

Appendix B

“Dead Marsh” BIOBLAST Overview Georgia Coastal Ecosystems (GCE) LTER Program

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Summary

On October 16, 2002, a team of scientists and graduate students from the Georgia Coastal Ecosystems LTER accompanied DNR Coastal Resource Division staff on a site visit to the Jericho River in Liberty County, GA, where the salt marsh is exhibiting signs of die-off (the so-called “dead marsh” phenomenon). The group performed transects at 3 sites exhibiting signs of die-back (one in an area that was completely devoid of vegetation and two that were only partially denuded) as well as at a nearby control site. Each transect was a total of 200 feet in length and samples were taken for analysis of both physical (water and soil) and biological (plants, animal, microbes) characteristics. Interstitial water samples are being analyzed for salinity, sulfide and sulfate levels, dissolved inorganic nitrogen concentrations, and potentially for metals. Soil samples are being analyzed for salinity and mineral content. Plants were identified, counted, classified as live (either tall or short shoots) or dead, and samples were obtained for plant tissue content (CNS, potentially metals). Infaunal animals were sampled with cores and field counts were made of crab holes and periwinkle snail density (*Littoraria irrorata*). Samples of both stems and rhizomes obtained from live and dead areas are being analyzed to characterize bacterial and fungal community composition. Samples were also obtained to perform two growth trials in a greenhouse: one to determine whether *Spartina alterniflora* rhizomes from the dead marsh are viable if given fresh water and the second to determine whether transplants from a nearby healthy marsh can survive in soil from the dead marsh site.

General Observations

The most extensive die-offs are upstream of I-95, but there are also abundant smaller die-offs downstream. The places where dead and dying marsh have been observed do not exhibit an obvious pattern. In areas where the main channel was curving, the die-offs appeared somewhat more extensive on the inside (accreting) side of the channel rather than on the outside (eroding) side.

We have made a preliminary classification into the following four categories: marshes where only the grass along the creekbank is dead; marshes where a live creekbank occurs in front of a fairly large patch of dead area; marshes that are completely denuded; and marshes where live and dead areas are interspersed that do not readily fit any of the above patterns (Figure 1). Many of the observations made on the ground along the Jericho River fall into the first category, where only the area where tall-form *Spartina* generally occurs along the creekbank has been

affected. The second type of marsh die-off pattern, where a live creekbank fronts a dead area, is readily obvious in aerial photographs but this type of pattern was not observed on the ground. In a few cases small patches of live tall-form *Spartina* on little mud islands (usually sections of creekbank that had slumped into the creek to form small bars) were observed, and there were also some areas where mid-marsh stands of *Spartina* were observed to be less dense than healthier creek areas. The third type of marsh die-off looks essentially like a mud flat, with areas where the marsh has started to visibly slough off into the water. The fourth type is more difficult to categorize except to say that there is no obvious pattern to the places where live and dead plants occur.

The plants that were affected by the die-off are *S. alterniflora* and *Juncus roemerianus*. Where both species were affected, *Juncus* was affected equally or more severely than *Spartina*. The upland border, with *Juncus* and shrubs, was not usually affected, and there have been observations of *Salicornia* species invading bare mud and also of live *Borrchia frutescens* in an otherwise bare area. Where *S. alterniflora* has been affected there is very little standing dead (or brown marsh), as has been described in Louisiana. However, large patches of standing dead plants were observed in the *Juncus* marsh.

Macroinvertebrates (*Littoraria irrorata*, *Geukensia demissa*, *Uca pugnax*) appeared reasonably abundant in both *Spartina*- and *Juncus*-dominated marshes, with few dead shells littering the marsh. At the *Juncus* marsh site (Figure 2), high numbers of *Melampus bidentatus* were massed in groups in small depressions at the base of plants. Most were large adults, and they were distributed all the way to the creekbank, which is generally not observed at the GCE marsh sites. Although densities appeared higher than normal, it may have been that the snails were aggregated and just easier to see due to the lack of plant cover. Plants at this site also showed signs of grasshopper grazing.

Methods:

Transects Three transects were performed at “dead marsh” sites along the Jericho River (Figure 2). Transect A was in an area that was completely denuded, although evidence of *S. alterniflora* was visible from the dead stubs remaining in the soil. Transects B and C were on the opposite sides of the marsh from transect A in areas where live and dead *S. alterniflora* was interspersed with bare mud. Transect D was a control and was performed in a marsh that contained live, healthy *Spartina* plants (estimated at > 95%) and was considered unaffected. Note that none of these transects were done in a *Juncus* marsh.

Each transect was 200 feet long and was permanently marked with PVC poles at the creekbank end and the inland end. Water, soil and plant samples were taken at 5 points (every 50 feet) along each transect. Epifaunal animal observations were recorded at each sample point and cores for infaunal animals were collected at 0, 100, and 200 feet along each transect. Samples were also collected for a comparative analysis of microbial (bacterial and fungal) composition in live and dead areas near Transect A and in the healthy site near Transect D.

Interstitial water samples were collected with PVC sippers at a depth of approximately 15 cm in the rooting zone of *S. alterniflora*. Salinity was measured with a refractometer. Sulfide samples (10 ml) were collected in acid-washed glass vials and immediately fixed with ZnAc and kept cold. The remaining water was returned to the laboratory, where aliquots were filtered through GF/F filters for analysis of dissolved inorganic nitrogen (NH_4 , $\text{NO}_2 + \text{NO}_3$). Additional water was filtered through 0.2- μm acrodisc filters and fixed with HNO_3 for sulfate analysis. It

may also be possible to perform additional analyses on these samples for dissolved minerals and heavy metals.

Soil samples were collected from a depth of approximately 15 cm in the *S. alterniflora* rooting zone. Each sample was separated into two aliquots: one for measurement of interstitial salinity (using the dry/wet weight method routinely used in the GCE) and one for routine analysis of salts, metals, etc.

Plants were identified, counted, and classified as live or dead. Dead plants were quantified by counting vegetation stubs (no standing dead plants were observed). Live plants were categorized as either tall (> 15 cm) or short (< 15 cm) shoots. Tissue samples were obtained for plant tissue CNS content and potentially for metals.

Infaunal animals were sampled with cores (10 cm diameter x 15 cm deep), refrigerated until they could be washed through a 500 μm mesh sieve, and fixed in 10% formalin with Rose Bengal stain. Organisms were sorted from organic material and debris and transferred to 70% ethanol for preservation. Aliquots of the material passing through the 500 μm mesh screen were subsequently washed over a 63 μm mesh sieve to collect meiofaunal organisms. These samples were preserved as above. Animals in each core will be identified to the lowest taxonomic level possible and counted. Field counts were made of crab holes and periwinkle snail density (*L. irrorata*) in a manner similar to that being performed as part of the LTER invertebrate sampling protocol. The quadrat size used for the crab hole counts was 500 cm^2 and that for the snail counts was 2500 cm^2 . No *M. bidentatus* were observed along these transects.

Microbial community composition is being analyzed on samples obtained from *S. alterniflora* leaves and rhizomes collected at the “Dead” marsh from patches of live *S. alterniflora* adjacent to Transect A (at a distance of approximately 400 feet from the creekbank) and at the reference site (Transect D). Both bacterial and fungal DNA is being extracted from these samples for determination of community composition via molecular methods. Samples are also being examined microscopically to identify fungi.

Additional field work *S. alterniflora* “scalloped” edges (borders where live *Spartina* and dead marsh are clearly defined, typically along a creekbank) were flagged adjacent to Transects B and C to follow future changes in border position on the creekbank.

Greenhouse trials Samples were also obtained to perform two growth trials in a greenhouse: one to determine whether *S. alterniflora* rhizomes from the dead marsh are viable if given fresh water and the second to determine whether transplants from a nearby healthy marsh can survive in soil obtained from the dead marsh site. For the rhizome viability trial, five blocks of soil (approximately 25 cm^2) were collected from both denuded areas and live areas near Transect A and transported back to the greenhouse in Athens. These pots are being watered regularly and any new growth will be monitored.

Soil samples for the transplant trial (25 cm^2 blocks) were collected from both the denuded marsh (Transect A) and the control site (Transect D). Healthy seedlings, ranging in size from 5 – 15 cm, were collected from the control site (Transect D). In the greenhouse, *S. alterniflora* was transplanted into 5 replicate pots from each site as well as 5 pots filled with a sand/peat moss (75/25) combination as a control. Two plants (one larger and one smaller) were transplanted into each pot. Survival and plant height are being monitored.

Results to date

Most of the analyses described here are ongoing. Results to date are limited to field observations of salinity, plant characteristics, and epifauna.

Salinity Salinity was measured two ways: on water samples obtained via PVC sippers and by rehydrating dried soil samples with a known amount of deionized water. Salinity in the water samples ranged from 26 to 35. These were approximately the same as salinities obtained in the soil samples, which ranged from 21 to 36 (Table 1).

Table 1. Salinities (PSU) along each transect, obtained by measuring interstitial water or soil samples.

Distance (ft)	0	50	100	150	200
Transect A - water	26	31	NA	30	32
- soil	25	24	25	29	31
Transect B - water	27	30	30	34	NA
- soil	33	23	36	32	30
Transect C - water	NA	28	33	35	34
- soil	21	31	34	28	24
Transect D - water	30	33	NA	28	27
- soil	28	34	26	36	28

Plants *S. alterniflora* was the dominant grass in all transects. (*J. roemerianus* was observed near Transect A but was not part of the transect). *S. alterniflora* characteristics varied among the transects (Figure 3). In Transect A (the denuded marsh), the area contained mostly dead vegetation stubs and no live stems were observed. In Transect B live plants were observed over the first 100 feet of the transect, and dead stubs were observed between 50 and 200 feet. Transect C was patchy, with high densities of dead stubs observed in the middle of the transect. Transect D, the healthy marsh, had a fairly even distribution of live plants (averaging 12.6 ± 3.4 plants per 500 cm^2). No dead stubs were recorded at this site, but they may have been covered by the incoming tide. The proportion of tall ($> 15 \text{ cm}$) versus short ($< 15 \text{ cm}$) shoots was fairly similar in all locations where live plants were observed (Figure 4).

Epifauna The density of *L. irrorata* showed interesting differences among the four transects (Figure 5). In Transect A, where no live plants were present, no snails were found at all. In transects B and C, snail densities were highest at the 0 and 50 foot sampling sites (where they ranged between 20 and 48 snails per m^2). These locations did not correspond to the highest densities of either live or dead plants. In the reference Transect (D), snail densities were much lower. For comparison, *L. irrorata* densities observed across GCE sites in October 2001 averaged 17 m^{-2} at creekbank sites and 181 m^{-2} at mid-marsh sites.

The number of crab holes was highest at the creekbank in the dead marsh site (Figure 6). Crab hole density was considerably lower in the other two impacted sites, ranging from 0 to 9 holes per 500 cm^2 . Crab holes were not counted at Transect D because the flooding tide had submerged the site and holes could not be located. During the concurrent GCE sampling, crab hole density averaged 13 ± 9 per 500 cm^2 and ranged from 0 to 70.

Figure 1. Dead marsh types. Photographs taken along the Georgia coast in spring 2002 show die-off categories discussed in the text: die-off concentrated at the creekbank (top left); die-off behind the creekbank (top right); die-off affects the entire marsh (bottom left); die-off pattern is erratic (bottom right).



Figure 2. Study site. Circles show the location of the sites along the Jerricho River that are discussed in the text. The site furthest upstream is the site of the *Juncus* marsh; sites labeled A through D are where transects were sampled.

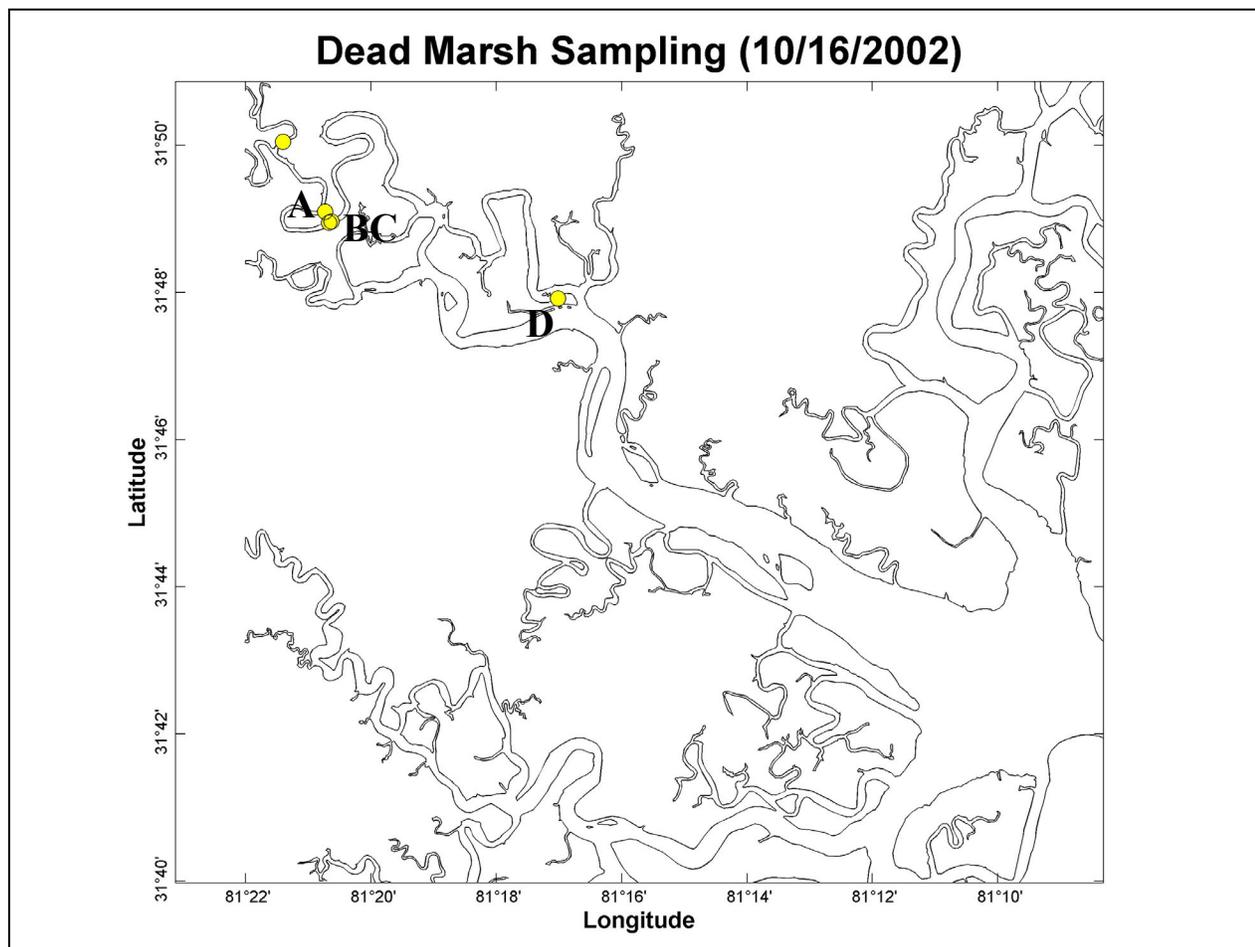


Figure 3. Distribution of *Spartina alterniflora*. The four graphs depict the number of live and dead *S. alterniflora* stems along Transects A through D.

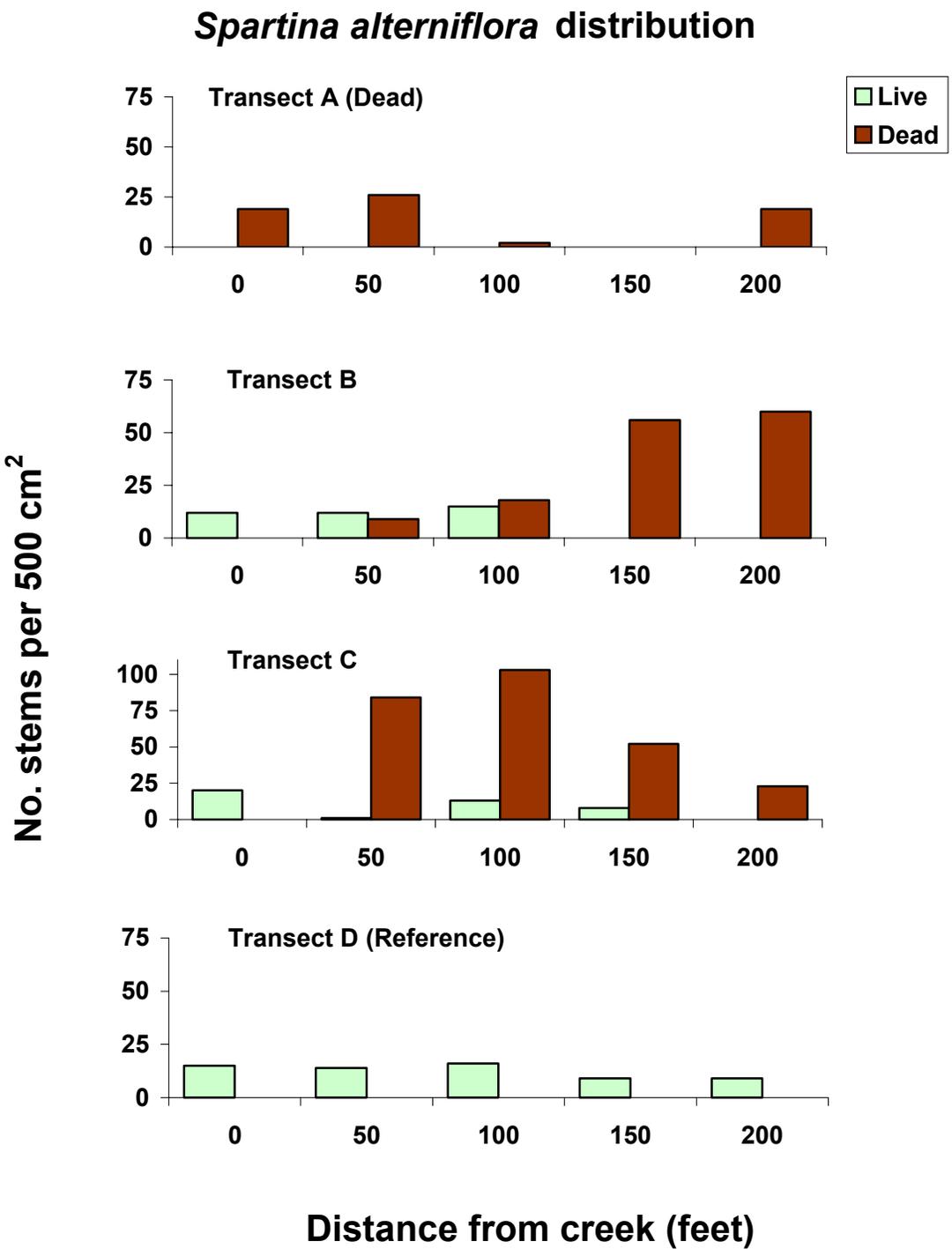


Figure 4. Classification of live *S. alterniflora*. The four graphs depict the number of live shoots classified as tall (> 15 cm) or short (< 15 cm) along each transect.

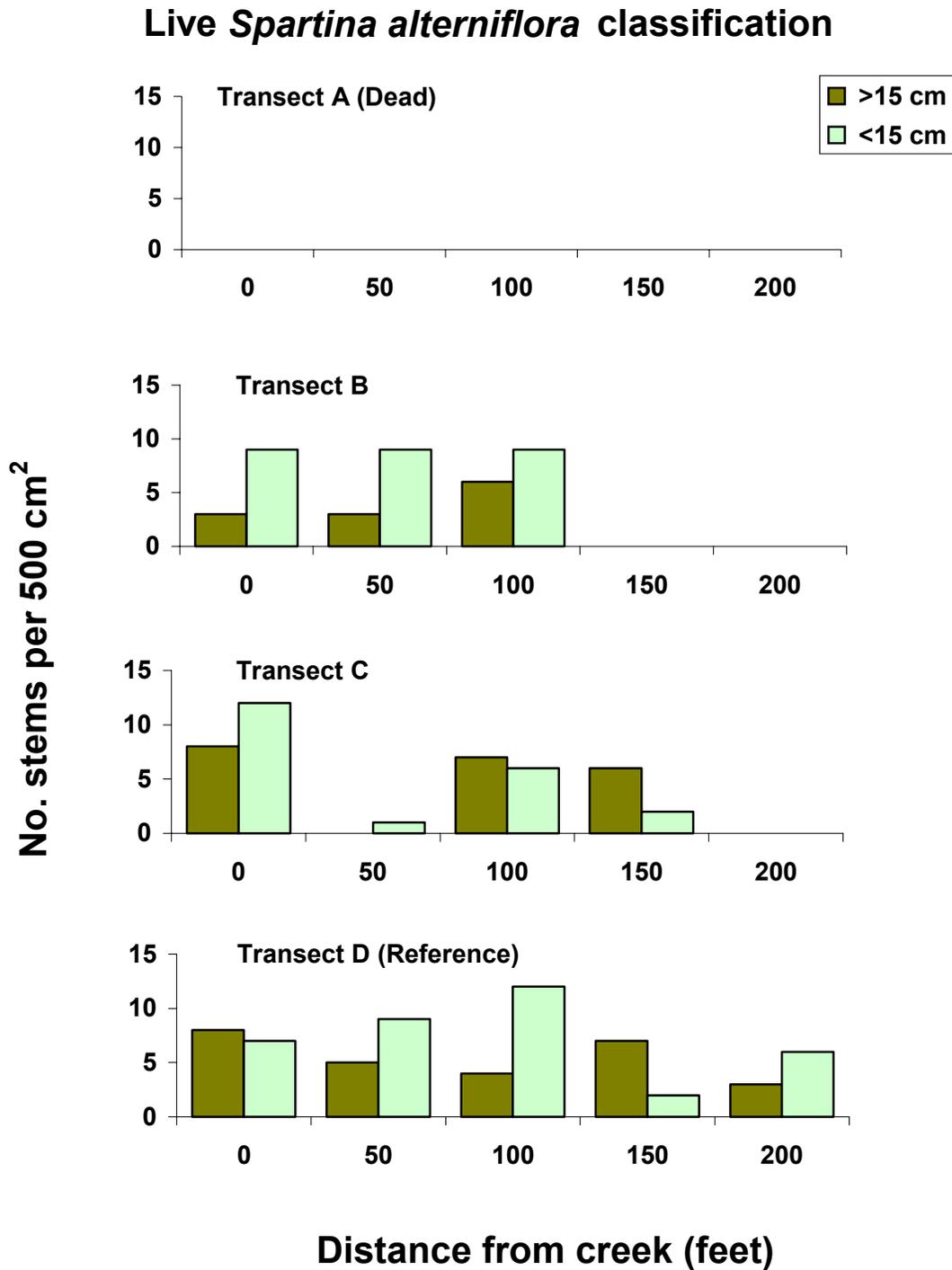


Figure 5. Distribution of *Littoraria irrorata*. The four graphs depict the density of *L. irrorata* along each transect.

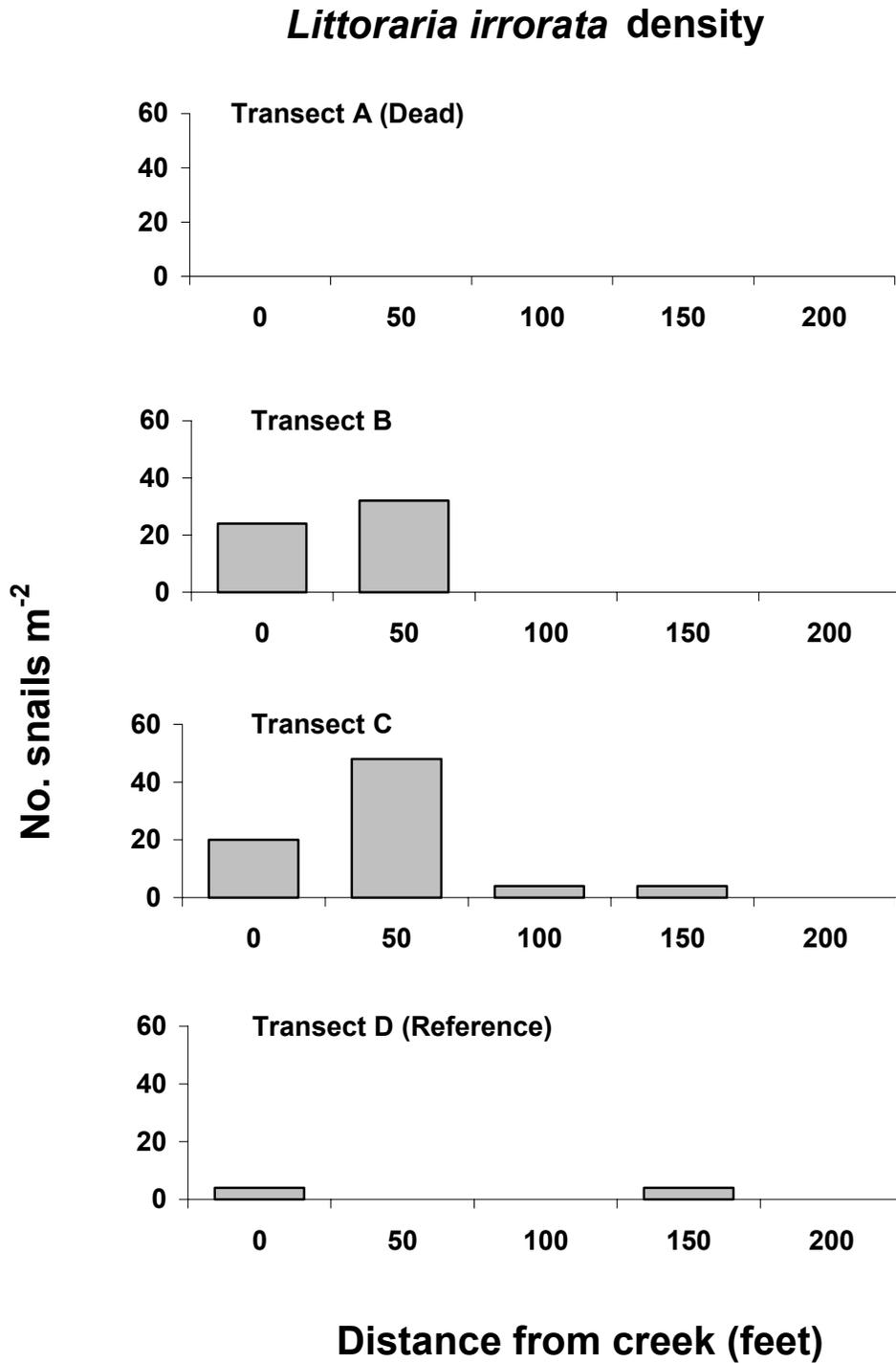


Figure 6. Distribution of crab holes. The four graphs depict the density of crab holes along each transect.

