogeneous substrate, then clumping on an array

of substrates may not be significant, and later test results would have been rendered meaningless. The change in dispersion pattern from random (as seen in the homogeneous substrate study) to clumped (as documented in the heterogeneous substrates experiment) suggests that in the former case individuals (of one or the other species) are not inherently attracted to each other and that in the latter case they passively allow other members of the same species to rest on the same substrate block.

Results of the main laboratory experiment (Figs. 7 and 8), in which individuals were presented a choice of substrates, are in agreement with the field-derived data. Following the same pattern seen in the field, in the laboratory setting *P. vulgaris* was most abundant on the wooden blocks occupying some of the cubicles, *P. pugio* on those covered with a mud veneer. In a similar study conducted by Arguin et al. (1989) both species exhibited a preference for wood, *P. vulgaris* moreso than *P. pugio*. The number of *P. pugio* (and *P. vulgaris*) on shell and sand was about the same in this and the present study, but *P. pugio* abundance on mud was considerably less (18%), and on wood more (39%), in the former experiment. A possible reason for this discrepancy is the fact that the mud used in the present experiment was more recently collected. This species is known to feed on selected mud meiofauna (Sikora, 1977; Bell and Coull, 1978; Smith and Coull, 1987) and detritus (Adams and Angelovic, 1970; Welsh, 1975); the "fresher" mud sample may have contained more food of this sort, thus rendering it more attractive. Also, in the Arguin et al. study the wooden blocks were positioned higher off the substrate (i.e., propped up a few cm on the partition edges in an effort to mimic the natural environment). Thus, more animals might have settled on the wood simply because, as a projection, it might have been encountered more often by animals swimming above the bottom. The presence of relatively large numbers of *P. pugio* on wood in both studies is consistent with Everett and Ruiz' (1993) observations (in the mesohaline Rhode River subestuary of Chesapeake Bay) of greater *P. pugio* densities around natural and experimental units of coarse woody debris (vs. areas lacking it).

The ANOVA (Table 2) showed that substrate selection in our experiment was influenced by salinity, a greater number of animals occurring on wood at 40 ppt than at 20 ppt, and significantly more animals being seen on mud at the lower salinity (Fig. 7). In mixed-species trials Arguin et al. (1989) found that at 20 ppt both species cohabited the wood in about equal numbers but at 40 ppt *P. vulgaris* was significantly more abundant on this substrate. From this they hypothesized that *P. vulgaris* is able to displace *P. pugio* from the "preferred" wood substrate at the higher salinity but not the lower one. In the present study, *P. vulgaris* predominated numerically over *P. pugio* on wood at both salinity levels. Differences in substrate selection attributable to both salinity and species were consistent but not statistically significant (P = 0.2285; Table 2) in our study.

According to the ANOVA, responses of *P. pugio* and *P. vulgaris* to a choice of substrates were not only significantly different, this choice was significantly influenced by the presence/absence of the other species, suggesting that some degree of interspecific competition occurs. Observations (Chambers, 1981, 1982; Ronald Gallin, unpublished data) of one-on-one interactions indicate that *P. vulgaris*, with stronger chelae, is indeed more aggressive and capable of displacing *P. pugio*. This may explain why a greater (average) number of *P. vulgaris* were seen on wood in

the mixed-species runs of our experiment (Fig. 8), i.e., it would be easier for individual *P. vulgaris* to "take over" the wooden blocks when half the individuals in the run were *P. pugio* rather than other *P. vulgaris*. Displacement of one species from a particular substrate by the other species, and reasons for it, could not be demonstrated in our study, in light of the results of the main experiment (significant factor interaction effects) and lack of documentation of the resources (e. g., types and amounts of food) they may compete for. However, displacement of *P. pugio* by *P. vulgaris* from shell to mud has been indicated in field and laboratory experiments carried out by Thorp (1976).

It is concluded that abundance and spatial distribution of *P. pugio* and *P. vulgaris* is strongly influenced by salinity and substrate, perhaps also temperature. Other factors such as tides and currents, water depth, light intensity, presence/absence of predators (e. g., the killifish *Fundulus*) and covering vegetation/woody debris, are also known to be important (Sikora, 1977; Heck and Orth, 1980; Coen et al., 1981; Kneib, 1984, 1987; Posey and Hines, 1991; Khan, 1992; Everett and Ruiz, 1993; Ruiz et al., 1993) but were not investigated in this study. Noting the decrease in *P. vulgaris* abundance (relative to *P. pugio*) with decreasing average salinities at the five stations (Fig. 5) and virtual absence of this species where ambient salinity was less than 13 ppt (Sta. 5), it is quite clear that salinity is an important abiotic factor limiting the distribution of *P. vulgaris* in Chesapeake Bay. In areas where *P. vulgaris* is absent, *P. pugio* is ubiquitous on wood as well as mud, sand, and shell. In areas of higher salinity, where the two species are sympatric, differential substrate selection may be an important aspect of resource partitioning, minimizing competition and promoting species coexistence. The reasons for the apparent preference for mud by *P. pugio*, and wood by *P. vulgaris*, as exhibited both in the field and in the laboratory experiment, have yet to be discerned. A mud surface offers an array of food types (meiofauna, detritus, etc.) favored by *P. pugio*. On the other hand, *P. vulgaris*, with stronger chelae, may be "attracted" to a wooden surface in order to prey on the epifauna harbored by algae, bryozoans, tunicates, and other encrusting organisms; or to seek protection from predation (as Everett and Ruiz, 1993, have demonstrated for *P. pugio*). Yet, in the laboratory experiments, *P. vulgaris* (and, to a lesser extent, *P. pugio*) frequented wood that was bare (i. e., bore no macroscopic periphyton), and in the field shrimp were commonly seen on pilings with little algal growth. Also, unanswered questions remain regarding the mechanism used by one species to displace the other, but our results support Thorp's (1976) contention that interference competition is an important separating mechanism for these two species where they are sympatric.

LITERATURE CITED

Adams, S. M., and J. W. Angelovic, 1970. Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeake Sci., 11:249-254.

Arguin, P. M., R. E. Knowlton, and T. A. Aldaghlas, 1989. Salinity and substrate as factors affecting seasonal abundance and spatial distribution of *Palaemonetes* spp. (grass shrimp). Am. Zool., 29:65A.

Anderson, G., 1985. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico) — Grass shrimp. U.S. Fish Wildl. Serv., Biol. Rep. 82 (11.35):19 pp.

Bell, S. S., and B. C. Coull, 1978. Field evidence that shrimp predation regulates meiofauna. Oecologia, 35:141-148.

Bowler, M. W., and A. J. Seidenberg, 1971. Salinity tolerance of the prawns, *Palaemonetes vulgaris* and *P. pugio*, and its relationship to the distribution of these species in nature. Va. J. Sci., 22:94.

Bryan, G. W., 1976. Heavy metal contamination in the sea. *In*: R. Johnston (ed.), Marine Pollution. Academic Press, London, pp. 185-302.

Chambers, R., 1981. Seasonal feeding and distribution of *Palaemonetes pugio* and *P. vulgaris* in Great Sippewissett salt marsh. Biol. Bull., 161:324.

Chambers, R. M., 1982. Partial niche partitioning and trophic analysis of the grass shrimps *Palaemonetes pugio* and *P. vulgaris* in Massachusetts salt marshes. M. S. special problems report, Univ. Massachusetts, Amherst. 54 pp.

Coen, L. D., K. Heck, Jr., and L. Abele, 1981. Experiments on competition and predation among shrimps of seagrass meadows. Ecology, 62:1484-1493.

Everett, R. A., and G. M. Ruiz, 1993. Coarse woody debris as a refuge from predation in aquatic communities. Oecologia, 93:475-486.

Ganz, H. H., and R. E. Knowlton, 1992. Species, seasonal, and latitudinal variations in grass shrimp (*Palaemonetes*) reproductive characteristics. Am. Zool., 32:72A.

Glantz, S. A., 1987. Primer of Biostatistics. McGraw-Hill, New York. 379 pp.

Heck, K. L., Jr., and R. J. Orth, 1980. Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay — Decapod Crustacea. Estuaries, 3:289-295.

Holthuis, L. B., 1952. A general revision of the Palaemonidae (Crustacea Decapoda Natantia) of the Americas. II. The subfamily Palaemonidae. Allan Hancock Found. Occas. Pap. No. 12: 396 pp.

Khan, R. N., 1992. Effects of substrate, macrophytic cover and predation upon the distribution of two sympatric species of grass shrimp, *Palaemonetes pugio* and *Palaemonetes vulgaris*. Ph. D. dissertation, George Washington Univ., Washington. 115 pp.

Kneib, R. T., 1984. Patterns of invertebrate distribution and abundance in the intertidal salt marsh: causes and questions. Estuaries, 7:392-412.

Kneib, R. T., 1985. Predation and disturbance by grass shrimp, *Palaemonetes pugio* Holthuis, in soft substratum benthic invertebrate assemblages. J. Exp. Mar. Biol. Ecol., 93:91-102.

Kneib, R. T., 1987. Seasonal abundance, distribution and growth of postlarval and juvenile grass shrimp (*Palaemonetes pugio*) in a Georgia, USA, salt marsh. Mar. Biol., 96:215-223.

Knowlton, R. E., and D. F. Kirby, 1984. Salinity tolerance and sodium balance in

the prawn *Palaemonetes pugio* Holthuis in relation to other *Palaemonetes* spp. Comp. Biochem. Physiol., 77A:425-430.

Knowlton, R. E., and R. H. Schoen, 1984. Salinity tolerance and sodium balance in the prawn *Palaemonetes vulgaris* (Say) compared with *P. pugio*. Comp. Biochem. Physiol. 79A:519-524.

Knowlton, R. E., and A. B. Williams, 1970. The life history of *Palaemonetes vulgaris* (Say) and *P. pugio* Holthuis in coastal North Carolina. J. Elisha Mitchell Sci. Soc., 86:185.

Morgan, M. D., 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol. Oceanogr., 25:896-902.

Nelson, W. G., 1981. Experimental studies of decapod and fish predation on seagrass macrobenthos. Mar. Biol. Prog. Ser., 5:141-149.

Nixon, S. W., and C. A. Oviatt, 1973. Ecology of a New England salt marsh. Ecol. Monogr., 43:463-498.

Odum, W. E., and E. J. Heald, 1972. Trophic analysis of an estuarine mangrove community. Bull. Mar. Sci., 22:671-738.

Posey, M. H., and A. H. Hines, 1991. Complex predator-prey interactions within an estuarine benthic community. Ecology, 72:2155-2169.

Ruiz, G. M., A. H. Hines, and M. H. Posey, 1993. Shallow water as a refuge habitat for fish and crustaceans in non-vegetated estuaries: an example from Chesapeake Bay. Mar. Ecol. Prog. Ser., 99:1-16.

SAS Institute, 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute, Inc., Cary, N. C. 956 pp.

Sikora, W. N., 1977. The ecology of *Palaemonetes pugio* in a southeastern salt marsh ecosystem with particular emphasis on production and trophic relationships. Ph. D. dissertation, Univ. South Carolina, Columbia. 122 pp.

Smith, L. D., and B. C. Coull, 1987. Juvenile spot (Pisces) and grass shrimp predation on meiobenthos in muddy and sandy substrata. J. Exp. Mar. Biol. Ecol., 105:123-136.

Sokal, R. R., and F. J. Rohlf, 1981. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Co., New York. 859 pp.

Thorp, J. H., 1976. Interference competition as a mechanism of coexistence between two sympatric species of the grass shrimp *Palaemonetes*. J. Exp. Mar. Biol. Ecol., 25:19-35.

Thorp, J. H., and D. E. Hoss, 1975. Effects of salinity and cyclic temperatures on survival of two sympatric species of grass shrimp (*Palaemonetes*), and their relationship to natural distributions. J. Exp. Mar. Biol. Ecol., 18:19-28.

Uguccioni, D. M., and M. H. Posey, 1992. Predation by grass shrimps, *Palaemonetes pugio*, on hard clams, *Mercenaria mercenaria*: size and substrate interactions. J. Elisha Mitchell Sci. Soc., 108:29-34.

Welsh, B. L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology, 56:513-530.

Williams, A. B., 1984. Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida. Smithsonian Inst. Press, Washington. 550 pp.

Wood, C. E., 1967. Physioecology of the grass shrimp, *Palaemonetes pugio*, in the Galveston Bay estuarine system. Univ. Texas Contrib. Mar. Sci., 12:54-79.