SALT MARSH DIEBACK: THE RESPONSE OF *SPARTINA ALTERNIFLORA* TO DISTURBANCES AND THE CONSEQUENCES FOR MARSH INVERTEBRATES

by

CAROLINE ROCHESTER MCFARLIN

(Under the Direction of Merryl Alber)

ABSTRACT

Spartina alterniflora is a foundation species that plays a disproportionately critical role in salt marshes, as it ameliorates chemical and physical stress to other plants and animals, provides essential habitat, protection from predators, and a source of organic matter to associated fauna. Disturbances including sudden dieback, herbivore overgrazing, and wrack deposition can lead to a loss of *Spartina* and thus, indirectly affect the invertebrate community. My goals were 1) to examine the effects on the invertebrate communities in 2 different geographical regions (GA, LA) and among 4 different disturbances within a region (GA), 2) to determine whether various disturbances would elicit a similar and predictable physiological response (the DMSO:DMSP ratio, and metal load) in *Spartina* that could be used as a sensitive and predictable indicator of stress among various disturbance types, and 3) to document the never before described long-term trajectory and patterns of recovery from sudden dieback in a *Spartina* and *Juncus roemerianus* marsh.

Spartina loss in GA and LA led to similar decreases in *Littoraria irrorata* (periwinkle snails), but there were strong differences in the responses of infauna between

the states and among years. These results suggested context-dependency in both the effect of foundation species within a geographical region and in the evaluation of the ecosystem service provided at the time of sampling. Overall and despite differing results, it was found that *Spartina* was ultimately was important in maintaining the invertebrate communities in both states. However, within a geographical region, both the physiological response of *Spartina* and the indirect response of the invertebrates to *Spartina* loss were similar and predictable among four different disturbances. The DMSO:DMSP ratio and metal loads were increased in affected *Spartina* plants often responsive in otherwise green leaves) and periwinkle snails and benthic macroinfauna (density, taxon richness, and diversity) were significantly decreased in affected areas, regardless of disturbance type. Vegetation recovery at sudden dieback is occurring slowly (on the order of a decade) via rhizomes extension from healthy areas, and thus understanding the effects to invertebrates is important, as disturbances such as these are expected to increase with climate change and anthropogenic effects.

INDEX WORDS: foundation species, salt marsh dieback, *Spartina alterniflora*, *Juncus roemerianus*, benthic macroinfauna, *Littoraria irrorata*, DMSP, DMSO, metals, chlorophyll *a*

SALT MARSH DIEBACK: THE RESPONSE OF *SPARTINA ALTERNIFLORA* TO DISTURBANCES AND THE CONSEQUENCES FOR MARSH INVERTEBRATES

by

CAROLINE ROCHESTER MCFARLIN

B.S.A., The University of Georgia, 2000

M.S., The University of Georgia, 2004

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2012

SALT MARSH DIEBACK: THE RESPONSE OF *SPARTINA ALTERNIFLORA* TO DISTURBANCES AND THE CONSEQUENCES FOR MARSH INVERTEBRATES

by

CAROLINE ROCHESTER MCFARLIN

Major Professor:	Merryl Alber
Committee:	T. Dale Bishop Jeb Byers William Fitt Steven C. Pennings

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2012

DEDICATION

To the loves of my life ~ Brady and Chip.

ACKNOWLEDGEMENTS

First, I would like to my family for their love and support – without them, this dissertation would not have been possible. I would also like to sincerely thank my advisor Merryl Alber who has been a great friend and mentor over the years. She is also an excellent editor, and although I didn't always look forward to writing yet another draft, she greatly improved this dissertation. I owe her a new pack of pencils! I thank my Alber Lab office mates past and present – Christine, Sylvia, Justine, and Natalie – for all of their encouragement and support over the years – they are the BEST and always keep me laughing! I thank Dale Bishop for patiently teaching me the infaunal invertebrate taxonomy and for good conversation, but mostly for the wonderful, gourmet meals that he shared with me while on Sapelo Island (especially after a long day of work in the marsh). I thank Joan Sheldon, Steve Pennings, and Susan Alber for statistical advice. I thank my Ph.D. committee for volunteering their time and for providing useful suggestions. I also thank all of those who have helped me with lab and field work along the way, especially Zane Cress, Ashley Rich-Robertson, Caitlin Yeager, Sylvia Schaefer, Christine Hladik, Galen Kaufman, Kristen Anstead, Jacob Shalack, Jenny Fenton, Galen Kaufman, and Trey Kenemer. I thank both the Moran and Whitman labs (and especially Leong-Keat Chan and Chris Reisch) for providing access to lab equipment and for helping me with methodology for *Spartina* DMSP analysis. I thank both John Fry and Doug Hoffman of Cumberland Island NPS for providing me with access to field sites. I

thank the Georgia Coastal Ecosystems (GCE) – LTER for funding my dissertation work. Last of all, I am thankful for Red Bull, Monster Energy, and Starbucks for providing me with the final push to the finish!

. . . Oh, what is abroad in the marsh and the terminal sea?

Somehow my soul seems suddenly free . . .

An excerpt from "The Marshes of Glynn"
Sydney Lanier, 1878

TABLE OF CONTENTS

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Background

Foundation species are those that single-handedly "create and define [the] entire ecological community or ecosystem" (Ellison et al. 2005). In a salt marsh, *Spartina alterniflora* serves this function by ameliorating soil and porewater conditions (Bertness 1991, Bertness and Shumway 1993), supplying a source of organic matter (Peterson et al. 1985, Currin et al. 1995), providing both above and belowground habitat (Rader 1984, Zimmerman et al. 1984, Healy and Walters 1994), and protecting organisms from predation (Kneib 2000, Silliman and Bertness 2002). Salt marsh benthic invertebrates depend heavily on *S. alterniflora,* and many of these species in turn form the basis of trophic transfers of salt marsh primary production to estuarine food webs (Kneib 2000).

The loss of an important foundation species such as *S. alterniflora* would be expected to dramatically affect the invertebrate community by altering habitat availability and environmental conditions (Pennings and Bertness 2001, Bruno et al. 2003, Ellison et al. 2005). The primary goal of this dissertation was to understand the consequences of *S. alterniflora* loss on invertebrates due to various disturbances that occur in low latitude salt marshes, including the recently described phenomenon of sudden dieback that occurred in the Gulf and Southeast. I was also interested in evaluating plants for signs of stress in disturbed marshes, and in documenting their recovery from disturbance. Below I

provide a brief review of disturbance and plant stressors in salt marshes, followed by an overview of the dissertation.

1.2. Marsh Disturbances

Bare areas in the marsh can be created by both biotic and physical disturbances. Wrack is probably the most common physical disturbance in southeastern marshes (Pennings and Bertness 2001). Wrack deposition typically causes damage when either the wrack mat is large (those from 100 m^2 to >1000 m^2) or resides on the marsh surface for a longer period of time (3-4 months has been reported to cause damage, regardless of mat thickness; Valiela and Rietsma 1995). Mats deposited higher in the marsh, therefore, typically cause the most damage as they become stranded by the tides (Valiela and Rietsma 1995). Bertness and Ellison (1987) monitored survivorship and recovery of wrack-induced bare patches in a northeastern marsh under experimental burial manipulations of 2-3 cm deep. Plants (*Spartina patens* and *Juncus gerardii*) survived for about 7 weeks underneath wrack coverage, and bare areas left behind took ~2-3 years for a full recovery to take place (Bertness and Ellison 1987). Other investigators have also noted a similar recovery time following wrack-induced bare patches (Reidenbaugh and Banta 1980, Tolley and Christian 1999).

Biotic disturbances caused by herbivore overgrazing can also lead to bare patches. Although early studies in the salt marsh suggested that herbivory is a minor factor controlling production as compared to bottom-up forces (e.g. nutrients, soil biogeochemistry, etc.) (Smalley 1959, 1960, Teal 1962), many recent studies argue that a loss of top predators, nutrient enrichment, and the introduction of invasive species have

allowed greater negative top-down impacts on primary producers (see the review by Gedan et al. 2009). For example, Jeffries and colleagues have documented long-term increases to the lesser snow goose population from ~0.8 to over 4 million between the late 1960's and the mid-1990's, as agricultural fields are a ready source of food (Abraham and Jeffries 1997, Jefferies and Rockwell 2002, Jefferies et al. 2003). The increased numbers of geese, largely unchecked by their herring gull predators, denude salt marshes in their Canadian summer feeding grounds and dig up roots and rhizomes before new growth has begun. These feeding events have caused secondary impacts and have set up a negative feedback loop for marsh recovery: plants are unable to resprout, thereby increasing erosion and evapotranspiration on the marsh surface, which creates stressful physicochemical conditions that further limit plant colonization success (Jeffries and Rockwell 2002). The introduction of other vertebrates (such as nutria, cattle, horses, and pigs) to coastal areas has led to decreases in salt marsh vegetation as well (Evers et al. 1998, Smith and Odum 1981, Turner 1987). For instance, horses introduced on Cumberland Island, GA tend to focus their grazing to the same patches of high marsh area so that biomass is low in these areas indefinitely (Turner 1987).

The increased consumption of marsh plants by native marsh invertebrates has been more recently documented in cases when predators are absent or plants are already experiencing stressful conditions (Silliman and Bertness 2002, Silliman et al. 2005, Holdredge et al. 2008). For instance, heavy grazing and burrowing disturbance by the *Sesarma* crab is proposed to have caused plants to die along the marsh creekbanks of Cape Cod, MA in 2004 due to loss of predators (tautog, night heron, blue crab) (Holdredge et al. 2008), and manipulation studies in GA have shown that high densities

of *Littoraria irrorata* ($\geq$ 600 ind m^{-2}) can lead to bare areas when blue crab predators are decreased (Silliman and Bertness 2002).

More recently, sudden dieback events have been described in both the Gulf and the Southeast. These events contrast to that of other disturbances in that they were characterized by a *sudden* loss of vegetation and had no obvious cause. The dieback progressed from yellowing and thinning vegetation to rhizome stubble, and eventually to bare areas (Figure 1.1.). The rapid onset of dieback in 2000-2002 was associated with a severe drought (as indicated by the NOAA's Palmer Drought Severity Index) in both regions, and affected >800 ha of marsh vegetation in Georgia (primarily *S. alterniflora*, but also *Juncus roemerianus*) and >100,000 ha Louisiana (*S. alterniflora*) (McKee et al. 2004, Ogburn and Alber 2006, Alber et al. 2008). Studies in both states showed that soil conditions (pH, salinity, redox potential, sulfides) following the dieback were similar to that of healthy areas (McKee et al. 2004, Ogburn and Alber 2006). McKee et al. (2004), however, did report elevated levels of metals in the soil and in standing dead plant tissues, which suggested that oxidation of the soils (due to extreme desiccation) could have initially resulted in a low pH at the time of dieback and led to the availability of metals (iron, aluminum) to plant tissues (2004). No standing dead plants were available in dieback areas in GA for a similar comparison (Ogburn and Alber 2006). Notably, however, there were references to dry, cracked soil surfaces in the dieback areas of both states that would be consistent with this idea. Since that time, there have been several new sudden dieback sites reported in GA that coincided with a drought in 2008 (Alber 2008, McFarlin, pers. obs.). There is also evidence that herbivores (periwinkle snails) can increase bare patches caused by the sudden dieback. In these cases high densities of

snails (at least >400 ind m^{-2}) are reported to move in "fronts" which expand the vegetation loss along the dieback border (Silliman et al. 2005).

Other accounts of bare areas in marshes can be linked to human disturbances. Anthropogenic inputs or activities that result in bare areas include oil spills (Pezeshki et al. 2000, Hester and Mendelssohn 2000), dams and water diversions (Turner 1990, Turner and Boyer 1997), canals (Boesch et al. 1994, Bass and Turner 1997), diking or ditching (Smith and Carullo 2007), dredging (Linthurst and Seneca 1980), construction of bridges, docks and causeways (Edwards and Frey 1977, Smith and Carullo 2007), and boating traffic (Smith and Carullo 2007). All of these modifications are likely to increase pressure on marshes, which can lead to lower resiliency to natural disturbances (Hughes et al. 2003, Gedan et al. 2009).

1.3. Plant Stressors in Salt Marshes

The above discussion describes disturbances that can result in the reduction or loss of marsh plants. However, plants may exhibit physiological responses long before there are visible signs of stress (Mendelssohn and McKee 1992). Dimethylsulfonio-propionate (DMSP) is a secondary metabolite commonly synthesized from the amino acid methionine by many marine algae, a few marine grasses, and sugarcane, although synthesis pathways vary (Kocsis et al., 1998), but the exact role of DMSP in *S. alterniflora* is not clear (Otte et al., 2004). Regardless of its function, research by Husband and Kiene (2007) showed that when *S. alterniflora* was under stress, there was direct conversion of DMSP to dimethylsulfoxide (DMSO), an oxidation product. They reported higher DMSO:DMSP ratios in senescing (yellowing) plants as compared to

healthy (green) plants and also in roots as compared to stems and leaves. Kiehn and Morris (2010) also found support for this idea, as DMSP concentrations of *S. alterniflora* were lowest near dieback areas and increased with distance from the dieback edge.

There is also evidence that metal concentrations could increase in the leaves of stressed plants. Toxic heavy metals (Fe, Al) become more soluble and bioavailable to vegetation in aerated marsh soils (Portnoy 1999), as one might expect in bare patches. Furthermore, McKee et al. (2004) showed that in drought-stricken sudden dieback areas, desiccated soils had increased in Al and Fe concentrations, which likely led to the increased concentrations of metals observed in *S. alterniflora* leaves there.

Other stress signals that have been looked at include altered concentrations of adenine nucleotides (and specifically, the adenylate energy charge ratio), proline concentrations, CO_2 uptake, water use efficiencies, alcohol dehydrogenase activities, and leaf spectral reflectances (Mendelssohn and McKee 1992, Ewing et al. 1995a,b, Mendelssohn et al. 2001, Hester et al. 2001). Most of these metrics have been evaluated under manipulated greenhouse conditions and have translated poorly as consistent signals of stress in the field (Ewing et al. 1995a, 1995b, 1997). Further, many are stressor-specific and are not appropriate measures for multiple types of disturbances. For instance, glutathione is often used to evaluate plants that are subject to metal contamination (Mendelssohn and McKee 1992, Pennings et al. 2002), and Ewing et al. found that although salinity stress was best indicated by altered proline concentration, nutrient stress was best indicated by leaf spectral reflectance, CO_2 uptake, or adenine nucleotide levels (Ewing et al. 1995 a,b). Therefore, it would be useful to find an indicator metric that responds consistently in multi-stressor field situations and across

multiple types of disturbance. A particularly valuable indicator would be able to detect plant stress prior to obvious symptoms (such as the loss of chlorophyll).

1.4. Overview of Dissertation

In Chapter 2 of this dissertation, I evaluated how the loss of foundation species (due to sudden dieback) would affect benthic invertebrates in two geographically distinct regions (GA and LA) that experience different hydrogeomorphic conditions. In Chapter 3, I evaluated the DMSO:DMSP ratio, chlorophyll concentration, and leaf metal concentration of *S. alterniflora* within disturbed areas as compared to healthy marsh, in order to see if the response is similar among four common salt marsh disturbances (sudden dieback, mammalian grazing, snail grazing, and wrack deposition), and thus predictable with stress. In Chapter 4, I compared the effect of a loss of *S. alterniflora* due to these same four disturbances on the invertebrate community. Lastly, in Chapter 5, I described the patterns of vegetation, invertebrate fauna, and soil porewater conditions in sudden dieback sites in GA over 7 years of following the disturbance.

References

Abraham, K.F., and R.L. Jefferies. 1997. High goose populations: causes, impacts and implications. In: B.D.J. Batt (ed.), *Arctic ecosystems in peril: a report of the Arctic Goose Habitat Working Group*. U.S Fish and Wildlife Service Washington, D.C., and Canadian Wildlife Service, Ottawa, Ontario.

Alber, M., Swenson, E.M., Adamowicz, S.C., and Mendelssohn, I.A., 2008. Salt marsh dieback: An overview of recent events in the US. *Estuarine, Coastal and Shelf Science* 80: 1-11.

Bass, A.S., and Turner, R.E., 1997. Relationships between salt marsh loss and dredged canals in three Louisiana estuaries. *Journal of Coastal Research* 13: 895-903.

Bertness, M.D. 1991. Interspecific interactions among high marsh perennials in a New England salt marsh. *Ecology* 72: 125:137.

Bertness, M.D. and A.M. Ellison. 1987. Determinants of pattern in a New England marsh plant community. *Ecological Monographs* 57: 129-147.

Bertness, M.D., and S.W. Shumway. 1993. Competition and facilitation in marsh plants. *The American Naturalist* 142 (4): 718-724.

Boesch, D.F., M.N. Josselyn, A.J. Mehta, J.T. Morris, W.K. Nuttle, C.A. Simenstad, D.J.P. Swift. 1994. Scientific assessment of coastal wetland loss, restoration and management in Louisiana. *Journal of Coastal Research*, Special Issue No. 20.

Bruno, J.F., J.J. Stachowicz, and M.D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18 (3): 119-125.

Currin, C.A., S.Y. Newell, and H.W. Paerl. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Marine *Ecology Progress Series* 121: 99-116.

Edwards, J.M., Frey, R.W.. 1977. Substrate characteristics within a Holocene salt marsh, Sapelo Island, Georgia. *Senckenbergiana Maritime* 9, 547–562.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliott, C.R. Ford, D.R. Foster, B.D. Kloeppel, J.D. Knoepp, G.M. Lovett, J. Mohan, D.A. Orwig, N.L. Rodenhouse, W.V. Sobczak, K.A. Stinson, J.K. Stone, C.M. Swan, J. Thompson, B. Von Holle, and J.R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3 (9): 479-486.

Evers, D.E., C.E Sasser, J.G. Gosselink, D.A. Fuller, and J.M. Visser. 1988. The impact of vertebrate herbivores on wetland vegetation in Atchafalaya Bay, Louisiana. *Estuaries* 21: 1-13.

Ewing, K., K.L. McKee, and I.A. Mendelssohn. 1997. A field comparison of indicators of sublethal stress in the salt-marsh grass *Spartina patens*. *Estuaries* 20 (1): 48-65

Ewing, K., K.L. McKee, I.A. Mendelssohn, and M. Hester. 1995 (a). A comparison of sub-lethal nutrient stress in the salt marsh grass, *Spartina patens*. *Environmental and Experimental Botany:* 35 (3): 331 343.

Ewing, K., K.L. McKee, I.A. Mendelssohn, and M. Hester. 1995 (b). A comparison of indicators of sublethal salinity stress in the salt marsh grass, *Spartina patens (Ait.)* Muhl. *Aquatic Botany* 52: 59-74.

Gedan, K.B., B.R. Silliman, and M.D. Bertness. 2009. Centuries of human-driven change in salt marsh ecosystems. *Annual Review of Marine Science* 1: 117-141.

Healy, B. and K. Walters, Oligochaeta in *Spartina* stems: the microdistribution of Enchytraeidae and Tubificidae in a salt marsh, Sapelo Island, USA. *Hydrobiologia* 278: 111-123.

Hester, M.W. and I.A. Mendelssohn, 2000. Long-term recovery of a Louisiana brackish marsh plant community from oil-spill impact: vegetation response and mitigating effects of marsh surface elevation. *Marine Environmental Research* 49, 233-254.

Hester, M.W., I.A. Mendelssohn, and K.L. McKee. 2001. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. *Environmental and Experimental Botany* 46: 277-297.

Holdredge, C., M.D. Bertness, and A.H. Altieri. 2008. Role of crab herbivory in die-off of New England Salt Marshes. *Conservation Biology* 23 (3):672-679.

Husband, J.D., and R.P. Kiene. 2007. Occurrence of dimethylsulfoxide in leaves, stems, and roots of *Spartina alterniflora*. *Wetlands* 27 (2): 224-229.

Jefferies, R.L., and R.F. Rockwell. 2002. Foraging geese, vegetation loss and soil degradation in an Arctic salt marsh. *Applied Vegetation Science* 5 (1): 7-16.

Jefferies, R.L., R.F. Rockwell, and K.F. Abraham. 2003. The embarrassment of riches: agricultural food subsidies, high goose numbers, and loss of Arctic wetlands – a continuing saga. *Environmental Reviews* 11 (4): 193-232.

Kiehn, WM, J.T. Morris. 2010. Variability in dimethylsulfoniopropionate (DMSP) concentrations in *Spartina alterniflora* and the effect on *Littoraria irrorata*. *Marine Ecology Progress Series* 406: 47-55.

Kneib, R.T., 2000. Salt marsh ecoscapes and production transfers by estuarine nekton in the southeastern United States. In: M.P. Weinstein and D.A. Kreeger (eds), *Concepts and Controversies in Tidal Marsh Ecology*, pp. 261-291.

Kocsis, M.G., K.D. Nolte, D. Rhodes, T.L. Shen, D.A. Gage, and A.D. Hanson. 1998. Dimethylsulfoniopropionate biosynthesis in *Spartina alterniflora* - Evidence that S-

methylmethionine and dimethylsulfoniopropylamine are intermediates. *Plant Physiology* 117 (1): 273-281.

Linthurst, R.A., and E.D. Seneca, 1980. Dieback of salt-water cordgrass (*Spartina alterniflora* Loisel.) in the lower Cape Fear Estuary of North Carolina: an experimental approach to re-establishment. *Environmental conservation* 7: 59-66.

McKee, K.L., I.A. Mendelssohn, and M.D. Materne. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecology and Biogeography* 13 (1):65-73.

Mendelssohn, I.A., and K.L. McKee. 1992. Indicators of environmental stress in wetland plants. In: D.H. McKenzie, D.E. Hyatt, and V.J. McDonald (eds.), *Ecological Indicators*, Vol. 1, pp. 603-624. Elseiver, New York.

Mendelssohn, I.A., K.L. McKee, and T. Kong. 2001, A comparison of physiolobical indicators of sublethal cadmium stress in wetland plants. *Environmental and Experimental Botany* 46: 263-275.

Ogburn, M.B., Alber, M., 2006. An investigation of salt marsh dieback in Georgia using field transplants. *Estuaries and Coasts* 29: 54–62.

Otte, M.L., G. Wilson, J.T. Morris, and B.M. Moran. 2004. Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants. *Journal of Experimental Botany* 55 (404): 1919-1925.

Pennings, S.C., and M.D. Bertness. 2001. Salt marsh communities. In: M.D. Bertness and M.E. Hay (eds.), *Marine community ecology*. Sunderland, Massachusetts: Sinauer.

Pennings, S.C., V.D. Wall, D.J. Moore, M. Pattanayek, T.L. Buck, and J.J. Alberts. 2002. Assessing salt marsh health: A test of the utility of five potential indicators. *Wetlands* 22 (2): 405-414.

Peterson, B.J., R.W. Howarth, and R.H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227: 1361-1363.

Pezeshki, S.R., M.W. Hester, Q. Lin, and J.A. Nyman. 2000. The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review. *Environmental Pollution* 108 (2): 129-139.

Portnoy, J.W. 1999. Salt marsh diking and restoration: Biogeochemical implications of altered wetland hydrology. *Environmental Management* 24(1): 111-120.

Rader, D.N. 1984. Salt marsh benthic invertebrates: small-scale patterns of distribution and abundance. *Estuaries* 7: 4 13-420.

Reidenbaugh, T.G., and W.C. Banta, 1980. Origin and effects of *Spartina* wrack in a Virginia salt marsh. *Gulf Research Reports* 6: 393-401.

Silliman, B.R. and M.D. Bertness, 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Sciences* 99(16): 10500-10505.

Silliman, B.R., J. van de Koppel, M.D. Bertness, L.E. Stanton, and I.A. Mendelssohn. 2005. Drought, snails, and large-scale die-off of Southern U.S. Salt Marshes. *Science* 310: 1803-1806.

Smalley, A.E., 1959. The role of two invertebrate populations, *Littorina irrorata* and *Orchelimum fidicinium* in the energy flow of a salt marsh ecosystem. Ph.D. thesis, University of Georgia, Athens.

Smalley, A.E., 1960. Energy flow of a salt marsh grasshopper population. *Ecology* 41: 672–77.

Smith, J.P., Carullo, M., 2007. Survey of potential marsh dieback sites in coastal Massachusetts. Massachusetts Bays National Estuary Program and Massachusetts Office of Coastal Zone Management. URL: http://www.mass.gov/czm/docs/pdf/marsh_dieback.pdf, 25 pp.

Smith, T.J., and W.E. Odum. 1981. The effects of grazing by snow geese on coastal salt marshes. *Ecology* 62 (1):98-106.

Teal, J.M. 1962. Energy flow in the salt marsh ecosystem of Georgia. *Ecology* 43:614–24.

Tolley, P.M., and R.R. Christian, 1999. Effects of increased inundation and wrack deposition on a high salt marsh plant community. *Estuaries*: 22: 944-954.

Turner, R.E. 1990. Landscape development and coastal wetland losses in the Northern Gulf of Mexico. *American Zoologist* 30 (1):89-105.

Turner, R.E., 1987. *Relationship between canal and levee density and coastal land loss in Louisiana*. U.S. Fish and Wildlife Service, Biological Report 85 (14), Washington, D.C.

Turner, R.E., and M.E. Boyer. 1997. Mississippi River diversions, coastal wetland restoration/creation and an economy of scale. *Ecological Engineering* 8 (2):117-128.

Valiela, I., and C.S. Rietsma. 1995. Disturbance of salt-marsh vegetation by wrack mats in Great-Sippewissett-Marsh. *Oecologia* 102 (1):106-112.

Zimmerman, R.J., T.J. Minello, and G. Zamora, 1984. Selection of vegetated habitat by brown shrimp, *Paneaus aztecus*, in a Galveston Bay salt marsh. *Fishery Bulletin* 82: 325-336.

Figure 1.1. The onset and progression of sudden dieback in the salt marsh from yellowing and thinning *S. alterniflora* to standing dead and rhizome stubble. Top: Thinning vegetation near the St. Simons in 2007 (photo by M. Alber). Bottom: Standing dead stems and rhizome stubble at near the Torres Causway in 2008 (photo by C. McFarlin).

CHAPTER 2

THE EFFECT OF SUDDEN MARSH DIEBACK ON THE BENTHIC INVERTEBRATE COMMUNITIES OF *SPARTINA ALTERNIFLORA* SALT MARSHES [1]

[1] McFarlin, C.R., T.D. Bishop, M. Hester, M. Alber. To be submitted to *Ecology*.

Abstract

Sudden dieback of the salt marsh grass *Spartina alterniflora* occurred in both GA and LA in 2000-2002. I used these dieback events as a natural experiment to examine the consequences of the loss of a foundation species on habitat provisioning for benthic invertebrates. During the fall of 2006-2008, I sampled infauna (meiofauna >63 μm, macroinfauna >500 μm) and epifauna (crabs, snails, bivalves) in bare (dieback) areas and nearby reference marshes on Sapelo Island, GA and in Port Fourchon, LA, as well as in transplanted plots that encompassed a range of *S. alterniflora* densities.

In GA, abundances of all invertebrate groups (epifauna, macroinfauna, meiofauna) were significantly lower in bare as compared to reference areas, as was taxon richness and diversity of macroinfauna. In LA, abundances of periwinkle snails were significantly lower in bare areas, but in contrast to GA, meiofauna densities were significantly higher in bare areas (there were no trends in the abundance of macroinfauna and infaunal crabs or in infaunal taxon richness and diversity). These results suggest that the idea of foundation species may not be "one-size fits all" for salt marsh ecosystems across their geographical range. In this case, the contrasting response to plant loss may be due to hydrogeomorphic differences between the two states: LA is microtidal and bare plots were persistently wetter than those in GA, which is macrotidal and bare plots occurred at a higher elevation. Additional physical disturbances in each state (drought in GA; a hurricane in LA) led to decreases in the density and taxon richness of all invertebrate groups (by 20-100%) in both bare and reference areas in 2008. Losses were larger in bare plots as compared to reference plots for the benthic infauna. These results

suggest that even when *S. alterniflora* does not provide habitat provisioning *per se*, it still functions as a buffer against additional disturbance.

*2.*1. Introduction

Foundation species play a disproportionately critical role in biological communities. These species (e.g. trees, corals, mangroves, seagrasses, and oysters) define the structure of a community, and facilitate important ecosystem processes such as nutrient cycling, sedimentation, carbon sequestration, and soil stabilization (Dayton 1972, Lawton 1994, Ellison et al. 2005). Through their presence, they add niche complexity, provide refugia, and ameliorate environmental stressors (i.e. by moderating abiotic conditions) (Bruno et al. 2001, Bruno et al. 2003, Ellison et al. 2005). Numerous studies have shown that foundation species increase faunal abundance and diversity in a variety of habitats: coral reefs (Taylor 1968, Sale 1977), mussel beds (Seed 1996, Norling and Kautsky 2008), kelp beds and forests (Christie 2009), tropical rain forests (Stork 1991, Terborgh 1992), hemlock forests (Rohr et al. 2009, Ellison et al. 2010), seagrass beds (Lee et al. 2001, Fredriksen et al. 2010), and oyster reefs (Boudreaux 2006, Quan et al. 2009). Additionally, many marine organisms rely on the presence of foundation species as larval settlement cues (Stockhausen and Lipicius 2003, Hadfield and Koehl 2004, Nakamura 2007, Laidig 2010).

When foundation species are lost, the consequences can be far-reaching (Ellison et al. 2005). Declines in faunal density and diversity have been reported where kelp deforestation (Graham 2004), tropical rain forest degradation (Vallan 2002), and coral reef exploitation (Wilson et al. 2006) have taken place. Because many of the organisms in these communities are highly interactive (via food webs, habitat creation and amelioration, and associational defenses), reduced species abundance and diversity in degraded habitats can result in a negative feedback to invertebrate communities (Bruno et

al. 2003, Hughes et al. 2009, Altieri and Bertness 2007). A recent meta-analysis study demonstrated this point, as it showed that for all threatened species of seagrasses, there were 10x as many associated faunal species potentially affected (Hughes et al. 2009). Even before a foundation species is completely lost there may be a functional loss where it cannot provide the same level of ecosystem services (Ellison et al. 2005). For instance, the densities of coral reef fish have declined where coral bleaching has occurred, despite the intact physical structure (Pratchett et al. 2008). Brooks et al. (1999) reported that associated species are quickly approaching extinction in areas where tropical rain forests are more fragmented.

Spartina alterniflora is considered a foundation species in salt marsh habitats, as it facilitates the establishment of the rest of the salt marsh community (Bertness 1991, Bertness and Shumway 1993, Pennings and Bertness 2001). Salt marshes are characteristically harsh environments due to the alternate exposure and flooding of the marsh surface during daily tidal cycles. When flooded, soils become anoxic and sulfides can build up to levels that are toxic to many organisms (900-3500 μM) (Hines et al. 1989). When exposed, evaporation can lead to increases in soil salinity (Adam 1990, de Leeuw et al. 1991). As a pioneer of salt marsh habitat, *S. alterniflora* is capable of colonizing inhospitable, submerged low intertidal locations where it binds, traps, and stabilizes sediment. Once established, *S. alterniflora* ameliorates chemical and physical stress to other plants and animals by oxygenating the soil and reducing sulfides (through aerynchyma and transpiration), decreasing soil salinity (through shading), and structurally dissipating wave and storm energy (Howes et al. 1986, Leonard and Luther 1995, Pennings and Bertness 2001, Bertness and Ewanchuk 2002). In addition to

providing suitable abiotic conditions, invertebrates rely on *S. alterniflora* as a source of food (Peterson and Howarth 1987, Currin et al. 1995), above and belowground habitat (Rader 1984, Zimmerman et al. 1984, Healy and Walters 1994), and protection from predation (Kneib 2000, Silliman and Bertness 2002). A loss of *S. alterniflora* would be expected to alter marsh function and faunal support dramatically (Pennings and Bertness 2001, Ellison et al. 2005).

In 2000-2002 (following record droughts), vast areas of *S. alterniflora* died back and degenerated to bare patches in GA (>800 ha) and LA (>100,000 ha) (McKee et al. 2004, Alber et al. 2008). In GA, salt marsh dieback of this extent had never been reported previously, although barren areas had been observed during periods of low rainfall (Basan and Frey 1977, Ogburn and Alber 2006). In LA, this sudden dieback event contrasted to the ongoing conversion of marsh to open water habitat in that the event occurred over a few months rather than gradually over several years, was widespread, and was not always associated with submergence (Mendelssohn and McKee 1988, McKee et al. 2004). In both states, there was a rapid progression from standing dead *S. alterniflora* to bare mud (McKee et al. 2004, Ogburn and Alber 2006, Alber et al. 2008). There were also signs of extreme desiccation, visible as dry and cracked soils that might be associated with drought (Alber et al. 2008). These dieback events provided a unique opportunity to study the effects of the loss of *S. alterniflora* on salt marsh communities in two very different settings.

The main objective of this study was to evaluate the effect of the loss of *S. alterniflora* on benthic invertebrate communities. As *S. alterniflora* is lost, both the physical structure of the habitat and the soil conditions (e.g. moisture, oxygen, salinity,

pH, etc) for benthic fauna can be diminished, and benthic microalgae (BMA) can flourish in bare marsh where more sunlight reaches the sediment (Whitcraft and Levin 2007). The lack of *S. alterniflora* also affects its capacity to provide a buffer against erosional forces (storms, winds, rainfall, tides), which can be important in protecting benthic invertebrates.

I expected to see a decrease in the density and diversity in benthic invertebrates in areas without *S. alterniflora* as compared to vegetated areas. With the decreases in invertebrates, I also expected an accompanying shift from subsurface to surface feeding types as soil conditions become physiologically more harsh in bare areas and the organic matter source shifts from *S. alterniflora* to BMA. I further expected that these responses would vary predictably along a range of *S. alterniflora* densities. Although I expected similar trends in both states, I expected to see a larger response in GA due to its greater tidal amplitude (3 m vs. < 1 m in LA), and thus increased soil exposure time during low tide.

2.2. Methods

2.2.1. Study Sites

This study was part of an EPA-funded project to compare the effects of sudden dieback in Georgia and Louisiana (Climate-linked alteration of ecosystem services in tidal salt marshes). In each state, six experimental sites were chosen based on the presence of dieback and nearby healthy *S. alterniflora* marsh. The study sites in GA were located along both the Duplin River and Doboy Sound, in a well-mixed tidal inlet next to Sapelo Island (31° 27' N 81° 15' W). The sites in LA were located at the

southernmost tip of LA in a river-dominated deltaic estuary near Port Fourchon (29° 7', 90° 12' W), which is the location of hundreds of offshore and deepwater oil rigs. In addition to differences in tidal inundation patterns (GA tides are semi-diurnal and ~ 3 m; LA tides are diurnal and < 1 m), elevation also differed greatly between the two states, with the sites in GA ranging from 0.75 m to 0.99 m and those in LA ranging from -0.01 m to 0.25 m above sea level.

Bare and reference marsh plots (each 60 m^2) were established within each of the 6 experimental sites per state (split-plot design) and these were used as the primary source of comparison in this study. Two additional bare plots (also 60 m^2) in each site were transplanted with *S. alterniflora* at two different target densities. The 24 plots in each state were accessed with an extensive boardwalk system of often >150 linear meters in order to minimize trampling.

2.2.2. Sample Collection and Processing

2.2.2.1. Epifauna

Epifauna (snails, crabs, bivalves) were sampled in bare and reference plots at all 6 sites during the fall of 2006-2008. Plants in the transplanted plots did not always thrive, but samples of each invertebrate group were opportunistically collected from well-established transplanted plots to provide observations at intermediate stem densities for regressions. I analyzed 3 replicate samples per plot for epifauna (see Table 2.1.). Snails (*Littoraria irrorata*, *Melampus bidentatus*, *Neritina usnea*) and bivalves (*Geukensia demissa*) were collected from within 2500 or 5000 cm^2 quadrats, preserved in 10% buffered formalin, and counted in the lab. Fiddler crab holes (>5 mm) were counted in

the field within a 625 cm^2 quadrat as a proxy for the number of crabs. These represent several species of *Uca* spp. (mostly *Uca pugnax* in GA and the ecological equivalent, *Uca rapax* in LA, Genoni 1991) as well as *Armases cinereum* and *Eurytium limosum*. The number of snail, mussel, and fiddler crab individuals were scaled to number per m^2. Blue crab (*Callinectes sapidus*) abundance was assessed in fall 2008 by deploying baited crab traps in reference and bare plots.

2.2.2.2. Infauna

Macroinfauna (>500 μm) and meiofauna (>63, but <500 μm) were sampled in bare and reference plots from each of 3-6 sites per state per year (Table 2.1). As with epifauna, samples from transplant plots were collected opportunistically. I analyzed 3 replicate samples per plot for macroinfauna and 1-2 replicates per plot for meiofauna. Infaunal samples were collected from each quadrat with a corer (diam. 5.2 cm x 5 cm depth). Samples were sieved, preserved in 10% buffered formalin or 100% ethanol, and stained with Rose Bengal dye. Density centrifugation with a colloidial silica (Ludox HS 40; density: 1.31 g cm^{-3}) was used in a ratio of 1:5 sample:Ludox to aid in separating meiofauna from the sediment (Burgess 2001). Meiofauna samples often had >1000 individuals in a single core. In these cases, samples were subsampled twice from a known slurry volume with a goal of attaining ~150-200 animals from each of the dominant groups (copepods and nematodes), and the 2 subsamples were averaged together and adjusted to core volume.

All meiofauna and macroinfauna individuals were identified to the lowest taxonomic level possible using a compound scope or a dissecting scope, respectively.

For macroinfauna, I determined the feeding mode for each taxon (based on classification by Craft and Sacco 2003) and calculated the percentage of surface, subsurface, and carnivorous feeders in bare and reference plots over years in each state. Meiofauna were scaled to no. per 10 cm^2 and macroinfauna were scaled to no. per 100 cm^2 to compare to other literature estimates. Taxon richness and Shannon H' diversity indices were calculated for each group.

2.2.2.3. Additional sampling

S. alterniflora stem density and the biomass of belowground soil, macro-organic matter, were both evaluated in all treatment and transplanted plots sampled in each state in 2007 and 2008. Stems were counted along with epifauna from within the 3 replicate quadrats sampled for epifauna (2500 or 5000 cm^2). Macro-organic matter (belowground biomass >500 μm) was collected from benthic invertebrate cores (discussed above), dried to a constant weight at 60°C, and weighed to the nearest 0.1 gram after all organisms were removed. These measurements were used in regression analyses in order to explore the variation of invertebrate density across the range of *S. alterniflora* density.

2.2.2.4. Isotopes

Tissues of dominant primary producers and consumers were collected from each state in 2008 to compare natural carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) isotopic ratios for determination of the food web structure in bare versus reference plots. However, there were a limited number of isotopic samples representative of organisms in both bare and

reference plots, and I was unable to make a strong comparison of the organic matter source between the treatments (Appendix A.).

2.2.3. Statistical analyses

Density and diversity indices of epifauna, macroinfauna, and meiofauna were compared among years and between bare and reference plots in each state. Each of the measured variables were analyzed using a 2-way repeated measures ANOVA for treatment (between-subjects factor), year (within-subjects factor), and the interaction of treatment*year effects. Pairwise differences among treatments and years were analyzed with a Tukey's post-hoc comparison test. Significant differences were assessed at the α=0.05 level.

Multiple regression analysis was used to explore relationships between invertebrate measurements (density, diversity), *S. alterniflora* stem density, and belowground macro-organic matter. Samples from bare and reference plots and from the intermediate transplanted density plots were used in the analysis. The ability for the independent variables (*S. alterniflora* density and macro-organic matter) to predict dependent variables (invertebrate density and diversity) was assessed using the individual *p*-value in a linear regression model. Variables where the individual *p*-value was >0.15 were removed from the model. All VIF scores were ≤1.0, indicating no collinearity. Prior to statistical testing with the ANOVA and linear regression models, variables were either natural log or square-root transformed as needed to meet assumptions of normality.

2.3. Results

2.3.1. Epifauna

2.3.1.1. Community composition

Perwinkle snails *Littoraria irrorata* and fiddler crabs *(Uca pugnax* in GA and *Uca rapax* in LA) were the dominant epifaunal species in both states (Table 2.2.). These species occurred in all 6 sites in both GA and LA, but they were not found in all plots. *Littoraria* occurred in 91% and 100% of reference plots in GA and LA, respectively, whereas none were observed in bare plots in either state. Fiddler crabs occurred in all reference (100%) and nearly all bare (98%) plots in GA, but were not as ubiquitous in LA where they occurred in only 50% of reference plots and 26% of bare plots. Taxon richness (and diversity) of the epifaunal communities were low as there were only 2 other species present in the plots of either state: the molluscs *Melampus bidentatus* (in GA only) and *Geukensia demissa* (GA and LA) and the arthropod *Callinectes sapidus* (blue crabs) (in LA only). As with the periwinkle snails, the two other mollusc species occurred only in the reference plots, but their presence within these plots was low (<50%) in each state. Blue crabs (assessed in 2008 only) were present in 100% of the LA sites, and occurred in both bare and reference plots. No blue crabs were observed in any GA site.

2.3.1.2. Density

In GA, periwinkle snail density was 0 m^{-2} in bare plots during all years and averaged from 142 ± 61 to 194 ± 68 m^{-2} in reference plots each year (overall avg. 167 ± 34 m^{-2}; Table 2.2., Figure 2.2.A.). Both treatment (p =0.0004) and year (p <0.03) were

significant sources of variation in snail density. Bare plots in GA had statistically fewer (zero) snails than reference plots in all years, and reference plots in 2008 had 17-26% fewer snails than the two previous years. The interaction term was also significant ($p <$ 0.03), indicating that the effect of treatment varied by year. In LA, periwinkle density was again 0 m^{-2} in bare plots during all years, but densities in reference plots were 4x lower than those in GA, averaging from 27 ± 7 to 64 ± 6 m^{-2} (overall avg. 41 ± 4 m^{-2}) (Table 2.2., Figure 2.2.C.). Treatment, year, and the interaction term (each $p < 0.0001$) were also significant sources of snail variation in LA. Bare *p*lots had fewer (zero) snails than reference plots, and reference plots in 2007 and 2008 had 50-56% fewer snails than in 2006.

In GA, fiddler crab density averaged from 277 ± 34 to 472 ± 61 $m\text{-}^{2}$ (overall avg. 381 ± 28 m^{-2}) in reference plots and from 147 ± 25 to 247 ± 37 $m\text{-}^{2}$ (overall avg. 198 ± 20 m^{-2}) in bare plots. Treatment (p =0.02) and the interaction term (year*treatment p =0.0004) were significant sources of variation, whereas year was not (Table 2.2., Figure 2.2.B.). Overall, bare plots had significantly fewer fiddler crabs than reference plots, but the magnitude of difference between the plots differed across years. In LA, fiddler crabs were 12x less numerous and much more variable across years than in GA, averaging from 16 ± 4 to 78 ± 14 $m\text{-}^{2}$ (overall avg. 33 ± 6 m^{-2}) in reference plots and from 4 ± 2 to 38 ± 11 $m\text{-}^{2}$ (overall avg. 14 ± 4 m^{-2}) in bare plots (Table 2.1., Figure 2.2.D.). Year and the interaction term (year*treatment) were significant sources of variation ($p < 0.0001$ each), whereas treatment was not (p=0.06) (Figure 2.2.D.). 2007 had decreased fiddler crab densities compared to 2006 and 2008, but differences between bare and reference plots were inconsistent and varied with year.

All other resident epifaunal species were 0 m^{-2} in bare plots and averaged ≤1 m^{-2} in reference plots of GA and LA in any given year, except for *Melampus bidentatus* in GA. *Melampus* ranged from 0.6 ± 0.3 to 46 ± 15 over the 3 years (overall avg. 19 ± 6 m^{-2}) in reference plots in GA (Table 2.2). Blue crab density, which was assessed with crab traps in 2008, was 0 in both treatments in GA, whereas in LA, there were significantly more crabs caught in traps in bare plots (7.0 ± 2.1 per plot) as compared to reference plots (2.7 ± 0.3 per plot).

2.3.2. Macroinfauna

2.3.2.1. Community composition

Macroinfauna occurred in all 6 sites in both states, but their occurrence was more widespread in the reference plots (reference plots: 86 ± 5%, GA and 72 ± 8%, LA; bare plots: 36 ± 8%, GA and 54 ± 9%, LA) (Table 2.2). There were also more macroinfaunal taxa present across sites in the reference plots of both states (9 taxa each),as compared to the bare plots, in which there were only 4 taxa present in GA and 6 in LA.

Annelid worms (polychaetes and oligochaetes) were the dominant infaunal organism in both states (Table 2.2.). In GA, oligochaete and polychaete worms were nearly equally abundant in each treatment plot (reference plots: 19 ± 6 vs. 21 ± 4 per 100 cm^2, respectively; bare plots: 0.8 ± 0.5 vs. 1.7 ± 0.4 per 100 cm^2, respectively). In LA, oligochaetes were much more abundant than polychaetes in each treatment plot (reference plots: 28± 7 vs. 1.3 ± 0.6 per 100 cm^2, respectively; bare plots: 43 ± 14 vs. 1.5 ± 0.9 per 100 cm^2, respectively. Other macroinfaunal organisms: nematodes, crustaceans (*Uca* sp., amphipods, tanaids, copepods), insect larvae (ceratopogonids, tabanids),

arachnids, and molluscs occurred in <15% of bare or reference plots in either state and had densities <<1 per 100 cm^2.

The average taxon richness (<3) and Shannon H' diversity (<0.6) was low in both states, but there were significant differences between reference and bare plots and among years (Figure 2.3.). In GA, treatment (p =0.006) and year (p = 0.0002) were significant sources of variation in taxon richness, with reference plots having 2x more taxa than bare plots and the year 2008 having 2x fewer taxa than the previous years. In LA, only year was a significant source of variation with 2008 having >3x fewer taxa than the previous years. The diversity index showed a similar pattern in both states, with bare plots being statistically less diverse than reference across all years and diversity in 2008 reduced compared to other years (Figure 2.3.C.,F.).

When macroinfaunal grou*p* s were classified by their feeding mode (surface, subsurface, and carnivorous feeders, as indicated in Table 2.1), there were shifts in the percentage presence of each group in bare versus reference plots in GA, but not in LA (Figure 2.4.). In GA, the percentage of subsurface feeders was lower and the percentage of surface feeders was higher in bare plots, as compared to reference plots. This was observed in both 2006 and 2007 (there were 0 macroinfauna in bare plots in 2008 for a comparison). In LA, there were no obvious shifts in the percentage of feeding types in bare vs. reference plots, and the proportion of surface and subsurface feeders were similar each year.

2.3.2.2. Density

In GA, total macroinfauna density averaged from 20 ± 6 to 65 ± 13 per 100 cm^2 in reference plots over sample years (overall avg. 42 ± 7 per 100 cm^2), and from 0 ± 0 to 3 ± 1 per 100 cm^2 (overall avg. 2.5 ± 0.6 per 100 cm^2) in bare plots (Table 2.1., Figure 2.3.A.). Both treatment ($p = 0.004$) and year ($p < 0.0001$) were significant sources of the variation in total macroinfauna density, with bare plots having statistically fewer macroinfauna than reference plots, and 2008 having 2x fewer organisms than the two previous years (Figure 2.3.A.).

In LA, total macroinfauna density did not show a strong difference between plots: reference plots averaged from 14 ± 11 to 42 ± 12 per 100 cm^2 over sample years (overall avg. 30 ± 7 per 100 cm^2), and bare plots averaged 1 ± 1 to 66 ± 24 per 100 cm^2 (overall avg. 45 ± 15 per 100 cm^2) (Table 2.1., Figure 2.3.B.). Year ($p <0.0001$) was a significant source of the variation in the macroinfauna density in LA, whereas treatment was not. There were 4x fewer individuals in 2008 than the two previous years.

2.3.3. Meiofauna

2.3.3.1. Community composition

All (100%) plots sampled in both states had meiofauna present (Table 2.2.). In GA, there were a total of 8 meiofauna taxa present in reference plots over the sample years, whereas 9 were present in bare plots. In LA, there were a total of 10 meiofauna taxa present across all reference plots, whereas only 7 taxa were present in bare plots. Nematodes, which were in all plots sampled, made up the largest proportion of the meiofauna (92% in GA and 85% in LA). The next most abundant group was copepods

(accounting for 5% of meiofauna in GA and 13% in LA). Copepods were present in all plots in 2006 and 2007, but in 2008 their presence was greatly reduced in bare plots in GA and both bare and reference plots in LA. Overall, copepods were present in 100% reference plots and 83% of bare plots in GA, and 78% of both reference and bare plots in LA[2]. Other species accounted for only ~3% of the meiofauna in each state and included juvenile oligochaetes and polychaetes, unidentified nauplii, insects (ceratopogonids, collembolans), mites (acari), ostracods, and molluscs (bivalves, hydrobiids). Of these only the nauplii and juvenile oligochaetes were present in >30% of plots altogether, and there was little difference in their presence in bare and reference plots.

The average taxon richness was <5 and Shannon H' diversity was <0.8 each year in both states, but there were significant differences between reference and bare plots and among years (Figure 2.5.). In GA, treatment (p =0.02) and year (p <0.0001) were significant sources of variation in the taxon richness, with reference plots having more taxa than bare plots and the year 2008 having ~2x fewer taxa than the previous years. Analysis of the Shannon H' index also indicated that treatment (p =0.0002) and year (p =0.0002) were significant sources of variation in diversity of meiofauna in GA, with a similar pattern for year (i.e. 2008 had the lowest diversity), but an opposite pattern for treatment as compared to the taxon richness (i.e. there was a higher diversity in bare plots). The increase in diversity in bare plots is likely due to the fact that diversity indices account for both species richness and evenness (J'), and despite fewer taxa in these plots, the ratio of dominant meiofauna (nematodes to copepods) was much more even (22:1 in reference plots vs. 5:1 in bare plots). In LA, only year was a significant

[2] In 2008, copepods were present in only 50% of bare plots in GA and in 33% of bare and reference plots in LA.

source of variation in taxon richness or diversity, with 2008 decreased compared to previous years (Figure 2.5.).

2.3.3.2. Density

In GA, total meiofauna density was 10x greater in reference plots (611 ± 157 per 10 cm^2) versus bare plots (67 ± 28 per 10 cm^2). Nematodes were the most abundant, averaging 575 ± 153 in reference plots and 51 ± 25 (per 10 cm^2) in bare plots, and were followed by harpacticoid copepods, which averaged 26 ± 7 in reference plots and 11 ± 4 (per 10 cm^2) in bare plots. Both treatment (0<0.0001) and year (p <0.0001) were significant sources of variation in nematode and copepod density, with much greater densities occurring in the reference plots, and reduced densities of each in 2008 (by ≥85%; Figure 2.5. A-B).

In LA, total meiofauna density was 3x greater in bare plots (409 ± 107 per 10 cm^2) as compared to reference plots (125 ± 30 per 10 cm^2). Nematodes were the most abundant overall, averaging 356 ± 96 in bare and 98 ± 21 per 10 cm^2 in reference plots, and were followed by harpacticoid copepods, which averaged 43 ± 13 per 10 cm^2 in bare and 25 ± 11 per 10 cm^2 in reference plots. Both treatment and year were significant sources of variation in nematode and copepod density, each significantly greater in bare plots as compared to reference plots. Densities were reduced in 2008 by ≥72%. (Figure 2.5. E-F).

2.3.4. *S. alterniflora* density and soil macro-organic matter

In GA, *S. alterniflora* density across treatment and transplanted plots ranged from 0-280 stems m^{-2}, and that in LA ranged from 0-340 stems m^{-2}. Macro-organic matter biomass ranged from 18-2511 g m^{-2} in GA and from 99-2874 g m^{-2} in LA. These data were used to explore the variation in invertebrate density, taxon richness, and diversity across treatment and transplanted plots. Snail densities increased with increasing stem densities in each state; regressions explained over 50% of the variation (GA: N =147, R^2=0.50, p <0.0001; LA: N=160, R^2=0.59, p <0.0001). In contrast, stem density was a poor predictor of fiddler crab density in each state (GA: N=147, R^2=0.001, p =0.6 and LA: N=160, R^2=0.07, p =0.002).

In GA, *S. alterniflora* stem density and macro-organic matter together predicted 21% of the variation in taxon richness (N=92, p <0.0001), 15% of the variation in diversity (N=92, p =0.0009), and 23% of the variation in density (N=92, p <0.0001), all in positive relationships. In LA, the same variables in a regression model were weakly and inversely related to macroinfaunal taxon richness and density (N=114, R^2=0.08, p =0.002; N=114, R^2=0.06, p =0.007, respectively), and did not predict macroinfaunal diversity at all (N=114, NS). In each state, oligochaetes were the primary driver of the significant relationships observed.

In GA, stem density predicted meiofaunal density in a positive relationship (N=37, R^2=0.23, p =0.003). In LA, stem density weakly predicted meiofaunal by an inverse relationship (N=42, R^2=0.10, p =0.04). In each state, nematodes drove these relationships. Stem density did not predict meiofaunal diversity or taxon richness in either state (GA: N=37, NS, each; LA: N=34, NS),

2.4. Discussion

2.4.1. Overview

A central principle in ecological studies is that through stabilizing abiotic condition, and adding habitat complexity, foundation species promote the presence and biological diversity of associated species in an ecosystem (Ellison et al. 2005). However, I found large unanticipated differences between GA and LA in the effect of *S. alterniflora* loss on salt marsh fauna. Although epifaunal snails were similarly absent in bare areas in both states, there was a strong contrast in the response of benthic infauna between the states. Abundances of both macroinfauna and meiofauna were significantly lower in bare as compared to reference (vegetated) plots in GA, whereas in LA there was no difference in macroinfauna abundance between plots, and meiofauna were significantly higher in bare as compared to reference plots. Both the taxon richness and diversity of benthic infauna were also significantly decreased in bare plots in GA, whereas there were no differences between plots in LA. The lower abundances and diversity in bare areas in GA is in agreement with previous general expectations of a reduction in faunal support when foundation species are lost (see introduction). However, in LA, the finding that many invertebrates were either unaffected or, in some cases (meiofauna), positively affected by the loss of *S. alterniflora* is contradictory. These results indicate that the effects of the loss of a foundation species can not necessarily be generalized to whole ecosystems (e.g. salt marshes, coral reefs, rainforests), and highlight the need for more context-dependent studies.

Another function of foundation species is that they protect against and dampen the effects of disturbances (de Groot et al. 2002). Coral reefs, mangrove forests and salt marshes all provide barriers against erosion and wave energy from storms, and vegetative cover in these habitats can negate the effects of floods and droughts (Moberg and Folke 1999, de Groot et al. 2002, Hopkinson et al. 2008). A loss of foundation species makes an area more susceptible to additional disturbances (Loya and Rinkevich 1980), and leads to diminished ecosystem functioning (forests: Bigler et al. 2005, Loo 2009; estuaries: Thrush et al. 2008; coral reefs: Nystrom et al. 2000). Understanding the effects of multiple disturbances has been highlighted as an important research direction for ecologists (Hughes and Petchy 2001). During this study, a second climatic disturbance in each state (a drought in GA and a hurricane in LA) provided me with a unique opportunity to examine whether the presence of a foundation species ameliorated the response to multiple disturbances. As described below, I found that salt marsh fauna were much more susceptible to disturbance without *S. alterniflora*.

Below I discuss the similarities and differences between the faunal responses to the loss of *S. alterniflora* in each state, followed by consideration of geographic setting and the effect of multiple disturbances on these systems.

2.4.2. Epifaunal Response to *S. alterniflora* loss in GA and LA

Epifaunal molluscs were dramatically affected by the loss of *S. alterniflora* in both states. Periwinkle snails were completely absent in the dieback (bare) plots of both states, whereas they were always present in nearby vegetated (reference) plots. I also found strong positive relationships between periwinkle snail abundance and *S.*

alterniflora densities in both states ($R^2 \geq 0.5$), as has been previously observed (Hutchens and Walters 2006, Kiehn and Morris 2010). Other less abundant molluscs such as the coffee-bean snail *Melampus* (GA only) and the ribbed mussel *Geukensia* (GA and LA), which were present in reference plots, were also absent in bare plots. These results are not surprising, as *S. alterniflora* is a principle source of food for *Littoraria* and *Melampus* in *S. alterniflora*-dominated marshes, and contributes to the diet of ribbed mussels (Haines and Montague 1979, Rietsma et al. 1988, Kreeger and Newell 2001, Silliman and Newell 2003, also see Appendix A.). *S. alterniflora* also provides necessary habitat and vertical refuge from predators (*Littoraria*) (Hamilton 1976, Silliman and Bertness 2002), as well as shade cover to minimize desiccation (*Melampus*) (Hutchens and Walters 2006, Lee and Silliman 2006). Ribbed mussels are involved in a facultative mutualism with *S. alterniflora*, whereby they positively facilitate the presence of one another through soil stabilization (both) and the addition of nutrients (mussels) (Bertness 1984, Stiven and Gardner 1992).

Fiddler crabs densities in GA (*U. pugnax*) were negatively affected by the loss of *S. alterniflora*, whereas crab densities in LA (*U. rapax*) were not significantly different in bare versus reference areas. Fiddler crabs have been shown to depend on *S. alterniflora* in many ways: as a source of food (Currin et al. 1995; also see Appendix A.), for structural burrow support (Bertness 1985), and shade to regulate temperature and prevent soil desiccation (Powers and Cole 1976, Nomann and Pennings 1998, Kenemer and McFarlin, unpublished data). The response in GA fits these observations. However, regression analysis indicated that *S. alterniflora* density explained very little of the variation in fiddler crab densities in either state ($R^2 \leq 0.06$); it may be that at very high

densities, plants can be prohibitive to burrowing through greater root mat coverage (Bertness 1985). It is possible that the numbers of fiddler crabs in LA were too low (overall avg. 25 ± 5.5 m^{-2}) to see a treatment response. Most fiddler crabs were seen along the elevated, unsubmerged mangrove berm, and it may be that the crabs preferred to feed on nearby mangroves or retreated to mangroves to avoid the blue crab predators in the *S. alterniflora* marsh.

2.4.3. Infaunal Response to *S. alterniflora* loss in GA and LA

Benthic infauna, macroinfauna (density, taxon richness, diversity) and meiofauna (density, taxon richness) community characteristics in GA decreased significantly in response to the loss of *S. alterniflora*. These results are again in keeping with the literature, showing that benthic infauna incorporate *S. alterniflora* into their diets (Carmen and Fry 2002, Galván et al. 2008), are often increased in density near culms of *S. alterniflora* (Rader 1984, Levin and Talley 2000), and respond positively to belowground macro-organic matter mass (the live and dead portion of roots and rhizomes; Craft and Sacco 2003). In regression analyses in this study, macroinfauna community responses in GA were positively related to increased stem density and soil-macro-organic matter, and the meiofauna density response was predicted by increased stem density.

In contrast, infauna in LA were either largely unaffected (macroinfauna density, taxon richness, and diversity; meiofauna taxon richness and diversity) or significantly increased (meiofauna density) in bare plots as compared to reference plots, and regression analyses showed a weak inverse relationship with *S. alterniflora* stem density.

A review of the literature on infaunal densities in salt marshes reveals that many studies have found little response of infauna to variation in vegetation coverage (Levin et al. 1996, Levin and Talley 2000, Johnson et al. 2007). For instance, in a created marsh in North Carolina, the densities and species richness of macroinfauna were similar between vegetated and unvegetated plots at both higher (37 cm below MHW) and lower elevations (57 cm below MHW) (Levin et al. 1996). There have also been reports of *increased* densities and diversities in unvegetated areas as compared to vegetated areas in New England marshes (Johnson et al. 2007). In a comprehensive review Levin and Talley (2000) compiled benthic infauna responses to vegetation presence, and hypothesized that the association with vegetation likely becomes increasingly positive in areas that are more physically stressful, where the soil amelioration provided by the vegetation becomes more important. As described below, this may be the explanation for the differences in infaunal response between the states.

2.4.4. Geographic differences between GA and LA

There were numerous differences between the states. The larger, semidiurnal tides in GA mean that soils were exposed more frequently than those in LA and subject to increased variability in terms of soil moisture. The GA sites were also at a higher elevation than those in LA, which meant that the LA sites remained consistently wetter than those in GA. In GA dieback areas, soils were very dry and often cracked (see inset of Fig. 1). Similar evidence of soil drying was not observed in LA (McFarlin, pers. obs.). LA sites also had increased concentrations of porewater ammonium and sulfide in LA (M. Joye and P. Baas, unpublished data), which is characteristic of waterlogged areas

where soil conditions are reducing (Stagg and Mendelssohn 2010). LA sites also had a greater concentration of BMA. BMA chlorophyll *a* concentrations increased to ~ 300 mg m^{-2} in bare plots as compared to <30 mg m^{-2} in vegetated plots, whereas in GA, BMA concentrations were similar in both treatments (<30 mg m^{-2}) (M. Joye and P. Baas, unpublished data). In LA, the combination of persistent wetness (lower elevation) and increased sunlight in the bare plots led to increased algal growth.

The fact that LA sites were at a lower elevation may have allowed increased access by predators; blue crabs access the marsh during submerged periods and are known to limit epifaunal densities (Silliman and Bertness 2002, Lewis and Eby 2002, Johnson and Eggleston 2010). The number of blue crab predators was much greater in LA (~10 blue crabs per 100 m^2 versus 0 in GA), thus predation likely accounts for differences in the epifaunal densities between the states. Periwinkle snails and fiddler crabs were 4x and 10x lower, respectively, in LA than in GA, despite similar *S. alterniflora* densities between the states (112 ± 7 stems m^{-2} in LA vs. 118 ± 12 stems m^{-2} in GA).

The differences in flooding can also account for the differential responses of benthic infauna. The lower elevation (and increased submergence) of the LA marshes would likely contribute to physiological suitability of the soil to benthic infauna (Rader 1984, Levin and Talley 2000). Infaunal invertebrates require moisture to prevent desiccation, to accommodate movement, respiratory function, and osmotic regulation (Brusca and Brusca 2003). Densities of benthic infaunal invertebrates are typically greater in the low marsh where soil conditions (salinity, tidal flushing, soil oxygen, soil moisture) are physiologically less harsh and more stable (Rader 1984, Johnson et al.

2007, Levin and Talley 2000). In GA, *S. alterniflora* in reference plots prevented soil desiccation through canopy coverage, thereby promoting the increased abundance of benthic infauna observed there, as compared to bare areas. It would be very unlikely that infauna could tolerate the dry conditions characteristic of the dieback areas in GA for sustained periods of time.

I saw a shift in the macroinfaunal feeding groups in the bare plots in GA, with decreases in the percentage of subsurface feeders and increases in the percentage of surface deposit feeders as compared to reference plots. Macroinfaunal feeding groups can shift in response to food resources, but also in response to habitat alteration (increase in temperature, dehydration, soil hardness) with a decrease in canopy coverage by plants (Whitcraft and Levin 2007). A general finding has been that subsurface feeders (especially oligochaetes) are dominant in vegetated habitat, where plant cover ameliorates soil conditions (Levin and Talley 2000, Moseman et al. 2004, Whitcraft and Levin 2007). Craft and Sacco (2003) also reported such a shift from subsurface to surface feeders in constructed marshes where macro-organic matter was below 500 g m^{-2}. Macro-organic matter between treatment plots at GA study sites did not vary considerably (bare plots: 811 ± 138 g m^{-2}; reference plots: 865 ± 106 g m^{-2}), thus it is more likely that the increased evaporation and deceased soil moisture (which could prevent burrowing) led to this shift. I did not see a shift in the percentage of subsurface and surface feeders in the LA sites, but this is likely because plots remained persistently wet regardless of vegetation status and provided physiologically suitable habitat for subsurface feeders[3].

[3] Macro-organic matter in LA was well above 500 g m^{-2} in each plot, as well (bare plots: 657 ± 84 g m^{-2}; reference plots: 944 ± 69 g m^{-2}).

The increased densities of meiofauna in bare areas in LA may have been in response to the increase in BMA, as it is a dominant food source of meiofauna (Carmen and Fry 2002, Maddi 2003, Galván et al. 2008). In GA, benthic infauna were likely physiologically excluded from dieback areas rather than limited by benthic microalgae, which was similar in both plots.

2.4.5. Effects of multiple disturbances

In GA, a drought in 2007-2008 led to extremely low river discharge rates: streamflow to the coast was reduced by 57% in 2007 and 49% in 2008 (Altamaha River at Doctortown, GA; USGS 2011). These 2 years ranked 71st and 78th out of 79 annual observations in terms of flow conditions. *S. alterniflora* densities in reference plots of this study were reduced from 145 ± 18 stems m^{-2} in 2007 to 91 ± 12 stems m^{-2} in 2008. In LA, Hurricane Ike in 2008 scoured the sediment of the study sites, especially in bare areas (J. Baustian and I. Mendelssohn, *pers. comm.*). Root mats were exposed along the border between dieback and vegetated areas, providing evidence for a loss of sediment within the bare area (C. McFarlin, pers. obs.). In contrast, roots were not exposed within vegetated (reference) plots, which indicated that these areas were protected from erosion during the hurricane. There was also a decrease in the benthic microalgal concentrations in bare plots in 2008 (M. Joye and P. Baas, unpublished data), and in some areas algal mats were physically removed along with eroded sediment. These separate disturbances in GA and LA both led to an overall decrease in invertebrate densities in 2008. In both states, disturbances affected the less mobile invertebrates: macroinfauna were reduced by 59-84% and meiofauna by 88-93%, regardless of treatment.

I compared the densities of invertebrates in bare and reference plots in 2008 to previous years, in order to assess whether the presence of *S. alterniflora* served to lessen the effect of these disturbances on the invertebrate communities. Both the density and taxon richness of macroinfauna in bare plots showed a greater response to these disturbances than did those in reference plots, suggesting that they were more susceptible to the additional disturbance when vegetation was absent. In GA, macroinfauna density and taxon richness in bare plots were each decreased by 100% following the second drought in 2008, compared to a much lower decrease in each in the reference plots (62% and 15%, respectively) as compared to previous years. In LA, following the hurricane in 2008, there was also a much greater decrease in the macroinfaunal density and diversity (99% and 92%, respectively) in bare plots as compared to that in nearby reference plots (which were decreased by 61% and 22%, respectively). The meiofauna community also showed a greater effect in the bare plots following the disturbances. In GA, the reduction in meiofaunal density in 2008 led to an extremely low average of only 11 organisms per 10 cm^2 in bare plots (compared to 10x that in reference plots). In LA, the taxon richness was reduced by a greater percentage in the bare plots (68% vs. 54% in reference plots) after the hurricane. Epifaunal snails could not be compared since there were never any in bare plots, and fiddler crabs showed a mixed response (increasing in both bare and reference plots in LA, and decreasing in reference plots in GA).

The results of the infaunal community support the notion that the presence of foundation species can promote resiliency to disturbances. In GA, the second drought caused a decrease in soil moisture in bare plots, further reducing the suitable habitat available to the benthic infauna. The presence of *S. alterniflora* likely ameliorated these

conditions by providing refuge from the effects of desiccation during the drought. If this is the case, the service provided by the plants was that of habitat provisioning, which was the same service provided by *S. alterniflora* during the 2000-2002 droughts. In LA, the first disturbance (drought) did not necessarily result in a loss of suitable habitat, but the second disturbance (Hurricane Ike) led to a physical removal of invertebrates (and reduced taxon richness) in bare plots which were not buffered by *S. alterniflora.* The presence of *S. alterniflora* likely decreased turbulent flow energies (Leonard and Luther 1995), thus limiting erosion due to the hurricane. If this is the case, the service provided by the plants was that of storm buffering. Thus in LA, I was able to evaluate the importance of *S. alterniflora* in providing habitat provisioning following the initial *S. alterniflora* dieback (2006 and 2007) and storm buffering following Hurricane Ike (2008). Although I did not see an increase in benthic invertebrate abundance or diversity in reference areas when habitat provisioning was the primary function, there was an overwhelmingly positive response when storm buffering was the primary function. These results highlight the importance of distinguishing among ecosystem services when examining the importance of foundation species in an ecosystem.

2.5. Conclusions

Many foundation species are experiencing declines due to combined impacts of habitat fragmentation, eutrophication, and climate change (Nystrom et al. 2000, Scheffer et al. 2001, Gedan et al. 2011). In this study, I compared the density and diversity of salt marsh invertebrates in bare and reference marsh plots in GA and LA. I expected to see similar decreases in invertebrates with *S. alterniflora* loss in each state, as studies of

foundation species have predicted, with a potentially greater effect in GA because of its larger tidal amplitude. I found that snails, which depend heavily on *S. alterniflora* for habitat, food, and refugia, responded to the loss as expected, as none were observed in either state when *S. alterniflora* was absent. However, I found the opposite trends in benthic infauna in the two states. In GA, infaunal density and diversity was reduced in bare areas, whereas in LA infauna was not different (macroinfauna) or greatly increased (meiofauna) in bare areas as compared to reference (vegetated) areas. Differing hydrogeomorphic (microtidal vs. macrotidal) conditions and the fact that LA sites were at a lower elevation and remained wetter than GA sites may be the reason for this difference. In GA, bare plots were often dehydrated as marsh elevation and aerial exposure was increased between tides, and thus *S. alterniflora* became essential to the provisioning of habitat (through shading). In LA, bare plots not only remained moist but also had much greater food availability (BMA), and thus infaunal invertebrates were not affected by the loss of *S. alterniflora*. A shift toward a higher proportion of surface feeding groups (and a decrease in the proportion subsurface feeding groups) in bare plots in GA as compared to LA also supports the idea of differential habitat. These results indicate the danger of generalizing about the functions of foundation species.

I was also able to evaluate whether the presence of a foundation species affected the ability of these sites to withstand additional disturbance. A second drought (GA) in 2007-2008 and a hurricane (LA) in 2008 affected the study sites, and these led to overall decreases in the density and taxon richness of the sessile fauna (snails and benthic infauna) in each state. I found greater decreases to the benthic infaunal community in the bare plots as compared to reference plots in each state following the disturbances. These

findings show that not only is the role of foundation species context-dependent in terms of geography, but it is also dependent upon the function that *S. alterniflora* is providing (amelioration of habitat vs. physical buffer). Overall, these results support the notion that healthy densities of *S. alterniflora* are critical to the resiliency of invertebrates to multiple disturbances.

2.6. Acknowledgments

This project would not have been possible without the help of many friends. I thank Kristen Anstead, Allen Bailey, Alphonso Bailey, Jenny Fenton, Christine Hladik, Galen Kaufman, Bernard Kenemer, Theron Menkon, Harlan Miller, Ashley Rich-Robertson, Shelly Robertson, Erin Romer, Daniel Saucedo, Jennie Seay, Sylvia Schaefer, Jacob Shalack, and Caitlin Yeager for their help in the field. I also thank Ashley Rich-Robertson, Zane Cress, and Caitlin Yeager for help in invertebrate preservation and processing in the lab. Funding was provided by EPA and GCE-LTER.

References

Adam, P. 1990. *Saltmarsh Ecology*. Cambridge University Press, Cambridge.

Alber, M., E.M. Swenson, S.C. Adamowicz, and I.A. Mendelssohn. 2008. Salt marsh dieback: An overview of recent events in the US. *Estuarine, Coastal and Shelf Science* 80: 1-11.

Altieri, A.H., B.R. Silliman, and M.D. Bertness. 2007. Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. *The American Naturalist* 169 (2): 195-206.

Basan, P.B., and R.W. Frey, 1977. Actual-palaeontology and neoichnology of salt marshes near Sapelo Island, Georgia. In:.P. Crimes and J.C. Harper (eds.), *Trace Fossils 2*, *Journal of Geology, Spec. Issue 9*, Seel House Press, Liverpool, pp. 41-70.

Bertness, M.D. 1984. Ribbed mussels and the productivity of *Spartina alterniflora* in a New England salt marsh. *Ecology* 65: 1794-1807.

Bertness, M.D. 1985. Fiddler crab regulation of *Spartina alterniflora* production on a New England salt marsh. *Ecology* 66(3): 1042-1055.

Bertness, M.D. 1991. Interspecific interactions among high marsh perennials in a New England salt marsh. *Ecology* 72: 125-137.

Bertness, M.D., and P.J. Ewanchuk. 2002. Latitudinal and climate-driven variation in the strength and nature of biological interactions in New England salt marshes. *Oecologia* 132 (3): 392-401.

Bertness, M.D., and S.W. Shumway. 1993. Competition and facilitation in marsh plants. *The American Naturalist* 142 (4): 718-724.

Bigler, C., D. Kulakowski, and T.T. Veblen. 2005. Multiple disturbance interactions and drought influence fire severity in Rocky Mountain subalpine forests. *Ecology* 86 (11): 3018-3029.

Boudreaux, M.L., J.L. Stiner, and L.J. Walters. 2006. Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in Mosquito Lagoon, Florida. *Journal of Shellfish Research* 25 (3): 1079-1089.

Brooks, T.M., S.L. Pimm, and J.O. Oyugi. 1999. Time lag between deforestation and bird extinction in tropical forest fragments. *Conservation Biology* 13 (5): 1140–1150.

Bruno, J.F. and M.D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. In: Bertness M.D., M.E. Hay, and S.D. Gaines (eds.) *Marine Community Ecology*. Sinauer, Sunderland, MA, pp. 201-218.

Bruno, J.F., J.J. Stachowicz, and M.D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18 (3): 119-125.

Brusca, R.C., and G.J. Brusca. 2003. *Invertebrates*, 2nd ed. Sinauer Associates, Sundeland, MA.

Burgess, R. 2001. An improved protocol for separating meiofauna from sediments using colloidal silica sols. *Marine Ecology Progress Series* 214: 161–165.

Carman, K.R., and B. Fry. 2002. Small-sample methods for δ13C and δ15N analysis of the diets of marsh meiofaunal species using natural abundance and tracer-addition isotope techniques. *Marine Ecology Progress Series* 240: 85–92.

Christie, H., K.M. Norderhaug, and S. Fredriksen. 2009. Macrophytes as habitat for fauna. *Marine Ecology Progress Series* 396: 221-233.

Craft C.B., and J.N. Sacco. 2003. Long-term succession of benthic infauna communities on constructed *Spartina alterniflora* marshes. *Marine Ecology Progress Series* 257: 45-58.

Currin, C.A., S.Y. Newell, and H.W. Paerl. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Marine *Ecology Progress Series* 121: 99-116.

Dame, R., M. Alber, D. Allen, M. Mallin, C. Montague, A. Lewitus, A. Chalmers, R. Gardner, C. Gilman, B. Kjerfve, J. Pinckney, and N. Smith. 2000. Estuaries of the South Atlantic Coast of North America: Their geographical signatures. *Estuaries and Coasts* 23 (6): 793-819.

Dayton PK 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In Parker BC (Ed). *Proceedings of the colloquium on conservation problems in Antarctica*. Lawrence, KS Allen Press.

de Groot, R.S., M.A. Wilson, and R.M.J. Boumans. 2002. A typology for the classification, description and valuation of ecosystem functions, goods, and services. *Ecological Economics* 41: 393-408.

de Leeuw, J., A. van den Dool, W. de Munck, J. Nieuwenhuize, and W.G. Beeftink. 1991 Factors influencing the soil salinity regime along an intertidal gradient. *Estuarine, Coastal and Shelf Science* 32(1): 87-97.

Deegan, LA, and R.H. Garritt. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147 (1-3): 31-47.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliott, C.R. Ford, D.R. Foster, B.D. Kloeppel, J.D. Knoepp, G.M. Lovett, J. Mohan, D.A. Orwig, N.L. Rodenhouse, W.V. Sobczak, K.A. Stinson, J.K. Stone, C.M. Swan, J. Thompson, B. Von Holle,

and J.R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3 (9): 479-486.

Fischlin, A., G.F. Midgley, J.T. Price, R. Leemans, B. Gopal, C. Turley, M.D.A. Rounsevell, O.P. Dube, J. Tarazona, and A.A. Velichko. 2007. Ecosystems, their properties, goods, and services. In: M.L. Parry, O.F. Canziani, J.P. Palutikof,P.J. van der Linden and C.E. Hanson (eds), *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, pp. 211-272.

Fredriksen, S., A. De Backer, C. Bostrom, and H. Christie. 2010. Infauna from *Zostera marina* L. meadows in Norway. Differences in vegetated and unvegetated areas. *Marine Biology Research* 6 (2): 189-200.

Galván, K., J.W. Fleeger, B. Fry. 2008. Stable isotope addition reveals dietary importance of phytoplankton and microphytobenthos to saltmarsh infauna. *Marine Ecology Progress Series* 359: 37-49.

Gedan, K.B., A.H. Altieri, and M.D. Bertness. 2011. Uncertain future of New England salt marshes. *Marine Ecology Progress Series* 434: 229-237.

Genoni, G.P. 1991. Increased burrowing by fiddler crabs *Uca rapax* (Smith) (Decapoda: Ocypodidae) in response to low food supply. *Journal of Experimental Marine Biology and Ecology* 147 (2): 267-285.

Graca, M.A., S.Y. Newell, and R.T. Kneib. 2000. Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. *Marine Biology* 136 (2): 281-289.

Graham, M.H. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. *Ecosystems* 7: 341-357.

Hadfield, M.G., and M.A.R. Koehl. 2004. Rapid behavioral responses of an invertebrate larva to dissolved settlement cue. *Biological Bulletin* 207 (1): 28-43.

Haines, E.B., and C.L. Montague. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. *Ecology* 60 (1): 48-56.

Hamilton, P.V. 1976. Predation on *Littorina irrorata* (Mollusca: Gastropoda) by *Callinectes sapidus* (Crustacea: Portunidae). *Bulletin of Marine Science* 26 :403-409.

Healy, B. and K. Walters, Oligochaeta in *Spartina* stems: the microdistribution of Enchytraeidae and Tubificidae in a salt marsh, Sapelo Island, USA. *Hydrobiologia* 278: 111-123.

Hines, M.E., A.I. Knollmeyer, and J.B.Tugel. 1989. Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh. *Limnology and Oceanography* 34: 578-590.

Hopkinson, C.S., A.E. Lugo, M. Alber, A.P. Covich, and S.J. Van Bloem. 2008. Effects of sea-level rise and windstorms on coastal and inland ecosystems. *Frontiers in Ecology and the Environment* 6 (5): 255-263.

Howes, B.L., J.W.H. Dacey, and D.D. Goehringer. 1986. Factors controlling the growth form of *Spartina alterniflora*: Feedbacks between above-ground production, sediment oxidation, nitrogen, and salinity. *Journal of Ecology* 74 (3): 881-898.

Hughes, A.R., S.L. Williams, C.M. Duarte, K.L. Heck, and M.Waycott. 2009. Associations of concern: declining seagrasses and threatened dependent species. *Frontiers in Ecology and the Environment* **7**: 242–246.

Hughes, J.B., and O.L. Petchy. 2001. Merging perspectives on biodiversity and ecosystem functioning (meeting report). *Trends in Ecology and Evolution* 16 (5): 222-223.

Hutchens, JJ, and K. Walters. 2006. Gastropod abundance and biomass relationships with salt marsh vegetation within ocean-dominated South Carolina, USA estuaries. *Journal of Shellfish Research* 25 (3): 947-953.

Johnson and Eggleston 2010. Population density, survival and movement of blue crabs in estuarine salt marsh nurseries. *Marine Ecology Progress Series* 407: 135–147.

Johnson, D.S., J.W. Fleeger, K.A. Galvan, and E.B. Moser. 2007. Worm holes and their space-time continuum: spatial and temporal variability of macroinfaunal annelids in a northern New England salt marsh. *Estuaries and Coasts* 30 (2): 226-237.

Jones, C.G., J.H. Lawton, and M. Shachak. 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* 78 (7): 1946-1957.

Kneib, R.T., 2000. Salt marsh ecoscapes and production transfers by estuarine nekton in the southeastern United States. In: M.P. Weinstein and D.A. Kreeger (eds), *Concepts and Controversies in Tidal Marsh Ecology*, pp. 261-291.

Kreeger, D.A., and R.I.E. Newell. 2001. Seasonal utilization of different seston carbon sources by the ribbed mussel, *Geukensia demissa* (Dillwyn) in a mid-Atlantic salt marsh. *Journal of Experimental Marine Biology and Ecology* 260: 71-91.

Laidig, T.E. 2010. Influence of ocean conditions on the timing of early life history events for blue rockfish (*Sebastes mystinus*) off California. *Fishery Bulletin* 108 (4): 442-449.

Lawton, J.H. 1994. What do species do in ecosystems? *Oikos* 71 (3): 367-364.

Lee, S.Y., C.W. Fong, and R.S.S. Wu. 2001. The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. *Journal of Experimental Marine Biology and Ecology* 259 (1): 23-50.

Lee, SC; B.R. Silliman. 2006. Competitive displacement of a detritivorous salt marsh snail. *Journal of Experimental Marine Biology and Ecology* 339 (1): 75-85.

Leonard, L.A., and M.E. Luther. 1995. Flow hydrodynamics in tidal marsh canopies. *Limnology and Oceanography* 40 (8): 1474-1484.

Levin, L.A., and T.S. Talley. 2000. Influences of vegetation and abiotic environmental factors on salt marsh invertebrates. In: M.P. Weinstein and D.A. Kreeger (eds), *Concepts and Controversies in Tidal Marsh Ecology*, Kluwer Academic Publishers, Boston, MA.

Levin, L.A., D. Talley and G. Thayer. 1996. Succession of macrobenthos in a created salt marsh. *Marine Ecology Progress Series* 141: 67-82.

Lewis, D.B., and L.A. Eby. 2002. Spatially heterogeneous refugia and predation risk in intertidal salt marshes. *Oikos* 96:119–129

Lindbladh, M., and D.R. Foster. 2010. Dynamics of long-lived foundation species: the history of *Quercus* in southern Scandinavia. *Journal of Ecology* 98: 1330-1345.

Loo, J.A. 2009. Ecological impacts of non-indigenous invasive fungi as forest pathogens. *Biological Invasions* 11 (1): 81-96.

Maddi, P. 2003. Use of primary production by benthic invertebrates in a Lousiana salt-marsh food web. M.S. Thesis, Lousiana State University, Baton Rouge, LA, 81 pp.

McKee, K.L., I.A. Mendelssohn, and M.D. Materne. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecology and Biogeography* 13 (1): 65-73.

Mendelssohn, I.A., and K.L. McKee, 1988. *Spartina alterniflora* die-back in Louisiana: Time course investigation of soil water-logging effects. *Journal of Ecology* 76: 509-521.

Moberg, F., and C. Folke. 1999. Ecological goods and services of coral reef ecosystems. *Ecological Economics* 29: 215-233.

Moseman, S.M., L.A. Levin, C. Currin, C. Fordera. 2004. Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California. *Estuarine, Coastal and Shelf Science* 60: 755-770.

Nakamura, Y., H. Kawasaki, M. Sano. 2007. Experimental analysis of recruitment patterns of coral reef fishes in seagrass beds: Effects of substrate type, shape, and rigidity. *Estuarine Coastal and Shelf Science* 71 (3-4): 559-568.

Nomann, B.E., and S.C.Pennings. 1998. Fiddler crab-vegetation interactions in hypersaline habitats. *Journal of Experimental Marine Biology and Ecology* 225: 53-68.

Norling,P., and N. Kautsky. 2008. Patches of the mussel *Mytilus* sp. are islands of high biodiversity in subtidal sediment habitats in the Baltic Sea. *Aquatic Biology* 4: 75–87.

Nystrom, M., C. Folke, and F. Moberg. 2000. Coral reef disturbance and resilience in a human-dominated environment. *Trends in Ecology and Evolution* 15 (10): 413-417.

Loya, Y., and B. Rinkevich. 1980. Effects of oil pollution on coral reef communities. *Marine Ecology Progress Series* 3: 167-180.

Ogburn, M.B., and M. Alber. 2006. An investigation of salt marsh dieback in Georgia using field transplants. *Estuaries and Coasts* 29: 54–62.

Pennings, S. C., and M. D. Bertness. 2001. Salt Marsh Communities, Chapter 11, pp. 289-316. In: M.D. Bertness, S.D. Gaines, and M.D. Hay (eds.), *Marine Community Ecology*. Sinauer Associates Inc., Sunderland, MA.

Peterson, B.J., and R.W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography* 32 (6): 1195-1213.

Powers, L.W., and J.F. Cole. 1976. Temperature variation in fiddler crab microhabitats. *Journal of Experimental Marine Biology and Ecology* 21: 141-157.

Pratchett, M.S., P.L. Munday, S.K. Wilson, N.A.J. Graham, J.E. Cinner, D.R. Bellwood, G.P. Jones, N.V.C. Polunin, and T.R. Mcclanahan. 2008. Effects of climate-induced coral bleaching on coral-reef fishes - Ecological and economic consequences. *Oceanography and Marine Biology: An Annual Review* 46: 251-296.

Quan, W.M., J.X. Zhu, Y. Ni, L.Y. Shi, and Y.Q. Chen. 2009. Faunal utilization of constructed intertidal oyster (*Crassostrea rivularis*) reef in the Yangtze River estuary, China. *Ecological Engineering* 35 (10): 1466-1475.

Rader, D.N. 1984. Salt marsh benthic invertebrates: small-scale patterns of distribution and abundance. *Estuaries* 7: 4 13-420.

Rietsma, C., I. Valiela, and R. Buchsbaum. 1988. Detrital chemistry, growth, and food choice in the salt-marsh snail (*Melampus bidentatus*). *Ecology* 69: 261-266.

Rohr, J.R., C.G. Mahan, and K.C. Kim. 2009. Response of arthropod biodiversity to foundation species declines: The case of the eastern hemlock. *Forest Ecology and Management* 258: 1503–1510.

Sale, P.F. 1977. Maintenance of high diversity in coral reef fish communities. *The American Naturalist* 111 (978): 337-359.

Scheffer, M., S. Carpenter, J.A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. *Nature* 413: 591-596.

Seed, R. 1996. Patterns of biodiversity in the macro-invertebrate fauna associated with mussel patches on rocky shores. *Journal of Marine Biological Association of the United Kingdom* 76 (1): 203- 210.

Silliman, B.R., and M.D. Bertness. 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Science* 99: 10500-10505.

Silliman, B.R., and S.Y. Newell. 2003. Fungal farming in a snail. *Proceedings of the National Academy of Science* 100 (26): 15643-15648.

Spelke, J.A., P.E. Fell, and L.L. Helvenston. 1995. Population-structure, growth and fecundity of *Melampus bidenatus* (Say) from 2 regions of a tidal marsh complex in Connecticut. *Nautilus* 108 (2): 42-47.

Stagg, C.L., and I.A. Mendelssohn. 2010. Restoring ecological function to a submerged salt marsh. *Restoration Ecology* 18: 10–17.

Stiven, A.E., and S.A. Gardner. 1992. Population processes in the ribbed mussel *Geukensia demissa* (Dillwyn) in a North Carolina salt marsh tidal gradient: spatial pattern, predation, growth and mortality. *Journal of Experimental Marine Biology and Ecology* 160 (1): 81–102.

Stockhausen, W.T., and R.N. Lipicious. 2003. Simulated effects of seagrass loss and restoration on settlement and recruitment of blue crab postlarvae and juveniles in the York River, Chesapeake Bay. *Bulletin of Marine Science* 72 (2): 4009-422.

Stork, N.E. 1991. The composition of the arthropod fauna of Bornean lowland rain forest trees. *Journal of tropical ecology* 7: 161-180.

Taylor, J.D. 1968. Coral reef and associated invertebrate communities (mainly molluscan) around Mahe, Seychelles. Philosophical Transactions of the Royal Society of London. Series B, *Biological Sciences* 254 (793): 129-206.

Terborgh, John. 1992. *Diversity and the Tropical Rainforest*. New York: Scientific American Library. 242 pp.

Thrush, S.F., J. Halliday, J.E. Hewitt, and A.M. Lohrer. 2008. The effects of habitat loss, fragmentation, and community homogenization on resilience in estuaries. *Ecological Applications* 18: 12-21.

USGS (U.S. Geological Survey). 2011. National Water Information System. Surface-Water Annual Statistics for the Nation: USGS 02226000, Altamaha River at Doctortown, GA. http://waterdata.usgs.gov/nwis/annual/

Vallan, D. 2002. Effects of anthropogenic environmental changes on amphibian diversity in the rain forests of eastern Madagascar. *Journal of Tropical Ecology* 18 (5): 725-742.

Whitcraft, C.R., and L.A. Levin. 2007. Regulation of benthic algal and animal communities by salt marsh plants: impact of shading. *Ecology* 88 (4): 904–917.

Wilson, S.K., N.A.J. Graham, M.S. Pratchett, G.P. Jones, and N.V.C. Polunin. 2006. Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? *Global Change Biology* 12: 2220–2234.

Zimmerman, R.J., T.J. Minello, and G. Zamora, 1984. Selection of vegetated habitat by brown shrimp, *Peneaus aztecus*, in a Galveston Bay salt marsh. *Fishery Bulletin* 82: 325-336.

Table 2.1. Total number (*N*) of epifaunal, macroinfaunal, and meiofaunal samples collected each year in bare and reference plots in GA and LA. The number of sites from which samples were collected from is indicated in parenthesis for bare and reference plots. For epifauna and macroinfauna, 3 subsamples were collected per site. For meiofauna, 2 subsamples were collected per site in 2006 and 2008 and 1 subsample per site in 2007. Samples were collected opportunistically in transplanted plots each year.

		Epifauna (N)		
State	**Year**	**Bare**	**Reference**	**Transplanted**
GA	2006	18 (6 sites)	18 (6 sites)	12
	2007	18 (6 sites)	18 (6 sites)	12
	2008	18 (6 sites)	18 (6 sites)	36
LA	2006	18 (6 sites)	18 (6 sites)	36
	2007	18 (6 sites)	18 (6 sites)	36
	2008	18 (6 sites)	18 (6 sites)	36
		Macroinfauna (N)		
GA	2006	12 (4 sites)	12 (4 sites)	11
	2007	18 (6 sites)	18 (6 sites)	20
	2008	9 (3 sites)	12 (4 sites)	18
LA	2006	9 (3 sites)	9 (3 sites)	18
	2007	18 (6 sites)	18 (6 sites)	34
	2008	9 (3 sites)	9 (3 sites)	18
		Meiofauna (N)		
GA	2006	6 (3 sites)	6 (3 sites)	---
	2007	6 (6 sites)	6 (6 sites)	7
	2008	6 (3 sites)	6 (3 sites)	---
LA	2006	6 (3 sites)	6 (3 sites)	---
	2007	6 (6 sites)	6 (6 sites)	12
	2008	6 (3 sites)	6 (3 sites)	---

Table 2.2. The mean density of fauna across all plots and the percentage of plots with fauna present in bare and reference marsh during 2006-2008 in Georgia and Louisiana. The total mean (SE) for each taxon grouping is shaded in grey. Feeding modes for macroinfauna taxa are listed as SF=surface feeder, SSF=subsurface feeder, C=carnivore. P= polychaete species.

	Georgia				***Louisiana***			
	Density in Plots		**Percentage of Plots**		**Density in Plots**		**Percentage of Plots**	
	Bare	**Reference**	**Bare**	**Reference**	**Bare**	**Reference**	**Bare**	**Reference**
Crabs (Uca spp.), # m^{-2}	**198.22 ± 20.15**	**381.33 ± 28.24**	**98 ± 2%**	**100 ± 0%**	**14.22 ± 4.45**	**32.59 ± 6.48**	**26 ± 6%**	**50 ± 7%**
Molluscs, # m^{-2}	**0**	**187.52 ± 33.79**	**0%**	**91 ± 4%**	**0**	**41.11 ± 4.33**	**0%**	**100 ± 0%**
Littoraria irrorata	---	167.30	---	91%	---	40.82	---	100%
Melampus bidentatus	---	19.48	---	41%	---	0.11	---	4%
Geukensia demissa	---	0.74	---	13%	---	0.19	---	4%
Macrofauna (>500 µm), # 100 cm^{-2}	**2.54 ± 0.65**	**41.69 ± 7.06**	**36 ± 8%**	**86 ± 5%**	**45.18 ± 14.82**	**30.29 ± 7.12**	**54 ± 9%**	**72 ± 8%**
Oligochaeta, SSF	0.85	19.44	8%	52%	43.02	28.45	49%	69%
Capitella capitata , SSF (P)	1.21	19.44	23%	69%	0.54	0.52	9%	8%
Neanthes succinea , SF (P)	0.36	0.79	8%	7%	---	---	---	---
Streblospio benedicti , SF (P)	0.12	0.67	3%	10%	0.94	0.52	---	6%
Uca sp. , SF	---	0.34	---	7%	0.13	0.13	3%	3%
Ceratopogonidae, SF	---	0.34	---	7%	0.13	---	3%	---
Nematoda, SSF	---	0.34	---	5%	1.08	8.13	14%	6%
Caprellidae (amphipod), SF	---	0.11	---	2%	---	---	---	---
Tabanidae, C	---	0.11	---	2%	---	---	---	---
Harpacticoida (copepod), SF	---	---	---	---	0.13	---	3%	---
Tanaidae, SF	---	---	---	---	---	---	---	---
Bivalva (mollusc), SF	---	---	---	---	---	0.13	---	3%
Arachnida, C	---	---	---	---	---	0.13	---	3%
Manyunkia speciosa , SF (P)	---	---	---	---	---	0.13	---	3%
Stenoninereis martini , SF (P)	---	---	---	---	---	0.13	---	3%
Meiofauna (>63 µm), # 10 cm^{-2}	**67.46 ± 28.36**	**611.07 ± 156.83**	**100 ± 0%**	**100 ± 0%**	**408.61 ± 106.58**	**125.13 ± 30.35**	**100 ± 0%**	**100 ± 0%**
Nematoda	51.32	574.60	100%	100%	356.40	97.91	100%	100%
Copepoda	10.57	26.33	83%	100%	42.87	24.65	78%	78%
Oligochaeta	3.56	5.10	22%	44%	2.33	1.50	56%	50%
Nauplii (Crustacea)	1.52	4.06	50%	39%	6.61	0.73	56%	33%
Acari	0.29	0.29	11%	11%	---	0.03	---	6%
Ceratopogonidae	0.03	0.34	6%	22%	---	0.03	---	6%
Capitella capitata (P)	0.13	0.13	6%	6%	---	0.03	---	6%
Hydrobiidae	0.03	0.13	6%	6%	---	---	---	---
Bivalva	0.03	---	6%	---	---	---	---	---
Crab Zoea	---	---	---	---	0.10	0.03	11%	6%
Kinorhynca	---	---	---	---	0.10	0.19	11%	17%
Ostracod	---	---	---	---	0.10	---	11%	---
Collembola	---	---	---	---	---	0.03	---	6%

Table 2.3. Summary of results of multiple regression models describing variation in invertebrate variables as predicted by stem density and macro-organic matter across plots in each state. Symbols (*, ** and *** indicate $P \leq 0.05$, <0.01, <0.001 respectively, and no asterisks for $0.15 > P > 0.05$) indicate significance of individual terms in the model. Overall model R^2, adjusted R^2, *P*-values, sample size (N), and the model coefficient constants are given. Acronyms: SD=stem density, MOM=macro-organic matter.

State	Dependent Variable	Independent Variables	R^2	Adj. R^2	*P*- value	N	Constant (Ln-scale)
GA	*Littoraria* density	+SD***	0.5	0.5	<0.0001	147	0.231
	Fiddler crab density	---	---	---	NS	147	---
	Macroinfauna taxon richnes	+SD,+MOM***	0.21	0.19	<0.0001	92	-0.001
	Macroinfauna diversity	+SD,+MOM**	0.15	0.12	0.0009	92	-0.048
	Macroinfauna density	+SD**,+MOM***	0.25	0.24	<0.0001	92	-0.180
	(oligochaetes)	+SD**,+MOM***	0.26	0.24	<0.0001	92	-0.592
	(polychaetes)	+MOM**	0.11	0.09	0.003	92	0.152
	Meiofauna taxon richness	---	---	---	NS	37	---
	Meiofauna diversity	---	---	---	NS	37	---
	Meiofauna density	+SD**	0.23	0.23	0.003	37	3.699
	(nematodes)	+SD**	0.24	0.24	0.0012	37	3.440
	(copepods)	---	---	---	NS	37	---
LA	*Littoraria* density	+SD***	0.59	0.59	<0.0001	160	0.584
	Fiddler crab density	+SD**	0.07	0.06	0.0008	160	0.674
	Macroinfauna taxon richnes	-SD**	0.08	0.07	0.002	114	0.593
	Macroinfauna diversity	---	---	---	NS	114	---
	Macroinfauna density	-SD**	0.06	0.06	0.007	114	2.420
	(oligochaetes)	-SD**	0.06	0.05	0.008	114	2.350
	(polychaetes)	---	---	---	NS	114	---
	Meiofauna taxon richness	---	---	---	NS	34	---
	Meiofauna diversity	---	---	---	NS	34	---
	Meiofauna density	-SD*	0.10	0.08	0.04	34	5.220
	(nematodes)	-SD*	0.10	0.08	NS	34	5.020
	(copepods)	---	---	---	NS	34	---

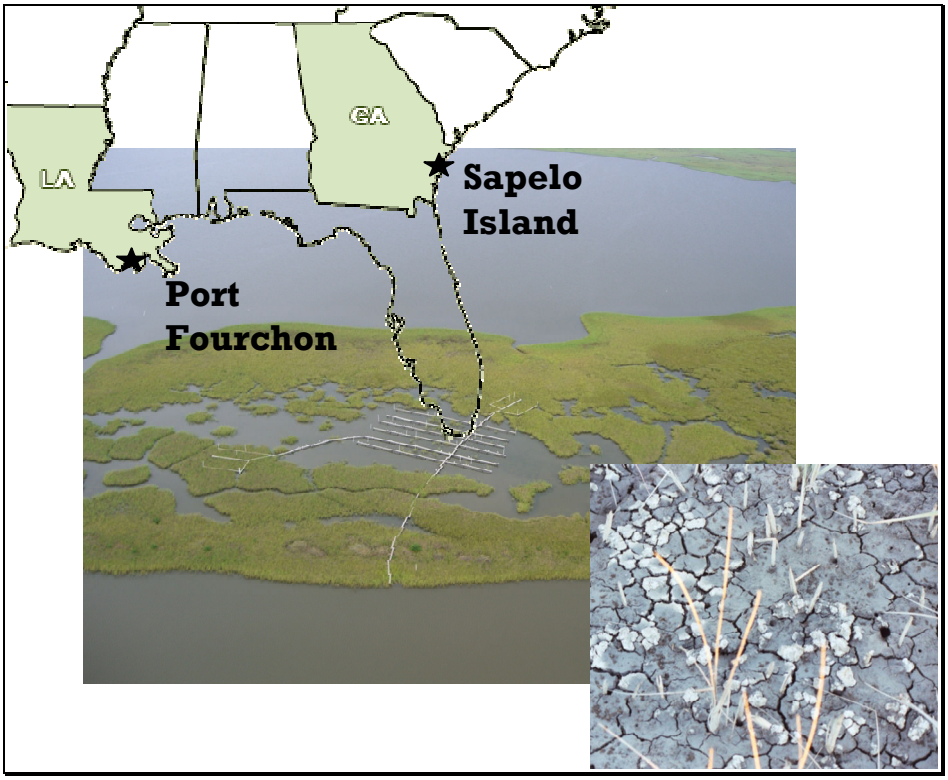


Figure 2.1. Location map of study sites in Port Fourchon, LA and Sapelo Island, GA. The aerial photograph shows a dieback area in Port Fourchon, LA (2 blocks), and the boardwalk system used to access plots in each state (courtesy of Mark Hester). Inset shows a close-up photograph of the marsh soil at the Sapelo Island, GA dieback area (courtesy of Dale Bishop).

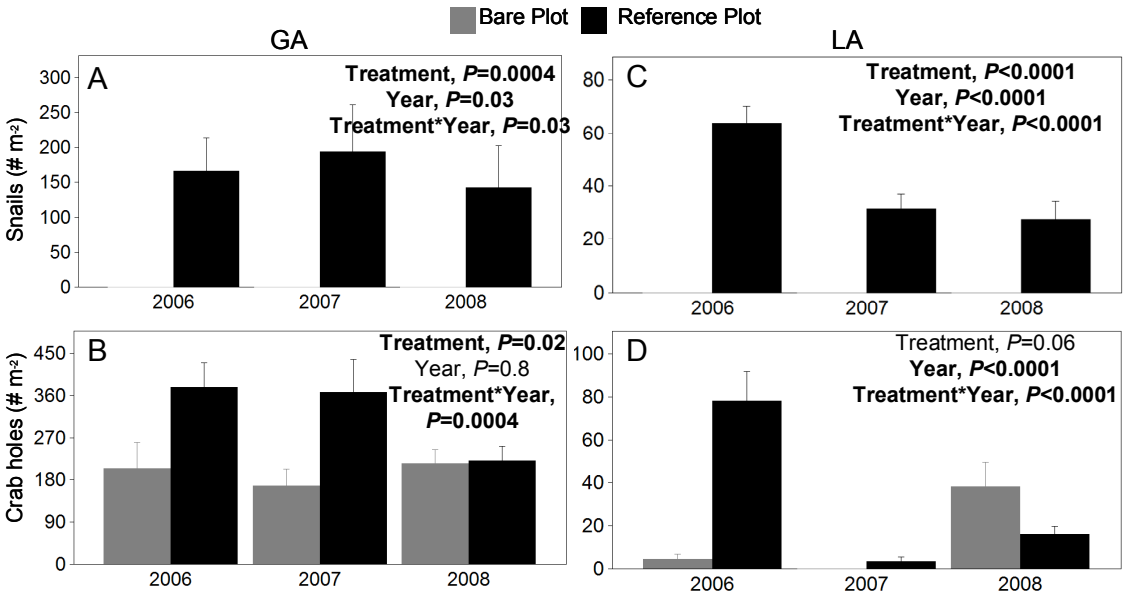


Figure 2.2. Epifaunal density (individuals m^{-2}) in Georgia and Louisiana in bare and vegetated plots from 2006-2008. GA snails (*Littoraria irrorata*) (A) and fiddler crabs (primarily *Uca pugnax*) (B), and LA snails (*Littoraria irrorata*) (C) and fiddler crabs (primarily *Uca rapax*) (D). Data are averaged across replicates for an overall mean $treatment^{-1}$ ± SE (*n*=18 per bar).

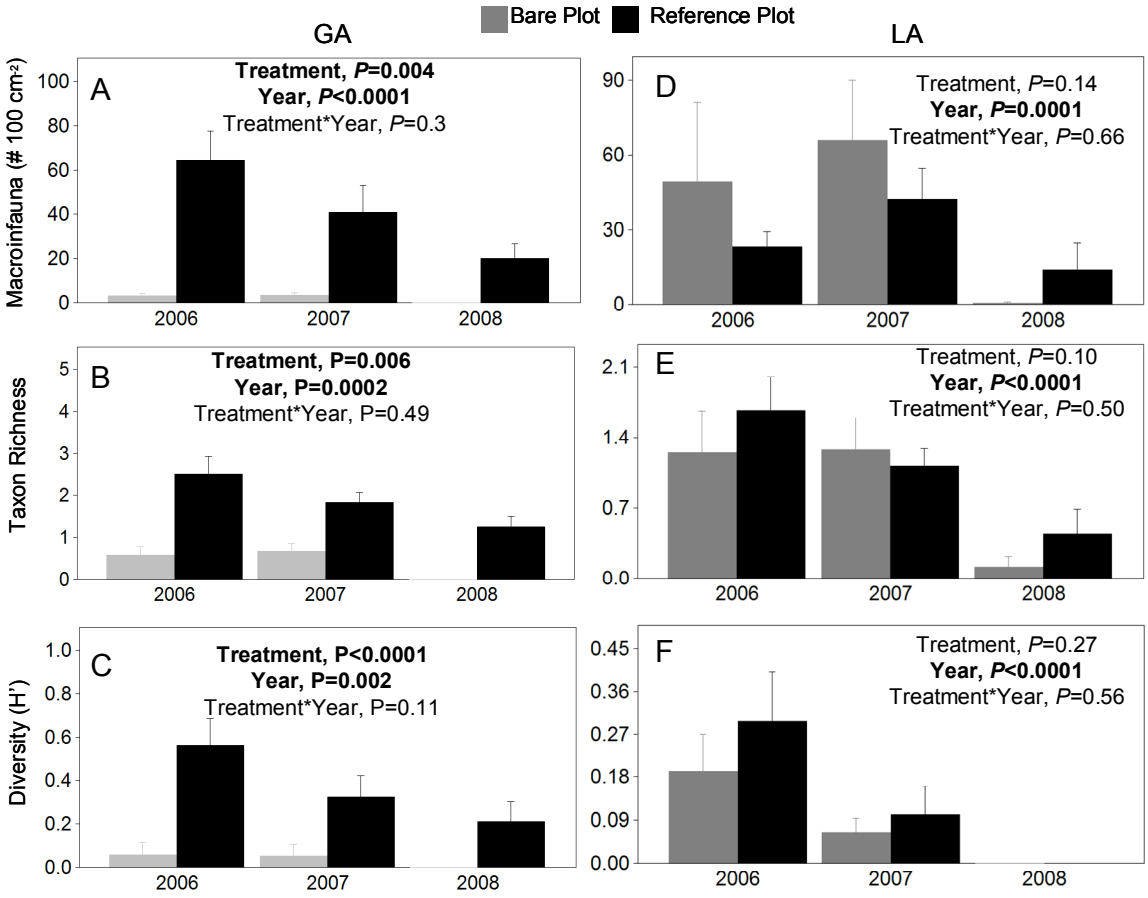


Figure 2.3. Macroinfauna density, taxon richness, and diversity (Shannon H') in Georgia (A-C) and Louisiana (D-F) in bare and vegetated plots from 2006-2008. Data are averaged across replicates for an overall mean treatment^{-1} ± SE (n=18 per bar).

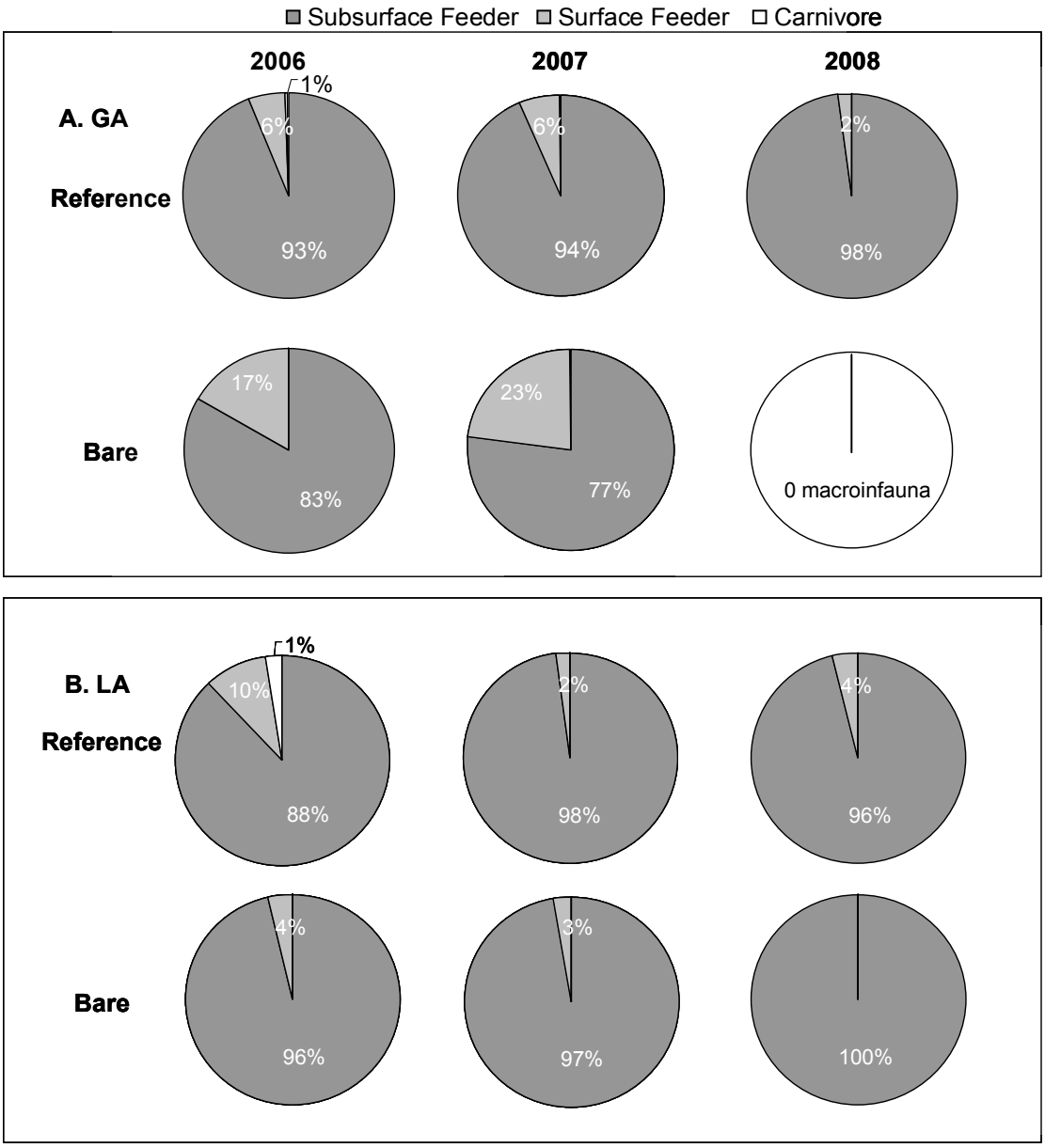


Figure 2.4. The percent frequency of subsurface, surface, and carnivorous macroinfauna feeders in bare and reference marsh in A) GA and B) LA.

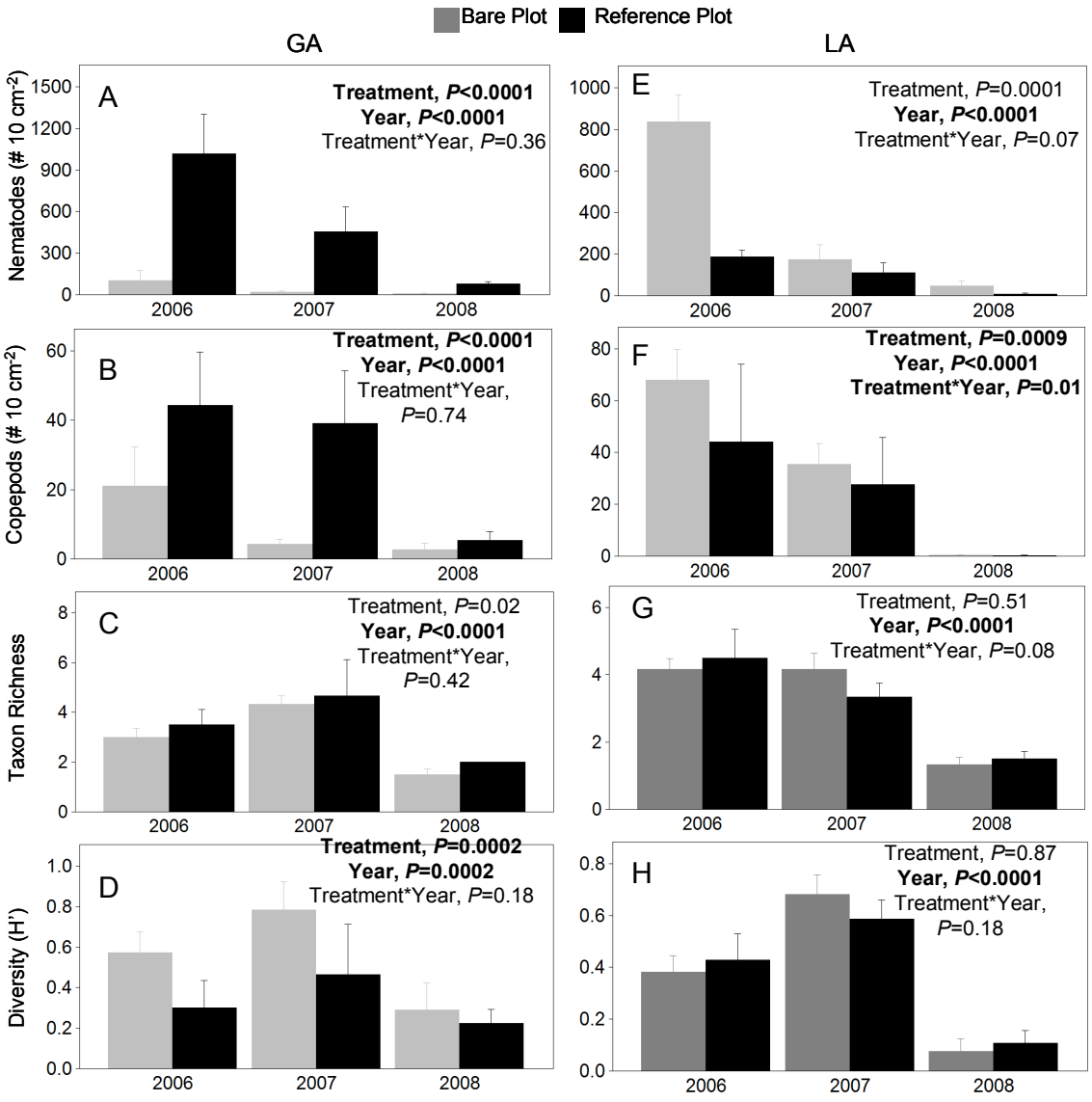


Figure 2.5. Nematode density, copepod density, and taxon richness, diversity (Shannon H'), in Georgia (A-D) and Louisiana (E-H) in bare and vegetated plots from 2006-2008. Data are averaged across replicates for an overall mean treatment^{-1} ± SE (n=18 per bar).

CHAPTER 3

DMSO:DMSP RATIOS AND METAL CONTENT AS POTENTIAL INDICATORS OF STRESS IN *SPARTINA ALTERNIFLORA*[4]

[4] McFarlin, C.R., and M. Alber. To be submitted to *Wetlands*.

Abstract

The most obvious and frequently studied outcome of a disturbance to salt marsh vegetation is change in biomass, but physiological responses can occur long before there are visible signs of stress. This study evaluated two potential indicators of stress (DMSO:DMSP ratio and foliar metals) in *Spartina alterniflora* collected from areas affected by wrack, increased snail densities, dieback, and horse disturbances in 20 marshes in GA. DMSP concentrations of leaves and roots were decreased in affected areas at all disturbances as compared to healthy areas, and concentrations in stems were also lowest in affected areas at all but wrack sites. DMSO concentrations were reduced in all plant sections but their patterns were variable across zones within each disturbance type. The DMSO:DMSP ratio was a stronger, more consistent indicator of disturbance than DMSP or DMSO concentration alone, and was significantly higher in the leaves and stems of plants collected from the affected areas as compared to healthy areas at all of the disturbance types. Foliar metal concentrations also differed in disturbed as compared to healthy areas: concentrations of nearly all 20 metals evaluated were increased in leaves collected from affected areas. Some metals (especially, Al, As, and Pb) were highly correlated with one another in the leaf tissues regardless of zone indicating that they may be taken up simultaneously, whereas correlations between other metals (i.e. Fe, K) varied in magnitude and direction depending on zone. Multidimensional scaling using the entire suite of metals showed that there was clear separation between plants from affected and healthy areas, but no difference among disturbance types. In contrast, chlorophyll *a* concentrations were not significantly different between affected and healthy areas. These results suggest that the DMSO:DMSP ratio and foliar metal suite are sensitive indicators

of sublethal stress in *S. alterniflora*, capable of identifying stress before there are visible signs such as chlorophyll loss. The fact that both indicators were consistent across a variety of disturbance types suggests that they may be broadly useful tools for evaluating the health of salt marsh habitat in the field.

3.1. Introduction

Salt marshes are subject to a wide variety of natural and anthropogenic disturbances, including wrack deposition, sedimentation and erosion from hurricanes and storms, herbivore overgrazing, as well as effects from agricultural and mining activities, the construction of water diversion structures, and urban development (Adam 2002, Laegdsgaard 2006, Gedan et al. 2009). Even without disturbance, marsh plants experience daily and seasonal fluctuations in salinity, inundation, and soil conditions resulting from the combined effects of tides and differences in elevation (Pennings and Bertness 2001). These multiple stressors may serve to enhance the effects of disturbances; Slocum and Mendelssohn (2008) found that salt marsh plants at higher elevations, where abiotic stress is increased, took longer to recover from disturbances than those at lower elevations.

The most obvious and frequently studied response to a disturbance to marsh vegetation is biomass loss (Baldwin and Mendelssohn 1998, Hartman 1988, Ewanchuk and Bertness 2003); however, physiological responses can occur long before there are visible signs of stress (Mendelssohn and McKee 1992). Moreover, the effects of many disturbances, such as increases in flooding frequency, pollutant contamination, and introduced species, can be gradual and difficult to detect (Mendelssohn and McKee 1992, Bertness et al. 2002, Laegdsgaard 2006, Weilhoefer 2011). If we can identify early signs of stress, we will be in a better position to identify areas that are at risk and potentially preserve valuable habitat.

Many investigators have evaluated the response of salt marsh vegetation (and *Spartina* spp. in particular) to altered environmental conditions (Mendelssohn and

McKee 1992). Early studies focused on changes in long-term growth and productivity of *S. alterniflora* in response to gradients in various edaphic conditions such as salinity, pH, redox potential, nutrients, metals, etc. (Smart and Barko 1978, Linthurst and Seneca 1981, Burdick et al. 1989). More recent studies have examined shorter-term physiological responses that occur at sublethal levels, such as altered concentrations of adenine nucleotides, adenylate energy charge ratio, proline concentrations, water use efficiencies, alcohol dehydrogenase activities, and leaf spectral reflectances (Mendelssohn and McKee 1992, Ewing et al. 1995 a,b; Mendelssohn et al. 2001, Hester et al. 2001). These short-term studies have generally been conducted under controlled greenhouse settings.

Only a handful of studies have looked at *S. alterniflora*'s responses in multi-stressor situations that might be encountered in the field. Ewing et al. (1997) tested whether the physiological indicators (CO_2 uptake, proline concentration, leaf spectral reflectance, adenine nucleotide level) that consistently responded to single stressors in earlier greenhouse experiments (Ewing et al. 1995 a, b) would also respond in the field. They found that field responses were varied and less predictable than in the greenhouse, likely because of the complex interactions between stressors in the field (Ewing et al. 1997). Padinha et al. (2000) found that concentrations of metal-chelating thiolic proteins (including glutathione) were often higher and the leaf adenylate energy charge and photosynthetic efficiency lower in *Spartina* spp. at sites closest to urban pollution sources in the Ria Formosa lagoon of Portugal. However, Pennings et al. (2002) evaluated some of the same indices as Padinha et al. (gas exchange measurements, glutathione concentrations), as well as other potential indices (peroxidase activity), and found no

difference in *S. alterniflora* taken from healthy and polluted (metals, PAHs) marshes in South Carolina. Thus, there is not currently a consistent, sensitive measure to indicate *S. alterniflora* stress under field conditions, and no study to date has explicitly compared responses to different types of disturbances.

Two common disturbances that affect salt marshes are wrack deposition and herbivore overgrazing (Pennings and Bertness 2001). The deposition of wrack onto the marsh surface can result from storms or from physical barriers to movement such as docks (Hackney and Bishop 1979, Reidenbaugh and Banta 1980, Tolley and Christian 1999). Wrack deposits are capable of completely killing *S. alterniflora* in seven to eight weeks, and the bare patches can persist for approximately one to three years (Bertness and Ellison 1987, Hartman 1988). Damage and plant death is most likely to occur when materials are deposited and remain higher in the marsh, away from tidal flow (Bertness and Ellison 1987, Reitsma and Valiela 1995). There are numerous examples of overgrazing leading to bare patches in the marsh. Some of these result from the introduction of non-native species (nutria: Evers et al. 1998, Taylor and Grace 1995, Taylor et al. 1997; feral horses: Turner 1987 and 1988, Furbish and Albano 1994; feral cattle: Martin 2003), others from the absence or reduction of predators, leading to an increase in herbivore populations (littorinid snails: Silliman and Bertness 2002; sesarmid crabs: Holdredge et al. 2008), and others from agricultural subsidies that increases herbivore populations (geese: Jefferies et al. 2003). Beyond the vegetation loss resulting from the initial grazing, herbivores can also exacerbate erosion and hamper wetland recovery because they continuously uproot, clip, trample, and/or focus their grazing on

new growth (Evers et al. 1998, Taylor and Grace 1995, Turner 1987, McFarlin *pers. obs*.).

Sudden dieback is another disturbance that results in the loss of salt marsh vegetation. There have been reports of dieback along the entire Eastern Atlantic Seaboard and Gulf Coasts since 2000 (reviewed in Alber et al. 2008; GCRC 2011). The onset of sudden dieback is indicated by a rapid yellowing and browning of *S. alterniflora* in standing position followed by a complete loss of vegetation (over the course of a few months) (McKee et al. 2004, Alber et al. 2008). To date, no single factor has been linked to sudden dieback; rather it has been described as a multi-stressor disturbance associated with drought (McKee et al. 2004, Silliman et al. 2005, Alber et al. 2008).

Most studies of salt marsh disturbance document reductions in plant biomass in affected areas, but few have looked for shorter-term indicators of stress. However, studies in sudden dieback areas, however, have reported two different types of physiological responses in *S. alterniflora* plants that may reflect physiological stress. McKee et al. (2004) reported increased concentrations of metals (Fe, Al) in the leaves of visibly affected *S. alterniflora* collected near dieback areas in LA. These metals often become more soluble and bioavailable to vegetation in drained, aerated marsh soils (Portnoy 1999) or when there has been a change in soil biochemistry (especially of pH and Eh) (Kashem and Singh 2001). More recently, Kiehn and Morris (2010) found that the tissue dimethylsulfoniopropionate (DMSP) concentrations of *S. alterniflora* in South Carolina marshes were lowest near dieback areas and increased with distance from the dieback edge. DMSP is a secondary metabolite synthesized by marine algae, a few wetland plants, and sugar cane (Kocsis et al. 1998). Although its exact function in *S.*

alterniflora is not clear, it has been speculated to be an herbivore deterrent, a sulfur detoxifying agent, and, more recently, an antioxidant (Sunda et al. 2002, Otte et al. 2004, Husband and Kiene 2007). Husband and Kiene (2007) found that under oxidation stress, there was direct conversion of *S. alterniflora* DMSP to its oxidation product dimethylsulfoxide (DMSO), supporting the idea of an antioxidant function. They also reported higher DMSO:DMSP ratios in senescing (yellowing) plants as compared to healthy (green) plants, as well as in roots as compared to stems and leaves (Husband and Kiene 2007). It is unclear whether these two types of indicators (metal concentration, and DMSP/DMSO concentration) may also apply to situations where *S. alterniflora* is stressed.

In a natural field experiment, I evaluated metal concentrations, DMSP and DMSO concentrations, and chlorophyll concentrations in *S. alterniflora* collected from areas subject to four different types of disturbance: sudden dieback, wrack deposition, herbivory by littorinid snails, and herbivory by horses. My goals were to test whether any of these measurements were useful as an indicator of stress under field conditions, and whether the response was consistent among the four types of disturbances.

3.2. Methods

3.2.1. Study sites

In the fall of 2008 and 2009, I sampled 20 salt marshes along the GA coast that had areas experiencing a loss of *S. alterniflora*: 5 with a high snail density, 5 with wrack accumulation, 5 with damage by horses, and 5 sudden dieback sites. Sites were located on Sapelo Island (5 snail, 3 wrack, and 3 sudden dieback), Cumberland Island (5 horse),

and in Meridian and Brunswick, GA (2 wrack and 2 sudden dieback, respectively) (Figure 3.1). All disturbed areas were located within a monoculture of *S. alterniflora*. Snail sites had unusually heavy snail densities in the disturbed areas (overall site mean 452 ± 117 m^2 (SE)), which was close to the levels that had been previously reported to lead to loss of vegetation in GA (~600 snails per m^2, Silliman and Bertness 2002). Wrack sites were areas that had visible plant debris accumulated on the salt marsh surface (~ 5 cm thick), with no other known disturbance factors. Horse sites were located in areas frequently grazed by horses, based on observations by NPS rangers at Cumberland National Park. Sudden dieback sites were locations on Sapelo Island known to have died (based on conversation with GCE-LTER research technicians), or those that had been reported to GA-DNR, Coastal Resources Division (Brunswick) following the 2000-2001 droughts in GA.

3.2.2. Sample collection

I sampled plants in three zones at each site: the affected area, along the edge of the affected area, and a nearby healthy area (generally ~10 m away from the edge of the affected area, where there was no visible disturbance). The rationale for including the "edge zone" was to examine *S. alterniflora* in an area that did not appear visibly stressed, yet might still experience negative effects from the nearby disturbed areas (for instance, through rhizomes of *S. alterniflora* or through the loss of neighboring plants that typically ameliorate edaphic stressors) (Bertness and Shumway 1993). In addition, *S. alterniflora* leaves and stems were already completely lost in the "affected zone" at all dieback sites (except for one stem at one site) and *S. alterniflora* leaves were lost in the "affected

zone" at two of the snail sites, so the "edge zone" also provided an intermediate level of a disturbance where I could sample plant tissues.

At each site, three intact *S. alterniflora* plants were haphazardly selected from each zone, except where plants were absent in the disturbed zone. Plants were dug up and then washed thoroughly in the lab to remove bacterial and algal growth. Samples of leaves, stems, and roots were clipped from each plant as follows: for measurements of foliar DMSP, DMSO, and chlorophyll concentrations, a small section of leaf (~0.5 cm length) was clipped from the middle of the youngest fully expanded leaf (typically the second or third leaf from the top), a small section of stem (~0.5 cm length) was clipped at mid-height of the plant, and roots were clipped near the attachment to the rhizome. I clipped samples from 2 of the 3 plants collected from each zone for these analyses. Because the physical condition of plants varied, I clipped the best leaf available based on color and vigor and noted the leaf color. Chlorophyll samples (also ~0.5 cm length) were clipped from leaf and stem areas directly next to samples used for DMSO and DMSP analysis to quantify condition (i.e. from the second youngest fully expanded leaf and at the mid-height of the stem). DMSP, DMSO, and chlorophyll samples were stored at -80° C until analysis.

For analysis of metal content, only living leaves (at least 75% green) were used as previous studies showed that metals (Cu, Pb, Zn) accumulate as *S. alterniflora* leaves get older and senesce, and can also vary greatly from plant to plant (Weis et al. 2003). Where possible, I used the portion of leaf that remained after clipping for DMSO, DMSP and chlorophyll analysis, as well as the entire length of the next youngest fully expanded green leaf. Leaves and stems were pooled separately across the two replicate plants

collected per zone at each site. Samples were dried at 60° C.

3.2.3. Foliar DMSP, DMSP, and DMSO:DMSP concentrations

Samples of plant pieces (typically 10.0-50.0 mg) were thawed and then weighed to the nearest tenth of a milligram for analysis of DMSP or DMSO concentration. In each case, two subsamples were analyzed as independent analytical replicates from a single plant sample and averaged. Samples were placed into 30-mL serum vials (Wheaton, 37.4 mL of headspace), which were capped with gas-tight septa and sealed with aluminum crimp tops. Cellular DMSP was converted to DMS gas by injecting 1 mL of 5 M NaOH into the serum vials. Samples were incubated upside-down in the dark for a period of 24 hrs at 30 C (without shaking) for the liberation of foliar DMSP to DMS gas. The process for DMSO was similar, except that 0.5 mL of 20% $TiCl_3$ was added to vials and incubation was for a period of 2 hours at 50° C for the liberation of DMSO to DMS gas. Following incubation, 0.2 mL (for DMSP analysis) or 0.5 mL (for DMSO analysis) of headspace gas from the serum vials was injected into a flame photometric detector gas chromatograph (an SRI 8610-C with a Chromosil 330 column with nitrogen as the carrier gas) and analyzed for DMS area using the PeakSimple Program.

Standard curves to relate peak area to DMS gas were obtained by injecting the GC with DMS gas liberated from known amounts of DMSP or DMSO standard stocks that were converted to DMS gas using similar volumes of NaOH and $TiCl_3$ as used for samples (Appendix C.1.). It was assumed that all DMS produced as headspace gas or dissolved in the liquid volume of the serum vial was due to direct liberation of foliar DMSP and DMSO. The foliar concentration of DMSP and DMSO (in nmol g^{-1} plant

tissue) was determined by dividing the concentration of DMS gas in the serum vial by the weight of plant tissue. Blank controls for each were *Ophiopogon japonicus* (monkey grass) and DI water, and positive controls of DMS gas were liberated from DMSO standards. Controls were treated and injected identically to samples.

3.2.4. Chlorophyll *a* and Phaeophytin *a* Concentrations

Samples of plant pieces (typically 10.0-50.0 mg) were thawed and then weighed to the nearest tenth of a milligram for analysis of pigment concentration. Samples of both leaf and stem tissue were analyzed for chlorophyll *a* and phaeophytin *a* using EPA Method 445.0 (used for marine algae, Arar and Collins 1997), which was modified for *S. alterniflora* tissue. Samples were macerated with a tissue grinder, chlorophyll was extracted in 90% acetone, centrifuged, and the supernatant measured before and after acidification with 10% HCl on a Turner Designs 10-AU Fluorometer. High and low-value liquid chlorophyll standards with certified spectrophotometer (Abs) readings were used to assign concentration values to fluorometer measurements. The readings from the fluorometer were converted to chlorophyll *a* and phaeophytin *a* using the equations detailed in the EPA 445.0 manual (Arar and Collins 1997) and converted to per gram of tissue (fresh weight). In each case, two subsamples were analyzed as independent analytical replicates and then averaged.

3.2.5. Leaf Metals

To analyze samples for metal concentrations, dried leaves were ground using a Wiley mill (mesh #40), and then burned in a muffle furnace at 500° C for 4 hours. A

plant buffer solution (30% HCl, v/v; 10% HNO_3, v/v; and 20 ppm of Molybdenum) was added to the ash at a ratio of 1:10, sample:buffer. Samples were then analyzed for a suite of 20 elemental constituents (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si, Sr, Zn) with an ICP spectrometer (Jarrell-Ash 965 Inductively Coupled Plasma-Optical Emission Spectrograph) at the University of Georgia's Chemical Analysis Lab using the EPA analytical method 6010 C. NIST plant standards (apple leaves) were used to confirm the proper calibration for the matrix (Appendix C.3.).

3.2.6. Statistical Analysis

Chlorophyll *a*, DMSP, DMSO, the DMSO:DMSP ratio were compared among the four disturbance types and between the three marsh zones. Each measure was analyzed using a 2-way split-plot (partially nested) ANOVA, where disturbance type and zone were the between-plot and within (split)-plot fixed effects, respectively, and sites were considered the unit of replication. The significance of disturbance type was evaluated against the whole-plot error term (sites within disturbance type). The significance of zone and the interaction term zone x disturbance type were evaluated against the split-plot error term (sites within disturbance type x zone, i.e. the residual). The interaction term was used to evaluate the main (null) hypothesis in this study, which was that the effect of zone would be similar regardless of disturbance type (i.e. a non-significant effect supports the hypothesis). Because the split-plot model requires a complete dataset with no missing values, there were a few cases where values were filled in. In the case of affected areas where no plants were collected (all dieback sites, two snail sites), I used the "edge" zone value for the missing "affected" zone. These were considered

conservative in that concentrations of DMSP, DMSO, and chlorophyll *a* were typically lower in the affected than in the edge zones of the other 13 sites. Tukey's multiple comparison post-hoc tests were used to evaluate pairwise differences among disturbance, zone, and disturbance x zone factors. Factors and pairwise differences were considered significant when $p \leq 0.05$.

In order to examine the full suite of elemental composition of *S. alterniflora* leaves, I used non-metric multi-dimensional scaling (NMDS) (R statistical package, R Foundation 2011) to view how zones and disturbance types were separated based on Bray-Curtis distances. Analysis of similarity (ANOSIM) was used to detect whether there were significant overall differences in the group clustering of zone and disturbance type based on 1000 permutations of the data. A sequential Bonferroni significance post-hoc test was used to examine differences within each factor (PAST statistical package, Hammer et al. 2001). Metal constituents were further analyzed using a Pearson correlation coefficient matrix, in order to examine the relationships between individual metals within the foliar tissue.

3.3. Results

3.3.1. DMSP and DMSO

DMSP concentrations in leaves (overall range, 7.4-42.8 μmol g^{-1} fresh weight) and stems (overall range, 2.1-26.2 μmol g^{-1} fresh weight) of healthy *S. alterniflora* plants were within the range of concentrations reported previously (Otte and Morris 1994, Otte et al. 2004, Husband and Keine 2007, Kiehn and Morris 2010; Table 3.1.). DMSO concentrations in leaves (overall range, 0.22-5.08 μmol g^{-1} fresh weight) and stems

(overall range, 0.15-2.68 μmol g^{-1} fresh weight) in the healthy areas were nearly 10x higher than those previously reported (~0.4-0.8 μmol g^{-1} fresh weight for leaves, ~0.3-0.4 μmol g^{-1} fresh weight for stems, Husband and Kiene 2007; Table 3.1.). DMSP and DMSO in the roots of *S. alterniflora* were fairly low with means ranging from 0.09 to 1.55 and 0.01 to 0.90 μmol g^{-1} fresh weight, respectively, in healthy areas (Appendix C.2.). The average observed concentration of DMSP in roots was ~10x lower than other reports (Dacey et al. 1987, Husband and Kiene 2007), whereas the DMSO concentration in roots was slightly higher than the one previous report (Husband and Kiene 2007).

The DMSP concentrations in both leaves and stems were lowest in the affected zones at all disturbance types, except for in stems at the wrack sites where DMSP was highest in the affected area (Table 3.1., Table 3.2.). Zone was a significant source of variation for both leaf ($p = 0.003$) and stem ($p = 0.01$) DMSP concentrations, with significantly higher concentrations in healthy as compared to affected areas (edge areas were intermediate). There was no overall difference in the effect of disturbance type, which indicated that mean concentrations (averaged across zone) of DMSP in leaves and stems were similar among the various disturbance types. There was also no difference in the effect zone x disturbance type, indicating and that the effect of zone on the DMSP concentrations of leaves and stems was similar, regardless of disturbance type.

The DMSO concentrations were approximately an order of magnitude lower than DMSP concentrations in leaves and stems, but the patterns of DMSO concentrations in leaves and stems was often similar to those observed in DMSP concentrations (Table 3.1.). This was especially true for stems: that is, in zones where stem DMSP concentrations were highest (bold terms), stem DMSO concentrations were also highest

in each of the four disturbance types (this pattern also held in two of four disturbance types for leaves). However, neither zone nor disturbance type were significant sources of variation in DMSO concentrations in leaves or stems (Table 3.2.). There was no effect of the interaction term (zone x disturbance type) on DMSO concentrations in leaves, but the effect was significant for stems (likely because stem DMSO concentrations exhibited greater variability in the snail sites, as compared to the variation between zones at other disturbance types). The concentration of DMSP was a significant predictor of the DMSO concentrations in leaves ($p < 0.0001$, $R^2 = 0.17$) and stems ($p < 0.0001$, $R^2 = 0.17$), but the trend was much stronger when only the healthy zones were analyzed (healthy leaves: $p < 0.0001$, $R^2 = 0.63$; healthy stems: $p < 0.0001$, $R^2 = 0.24$).

The ratio of DMSO:DMSP was used by Husband and Kiene (2007) as a way to evaluate changes in the proportions of these constituents. In this study, DMSO consistently represented about ~5% of DMSP fraction in leaves and ~8% of DMSP in stems of healthy zones (Table 3.1., Figure 3.2.). The proportion of DMSO and thus, the ratio of DMSO:DMSP increased in affected zones of both leaves and stems at horse, snail, and wrack sites (Figure 3.2). In the case of dieback, where there were no leaves in the affected zone for comparison, the ratio was increased in plants from the edge zone. Zone was a significant source of variation in the DMSO:DMSP ratio of leaves ($p < 0.0001$) and stems ($p = 0.006$). This pattern was strongest in the leaves where concentrations of DMSP tended to be highest (DMSO concentrations were similar in leaves and stems), and thus the ratio had a greater variation between zones.. There was no effect of disturbance type or zone x disturbance type, indicating that the ratio of DMSO:DMSP in leaves and stems was similar across marsh sites (i.e. mean of each of

the disturbance types) and that the effect of zone was similar, regardless of disturbance type.

Roots had relatively low concentrations of both DMSP and DMSO, and the patterns of DMSO:DMSP were not as strong or consistent for roots as they were for leaves and stems (Appendix C.2.).

3.3.2. Chlorophyll

The chlorophyll *a* concentrations measured for *S. alterniflora* ranged from 0.23-0.80 mg g^{-1} fresh weight for leaves. The chlorophyll *a* content found in healthy leaves was similar to field values reported previously (0.6 mg g^{-1} fresh wt., Seneca and Broome 1972; 0.76 mg g^{-1} fresh wt., Piceno and Lovell 2000), but slightly lower than those reported from plants grown in the greenhouse (Seneca and Broome 1972, Pezeshki et al. 1993) (Table 3.1).Chlorophyll *a* content of stems was consistently 12-20% that of leaves, ranging from 0.05-1.08 mg g^{-1} fresh weight.

Chlorophyll *a* content was highest in stems and leaves in the healthy zones at all of the disturbance types, except for horse sites where concentrations were highest in the affected zone (no measurements were made in affected areas at dieback sites due to unavailability of plants). However, chlorophyll differences among zones within each disturbance type were typically fairly small, and zone was not significant for either leaves or stems (Table 3.2.). Disturbance type was a significant source of variation in leaf chlorophyll, with the horse sites having a higher overall mean concentration of chlorophyll (0.75 mg g^{-1} fresh wt.) as compared to other disturbance types (which were

all ≤ 0.46 mg g^{-1} fresh wt.). There was no significant interaction effect of zone x disturbance type.

Because Husband and Kiene (2007) found that the yellowing plants had higher ratios of DMSO:DMSP, I explored the relationship between chlorophyll *a* and DMSO:DMSP using linear regression. Variation in chlorophyll *a* concentrations did not predict leaf ($N = 32$; $R^2 = 0.06$, NS) or stem ($N = 32$; $R^2 = 0.03$, NS) DMSO:DMSP ratios.

3.3.3. Elemental Composition (metals)

The elemental composition of green *S. alterniflora* leaves generally fell within range of previous reports (Table 3.3.,Table 3.4.), except for B, Cd, and Co, which were approximately an order of magnitude higher than the limited number of previous reports.

The concentration of the 20 elemental trace metals examined exhibited a strikingly similar pattern among all of the disturbance types; the affected and edge zones had the highest concentration for 20 out of 20 metal constituents in the dieback and horse sites, and for 19 of 20 metal constituents (all but K) in the snail and wrack sites (Table 3.3.). I also examined the ratio of metal concentration to potassium, in order to make a comparison to the metal:K ratios reported by McKee et al. (2004) in dieback areas of LA (Table 3.5.). Generally, the pattern was similar to that observed in the raw metal concentrations. The ratios were increased in edge and affected zones for 19 of 19 metals in dieback, horse, and wrack sites, and for 17 of 19 in snail sites. Zone was a significant source of variation in about 13% of the metal ratios, and in these cases the affected zone (~80% of the time) or edge zone (~20% of the time) was always statistically increased in the metal ratio as compared to the healthy area.

In order to examine the overall pattern of total metal composition in *S. alterniflora*, I used an NMDS to view how zones and disturbance types grouped (Figure 3.3.). The ordination was 3-dimensional as determined by a scree plot, which showed the stress versus the number of dimensions in the model (Appendix C.4.). Zones (especially the affected and healthy zones) were distinctly separate groups, whereas disturbance types were not separate (ANOSIM: zone, $p = 0.002$, $R = 0.33$; disturbance type, $p = 0.14$, $R = 0.18$). In post-hoc multiple comparisons, the healthy zone was significantly different from the edge and affected zones, whereas the edge and affected zones were not different from one another (using sequential Bonferroni significance).

I used Pearson correlation coefficient matrices to further examine patterns of individual metal concentrations within each zone (healthy, edge, affected) (Appendix C.5.-C.7.). A majority of the metals were positively correlated with one another in the foliar tissue in each zone. The healthy zone had the greatest number of positive correlations (72%), the affected zone had the least (60%), and the edge zone was intermediate (68%). Statistically significant associations between foliar elements was greatest in the edge zone (48 of 190 comparisons, all positive), followed by the healthy (25 of 190 comparisons, 24 positive) and affected zones (15 of 190 comparisons, 11 positive). There were only 5 significant negative correlations among the elements, and 4 of these were in the affected zone and associated with K (Al, As, Mg, Pb). Several comparisons were strongly correlated, regardless of zone: Al with As ($r > 0.95$) and Sr with Ca ($r > 0.97$). The strong positive correlation between Al and As likely drove many of the other significant associations as well. Among these, Al and As were consistently positively correlated to Pb ($r > 0.71$) in all zones.

Other significant correlations driven by the Al-As association varied with zone, with healthy and edge zones tending to be more similar. Most notably, Al and As were both positively correlated to Fe in the healthy ($r = 0.86$, $r = 0.80$, respectively) and edge zones ($r = 0.90$, $r = 0.94$, respectively), but not in the affected zone ($r = 0.31$, and $r = 0.44$, respectively). On the other hand, both Al and As had significant negative correlations to K in the affected zone ($r = -0.94$, $r = -0.93$, respectively), whereas this relationship was opposite (positive) and not significant in the healthy and edge zones. Other elements significantly correlated in the healthy and edge zones were Pb and Fe with one another ($r > 0.89$), which were each also well-correlated to Co ($r > 0.81$) in these zones. In the affected zone, none of these were significantly correlated with one another. Unpublished data from my other studies also showed relationships among metals (Appendix D., Table D.1.).

3.4. Discussion

I examined the variation of DMSP, DMSO, and metal concentrations in *S. alterniflora* as a measure of physiological stress response to various disturbances. In all 4 disturbance types examined here (sudden dieback, horse overgrazing, high snail density, and wrack), both foliar DMSO:DMSP ratios and the metal composition of *S. alterniflora* were significantly higher in the affected zone, as compared to the healthy areas. Because these responses varied by zone (a proxy for degree of stress) and not by disturbance type, the DMSO:DMSP ratio and metal composition in *S. alterniflora* variables appear to be sensitive indicators that are capable of detecting a generic stress response. In contrast, the individual components (DMSP, DMSO, or single metal species alone) were not as

consistently different among zones. Chlorophyll *a* concentrations, typically used as a visible sign of stress, were the least sensitive of all measures to stress. Below I discuss these results in the context of previous literature.

3.4.1. DMSP response to stress

Several previous studies have examined the concentration of DMSP in *S. alterniflora* with respect to how it varies with salinity, sulfides, and nitrogen (Otte and Morris 1994, Colmer et al. 1996, Mulholland and Otte 2000 and 2001). However, they found that DMSP concentrations were not consistently related to any of these variables, indicating that it was not acting as a compatible solute or sulfur detoxicant. Otte and Morris (1994) suggested that DMSP might potentially function as a methylating agent, an herbivore deterrent, an intermediate in the synthesis of acrylic acid or other compounds, or as a combination of these. There is also some evidence that DMSP has an antioxidant role in both phytoplankton and *S. alterniflora* (Sunda et al. 2002, Husband and Kiene 2007).

More recently, foliar DMSP was found to increase in concentration with distance from dieback areas in SC (Kiehn and Morris 2010). These dieback areas were associated with drought and the multiple associated stressors, suggesting that DMSP could have been a response to generic stress, rather than to a specific stressor. This is in keeping with Husband and Kiene's report (2007) that DMSP concentrations were lower in visibly stressed (yellowing) *S. alterniflora* in the field. I observed a similar pattern of DMSP at the GA dieback sites examined here: leaves and stems taken from healthy zones, located approximately 10 m from the dieback, had higher concentrations of DMSP than did those

collected from the edge zones. DMSP concentrations in both leaves and stems were also significantly decreased in the affected zones of the other three disturbance types, except for stems at wrack sites (it is possible that *S. alterniflora* was not as stressed at the wrack sites).

3.4.2. DMSO response to stress

DMSO concentration alone was not an effective indicator of stress because it occurred at a very low concentration and the variation among zones was inconsistent or insignificant (Table 3.1., Appendix C.2.). The one other study to measure DMSO concentrations in *S. alterniflora* reported even lower mean concentrations, by ~25% for leaves, ~50% for stems, and ~35% for roots than those observed here (Husband and Kiene 2007). Although it is possible that *S. alterniflora* DMSO is highly variable, methodological differences may account for the discrepancies between the two studies. Husband and Kiene (2007) estimated DMSO from within the same serum vial (same plant sample) that was used to estimate DMSP, which required an additional degassing of DMS, and neutralization (with HCl) of the NaOH reagent (used to oxidize DMSP) in order for DMSO reduction to take place with $TiCl_3$. If the NaOH is not fully neutralized, then the $TiCl_3$ can react with it instead of reducing the DMSO, thereby leading to an underestimate of DMSO. It is also possible that the conversion efficiency of DMSO to DMS was greater in the particular batch of $TiCl_3$ reagent that we used. Kiene and Gerard (1994) noted that when the reduction efficiency was low, $TiCl_3$ often yielded as much as 30% less DMS from DMSO standards.

Regardless of differences in the absolute value of DMSO, its concentrations were related to those of DMSP in healthy *S. alterniflora* plants as was also noted by Husband and Kiene (2007). Because DMSO is an oxidation product of DMSP (Sunda et al. 2002, Husand and Kiene 2007), it is not unexpected for it to account for some percentage of the DMSP. I found that DMSO typically accounted for about ~3-8% of leaf DMSP and ~8-9% of stem DMSP. In contrast to healthy zones, DMSP was not well-correlated with DMSO concentrations in the edge and affected zones. This suggests that disturbances likely affect the proportion of foliar DMSP that gets converted to DMSO (Husband and Kiene 2007).

3.4.3. DMSO:DMSP ratio response to stress

If the proportion of DMSO varies with stress, then the ratio of DMSO:DMSP provides a useful way to make comparisons. Husband and Kiene (2007) showed that the DMSO:DMSP value was higher in yellow and spotty, presumably stressed, leaves than in nearby healthy leaves. They suggested that the ratio may increase with senescence and the loss of plant pigment (yellowing). I found that the DMSO:DMSP ratio was significantly greater in the affected zone of leaves and stems, and that this effect did not vary with disturbance type. This finding supports Husband and Kiene's (2007) original idea that stress increases the proportion of foliar DMSO, but also shows that it did not matter what caused the stress to *S. alterniflora*. However, that fact that I did not find a significant relationship between the DMSO:DMSP ratio and chlorophyll *a* concentration suggests that the DMSO:DMSP ratio is responding to something other than senescence alone (as was originally proposed).

3.4.4. Foliar metal concentration response to stress

This is one of the first studies to view metal uptake as a symptom of stress, rather than the cause. Of the 19 metals and phosphorus evaluated in foliar tissues of *S. alterniflora*, nearly all cases (77 of 80) were higher in either the edge or affected zone as compared to the healthy zone. Only K was higher in the healthy zone. The NMDS analysis showed clear differences between the affected and healthy zones in their overall metal load, but there were no difference among disturbance-types. This finding also suggests that the stress response of *S. alterniflora* was similar, regardless of the initial cause of stress.

Past studies have looked at metals as a source rather than an indicator of stress. These have either reported the effects of metal toxicity on *S. alterniflora* in the greenhouse (Carbonell et al. 1998, Mendelssohn et al. 2001, Mateos-Naranjo 2008a and 2008b) or have studied metal accumulation in the field at polluted sites (Hempel et al. 2008, Cambrollé et al. 2011, Salla et al. 2011). Most of these efforts have focused on a single or just a few specific metals of interest, rather than a suite. Across these studies, *S. alterniflora* has shown itself as an excellent phytoremediator, as it is able to hyperaccumulate metals (Salla et al. 2011). Plants that are capable of phytoremediation often scavenge metals into vacuoles (Tang et al. 2009, Xu et al. 2011) or use calcium to block toxicity (Skorzynska-Polit and Baszynski 2000). *S. alterniflora* can also tolerate metals by excluding them through salt glands (Rozema et al. 1991, Burke et al. 2002, Weis et al. 2002) or exporting them to senescing leaves (Weis et al. 2003). Because my sites were located in pristine areas (at a National Estuarine Research Reserve - Sapelo Island, and a National Park - Cumberland Island), contamination was unlikely. Very few

of the metal concentrations observed here (B, Cd, and Co) were elevated compared to other studies that have reported foliar metals in *S. alterniflora* (Table 3.3., Table 3.4.), and none of these exceeded the amounts that would be expected to cause toxicity (Mendelssohn et al.2001, Plank and Kissel 2011).

Mobility and bioavailability of metals in the soil can increase with decreased soil pH (≤5) and an oxidizing environment (Portnoy 1999, Kabata-Pendias 2004). McKee et al. (2004) found increased Al and Fe accumulation in *S. alterniflora* in response to sudden dieback in LA, and suggested that drought conditions could have led to a decrease in soil pH (~5), and that desiccation could have resulted in oxidizing conditions. However, no unusual pH or redox values were observed during sampling (the mean pH was 7.16 and the mean redox value was -180 mV in affected areas across all 4 disturbance types (Chapter 4, Table 4.2.). These results support the idea that metals were not a cause of stress, but rather were a symptom wherein plants accumulated metals under stressful conditions.

Another interesting result observed in the elemental analysis of *S. alterniflora* leaves was that many of the metals were increased simultaneously. A Pearson's correlation matrix showed that most metals were positively correlated with one another, with the highest percentage in the healthy zone. Al and As were correlated with one another in all zones, and each were positively well-correlated with Pb across all zones, and with Fe in the healthy and edge zones. Arsenic is often found in the clay fraction of soils, associated with aluminum and other cations (Ca, Mn, Mg, Pb, Zn) (National Research Council 1977, Walsh et al. 1977, ATSDR 2007) so these results are not surprising. A few studies have observed these metals surrounding the roots of

macrophytes as oxidized plaque accumulations, which could provide a mechanism for their simultaneous uptake (Sunby et al. 1998, Taggert et al. 2009). It is possible that this is the reason for many of the positive associations among metals that were found within leaves of *S. alterniflora*.

There were also variations in relationships among zones. For example, there was a strong negative correlation of Al and As to K in the affected zone, whereas the relationship was positive in the healthy zone and there was no relationship in the edge zone. The opposite response to K and the fact that Fe was not well correlated to Al and As in the affected area may point to a difference in the way that *S. alterniflora* accumulates metals in stressed areas.

One possible scenario to explain the increased concentrations of metals in the disturbed areas is that under stress, plants often close stomata (Mittler et al. 2002, Maricle et al. 2007). This could lead to reduced metal exclusion through the salt glands (hydathodes) and thus increased metals in *S. alterniflora* tissues. The fact that some correlations varied among zones, in the affected area especially (K, for instance), could be due to the inability to control ion balance under stress. Because correlations among metals within *S. alterniflora* have not been previously evaluated, more research is needed to help interpret these results. Nevertheless, and regardless of the mechanism by which metals increase in *S. alterniflora* tissues, this study suggests that evaluating the metal suite could identify stress.

3.4.5. Chlorophyll *a* response to stress

It was surprising how little the disturbances altered the chlorophyll concentration in affected zones as compared to healthy zones (zone was only a significant source of variation at snail sites), as chlorophyll content is often used in research studies as a symptom of stress (Castillo et al. 2000, Mateos-Naranjo 2008a and 2008b, Williams et al. 2009, Li et al. 2010). Chlorophyll concentrations measured in the affected zones (or edge zones for dieback) were reduced by 47%, 19%, and 1% in snail, dieback, and wrack disturbed sites, respectively, in comparison to that measured in the healthy zones. These results suggest that a reliance on chlorophyll content to indicate stress is not necessarily appropriate. This has also been observed in evaluations of salinity stress (Pezeshki and Delaune 1993, Mateos-Naranjo et al. 2010), redox stress (Pezeshki et al. 1993), and CO_2 stress (Mateos-Naranjo et al. 2010). I also found that *S. alterniflora* plants disturbed by horses had increased chlorophyll content as compared to the healthy zones at those sites, a trend opposite to that of the other disturbance types. In this case, it is likely that leaves collected from the affected zone were younger than those in healthy areas due to continuous grazing; younger leaves typically have greater chlorophyll content than older leaves (Šesták 1963, Aslam et al. 1977). Piceno and Lovell (2000) also found a similar effect of increased chlorophyll content in *S. alterniflora* leaves that had been experimentally clipped.

3.5. Conclusions

Many indicators of stress that have been suggested for *S. alterniflora* have been stressor-specific and therefore of limited utility. Proline concentration is a good indicator of salt stress (Mendelssohn and McKee 1992), and glutathione may indicate metal contamination (Pennings et al. 2002). However, indicators capable of detecting stress in many situations, as well as under multi-stressor scenarios, would be much more useful tools for identifying areas potentially at risk. In this study, I found that both the leaf DMSO:DMSP ratio and the overall elemental composition were good integrative indicators of stress in the field, and that both responded consistently to different disturbance types and across multiple field sites. Because the DMSO:DMSP ratio was more than just a simple function of chlorophyll concentration (a proxy for senescence), and the metal composition was responsive in otherwise apparently healthy (green) leaves, both were also sensitive early indicators of stress. It may be that both the DMSO:DMSP ratio and the metal composition of *S. alterniflora* are responding to oxidative stress that can be caused by a wide range of disturbances, but more research is needed to understand the underlying mechanisms of these stress responses. Regardless of the mechanism, these results provide two potential early indicators of stress that can be used in the field.

References

Adam, P. 2002. Saltmarshes in a time of change. *Environmental Conservation* 29 (1)-39-61.

Agency for Toxic Substances and Disease Registry (ATSDR). 2007. Toxilogical Profile for Arsenic. Center for Diesease Control, Applied Toxicology Branch. http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf

Alber, M., Swenson, E.M., Adamowicz, S.C., and Mendelssohn, I.A., 2008. Salt marsh dieback: An overview of recent events in the US. *Estuarine, Coastal and Shelf Science* 80: 1-11.

Alberts, J.J., M.T. Price, and M. Kania. 1990. Metal concentration in tissues of *Spartina alterniflora* (Loisel) and sediments of Georgia salt marshes. *Estuarine, Coastal and Shelf Science* 30: 47-58.

Arar, E.J., and G.B. Collins. 1997. *In vitro* determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence, Method 445.0. National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency.

Aslam, M. S.B. Lowe, and L.A. Hunt. 1977. Effect of leaf age on photosynthesis and transpiration of Cassava (*Manihot esculenta*). *Canadian Journal of Botany* 55 (17): 2288-2295.

Baldwin, A.H., and I.A. Mendelssohn. 1998. Response of two oligohaline marsh communities to lethal and nonlethal disturbance. *Oecologia* 116: 543-555.

Bertness, M. D., and S. W. Shumway. 1993. Competition and facilitation in marsh plants. *American Naturalist* 142:718-724.

Bertness, M. D., P. J. Ewanchuk, and B. R. Silliman. 2002. Anthropogenic modification of New England salt marshes. *Proceedings of the National Academy of Sciences* 99: 1395-1398.

Bertness, M.D. and A.M. Ellison. 1987. Determinants of pattern in a New England marsh plant community. *Ecological Monographs* 57: 129-147.

Bradley, P.M., and J.T. Morris. 1991. Relative importance of ion exclusion, secretion and accumulation in *Spartina alterniflora* Loisel. *Journal of Experimental Botany* 42 (245): 1525-1532.

Broome, S.W., W.W. Woodhouse, Jr., and E.D. Seneca. 1975. The relationship of mineral nutrients to growth of *Spartina alterniflora* in North Carolina: I. Nutrient

status of plants and soils in natural stands. *Journal Series of the North Carolina Agricultural Experiment Station*, North Carolina State University, pp. 295-301.

Burdick, D.M., I.A. Mendelssohn, and K.L. McKee. 1989. Live standing crop and metabolism of the marsh grass *Spartina patens* as related to edaphic factors in a brackish, mixed marsh community in Louisiana. *Estuaries* 12 (3): 195-204.

Burke, D.J., J.S. Weis, and P. Weis. 2002. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuaries, Coastal, and Shelf Science* 51: 153-159.

Cambrollé, E. Mateos-Naranjo, S. Redondo-Gómez, T. Luque, and M.E. Figueroa. 2011. The role of two *Spartina* species in phytostabilization and bioaccumulation of Co, Cr, and Ni in the Tinto-Odiel estuary (SW Spain). *Hydrobiologia* 671: 95-103.

Carbonell, A.A., M.A. Aarabi, R.D. DeLaune, R.P. Gambrell, and W.H. Patrick, Jr. 1998. Arsenic in wetland vegetation: Availability, phytotoxicity, uptake and effects on plant growth and nutrition. *The Science of the Total Environment* 21: 189-199.

Castillo, J.M., L. Fernandez-Baco, E.M. Castellanos, C.J. Luque, M.E. Figueroa, and A.J. Davy. 2000. Lower limits of *Spartina densiflora* and *S. maritima* in a Mediterranean salt marsh. Journal of Ecology 88(5): 801-812.

Colmer, T.D., T.W.M. Fan, A. Lauchli, and R.M. Higashi. 1996. Interactive effects of salinity, nitrogen, and sulphur on the organic solutes in *Spartina alterniflora* leaf blades. *Journal of Experimental Botany* 47: 369–375.

Dacey, J.W.H., G.M. King, and S.G. Wakeham. 1987. Factors controlling emission of dimethylsulphide from salt marshes. *Nature* 330:643–45.

Evers, D.E., C.E Sasser, J.G. Gosselink, D.A. Fuller, and J.M. Visser. 1988. The impact of vertebrate herbivores on wetland vegetation in Atchafalaya Bay, Louisiana. *Estuaries* 21: 1–13.

Ewanchuk, P.J., and M.D. Bertness. 2003. Recovery of a northern New England salt marsh plant community from winter icing. *Oecologia* 136 (4): 616-626.

Ewing, K., K.L. McKee, and I.A. Mendelssohn. 1997. A field comparison of indicators of sublethal stress in the salt-marsh grass *Spartina patens*. *Estuaries* 20 (1): 48-65

Ewing, K., K.L. McKee, I.A. Mendelssohn, and M. Hester. 1995 (a). A comparison of sub-lethal nutrient stress in the salt marsh grass, *Spartina patens*. *Environmental and Experimental Botany:* 35 (3): 331 343.

Ewing, K., K.L. McKee, I.A. Mendelssohn, and M. Hester. 1995 (b). A comparison of indicators of sublethal salinity stress in the salt marsh grass, *Spartina patens (Ait.)* Muhl. *Aquatic Botany* 52: 59-74.

Furbish, C.E., and M. Albano. 1994. Selective herbivory and plant community structure in a Mid-Atlantic salt marsh. *Ecology* 75(4): 1015-1022.

Gallego, S.M., M.P. Benavides, and M.L. Tomaro. 1996. Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. *Plant Science* 121 (2): 151-159.

Gedan, K.B., M.D. Bertness, and B.R. Silliman. 2009. Centuries of human-derived change in salt marsh ecosystems. *Annual Review of Marine Science* 1: 117–141.

Georgia Coastal Research Council (GCRC). 2011. *Focus Areas: Marsh Dieback*. URL: http://www.gcrc.uga.edu/FocusAreas/marsh_dieback.htm. Accessed September 12, 2011.

Hackney, C.T., and T.D. Bishop. 1979. The effect of Hurricane Bob (July 11, 1979) on the St. Louis Bay tidal marshes: Transport and relocation of debris. Mississippi-Alabama Sea Grant Consortium. Publication no. MASGP-79-015.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: Paleontological Statistics software package for education and data analysis. Version 2.10. *Paleontologia Electronica* 4(1): 9 pp.

Hartman, J.M. 1988. Recolonization of small disturbance patches in a New England salt marsh. *American Journal of Botany* 75 (11): 1625-1631.

Hempel, M., S.E. Botté, V.L. Negrin, M.N. Chiarello, and J.E. Marcovecchio. 2008. The role of the smooth cordgrass *Spartina alterniflora* and associated sediments in the heavy metal biogeochemical cycle within Bahía Blanca estuary salt marshes. *Journal of Soils and Sediments* 8:289–297.

Hester, M.W., G.P. Shaffer, J. M. Willis, and D.J. DesRoches. 2002. Restored drill cuttings for wetland creation: results of a mesocosm approach to emulate field conditions under varying salinity and hydrologic conditions. Final Report. US Department of Energy, National Petroleum Technology Office.

Hester, M.W., I.A. Mendelssohn, and K.L. McKee. 2001. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. *Environmental and Experimental Botany* 46: 277-297.

Holdredge, C., M.D. Bertness, and A.H. Altieri. 2008. Role of crab herbivory in die-off of New England Salt Marshes. *Conservation Biology* 23 (3):672-679.

Husband, J.D., and R.P. Kiene. 2007. Occurrence of dimethylsulfoxide in leaves, stems, and roots of *Spartina alterniflora*. *Wetlands* 27 (2): 224-229.

Jefferies, R.L., R.F. Rockwell, and K.F. Abraham. 2003. The embarrassment of riches: agricultural food subsidies, high goose numbers, and loss of Arctic wetlands – a continuing saga. *Environmental Reviews* 11 (4):193-232.

Kabata-Pendias, A. 2004. Soil-plant transfer of trace elements – an environmental issue. *Geoderma* 122: 143-149.

Kashem, M.A., and B.R. Singh. 2001. Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. *Nutrient Cycling in Agroecosystems* 61: 247-255.

Kiehn, WM, J.T. Morris. 2010. Variability in dimethylsulfoniopropionate (DMSP) concentrations in *Spartina alterniflora* and the effect on *Littoraria irrorata*. *Marine Ecology Progress Series* 406: 47-55.

Kiene, R.P. and G. Gerard. 1994. Determination of trace levels of dimethylsulfoxide (DMSO) in seawater and rainwater. *Marine Chemistry* 47: 1-12.

Kocsis, M.G., K.D. Nolte, D. Rhodes, T.L. Shen, D.A. Gage, and A.D. Hanson. 1998. Dimethylsulfoniopropionate biosynthesis in *Spartina alterniflora* - Evidence that S-methylmethionine and dimethylsulfoniopropylamine are intermediates. *Plant Physiology* 117 (1): 273-281.

Laegdsgaard, P. 2006. Ecology, disturbance and restoration of coastal saltmarsh in Australia: a review. *Wetlands Ecology and Management* 14: 379-399.

Li, R., F. Shi, and K. Fukuda. 2010. Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte *Spartina alterniflora* (Poaceae). *Environmental and Experimental Botany* 68(1): 66-74.

Lichtenthaler, H.K. (1987) Chlorphylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* 148: 350-382.

Linthurst, R.A., and E.D. Seneca. 1981. Aeration, nitrogen, and salinity as determinants of *Spartina alterniflora* Loisel. growth response. *Estuaries* 4(1): 53-63.

Mahon, S., and K.R. Carman. 2008. The influence of salinity on the uptake, distribution, and excretion of metals by smooth cordgrass, *Spartina alterniflora* (Loisel.), grown in sediment contaminated by multiple metals. *Estuaries and Coasts* 31: 1089-1097.

Maricle, B.R., D.R. Cobos, and C.S. Campbell. 2007. Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses. *Environmental and Experimental Botany* 60: 458-467.

Martin, J.L. 2003. The effect of cattle grazing on the abundance and distribuation of selected macroinvertebrates in West Galveston Island salt marshes. M.S. Thesis, Texas A&M University, 74 p.

Mateos-Naranjo, E., Redondo-Gómez, J. Cambrollé, and M. E. Figueroa. 2008 (a). Growth and photosynthetic responses to copper stress of an invasive cordgrass, *Spartina densiflora*. *Marine Environmental Research* 66: 459–465.

Mateos-Naranjo, E., Redondo-Gómez, J. Cambrollé, T. Luque and M. E. Figueroa. 2008 (b). Growth and photosynthetic responses to zinc stress of an invasive cordgrass, *Spartina densiflora*. *Plant Biology* 10: 754–762.

Mateos-Naranjo, E., S. Redondo-Gómez, L. Andrades-Moreno, and A.J. Davy. 2010. Growth and photosynthetic responses of the cordgrass *Spartina maritima* to CO2 enrichment and salinity. *Chemosphere* 81: 725–731.

McKee, K.L., I.A. Mendelssohn, and M.D. Materne. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecology and Biogeography* 13 (1): 65-73.

Mendelssohn, I.A., and K.L. McKee. 1992. Indicators of environmental stress in wetland plants. In: D.H. McKenzie, D.E. Hyatt, and V.J. McDonald (eds.), *Ecological Indicators*, Vol. 1, pp. 603-624. Elseiver, New York.

Mendelssohn, I.A., K.L. McKee, and T. Kong. 2001, A comparison of physiolobical indicators of sublethal cadmium stress in wetland plants. *Environmental and Experimental Botany* 46: 263-275.

Miller, W.D., S.C. Neubauer, and I.C. Anderson. 2001. Effects of sea level induced disturances on high salt marsh metabolism. *Estuaries* 24 (3): 357-367.

Mittler, R. 2002. Oxidative stress, antioxidants, and stress tolerance. *Trends in Plant Science* 7 (9): 405-410.

Mulholland M.M., and M.L. Otte. 2000. The effects of varying sulphate and nitrogen supply on DMSP and glycine betaine levels in *Spartina anglica*. *Journal of Sea Research* 43: 199–207.

Mulholland M.M., and M.L. Otte. 2001. The effects of nitrogen supply and salinity on [DMSP], [glycine betaine] and [proline] concentrations in leaves of *Spartina anglica*. *Aquatic Botany* 71: 63-70.

National Research Council (Committee on Medical and Biological Effects of Environmental Pollutants). 1977. *Arsenic: Medical and Biological Effects of Environmental Pollutants.* National Academy of Sciences, Washington, D.C.

Ornes, W.H., and D.I. Kaplan. 1989. Macronutrient status of tall and short forms of *Spartina alterniflora* in a South Carolina salt marsh. *Marine Ecology Progress Series* 55: 63-72.

Otte, M.L., and J.T. Morris. 1994. Dimethylsuphoniopropionate (DMSP) in *Spartina alterniflora* Loisel. *Aquatic botany* 48: 239-259.

Otte, M.L., G. Wilson, J.T. Morris, and B.M. Moran. 2004. Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants. *Journal of Experimental Botany* 55 (404): 1919-1925.

Padinha, C., R. Santos, and M.T. Brown. 2000. Evaluating environmental contamination in Ria Formosa (Portugal) using stress indexes of *Spartina maritima*. *Marine Enviornmental Research* 49: 67-78.

Pennings, S.C. and M. D. Bertness. 2001. Salt Marsh Communities. In: M. D. Bertness, S.D. Gaines, and M. Hay (eds), *Marine Community Ecology*. Sinauer, Sunderland MA.

Pennings, S.C., V.D. Wall, D.J. Moore, M. Pattanayek, T.L. Buck, and J.J. Alberts. 2002. Assessing salt marsh health: A test of the utility of five potential indicators. *Wetlands* 22 (2): 405-414.

Pezeshki, S.R., and R.D. Delaune. 1993. Effects of soil hypoxia and salinity on gas exchange and growth of *Spartina patens*. *Marine Ecology Progress Series* 96: 75-81.

Pezeshki, S.R., J.H. Pardue and R.D. DeLaune. 1993. The influence of soil oxygen deficiency on alcohol dehydrogenase activity, root porosity, ethylene production and photosynthesis in *Spartina patens*. *Environmental and Experimental Botany* 33(4): 565-573.

Piceno, Y.M., and C.R. Lovell. 2000. Stability in natural bacterial communities: II. Plant resource allocation effects on rhizosphere diazotroph assemblage composition. *Microbial Ecology* 39: 41-48.

Plank, C.O., and D.E. Kissel. 2011. *Plant Analysis Handbook for Georgia*. The Cooperative Extension Service, The University of Georgia, College of Agricultural and Environmental Sciences. URL: http://aesl.ces.uga.edu/publications/plant/. Accessed October 15, 2011.

Portnoy, J.W. 1999. Salt marsh diking and restoration: Biogeochemical implications of altered wetland hydrology. *Environmental Management* 24(1): 111-120.

R Foundation for Statistical Computing. 2011. R: A Language and Environment for Statistical Computing (version 2.13.1). R Development Core Team: Vienna, Austria, http://www.R-project.org.

Reidenbaugh, T.G., and W.C. Banta. 1980. Origin and effects of *Spartina* wrack in a Virginia salt-marsh. *Gulf Research Reports* 6(4): 393-401.

Rietsma, C.S., and I. Valiela. 1995. Disturbance of salt-marsh vegetation by wrack mats in Great-Sippewissett Marsh. *Oecologia* 102 (1): 106-112.

Rozema, J., E. Buys, M.L. Otte, R.A. Broekman, and W. Ernst. 1991. Ion content and ion excretion of *Spartina anglica* in relation to salinity and redox potential of salt marsh soil. *Journal of Plant Nutrition and Soil Science* 154 (4): 307-313.

Salla, V., C.J. Hardaway, and J. Sneddon. 2011. Preliminary investigation of *Spartina alterniflora* for phytoextraction of selected heavy metals in soils from Southwest Louisiana. *Microchemical Journal* 97: 207-212.

Seneca, E.D., and S.W. Broome 1972. Seedling response to photoperiod and temperature by smooth cordgrass, *Spartina alterniflora,* from Oregon Inlet, North Carolina. *Chesapeake Science* 13 (3): 212-215.

Šesták, Z. 1963. Changes in the chlorophyll content as related to photosynthetic activity and age of leaves. *Photochemistry and Photobiology* 2 (2): 101-110.

Silliman, B.R. and M.D. Bertness, 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Sciences* 99 (16): 10500-10505.

Silliman, B.R., J. van de Koppel, M.D. Bertness, L.E. Stanton, and I.A. Mendelssohn. 2005. Drought, snails, and large-scale die-off of Southern U.S. Salt Marshes. *Science* 310 (5755): 1803-1806.

Skorzynska-Polit, E., and T. Baszynski. 2000. Does Cd2+ use Ca2+ channels to penetrate into chloroplasts? - a preliminary study. *Acta Physiologiae Plantarum* 22 (2): 171-178.

Slocum, M.G., and I.A. Mendelsoohn. 2008. Use of experimental disturbances to assess resilience along a known stress gradient. *Ecological Indicators* 8: 181-190.

Smart, R.M., and J.W. Barko 1978. Influence of sediment salinity and nutrients on the physiological ecology of selected salt marsh plants. *Estuarine and Coastal Marine Science* 7: 487-495.

Sunda, W., D. J. Kieber, R. P. Kiene, and S. Huntsman. 2002. An antioxidant function for DMSP and DMS in marine algae. *Nature* 418: 317–20.

Sundby, B, C. Vale, I. Cacador, F. Catarino, M.J. Madureira, and M. Caetano. 1998. Metal rich concretions on the roots of salt marsh plants: Mechanism and rate of formation. *Limnology and Oceanography* 43 (2): 245-252.

Taggart, M.A., R. Mateo, J.M. Charnock, F. Bahrami, A.J. Green, and A.A. Megarg. 2009. Arsenic rich plaque on macrophytes roots – an ecotoxilogical risk? *Environmental Pollution* 157: 946–954.

Tang, C.F., R.Q. Zhang, S.Z. Wen, C.F. Li, X.F. Guo, and Y.G. Liu. 2009. Effects of exogenous spermidine on subcellular distribution and chemical forms of cadmium in *Typha latifolia* L. under cadmium stress. *Water Science and Technology* 59 (8): 1487-1493.

Taylor, K.L., and J.B. Grace. 1995. The effects of vertebrate herbivory on plant community structure in the coastal marshes of the Pearl River, Louisiana, USA. *Wetlands* 15 (1): 68-73.

Taylor, K.L., J.B. Grace, and B.D.Marx. 1997. The effects of herbivory on neighbor interactions along a coastal marsh gradient. *American Journal of Botany* 84 (5): 709-715.

Tolley, P.M., and R.R. Christian. 1999. Effects of increased inundation and wrack deposition on a high salt marsh plant community. *Estuaries* 22 (4): 944-954.

Turner, M.G. 1987. Effects of grazing by feral horses, clipping, trampling, and burning in a Georgia salt marsh. *Estuaries* 10 (1): 54-60.

Turner, M.G. 1988. Simulation and management implications of feral horse grazing on Cumberland Island, Georgia. *Journal of Range Management* 41 (5): 441-447.

Walsh, L.M., D.R. Keeney, and M.E. Sumner. 1977. Occurrence and distribution of arsenic in soils and plants. *Environmental Health Perspectives* 19: 67-71.

Weilhoefer, C.L. 2011. A review of indicators of estuarine tidal wetland condition. *Ecological Indicators* 11: 514-525.

Weis, J.S., L. Windham, and P. Weis. 2003. Patterns of metal accumulation in leaves of the tidal marsh plants *Spartina alterniflora* Loisel and *Phragmites australis* Cav. Trin Ex Steud. over the growing season. *Wetlands* 23 (2): 469-465.

Weis, P., L. Windham, D.J. Burke, and J.S. Weis. 2002. Release into the environment by two vascular salt marsh plants. *Marine Environmental Research* 54: 325-329.

White, S.N. 2004. *Spartina* species zonation along an estuarine gradient in Georgia: exploring mechanisms controlling distribution. Ph.D. Thesis, University of Georgia, 206 p.

Williams, S.L., A. Carranza, J. Kunzelman, S. Datta, and K.M. Kuivila. 2009. Effects of the herbicide diuron on cordgrass (*Spartina foliosa*) reflectance and photosynthetic parameters. *Estuaries and Coasts* 32 (1): 146-157.

Windham, L., J.S.Weis, and P. Weis. 2003. Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). *Estuarine, Coastal and Shelf Science* 56: 63-72.

Xu, W., W. Shi, F. Yan, B. Zhang, J. Liang. 2011. Mechanisms of cadmium detoxification in cattail (*Typha angustifolia* L.) *Aquatic Botany* 94: 37-43.

Table 3.1. Mean (SE) of chlorophyll *a*, DMSP, and DMSO concentrations in leaves and stems of *S. alterniflora* collected in healthy, edge, and affected zones at dieback, horse, snail, and wrack disturbance types. The highest concentration of chlorophyll *a*, DMSP, or DMSO per zone is shown in bold, in order to highlight trends. Each mean represents *N*=15 for chlorophyll and *N*=10 for DMSP and DMSO in leaves and stems, except at wrack sites (where *N*=9 for chlorophyll and *N*=6 for DMSP and DMSO). --- = no sample, † = one sample.

Site-Type	Zone	Leaf Chl a (SE)	Stem Chl a (SE)	Leaf DMSP (SE)	Stem DMSP (SE)	Leaf DMSO (SE)	Stem DMSO (SE)
		(mg g^{-1} fresh wt)		(µmol g^{-1} fresh wt)			
dieback	Affected	---	---	---	0.36 (0)†	---	0.11 (0)†
	Edge	0.389 (0.052)	0.073 (0.020)	13.45 (3.62)	8.35 (1.67)	**1.06 (0.30)**	0.68 (0.09)
	Healthy	**0.481 (0.055)**	**0.075 (0.010)**	**17.44 (2.41)**	**12.27 (2.14)**	1.05 (0.28)	**1.05 (0.29)**
horse	Affected	**0.803 (0.045)**	**0.108 (0.024)**	11.32 (0.97)	4.37 (0.76)	1.25 (0.36)	0.61 (0.16)
	Edge	0.744 (0.054)	0.102 (0.017)	12.56 (1.53)	6.66 (1.36)	0.63 (0.09)	047 (0.11)
	Healthy	0.688 (0.078)	0.095 (0.013)	**21.74 (2.48)**	**9.43 (1.59)**	**1.66 (0.42)**	**0.86 (0.23)**
snails	Affected	0.231 (0.069)	0.049 (0.013)	10.71 (3.48)	5.34 (1.79)	**0.90 (0.20)**	0.26 (0.06)
	Edge	0.43 (0.052)	0.059 (0.010)	**15.61 (1.27)**	8.04 (1.60)	0.50 (0.08)	0.37 (0.08)
	Healthy	**0.438 (0.036)**	**0.084 (0.014)**	14.97 (1.22)	**11.16 (1.58)**	0.57 (0.08)	**1.06 (0.30)**
wrack	Affected	0.479 (0.042)	0.063 (0.010)	10.13 (1.64)	**12.21 (2.25)**	0.80 (0.26)	**1.93 (0.88)**
	Edge	0.422 (0.030)	0.058 (0.014)	**17.81 (3.04)**	11.99 (1.58)	**0.93 (0.37)**	0.76 (0.23)
	Healthy	**0.484 (0.030)**	**0.078 (0.014)**	15.22 (2.11)	10.30 (0.99)	0.67 (0.10)	0.51 (0.08)

Table 3.2. Statistical summary of split-plot ANOVAs for testing the main effects disturbance type and zone on the variation in chlorophyll *a*, DMSP, DMSO, and the DMSO:DMSP ratio in *S. alterniflora* leaves and stems of plants in the 20 survey sites. Values in bold are significant ($p < 0.05$).

Source of Variation	Leaves			Stems		
	d.f.	F	P	d.f.	F	P
		Chlorophyll a			*Chlorophyll a*	
Disturbance Type	3	10.99	**0.0004**	3	3.12	0.0555
Sites within disturbance type (whole-plot error term)	16			16		
Zone	2	0.61	0.5472	2	1.02	0.3704
Zone x Disturbance Type	6	1.44	0.2310	6	0.54	0.7757
Residual (Split-plot error term)	32			32		
		DMSP			*DMSP*	
Disturbance Type	3	0.29	0.8298	3	2.56	0.0917
Sites within disturbance (whole-plot error term)	16			16		
Zone	2	7.22	**0.0026**	1	5.02	**0.0127**
Zone x Disturbance Type	6	1.19	0.3383	3	1.04	0.7561
Residual (Split-plot error term)	32			16		
		DMSO			*DMSO*	
Disturbance Type	3	1.76	0.1947	3	2.2	0.1280
Sites within disturbance type (whole-plot error term)	16			16		
Zone	2	1.45	0.2496	1	1.54	0.2308
Zone x Disturbance Type	6	1.58	0.1837	3	3.77	**0.0060**
Residual (Split-plot error term)	32			16		
		DMSO:DMSP			*DMSO:DMSP*	
Disturbance Type	3	0.54	0.6622	3	1.02	0.4081
Sites within disturbance type (whole-plot error term)	16			16		
Zone	2	11.81	**<0.0001**	2	6.12	**0.0056**
Zone x Disturbance Type	6	1.27	0.2997	6	0.19	0.9771
Residual (Split-plot error term)	32			32		

Table 3.3. Mean tissue elemental composition of *S. alterniflora* leaves (µg g^{-1} leaf dry weight) in healthy, edge, and affected zones at dieback, horse, snail, and wrack disturbance types. The number of sites that were averaged for a mean per zone by site type is indicated. The highest concentration of each element per zone is shown in bold.

Site Type	Zone	N (sites)	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe
dieback	Healthy	3	1016	0.21	43.8	0.00	2870	2.82	0.84	2.80	1.56	459
	Edge	3	1623	0.29	**112.9**	0.00	**3529**	**3.44**	**1.05**	**3.59**	**2.98**	**690**
	Affected	1	**2368**	**0.37**	99.1	**3.56**	2705	2.84	0.91	3.42	2.83	593
horse	Healthy	2	1351	0.27	46.1	1.94	1583	2.78	1.10	3.68	2.15	704
	Edge	2	**1901**	**0.38**	**132.6**	**2.28**	**2493**	**4.40**	**1.75**	**5.85**	5.23	**1086**
	Affected	2	1596	0.33	121.0	2.14	2418	4.03	1.40	4.69	**7.73**	842
snails	Healthy	3	1545	0.28	43.1	1.00	3065	2.94	0.94	3.15	1.54	589
	Edge	3	1580	0.28	**59.8**	0.42	3088	3.15	0.97	3.28	1.98	613
	Affected	2	**2399**	**0.41**	52.3	**2.36**	**4192**	**4.30**	**1.39**	**4.24**	**2.43**	**640**
wrack	Healthy	2	1486	0.24	43	0.00	2471	2.28	0.87	3.75	1.76	552
	Edge	2	**2493**	**0.37**	35	0.18	2897	2.89	1.04	3.38	2.19	**808**
	Affected	2	2307	**0.37**	**109**	**0.45**	**4298**	**4.12**	**1.50**	**4.44**	**3.21**	542

Table 3.3. (continued) Mean tissue elemental composition of *S. alterniflora* leaves (µg g^{-1} leaf dry weight) in healthy, edge, and affected zones at dieback, horse, snail, and wrack disturbance types. The number of sites that were averaged for a mean per zone by site type is indicated. The highest concentration of each element per zone is shown in bold.

Site Type	Zone	N (sites)	K	Mg	Mn	Na	Ni	P	Pb	Si	Sr	Zn
dieback	Healthy	3	8670	2834	46.1	3720	2.51	1377	1.03	2935	38.1	6.3
	Edge	3	8588	**3892**	**89.9**	3915	**2.96**	1609	1.45	**4713**	**50.3**	11.7
	Affected	1	**11040**	3384	46.0	**6252**	2.34	**1985**	**1.65**	4588	43.0	**21.8**
horse	Healthy	2	11027	2503	25.8	3852	3.02	1023	1.34	5187	20.1	8.1
	Edge	2	9975	**4163**	**52.9**	4930	**7.24**	1707	**2.28**	**7815**	32.3	12.6
	Affected	2	**12930**	4022	48.5	**6405**	6.08	**2258**	1.80	7603	**35.6**	**18.6**
snails	Healthy	3	**11825**	3187	17.6	4456	2.85	1235	1.37	4100	43.6	5.9
	Edge	3	11247	3826	19.8	4180	**5.58**	**1285**	1.45	**4287**	44.6	6.8
	Affected	2	9035	**5678**	**19.9**	**7469**	3.55	1106	**2.36**	3691	**69.2**	**7.9**
wrack	Healthy	2	**11165**	2132	22	4232	2.7	1294	1.24	3326	33.9	4.7
	Edge	2	11145	3044	36	5843	**11.4**	1448	1.84	**4079**	44.0	6.4
	Affected	2	9733	**5050**	**161**	**14925**	3.4	**2358**	**1.92**	3578	**66.5**	**12.9**

Table 3.4. Reported means and ranges (μg g^{-1} dry weight) of tissue elemental composition in leaves of *S. alterniflora*, unless otherwise noted. Concentrations were taken from experimental control plants or from plants in natural field settings where possible, unless otherwise noted.

Source	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Si	Sr	Zn
Broome et al. 1975 (NC)[1]					3100-3200					530-702	7700-9500	2900-3400	20-31	22900-25000		900-1200				
Gallagher 1975 (GA)[1]			5-12		700-2100				5-14		6300-11200	4300	26-55			1400-1600			15-24	12-38
Linthurst 1979 (NC)[1]					1500-3000				5-12	221-555	10200-14300	2400-3000	28-141	17000-20000		1100-1200				20-53
Gallagher et al. 1980 (GA)[1]					~1500					~650	~10000	~3700	~110			~1700				
Linthurst & Seneca 1981 (NC)[1,a]					2500-3400				5-13	99-250	12100-14400	3000-4000	30-74	17000-21400		1800-2200				17-30
Broome et al. 1986 (NC)[1]					2500					623	7800	4660	36	29500		900				
Ornes & Kaplan 1989 (SC)[1]					2200-2600						12300-12900	3500				1600-1900				
Alberts et al. 1990 (GA)	119-471								4.2-6.0	128-385			30-103							6-14
Bradley & Morris 1991[1]					1603-2004						8602-14706	4133-8509		117708						
Ornes et al. 1998 (SC)[1]	200-5000		2-10						0.25-8	200-1750			20-125							3-25
Carbonell et al. 1998 (LA)		0.25-0.5			3600						18330	2230		4720		2630				
Hester 2002 (LA)[b]				~6-15																
Windham et al. 2003 (NJ)								1.25-4.0	3.7-6.5								0.75-3.10			22-40
White (2004) (GA)	1542				3385					939	9470	4774	257	9551		1667		2477	51.6	

Table 3.4. (continued) Reported means and ranges (μg g^{-1} dry weight) of tissue elemental composition in leaves of *S. alterniflora*, unless otherwise noted. Concentrations were taken from experimental control plants or from plants in natural field settings where possible, unless otherwise noted.

Source	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Si	Sr	Zn
Mahon and Carman 2008 (LA)								~0.5-1.0	~5-8						~2-5					~10-40
Hempel et al. 2008 (Argentina)[c]						0.1-0.8		<0.02	4-37	182-510			857		2-5		<0.12-2.1			18-103
Salla et al. 2011 (LA)								2.0-4.0	6.0-8.0	310-920							0.5-3.0			26-42
Cambrollé et al. 2011 (Spain)[d]							<0.1	1.4-14							<0.5					

[1] Orignally compiled by S. White (2004), edited by C. McFarlin (2011).
[a] Reporting elemental range of greenhouse plants grown under salinity of 15-30 ppt.
[b]Measurements represent the average elemental tissue compostion of *S. alterniflora*, *S. patens*, *S. cynosuroides*, *Avicennia germinans* in topsoil.
[c]Sites were suspected of contamination.
[d]Measurements were from *Spartina densiflora* and *S. maritima* in sites supected of metal contamination.

Table 3.5. Mean tissue ratio (multiplied by 100 for all, and by 1000 for As:K) of element to potassium concentration in *S. alterniflora* leaves in healthy, edge, and affected zones at dieback, horse, snail, and wrack disturbance types. The number of sites that were averaged for a mean ratio per zone by site type is indicated.

Site Type	Zone	N (sites)	Al:K	As:K	B:K	Ba:K	Ca:K	Cd:K	Co:K	Cr:K	Cu:K	Fe:K
dieback	Healthy	3	11.7	0.024	0.506	0.000	33.1	0.033	0.010	0.032	0.018	5.30
	Edge	3	18.9	**0.034**	**1.325**	0.000	**41.1**	**0.040**	**0.012**	**0.042**	**0.035**	**8.04**
	Affected	1	**21.4**	**0.034**	0.898	**0.032**	24.5	0.026	0.008	0.031	0.026	5.37
horse	Healthy	2	12.3	0.024	0.418	0.018	14.4	0.025	0.010	0.033	0.020	6.39
	Edge	2	**19.1**	**0.038**	**1.330**	**0.023**	**25.0**	**0.045**	**0.018**	**0.059**	0.052	**10.89**
	Affected	2	12.3	0.026	0.936	0.017	18.7	0.031	0.011	0.036	**0.060**	6.51
snails	Healthy	3	13.1	0.023	0.364	0.008	**25.9**	0.025	0.008	0.027	0.013	4.98
	Edge	3	14.0	0.025	0.532	0.004	27.5	0.028	0.009	0.029	0.018	5.45
	Affected	2	**26.5**	**0.045**	**0.579**	**0.026**	46.4	**0.048**	**0.015**	**0.047**	**0.027**	**7.08**
wrack	Healthy	2	13.3	0.022	0.383[a]	0.000	22.1	0.020	0.008	0.034	0.016	4.94
	Edge	2	22.4	0.033	0.316[a]	0.002	26.0	0.026	0.009	0.030	0.020	**7.25**
	Affected	2	**23.7**	**0.038**	**1.118[b]**	**0.005**	**44.2**	**0.042**	**0.015**	**0.046**	**0.033**	5.57

Table 3.5. (continued) Mean tissue ratio (multiplied by 100 for all, and by 1000 for As:K) of element to potassium concentration in *S. alterniflora* leaves in healthy, edge, and affected zones at dieback, horse, snail, and wrack disturbance types. The number of sites that were averaged for a mean ratio per zone by site type is indicated. The highest ratio of element to potassium per zone is shown in bold.

Site Type	Zone	N (sites)	Mg:K	Mn:K	Na:K	Ni:K	P:K	Pb:K	Si:K	Sr:K	Zn:K
dieback	Healthy	3	32.7	0.532	42.9	0.029	15.9	0.012	33.8	0.439	0.073
	Edge	3	**45.3**	**1.047**	45.6	**0.034**	**18.7**	**0.017**	**54.9**	**0.586**	0.136
	Affected	1	30.7	0.416	**56.6**	0.021	18.0	0.015	41.6	0.389	**0.198**
horse	Healthy	2	22.7	0.234	34.9	0.027	9.3	0.012	47.0	0.182	0.073
	Edge	2	**41.7**	**0.530**	49.4	**0.073**	17.1	**0.023**	**78.3**	**0.324**	0.126
	Affected	2	31.1	0.375	**49.5**	0.047	**17.5**	0.014	58.8	0.275	**0.144**
snails	Healthy	3	27.0a	0.149	37.7	0.024	10.4	0.012	34.7	0.369	0.050
	Edge	3	34.0a,b	0.176	37.2	**0.050**	11.4	0.013	38.1	0.396	0.060
	Affected	2	**62.8b**	**0.220**	**82.7**	0.039	**12.2**	**0.026**	**40.9**	**0.766**	**0.087**
wrack	Healthy	2	19.1	0.194	37.9	0.024	11.6	0.011	29.8	0.304	0.042
	Edge	2	27.3	0.321	52.4	**0.102**	13.0	0.017	36.6	0.394	0.057
	Affected	2	**51.9**	**1.650**	**153.4**	0.035	**24.2**	**0.020**	**36.8**	**0.683**	**0.132**

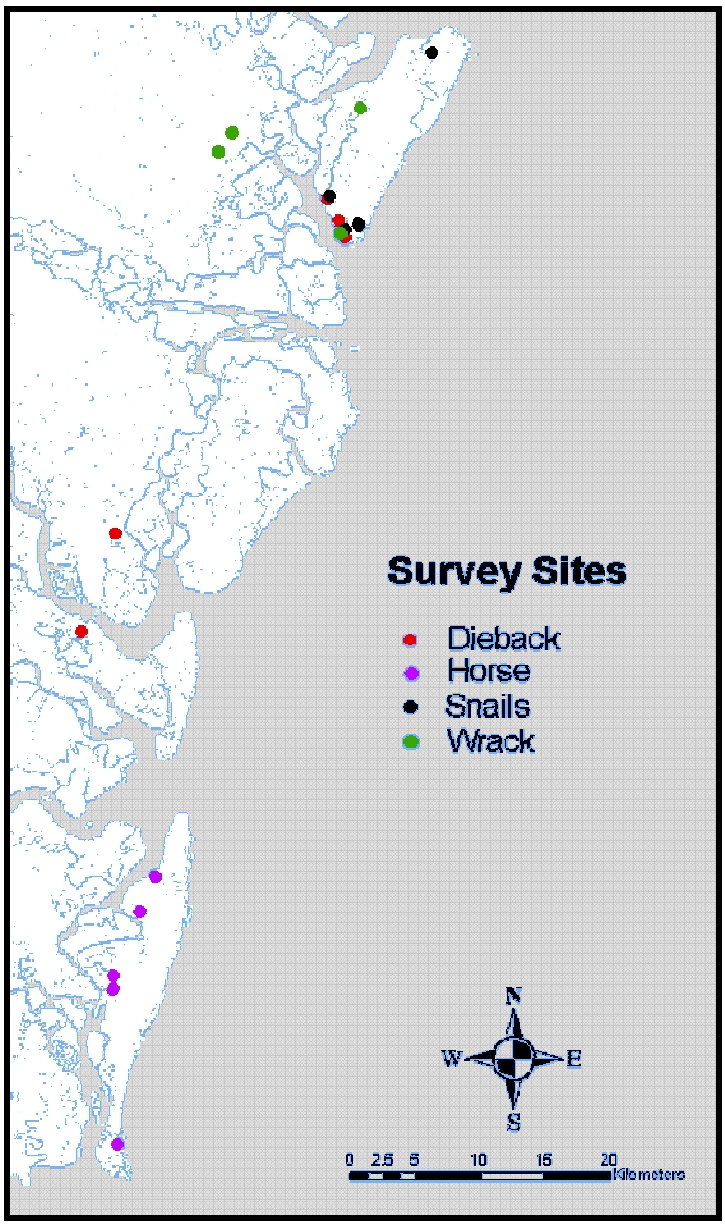


Figure 3.1. Location of the dieback, horse, snail, and wrack sites along the Georgia Coast (five sites per disturbance type). At each site, plants were sampled haphazardly to test tissue DMSP, DMSO, chlorophyll, and metal concentration, from within the healthy, edge of affected, and affected marsh.

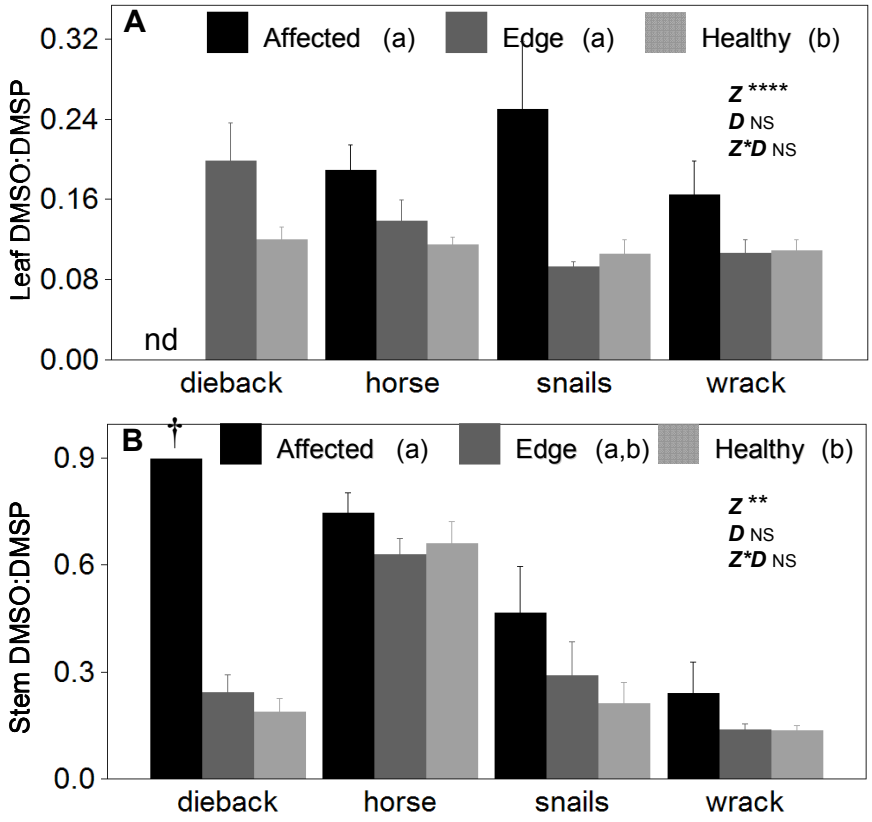


Figure 3.2. The ratio of DMSO:DMSP in A)*S. alterniflora* leaves and B) stems in healthy, edge, and affected zones of dieback, horse, snail, and wrack disturbance types (N=10 per bar, except at wrack sites where N=6 per bar). The significance (p-value) of the split–plot ANOVA factors zone (Z), disturbance type (D), and zone x disturbance type (Z*D) are indicated by asterisks, where *<0.5, **<0.01, ***<0.001, ****<0.0001, and NS=not significant, and different letters indicate pairwise differences among zones (Tukey's multiple comparison test). nd=no data, †=one sample (not included in statistical analysis).

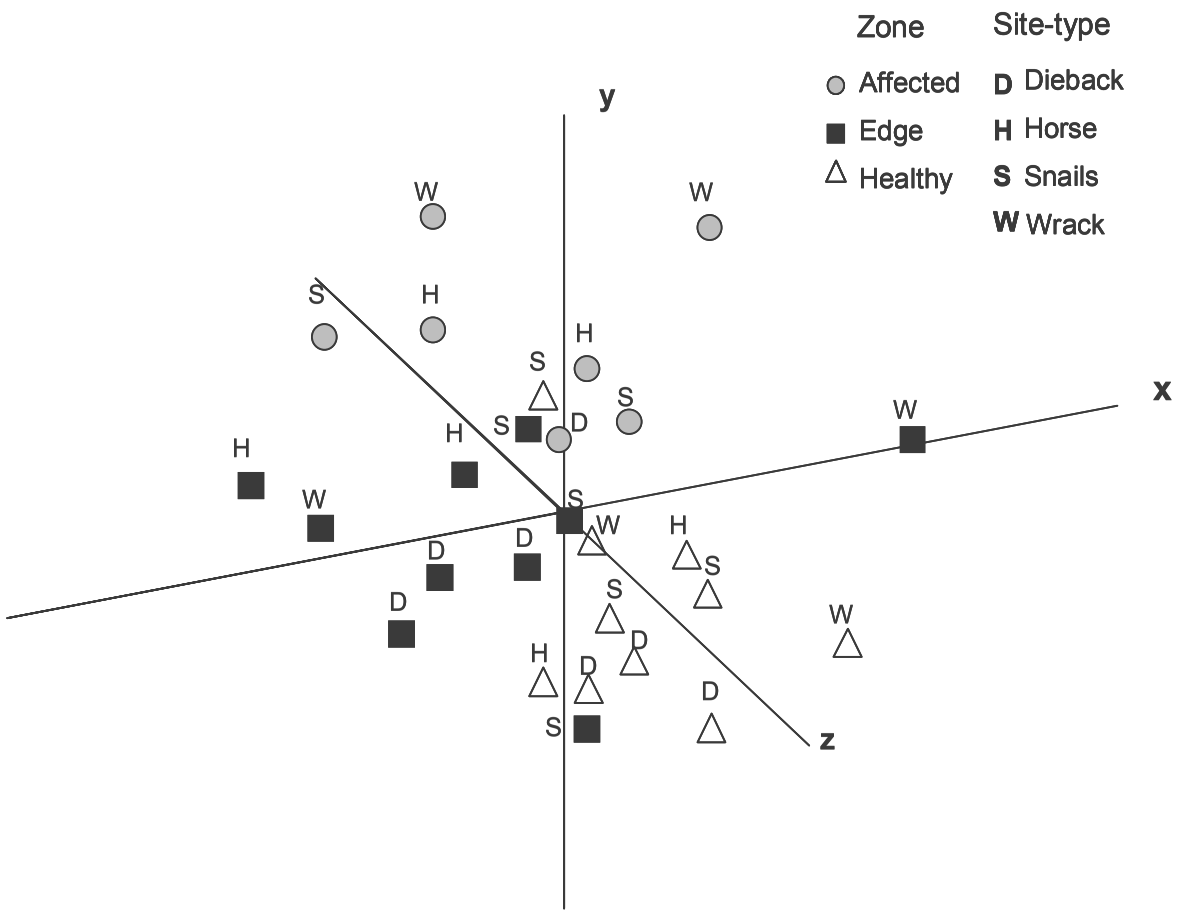


Figure 3.3. Three-dimensional ordination (non-metric multidimensional scaling) grouped by salt marsh zone and disturbance site-type based on similarities (Bray-Curtis) in elemental composition in *S. alterniflora* leaf tissues for the entire suite of constituents (20 elements). Stress: 0.07.

CHAPTER 4

THE EFFECT OF DISTURBANCE ON INVERTEBRATE ASSEMBLAGES IN GEORGIA SALT MARSHES[5]

[5] McFarlin, C.R., and M. Alber. To be submitted to *Estuaries and Coasts*.

Abstract

In salt marshes, *Spartina alterniflora* is a foundation species that provides essential habitat and ameliorates soil properties for benthic invertebrates. Disturbances leading to a loss of *S. alterniflora* can indirectly affect the invertebrate community. Twenty sites along the GA coast were chosen to represent sudden dieback, horse-, snail-, and wrack-disturbed areas (5 of each disturbance type). At each site, I assessed *S. alterniflora*, epifauna and infaunal organisms, and soil conditions along a transect through 3 zones (healthy, edge, affected). I evaluated the null hypothesis that there would be no zone x disturbance type interactions, that is, that the effect of *S. alterniflora* loss would be similar regardless of disturbance type. Although sites were quite different, varying greatly in marsh elevation, soil characteristics, and the nature of the disturbance itself, I found strong commonalities in the response of the invertebrate community. *S. alterniflora* density, height, and percent cover were significantly reduced in disturbed (affected) as compared to healthy areas. The periwinkle snail density and the benthic macroinfaunal community (density, taxon richness, and diversity) were significantly decreased in affected areas, regardless of disturbance type, whereas fiddler crab densities were not affected. There was also a shift in the benthic infaunal community to a greater proportion of surface feeders (and a decrease in subsurface feeders) in the affected zones. Soil pH and redox values were increased in the affected areas at all disturbance types, likely as a result of plant loss and the absence of amelioration effects. Multiple regression analyses were used to explore the variation in invertebrates across sites. *S. alterniflora* was the most important explanatory variable in the variation of the invertebrate groups (supporting the results across zone), with soil condition variables

(particularly pH) often increasing predictability of the models. Taken together, these finding suggest that any disturbance leading to a loss of *S. alterniflora* will have a similarly strong negative effect on the invertebrate community, through both the loss of habitat and the resulting altered soil environment when plants are absent.

4.1. Introduction

Spartina alterniflora is considered a foundation species in salt marshes. Foundation species create habitat, influence the local site hydrology and climate, and can provide ecosystem services such as carbon sequestration and erosion control (Lawton 1994, Soule et al. 2003, Ellison et al. 2005). Faunal density and diversity is often increased in association with *S. alterniflora* as it provides niche complexity, a high concentration of organic matter, protection from predators, and amelioration of porewater conditions (often decreasing salinity, increasing soil moisture, and providing an oxygen microhabitat near the roots; Rader 1984, Bertness 1984 and 1985, West and Williams 1986, LaSalle et al. 1991, Lana and Guiss 1992, Whitcraft and Levin 2007).

The introduction of *S. alterniflora* to the west coast of the U.S. provides an explicit example of how the presence of foundation species can modify the habitat and alter the macrofaunal community. *S. alterniflora* invaded mudflats, where it reduced light reaching the sediment surface, decreased soil salinities, increased peat content, and altered the local hydrology (Grosholz et al. 2009). Subsurface feeders (i.e. capitellids and oligochaetes) proportionally increased in response to the increase in belowground organic matter, while surface algal feeders decreased (Neira et al. 2005). Along the East Coast where *S. alterniflora* is not an invasive, subsurface feeding oligochaetes form a larger fraction of the invertebrate community in vegetated as compared to bare areas in the salt marsh (Minello et al. 1994, Levin et al. 1996).

Facilitative interactions may occur among salt marsh fauna when *S. alterniflora* is present, in the form of a "habitat cascade" that increases invertebrate abundance and biodiversity in a positive feedback loop (Bruno et al. 2003, Thomsen et al. 2010). For

example, *S. alterniflora* facilitates the presence of mussels in New England salt marshes, which in turn facilitates the presence of other invertebrates (e.g. barnacles and amphipods) through increased attachment and crevice space (Alteri et al. 2007). Thus the presence of *S. alterniflora* can dramatically affect the invertebrate assemblage in a salt marsh.

Disturbances in salt marshes can result in the loss of *S. alterniflora*. Documented disturbances include the deposition of wrack onto the marsh surface (Reidenbaugh and Banta 1980, Bertness and Ellison 1987, Valiela and Rietsma 1995, Alexander 2008), ice scouring of marsh sediments surfaces in high latitude marshes (Ewanchuk and Bertness 2003), and the headward erosion of tidal creekbanks caused by increases in flooding due to rising sea level (May 2002). Herbivore overgrazing by mammals, birds, and invertebrates can also lead to large bare patches. Nutria and muskrats in Atchafalaya Bay in LA (Evers et al. 1998, Keddy et al. 2009), horses on Cumberland Island, GA (Turner 1987), geese in the Hudson Bay, CAN (McLaren and Jeffries 2004), and sesarmid crabs in New England have all been shown to heavily graze salt marsh vegetation (Holdredge et al. 2008). Finally, sudden dieback can lead to widespread mortality of large expanses of salt marsh (McKee et al. 2004, Ogburn et al. 2006).

Disturbances can create bare patches in the marsh that last for varying amounts of time. Wrack deposition can often lead to bare patches that range from 1 to >1000 m^2 and remain unvegetated for as long as 3 years. Sudden dieback, which has been linked to drought, has affected larger patches (for instance ~240 ha at a single site in GA, Ogburn and Alber 2006) which remain unvegetated for much longer periods of time (>7 years, Chapter 5). Herbivore disturbances often occur when species are introduced and have no

natural predators or when there is a decrease in the number of predators. There is evidence that snails may cause bare patches when blue crab predator densities are low (Silliman et al. 2002). Horses introduced to Cumberland Island graze *S. alterniflora* in the upper marsh, such that biomass is typically <40 g m^{-2} (Turner 1987). These areas typically remain disturbed indefinitely, as horses concentrate their grazing efforts in the same patches (Turner 1987, Cumberland Island NPS, *pers. comm.*). These different types of disturbances all led to a loss of *S. alterniflora*, but it is unclear whether they will have similar effects on invertebrates. It is important to understand the effects of disturbances on invertebrates, especially because they have an important role in transferring primary production from the salt marsh to estuaries (Kneib 2000)

In the Chapter 3, I found that the DMSO:DMSP ratio and metal load increased similarly in foliar tissue of *S. alterniflora* in areas affected by 4 types of disturbances (dieback, wrack, and snail and horse grazing). Here I compare those same 4 disturbances in order to evaluate the effects of a loss of *S. alterniflora* on marsh fauna. Although *S. alterniflora* was lost or reduced in each case, these disturbances generally did not affect the marsh fauna directly. I therefore expected that the loss of this foundation species would have a similar indirect effect on invertebrates, regardless of the cause. I predicted that invertebrate density and diversity would decrease and that there would be a shift in the structure of the invertebrate community (i.e. an increase in the proportion of surface and a decrease in subsurface feeders) as *S. alterniflora* is lost.

4.2. Methods

4.2.1. Study sites

I investigated 20 salt marshes along the GA coast that were experiencing a loss of *S. alterniflora*: 5 with a high snail density, 5 with wrack on the surface, 5 with damage by horses, and 5 sudden dieback sites (Figure 4.1., Figure 4.2.). Snail-disturbed sites had snail densities of near ~600 snails m^2, which have been reported to contribute to *S. alterniflora* mortality (Silliman et al. 2002, 2005). Wrack-disturbed sites had a ~5 cm layer of wrack on the surface that covered >50 m^2 of marsh. Horse sites were located in areas frequently grazed by horses, based on observations by NPS rangers at Cumberland National Park. Sudden dieback sites were locations known to have experienced dieback through direct observation (Sapelo Island), or those that had been reported to GA-DNR, Coastal Resources Division (Brunswick) following the 2000-2001 droughts in GA.

Each of 20 sites was sampled once between July and September, either in 2008 and 2009. Six of these sites (two each affected by wrack, snails, and sudden dieback, all of which were located on Sapelo Island) were monitored approximately monthly from July 2008 to December 2008 and then revisited in July 2009 in order to compare disturbance effects over time.

At each site, a transect of six plots (0.25-m^2) was arranged through the center of the disturbed area, such that the two outside plots were located within the healthy marsh ("healthy zone"), two were located along the transition between the healthy and affected marsh ("edge zone"), and two were within the affected marsh ("affected zone") (Fig. 3.1., inset). At many sites, *S. alterniflora* was absent in the "affected zone" (i.e. snails, dieback), therefore the edge zone provided an intermediate level of disturbance.

4.2.2. Vegetation

Live and standing dead plants were counted from within the 0.25 m^2 plots and estimated for a 1 m^2 area for analysis. Tiller heights of all plants were measured from within the lower left corner (0.0625 m^2) area of the plot. If there were <5 plants within the smaller area, adjacent plants were randomly measured from within the larger plot until there were a total of five tiller heights (if possible). Percent live vegetative cover was measured by placing a 0.25 m^2 quadrat, which was divided by monofilament line into 100 5 x 5 cm squares, over the plot, and counting the number of squares that contained vegetation. The proportion of standing dead *S. alterniflora* was calculated as the number of standing dead stems divided by the total number (live + standing dead) of stems.

4.2.3. Fauna

Fauna sampled in each plot included epifauna (molluscs and crabs) and benthic infauna. Molluscs and crab holes greater than 5 mm (as a proxy for the number of crabs) were counted from within the plot (0.25 m^2) and scaled up to 1 m^2 for analysis. Molluscs included *Littoraria irrorata* (the periwinkle snail) and *Geukensia demissa* (the ribbed mussel), which were observed at most sites, and *Melampus bidentatus*, which was observed just once. Crabs were primarily species of *Uca* (generally, *Uca pugnax*), but likely included *Armases cinereum* and *Eurytium limosum* at some sites.

Six soil cores (21.2 cm^2 area) each were collected to a depth of 5 cm for analysis of benthic macroinfauna (>500 μm) from areas immediately adjacent to each plot (to

avoid disturbing plots that were sampled on multiple dates). Core contents were sieved with a 500 μm screen, preserved (10% formalin), and stained with rose bengal dye. Macroinfauna were removed from the plant debris, counted and identified to the lowest taxonomic level possible using a dissecting microscope, and scaled to no. per 100 cm^2. Taxon richness and Shannon H' diversity indices were determined for each core and overall by zone, and the former was also calculated for a total across zones by disturbance type (as the sum of 10 cores from 5 sites). The feeding mode for each taxon (based on classification by Craft and Sacco 2003) was determined and the percentage of surface, subsurface, and carnivorous feeders was calculated by zone for each of the 4 disturbances.

4.2.4. Soil Conditions

Soil biogeochemistry measurements (pH, salinity, redox) were collected opportunistically at each of the 20 sites and in the subset of 6 sites sampled over time. To ensure consistency and accuracy, samples were collected only during low tide and never immediately following rain events. In each case, 3 replicate measurements each of porewater pH, porewater salinity, and redox potential were collected in areas outside the plots but from within both the disturbed and the healthy marsh zones. Salinity (psu) and pH measurements of interstitial porewater were measured with a refractometer, and pH was measured with either a portable or desktop probe (in the case of the latter, samples were collected in glass vials and transported on ice). Redox measurements (mV) were collected by inserting a portable redox probe (Pt electrode, Ag-AgCl reference solution) into the soil to a depth of ~7 cm.

Soil macro-organic matter from each plot (or from areas adjacent to each plot in the case of sites monitored over time) was separated from the same cores collected to evaluate macroinfauna. Belowground material (>500 μm) was dried at 60 C to a consistent weight and reported to the nearest 0.1 gram per 100 cm^2.

4.2.5. Statistics

S. alterniflora, invertebrate, and soil condition variables were compared among disturbance types and between marsh zones for a total of 20 sites (5 per disturbance type). This included the 14 sites sampled once, as well as the August 2008 observations taken from the 6 sites sampled over time. Each variable was analyzed using a split-plot (partially nested) ANOVA where disturbance type and zone were the main effects and site was considered the unit of replication. Disturbance type was analyzed as the between plots, fixed factor, and evaluated against the whole-plot error term (sites within disturbance type). Zone and the interaction term zone x disturbance type were analyzed as the within plots, fixed factor, and evaluated against the split-plot error term (sites within disturbance type x zone, i.e. the residual). The interaction term was used to evaluate the main (null) hypothesis in this study, which was that the effect of zone would be similar regardless of disturbance type (i.e. a non-significant effect supports the hypothesis). A Tukey's multiple comparison post-hoc test was used to evaluate pairwise differences among disturbance, zone, and disturbance x zone factors. Significant differences were assessed at the α=0.05 level.

For the 6 sites monitored over time, *S. alterniflora*, invertebrate, and soil condition variables were analyzed separately by disturbance type (dieback, snail, or

wrack) using a split-plot (partially nested), repeated measures ANOVA where zone, sites, and date were the main effects, plot replicates within zone were considered the unit of replication, and individual plots within a site the unit of repeated measures. Zones and sites, in this case, were analyzed as between-plot, fixed factors, and each was evaluated against the whole-plot error term (plots within zone x site). Date and the interaction term date x zone were analyzed as within plot, fixed factors, and evaluated against the split-plot error term (plots within zone x site x replicates, i.e. the residual). In this case, the interaction term date x zone was used to evaluate whether the effect of zone was similar, regardless of date that a site was sampled. A Tukey's multiple comparison post-hoc test was used to determine pairwise differences among zone, site, date, zone x date, and site x date factors. Significant differences were assessed at the $\alpha = 0.05$ level.

Multiple regression analysis was used to explore variation of invertebrate measurements (density, diversity) as a function of *S. alterniflora* stem density and soil conditions (salinity, pH, redox, and MOM). All variables were averaged across plots for an overall zone mean by site prior to regression for a total of 60 observations (i.e. N = 3 zones x 20 sites). Results of backward elimination and forward regression were compared with best subsets regression analyses, and the best model was chosen by evaluating Mallow's Cp statistic (Mallows 1973, 1995), model adjusted R^2, and the overall model *p*-value. Variables where the individual *p*-value was >0.15 were removed from the model. All VIF scores were ≤1.0, indicating no collinearity.

Prior to statistical testing, variables were either natural log (ln (x +1); *S. alterniflora* density, *Littoraria*, macroinfauna variables) or square-root (sqrt (x +1); fiddler crabs) transformed as needed to improve normality. Bartlett's test of equal

variance was used to confirm homoscedascity among groups compared by ANOVA. In addition, because the split-plot ANOVA model requires a balanced data set, missing data was filled on one occasion (macroinfauna in all zones at 1 wrack site) using the means for a particular zone-disturbance pair. I confirmed that this did not affect the outcome of the statistical test by running a split-plot ANOVA following a listwise deletion of the missing data (4 sites were compared per disturbance).

4.3. Results

4.3.1. Variation among zones and disturbances

4.3.1.1. Vegetation

By definition, *S. alterniflora* was affected by the 4 disturbance types. *S. alterniflora* had a significantly decreased density (by 65%), height (by 69%), and percent of live cover (by 44%), and an increased proportion of standing dead stems (by 35%) in the affected as compared to the healthy marsh zones (Figure 4.3., Table 4.1.). Observations in the edge zone were intermediate and were either statistically different than both zones (height and percent of live cover) or statistically similar to the healthy zone (density and the proportion of standing dead). The type of disturbance was also a significant source of variation in terms of *S. alterniflora* density, height, and the proportion of standing dead: horse sites had denser stands of *S. alterniflora* and a lower proportion of standing dead than the other 3 disturbance types, and wrack sites had taller plants. The interaction term (zone x disturbance) was not significant for *S. alterniflora* density, height, or percent of live cover, indicating that there was a similar effect of zone on each variable within the 4 disturbance types. However, the interaction term was

significant for the proportion of standing dead stems as horse sites had a much lower proportion of standing dead *S. alterniflora* overall and within the affected zone as compared to the other disturbance types.

4.3.1.2. Soil Conditions

Soil pH (which ranged from 6.96 to 7.89) and redox potential (which ranged from -107 to -307 mV) varied among marsh zones, both of which were increased in the affected zone as compared to the healthy zone overall. These differences held for 4 of 4 and 3 of 4 disturbance types, respectively. Although not significant, there were trends towards increased salinity (which ranged from 30.3 to 45.8 psu) and decreased macro-organic matter content (which ranged from 9.0 to 25.4 g 100 cm^{-2}) in the affected zone overall, and within each of the 4 disturbance types. pH and redox values were also different among disturbance types, as horse sites had a significantly higher pH and lower redox value than the three other disturbance types. However, the interaction term (zone x disturbance) was not significant for any of the soil condition variables, indicating that there was a similar effect of zone (due to *S. alterniflora* loss) across the disturbance types.

4.3.1.3. Epifauna

Marsh zone was a significant source of variation of for the epifaunal snails, with a decreased density overall in the affected as compared to the healthy marsh zone (Table 4.3., Figure 4.4.). Snail density in the edge zone was statistically similar to the healthy marsh. Disturbance type was also a significant source of variation for epifaunal snails,

with much higher snail densities at the snail-disturbed sites (where snail density is high by definition) than in the other three disturbance types. The interaction term of zone x disturbance type was also significant because the effect of zone was opposite at the snail sites (where density was increased in the affected area), as compared to the other three disturbance types. When snail-disturbed sites were excluded from the ANOVA analysis, there was no effect of disturbance type or of the interaction term (zone x disturbance type), indicating a similar decrease in snail density in the affected zone at the dieback-, horse-, and wrack- disturbed sites (by 93%, 88%, and 79%, respectively).

Marsh zone was not a significant source of variation in fiddler crab density (Table 4.3., Figure 4.4.), but the densities were significantly increased in the wrack-disturbed sites as compared to the other three disturbance types. The interaction term, however, was not significant. Mussels did not vary significantly with marsh zone, disturbance type, or zone x disturbance type, likely because of the large standard errors.

The variation in the density of snails, crabs, and mussels across sites were each significantly related to both plant and soil conditions (Table 4.5.). The best model for snail density had a positive relationship with both standing dead and live *S. alterniflora* density, and a negative relationship to stem height and pH (R^2=0.43, p=0.001). The model for fiddler crab density had a positive relationship with stem height and a negative relationship with live *S. alterniflora* density (R^2=0.45, p<0.0001). The model for mussel density had a positive relationship with macro-organic matter content, and a negative relationship to salinity (R^2=0.24, p=0.01).

4.3.1.4. Benthic macroinfauna

Overall, there were only 9 infaunal taxa observed across the 20 sites: 4 polychaetes, which were identified to the species level, and 5 other taxa, which were identified to the lowest level possible with a dissecting scope (Table 4.4). Oligochaetes tended to comprise the largest fraction of macroinfauna at most sites, followed by *Capitella capitata* and then *Streblospio benediciti*.

Macroinfauna were observed 10-20% more frequently in plots located in the healthy marsh as compared to those in affected areas. Marsh zone was a significant source of variation in each of the macroinfauna variables, with a decrease in the overall density (by 73-100%), taxon richness (by 44-100%), and diversity (by 50-100%) in affected as compared to healthy areas (Table 4.3., Figure 4.4.). Disturbance type was also significant, with the horse-disturbed sites having an overall increased abundance and more diverse assemblage than other disturbances. There was no significant effect of the interaction term of zone x disturbance in any of the cases, indicating that the effect of zone on macroinfauna was similar, regardless of the type of disturbance. When macroinfauna were viewed based on the classification into surface, subsurface, and carnivorous feeding types (Figure 4.5.), there were substantial increases in the proportion of surface feeders and decreases in the proportion of subsurface feeders in the affected zone as compared to the healthy marsh for each disturbance type.

Macroinfauna density, taxon richness, and diversity were all related to *S. alterniflora* and soil condition variables (Table 4.5.). Macroinfauna density and taxon richness increased with live and standing dead *S. alterniflora* densities (R^2=0.34 and 0.40, respectively, and p<0.0001 each), whereas the diversity of macroinfauna increased with

S. alterniflora density and decreased with pH (R^2=0.23, p=0.01).

4.3.2. Temporal variation of disturbance effects

The results described above are from one time observations at each of 20 sites. I used data from the 6 sites where I had data on disturbance effects over time (2 each of dieback, snail, and wrack-disturbed sites) to evaluate whether the differences between marsh zone held on multiple dates. Multiple comparisons of zone (vegetation, soil conditions, fauna) on each date and the ANOVA interaction term "zone x date" (vegetation, fauna) were used to specifically evaluate the effect of zone over time (Table 4.6.). As described below, the patterns in vegetation, fauna, and porewater were generally consistent on most dates, such that the date used in the 20 site analysis was representative of the overall dataset.

4.3.2.1. Vegetation

In the dieback sites, *S. alterniflora* densities were significantly decreased in the affected marsh as compared to the healthy marsh zone for all sampling dates (significant for 7 of 7 dates, based on multiple comparisons; Figure 4.6., Table 4.6.). However, *S. alterniflora* densities in all zones declined beginning in December 2008 due to the onset of drought conditions, resulting in a significant effect of both date and the interaction term zone x date, as the edge zone densities became statistically similar to the affected zone. The effect of the drought, however, did not show up as a decline in *S. alterniflora* densities at the other disturbance types.

In the snail sites, there was a significant effect of zone on *S. alterniflora* density

and this pattern held over all sampling dates (i.e. both date and zone x date were NS). On each date, densities were decreased in the affected zone as compared to the healthy zone, with the edge zone statistically similar to the healthy zone.

In the wrack sites, there was a significant overall effect of zone and date on *S. alterniflora* density. The affected zone had significantly fewer plants than the healthy and edge zones, and this pattern held over time, although it was only significant during three middle sampling dates (based on multiple comparisons). Zones were not statistically different on the initial sampling dates because the wrack in the affected zone was still covering living *S. alterniflora*. Over time, *S. alterniflora* was decreased by the wrack disturbance, and the differences between zones became significant in September 2008, and were especially evident because this loss occurred when *S. alterniflora* density in the healthy zone peaked (i.e. in the fall). Wrack was apparently moved away from the affected areas by Tropical Storm Fay on August 18, 2008 (as observed on the August 27, 2008 sampling date), after which *S. alterniflora* in the affected zone began to recover and reached healthy densities by July 2009. Thus, the date effect was significant due to the changes in *S. alterniflora* density that occurred over time.

4.3.2.2. Soil Variables

In general, pH, salinity, and soil redox values fluctuated temporally at all sites (Figure 4.7.). In the dieback sites, the affected zone tended to have higher pH, salinity, and redox values on any given date, and this was significant on several occasions. Similar patterns were also observed in snail sites, although pairwise differences between zones were not significant. In wrack sites, pH also tended to be increased in the affected

zone, although this was not significant on individual dates, whereas salinity and redox values were not different between the healthy and affected zone.

4.3.2.3. Epifauna

In the dieback sites, snail densities were significantly decreased in the affected as compared to the healthy marsh zone (as observed for the 1-time survey), and this pattern held for all sampling dates (significant for 7 of 7 dates, based on multiple comparisons; Figure 4.6, Table 4.6.). There was no significant interaction effect of zone x date, further indicating that the effect of marsh zone over dates remained similar for all observations.

In the case of the 2 snail sites observed over time, there were very few snails in the affected areas (which were bare). Instead, snail densities were significantly higher in the edge zone over all sampling dates (this was significant for 4 of 7 dates). This is in contrast to the patterns observed in the 1-time survey because at the other three snail sites, *S. alterniflora* was present in the affected areas and snail densities (which were causing the disturbance) were increased. In the sites observed over time, snail densities decreased significantly from September 2008 onward. Because this occurred primarily in the edge plot, the effect of zone varied by date and, zone x date was significant, as well.

In the wrack sites, snail densities tended to be highest in the edge zone and lowest in the affected zone. The effect of zone was significant overall, but when individual dates were compared (as multiple comparisons), the edge zone differed on only 1 date. This was likely due to the large variation in snail density on individual dates, as compared to the smaller error when snail densities within zone are averaged across dates. There was no effect of zone x date, indicating that the effect of zone among sampling dates was the

same.

Fiddler crabs generally responded similarly at all sites across time. Date was significant in all disturbance types, but fluctuated seasonally in all zones, with peak crab hole density occurring in December 2008. There was no significant interaction effect of zone x date, indicating that the pattern of seasonal fluctuation was similar among the zones. At the dieback sites only, zone was a significant effect in fiddler crab density, with an increased number of crabs in the healthy as compared to the affected zone. This pattern held on all sampling dates, although it was significant for only one date (July 2008).

4.4. Discussion

There were striking similarities in the effect of disturbances on salt marshes in this study, despite the fact that it was conducted across 20 sites ranging in environmental characteristics, as well as in the nature (dieback, horse, snail, wrack) and duration of the disturbance affecting the area. There was an overall negative effect on *S. alterniflora* in the affected zones, which was not surprising. The loss of *S. alterniflora* was associated with changes in the environmental soil variables: there was a significant increase in the pH and redox potential in the affected marsh as compared to the healthy marsh. Vegetation loss has been shown to lead to increased soil oxidation (higher redox values) as a result of increased sediment exposure (Portnoy 1999, McKee et al. 2004). It is also possible that the increase in pH and redox values were due to increased benthic microalgal production in bare areas as a result of increased light penetration. Although

this was not quantified here, BMA production can lower soil CO_2 (raising the pH) and increase O_2 (raising the redox potential) (Pomeroy 1959).

S. alterniflora disturbance resulted in strong responses in the invertebrate community. The abundance of epifaunal snails and the abundance and diversity of benthic infauna were all decreased significantly in disturbed areas (with the exception of snails at snail-disturbed sites). The magnitude of this decrease was similar regardless of disturbance type, and therefore the null hypothesis was supported (i.e. no interaction effect of disturbance type x zone). Marsh sites monitored over a longer period of time (~1 year) continued to support the results found for the survey of 20 marsh sites. When disturbance types and zones were lumped in order to explore the spatial variation of invertebrates across sites, the variation in *S. alterniflora* was typically the most important explanatory variable, although soil variables helped to improve predictability. *S. alterniflora* is important for habitat structure, as an organic matter source, and for the provision of suitable environmental conditions. With the loss of this foundation species, both the resources available to and soil conditions for salt marsh organisms are altered from those in the healthy marsh. These finding indicate that the loss of *S. alterniflora* affected salt marsh invertebrates similarly, regardless of disturbance type.

Few other studies have explicitly compared the effects of different disturbance types on marine invertebrate communities. In a New England salt marsh, researchers examined the response of mobile invertebrates (terrestrial ants and semi-terrestrial fiddler crabs) to the simulated deposition of wrack and sand into the high marsh following storms (Brandt et al. 2010). They found that ants responded positively to sand, as it resulted in drier conditions, whereas fiddler crabs responded positively to wrack, as it

increased moisture and decreased evaporative stress. Their findings highlighted the fact that disturbances can have contrasting effects on different species. Whomersley et al. (2010) evaluated the direct effects of experimentally applied disturbances (burial, raking, and organic enrichment) on sessile organisms living in mudflats. They found differences in responses to the disturbance types at their two study sites and rejected their null hypothesis of no interaction of site x treatment at both the level of the community and of individual species. They suggested that the effects of disturbance were context-dependent, and thus difficult to predict (Whomersley et al 2010).

The above studies compared the community response of invertebrates to different habitat (sand vs. wrack in Brandt et al. 2010, experimental manipulation of the substrate in Whomersley et al. 2010). These examples focused on the direct effects of disturbances on the invertebrates. In contrast, I looked at a similar change (loss of *S. alterniflora*) due to various disturbances, and found that they all resulted in a similar response in the invertebrate community regardless of the reasons for the loss of the plants. Below I provide details about the response of each of the invertebrates to the loss of *S. alterniflora* and then explore several differences that occurred among disturbance types, regardless of zone.

L. irrorata is strongly associated with *S. alterniflora*. In this study, the density of these snails were significantly reduced in the affected zones of dieback, horse, and wrack sites, whereas, by definition, they were increased in the affected zones of snail-disturbed sites (if *S. alterniflora* was present). Periwinkle snails rely on *S. alterniflora* as their primary habitat, utilizing it as vertical refuge from predation and flooding, as plant cover to prevent dehydration, and as a source of organic matter. *S. alterniflora* is also used as

refuge and dehydration prevention for newly recruited juveniles, which exclusively inhabit the leaf furls (Hamilton 1976, Stiven and Hunter 1976, Silliman et al. 2005). Regression models across sites were able to significantly explain 37% of the variation in *Littoraria* density. *Littoraria* was increased with greater habitat availability (i.e. the live and standing dead *S. alterniflora*), which agrees with previous research (Kiehn and Morris 2009). Snails were also increased where pH was lower. This is difficult to explain, and could be an artifact of the lowest snail densities occurring on Cumberland Island, where pH was much higher regardless of zone.

Crab and mussel densities were not significantly different across zones, which indicate a looser association of crabs and mussels with *S. alterniflora* as compared to periwinkles. Fiddler crabs utilize *S. alterniflora* for predator protection, as a source of organic matter, and as shade to prevent desiccation (Currin et al. 1995, Nomann and Pennings 1998). However, they are often prohibited from burrowing in areas of dense root mats (Bertness 1985) and have been observed to construct burrows in bare areas (Kenemer et al. 2006, *pers. obs.*). Regression models explained 45% of the variation in fiddler crab density, which was negatively correlated to *S. alterniflora* density but positively correlated to *S. alterniflora* height: these results support the observation that crabs preferentially burrow in locations of taller *S. alterniflora,* which have a less dense root mat but still provide shade and structure (Bertness 1985, Nomann and Pennings1998). Mussels can benefit from the presence of *S. alterniflora* for soil stabilization and as a source of organic matter (Bertness 1985, Stiven and Kuenzler 1979), but often settle in bare areas and where *S. alterniflora* is less dense (Stiven and Gardner 1992). Regression models explained only 24% of the variation in mussel density

and did not include any *S. alterniflora* variables. Instead, mussels varied positively with macro-organic matter and negatively with salinity. It is likely that increased macro-organic matter in the soil provides adequate moisture, attachment substrate, and source of organic matter for the mussels whether or not *S. alterniflora* is present. Mussels were not found in any location (regardless of zone) when salinities were above 36 psu.

The density, taxon richness, and Shannon H' diversity of benthic macroinfauna were all significantly decreased in the affected as compared to the healthy marsh areas and the effect of this response did not vary among the disturbance types (i.e. no effect of the interaction term). That the benthic macroinfauna responded negatively to the loss of *S. alterniflora* was not surprising. Researchers have often found increased density and biodiversity of macroinfauna in association with *S. alterniflora* as it provides belowground habitat, oxygenated sediments, predator protection, and a source of their organic matter (Kneib 1984, Rader 1984, Lana and Guiss 1992). However, several other studies have reported no differences between vegetated and unvegetated habitat, or even increased densities in mudflats and bare areas (review by Levin and Talley 2000). To account for these differences, Levin and Talley (2000) suggest that increased infaunal densities are associated with vegetation in situations where the amelioration of stressful abiotic conditions in the soil becomes necessary. In this study, all of the sites were located in the mid to high marsh, which are regularly exposed during low tides and may not be inundated during some neap tides. It is therefore likely that the vegetation was important as a source of shade, and buffered soil conditions.

Regression models across sites significantly explained 34%, 40%, and 23% of the variation in macroinfauna density, taxon richness, and diversity, respectively. In each

case increased live *S. alterniflora* density was the most important explanatory variable in the regression, although inclusion of other variables improved predictability. Infaunal density and taxon richness were also both positively related to standing dead *S. alterniflora* density. It is likely that both the live and standing dead *S. alterniflora* may have increased the soil moisture (not measured) as a result of shade, and provided the infauna with a more physiologically stable habitat. Diversity was negatively related to pH. Because affected zones had increased pH, this observation suggests that *S. alterniflora* was ameliorating the belowground habitat for infauna. The addition of macro-organic matter did not improve these models, which was surprising as increased availability of belowground macro-organic matter has often been shown to enhance density and diversity of macroinfauna (Lana and Guiss 1992, Craft et al. 2003). That macro-organic matter was not in the models may be because there were no differences in the macro-organic matter content between the healthy and affected zones.

The decrease in the proportion of subsurface feeders in the affected zone observed here is similar to my other observations in dieback sites in GA (Chapter 2) and to reports from elsewhere. In a California marsh, Whitcraft and Levin (2007) observed increased subsurface feeders in the presence of artificial shade. These were primarily oligochaetes (as in this study), and the authors suggested that *S. alterniflora* plays a strong role in habitat amelioration for these groups. When shade was removed, subsurface feeders decreased whereas polychaetes and other surface algal feeders increased. Oligochaetes and other subsurface detrital feeders have also been observed to increase in response to invasion of *S. alterniflora* in California mudflats (Talley and Levin 1999, Neira et al. 2003).

Whitcraft and Levin (2007) attributed the shift away from subsurface feeders in bare areas to both harsher belowground conditions, such as increased salinity and temperature and decreased soil moisture, and an increase in benthic microalgae (for the surface algal feeders). These same factors could account for the differences I saw in the affected zones at the horse, snail and dieback sites, all of which were bare (or thinned) in comparison to healthy areas. However, wrack presents a source of shade, so one might expect to see the opposite trend in wrack disturbed areas. In fact, Rossi et al. (2002) saw an increase in infaunal subsurface feeders (oligochaetes and capitellids) when wrack was added to mudflats. In addition, several studies have noted a positive response of other fauna to wrack cover (Kneib 1984, Rossi and Underwood 2002). In this study, however, macroinfauna densities were low in the healthy and edge zones, and no organisms were collected in the affected zones. It is difficult to draw strong conclusions about how the infaunal community was affected by wrack since the densities were so low, but the lack of subsurface feeders in the affected areas could reflect low densities of subsurface feeders across all zones at these sites, whereas the lack of surface feeders may be because the wrack acted as a barrier to the recruitment of larval-dispersing infauna such as the polychaetes (as suggested by Rossi et al. 2002). Alternatively, it is possible that the infaunal invertebrates really were decreased in response to the loss of *S. alterniflora* and resulting increase in pH in the wrack affected areas, as was the case in the other disturbance types.

There were some examples where vegetation and fauna differed among the disturbance types, independent of zone effect. Most of these cases can be explained by geographical or elevational differences or a combination of both among the sites used to

represent the disturbances. For instance, although *S. alterniflora* density, height, and percent of live cover decreased, and the proportion of standing dead increased significantly in the affected zones across all sites, horse sites overall had a significantly increased density of *S. alterniflora* and decreased proportion of standing dead plants as compared to the other three disturbance types. These sites were all located in the high marsh on Cumberland Island, which may account for the denser stands of *S. alterniflora*. The horse sites were also significantly different than the other sites in terms of the environmental soil variables, as they had a higher overall pH and a lower redox value. Redox values and pH are often negatively correlated, with higher pH and lower redox values occurring in flooded soils (Giblin and Howarth 1984, Luther and Church 1988), so this may reflect poor drainage in the high marsh areas where these sites were located. The other difference in vegetation among disturbance types was that plant height in wrack sites was taller than in other sites. This may be due to elevation, as these sites were located lower in the marsh (in mid-marsh areas) than the other sites.

Fauna were also different among disturbance types. Periwinkles were significantly increased at snail disturbed sites within all zones, as these sites have increased densities of snails by definition. Fiddler crabs were increased at wrack disturbed sites as compared to the other site types. This again may have been a result of elevation because wrack sites occurred lower in the marsh where the fiddler crabs (especially *U. pugnax*) are often more numerous (Teal 1958). Macroinfauna (density and taxon richness) were significantly increased within all zones at horse disturbed sites. This may have been primarily due to the differences in soil characteristics observed at these sites, as described above. The lower redox values at these sites indicate that soil

moisture was likely higher, which may have, provided a better habitat for infauna. These sites were particularly high in oligochaetes which were numerically dominant (>66% in any zone) at these sites. Other studies have observed higher densities of oligochaetes in locations where soil moisture was higher and soil oxygen was low (Sarda et al. 1996).

The results described above were all for one-time observations that were compared in the survey. However, the findings from the survey were generally supported by observations from the 6 sites visited over time (7 observations over a period of 13 months). In these cases, *S. alterniflora* and periwinkle densities were statistically decreased in the affected zones on most dates. The one exception was at the wrack site, where the vegetation in the affected zone recovered once the wrack was removed by a storm. These results indicate that the negative response of invertebrates to disturbance was generally consistent over time.

4.5. Conclusions

This is the first study to explicitly compare the indirect effect of *S. alterniflora* loss due to differing disturbances. I found that the negative effects on the resource and environmental conditions due to *S. alterniflora* loss led to a negative response of the benthic invertebrates, regardless of which disturbance caused the initial vegetation loss. Density and diversity of the benthic infauna was greatly decreased by the loss of *S. alterniflora*. Epifaunal snails, which intimately depend on *S. alterniflora* for habitat, were absent when *S. alterniflora* was not present in affected areas. Much of the spatial variation of the snails and benthic infauna across sites was a function of the presence of *S. alterniflora*. More mobile species (i.e. fiddler crabs) and less obligately dependent

epifauna (i.e. mussels and fiddler crabs) did not have as strong a response, although mussels were positively related to macro-organic matter concentration and fiddler crabs were related to *S. alterniflora* height, both of which are provided by the presence of vegetation.

These results are important in light of the fact that salt marshes are vulnerable to increasing disturbances due to a combination of climate change and anthropogenic activities. Climate change is likely to lead to more variable and severe episodic events like flooding and drought, both of which may results in the loss of *S. alterniflora* (Scavia et al. 2002, Fischlin et al. 2007). Anthropogenic activities such as eutrophication, urbanization, and flow alteration are likely to stress the marsh and may lead to lower resiliency (Hughes et al. 2003, Silliman et al. 2009, Gedan et al. 2011). These may act in concert. For instance, wrack deposition may increase, not only due to the greater frequency and strength of storms (Miller et al. 2001, Scavia et al. 2002), but also with an increased number of structures such as docks, seawalls, and roads that can trap wrack (Bozek and Burdick 2005, Alexander 2008). In addition, increased agricultural or nutrient subsidies to herbivore populations, intentional or accidental introduction of species, and the loss of natural predators due to overfishing, habitat loss, and other factors can increase the effect that herbivores have on the marsh, potentially leading to overgrazing (Smith and Odum 1981, Turner 1987, Jeffries et al. 2003, Silliman et al. 2005, Holdredge et al. 2008). It is therefore important to understand how disturbances will affect benthic invertebrate communities in these important environments.

References

Alexander,C., 2008. Wrack assessment using aerial photography in coastal Georgia. Report to the Georgia Coastal Zone Management Program, Georgia Department of Natural Resources, Coastal Resources Division, Brunswick, GA. 20 p.

Altieri, A.H., B.R. Silliman, and M.D. Bertness. 2007. Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. *The American Naturalist* 169 (2): 195-206.

Bertness, M.D. 1984. Ribbed mussels and the productivity of *Spartina alterniflora* in a New England salt marsh. *Ecology* 65: 1794-1807.

Bertness, M.D. 1985. Fiddler crab regulation of *Spartina alterniflora* production on a New England salt marsh. *Ecology* 66 (3): 1042-1055.

Bertness, M.D. and A.M. Ellison. 1987. Determinants of pattern in a New England salt marsh plant community. *Ecological Monographs* 57 (2): 129-147.

Bozek, C.M., and D.M. Burdick. 2005. Impacts of seawalls on salt marsh plant communities in the Great Bay Estuary, New Hampshire USA. *Wetlands Ecology and Management* 13 (5): 553-568.

Brandt, M., K.B. Gedan, and E.A. Garcia. 2010. Disturbance type affects the distribution of mobile invertebrates in a high salt marsh community. *Northeastern Naturalist* 17 (1): 103-114.

Bruno, J.F., J.J. Stachowicz, and M.D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18 (3): 119-125.

Craft C.B., and J.N. Sacco. 2003. Long-term succession of benthic infauna communities on constructed *Spartina alterniflora* marshes. *Marine Ecology Progress Series* 257: 45-58.

Currin, C.A., S.Y. Newell, and H.W. Paerl. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Marine *Ecology Progress Series* 121: 99-116.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliott, C.R. Ford, D.R. Foster, B.D. Kloeppel, J.D. Knoepp, G.M. Lovett, J. Mohan, D.A. Orwig, N.L. Rodenhouse, W.V. Sobczak, K.A. Stinson, J.K. Stone, C.M. Swan, J. Thompson, B. Von Holle, and J.R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3 (9): 479-486.

Evers, D.E., C.E Sasser, J.G. Gosselink, D.A. Fuller, and J.M. Visser. 1988. The impact of vertebrate herbivores on wetland vegetation in Atchafalaya Bay, Louisiana. *Estuaries* 21: 1–13.

Ewanchuk, P.J., and M.D. Bertness. 2003. Recovery of a northern New England salt marsh plant community from winter icing. *Oecologia* 136 (4): 616-626.

Fischlin, A., G.F. Midgley, J.T. Price, R. Leemans, B. Gopal, C. Turley, M.D.A. Rounsevell, O.P. Dube, J. Tarazona, and A.A. Velichko. 2007. Ecosystems, their properties, goods, and services. In M.L. Parry, O.F. Canziani, J.P. Palutikof,P.J. van der Linden and C.E. Hanson (eds) *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, pp. 211-272.

Gedan, K.B., B.R. Silliman, and M.D. Bertness. 2009. Centuries of human-driven change in Salt Marsh ecosystems. *Annual Review of Marine Science* 1: 117-141.

Giblin, A.E., and R.W. Howarth. 1984. Porewater evidence for a dynamic sedimentary iron cycle in salt marshes. *Limnology and Oceanography* 29 (1): 47-63.

Grosholz, E.D., L.A. Levin, A.C. Tyler, and C. Neira. 2009. Changes in community structure and ecosystem function following *Spartina alterniflora* invasion of Pacific estuaries. In: Silliman, B.R., T. Grosholtz, and M.D. Bertness (eds), *Human Impacts on Salt Marshes: A Global Perspective*. University of California Press.

Holdredge, C., M.D. Bertness, and A.H. Altieri. 2008. Role of crab herbivory in die-off of New England Salt Marshes. *Conservation Biology* 23 (3): 672-679.

Hughes, T.P., A.H. Baird, D.R. Bellwood, M. Card, S.R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J.B.C. Jackson, J. Kleypas, J.M. Lough, P. Marshall, M. Nystrom, S.R. Palumbi, J.M. Pandolfi, B. Rosen, J. Roughgarden. 2003. Climate change, human impacts, and the resilience of coral reefs. *Science* 301: 929-933.

Jefferies, R.L., R.F. Rockwell, and K.F. Abraham. 2003. The embarrassment of riches: agricultural food subsidies, high goose numbers, and loss of Arctic wetlands — a continuing saga. *Environmental Reviews* 11: 193–232.

Keddy, P.A., L. Gough, J.A. Nyman, T. McFalls, J. Carter, and J. Siegrist. 2009. Alligator hunters, pelt traders, and runaway consumption of Gulf Coast marshes: A trophic cascade perspective on coastal wetland losses. In: Silliman, B.R., T. Grosholtz, and M.D. Bertness (eds), *Human Impacts on Salt Marshes: A Global Perspective*. University of California Press.

Kenemer, B., C. McFarlin, and M. Alber. 2006. Fiddler crabs dig it: A study of burrow dynamics in a salt marsh. Poster presented at the Southeastern Estuarine Research Society, Savannah, GA.

Kiehn, W.M., and J.T. Morris. 2009. Relationships between smooth cord-grass *Spartina alterniflora* (Loisel.) and the periwinkle snail *Littoraria irrorata* (Say) in a South Carolina salt marsh. *Wetlands* 29 (3): 818-825.

Kneib, R.T. 1984. Patterns of invertebrate distribution and abundance in the intertidal salt marsh: causes and questions. *Estuaries* 7 (4): 392-412.

Kneib, R.T., 2000. Salt marsh ecoscapes and production transfers by estuarine nekton in the southeastern United States. In: M.P. Weinstein and D.A. Kreeger (eds), *Concepts and Controversies in Tidal Marsh Ecology*, pp. 261-291.

Lana, P.C. and C. Guiss. 1992. Macrofauna - plant-biomass interactions in a euhaline salt marsh in Paranagua Bay (SE Brazil). *Marine Ecology Progress Series* 80: 57-64.

LaSalle, M.W., M.C. Landin, and J.G. Sims. 1991. Evaluation of the flora and fauna of a *Spartina alterniflora* marsh established on dredged material in Winyah Bay, South Carolina. *Wetlands* 11 (2): 191-208.

Lawton, J.H. 1994. What do species do in ecosystems? *Oikos* 71 (3): 367-364.

Levin, L.A., and T.S. Talley. 2000. Influences of vegetation and abiotic environmental factors on salt marsh invertebrates. In: M.P. Weinstein and D.A. Kreeger (eds), *Concepts and Controversies in Tidal Marsh Ecology*, Kluwer Academic Publishers, Boston, MA.

Levin, L.A., D. Talley, and G. Thayer. 1996. Succession of macrobenthos in a created salt marsh. *Marine Ecology Progress Series* 141: 67-82.

Luther, G.W., III., and T.M. Church. 1988. Seasonal cycling of sulfur and iron in porewaters of a Delaware salt marsh. *Marine Chemistry* 23: 295-309.

Mallows, C. 1973. Some comments on C_p. *Technometrics* 15: 661-675.

Mallows, C.L. 1995. More comments on Cp. *Technometrics* 37 (4): 362-372.

May, M.K., 2002. Patterns and processes of headward erosion in salt marsh tidal creeks. M.S. thesis, East Carolina University, Greenville, NC. 142 pp.

McKee, K.L., I.A. Mendelssohn, and M.D. Materne. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecology and Biogeography* 13 (1): 65-73.

McLaren, J.R., and R.L. Jeffries. 2004. Initiation and maintenance of vegetation mosaics in an Arctic salt marsh. *Journal of Ecology* 92: 648-660.

Miller, W.D., S.C. Neubauer, and I.C. Anderson. 2001. Effects of sea level on induced disturbances on high salt marsh metabolism. *Estuaries* 24 (3): 357-367.

Minello, T.J., R.J. Zimmerman, and R. Medina. 1994. The importance of edge for natant macrofauna in a created salt marsh. *Wetlands* 14 (3): 184-198.

Neira, C., L.A. Levin, E.D. Grosholz, and G. Mendoza. 2007. Influence of invasive *Spartina* growth stages on associated macrofaunal communities. *Biological Invasions* 9: 975-993.

Nomann, B.E., and S.C. Pennings. 1998. Fiddler crab–vegetation interactions in hypersaline habitats. *Journal of Experimental Marine Biology and Ecology* 225: 53–68.

Pomeroy, L.R. 1959. Algal productivitiy in salt marshes of Georgia. *Limnology and Oceanography* 4 (4): 386-397.

Portnoy, J.W. 1999. Salt marsh diking and restoration: Biogeochemical implications of altered wetland hydrology. *Environmental Management* 24 (1): 111-120.

Rader, D.N. 1984. Salt marsh benthic invertebrates: small-scale patterns of distribution and abundance. *Estuaries* 7: 4 13-420.

Reidenbaugh, T.G., and W.C. Banta, 1980. Origin and effects of *Spartina* wrack in a Virginia salt marsh. *Gulf Research Reports* 6: 393-401.

Rossi, F., and A.J. Underwood. 2002. Small-scale disturbance and increased nutrients as influences on intertidal macrobenthic assemblages: experimental burial of wrack in different intertidal environments. *Marine Ecology Progress Series* 241: 20-39.

Sardá, R., I. Valiela, and K. Foreman. 1996. Decadal shifts in a salt marsh macroinfaunal community in response to sustained long-term experimental nutrient enrichment. *Journal of Experimental Marine Biology and Ecology* 205: 63-81.

Scavia, D., J.C. Field, D.F. Boesch, R. W. Buddemeir, V. Burkett, D.R. Cayan, M. Fogarty, M.A. Harwell, R.W. Howarth, C. Mason, D.J. Reed, T.C. Royer, A.H. Sallenger, and J.G. Titus. 2002. Climate change impacts on U. S. coastal and marine ecosystems. *Estuaries* 25(2): 149-164.

Silliman, B.R. and M.D. Bertness, 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Sciences* 99 (16): 10500-10505.

Silliman, B.R., J. van de Koppel, M.D. Bertness, L.E. Stanton, and I.A. Mendelssohn. 2005. Drought, snails, and large-scale die-off of Southern U.S. Salt Marshes. *Science* 310: 1803-1806.

Silliman, B.R., T. Grosholtz, and M.D. Bertness (eds). 2009. *Human Impacts on Salt Marshes: A Global Perspective*. University of California Press.

Smith, T.J., and W.E. Odum. 1981. The effects of grazing by snow geese on coastal salt marshes. *Ecology* 62 (1): 98-106.

Soulé, M.E., J.A. Estes, J. Berger, and C.M. Del Rio. 2003. Ecological effectiveness: conservation goals for interactive species. *Conservation Biology* 17 (5): 1238–1250.

Stiven, A.E., and E.J. Kuenzler. 1979. The response of two salt marsh molluscs *Littorina irrorata* and *Geukensia demissa*, to field manipulations of density and *Spartina* litter. *Ecological Monographs* 49(2): 151-171.

Stiven, A.E., and S.A. Gardner. 1992. Population processes in the ribbed mussel *Geukensia demissa* (Dillwyn) in a North Carolina salt marsh tidal gradient: spatial pattern, predation, growth and mortality. *Journal of Experimental Marine Biology and Ecology* 160: 81-102.

Talley, T.S., and L.A. Levin. 1999. Macrofaunal succession and community structure in *Salicornia* marshes of southern California. *Estuarine, Coastal, and Shelf Science* 49: 713-731.

Teal, J.M. 1958. Distribution of fiddler crabs in Georgia salt marshes. *Ecology* 39 (2): 186-193.

Thomsen, M.S., T. Wernberg, A. Alteri, F. Tuya, D. Gulbransen, K.J. McGlathery, M. Holmer, and B.R. Silliman. 2010. Habitat cascades: The conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integrative and Comparative Biology* 50 (2): 158–175.

Turner, M.G. 1987. Effects of grazing by feral horses, clipping, trampling, and burning in a Georgia salt marsh. *Estuaries* 10(1): 54-60.

Valiela, I., and C.S. Rietsma. 1995. Disturbance of salt-marsh vegetation by wrack mats in Great-Sippewissett-Marsh. *Oecologia* 102 (1):106-112.

West, D.L., and A.H. William. 1986. Predation by *Callinectes sapidus* (Rathbun) within *Spartina alterniflora* (Loisel) marshes. *Journal of Experimental Marine Biology and Ecology* 100: 75-95.

Whitcraft, C.R., and L.A. Levin. 2007. Regulation of benthic algal and animal communities by salt marsh plants: Impact of shading. *Ecology* 88 (4): 904-917.

Whomersley, P., M. Huxham, S. Bolam, M. Schratzberger, J. Augley, and D. Ridland. 2010. Response of intertidal macrofauna to multiple disturbance types and intensities – An experimental approach. *Marine Environmental Research* 69: 297-308.

Table 4.1. Statistical summary of split-plot ANOVAs for testing the main effects disturbance and zone on *S. alterniflora* and soil condition variables in the 20 survey sites. Values in bold are significant ($P<0.05$).

Source of Variation	*Spartina* Variables			Soil Condition Variables		
	d.f.	*F*	*P*	d.f.	*F*	*P*
	Spartina Density			*Salinity*		
Disturbance	3	28.94	**<0.0001**	3	2.72	0.0787
Sites within disturbance (whole-plot error term)	16			16		
Zone	2	32.92	**<0.0001**	1	3.47	0.0809
Zone x Disturbance	6	1.12	0.3727	3	1.77	0.1924
Sites within disturbance x zone (Split-plot error term)	32			16		
	Spartina Height			*pH*		
Disturbance	3	9.34	**0.0008**	3	12.49	**0.0002**
Sites within disturbance (whole-plot error term)	16			16		
Zone	2	30.3	**<0.0001**	1	8.85	**0.0089**
Zone x Disturbance	6	1.04	0.4212	3	0.4	0.7561
Sites within disturbance x zone (Split-plot error term)	32			16		
	Proportion standing-dead Spartina			*Redox potential*		
Disturbance	3	10.66	**0.0004**	3	3.3	**0.0473**
Sites within disturbance (whole-plot error term)	16			16		
Zone	2	40.1	**<0.0001**	1	5.41	**0.0335**
Zone x Disturbance	6	8.24	**<0.0001**	3	1.87	0.1755
Sites within disturbance x zone (Split-plot error term)	32			16		
	Percent Cover of Spartina			*Macro-organic matter*		
Disturbance	3	0.81	0.5057	3	0.67	0.5846
Sites within disturbance (whole-plot error term)	16			16		
Zone	2	107.76	**<0.0001**	2	2.92	0.0684
Zone x Disturbance	6	1.17	0.3452	6	1.64	0.1674
Sites within disturbance x zone (Split-plot error term)	32			32		

Table 4.2. Mean (SE) salinity, pH, redox potential, and macro-organic matter within healthy, edge, and affected zones at dieback, horse, snail, and wrack disturbed sites. N represents the total number of replicates per zone as averaged across all disturbance sites for a zone mean. Letters indicate significant differences among disturbance types and zones based on pairwise comparisons (Tukey's multiple comparisons test).

Disturbance	Salinity (psu)		
	Healthy (N=54)	Edge (N=0)	Affected (N=54)
Dieback	37.3 (1.8)	---	45.8 (4.1)
Horse	30.3 (2.3)	---	33.7 (0.4)
Snail	38.8 (1.7)	---	42.1 (3.1)
Wrack	31.8 (1.2)	---	39.3 (0.8)
Zone mean	**34.9 (1.1)**		**38.6 (1.6)**
	pH		
	Healthy (N=54)	Edge (N=0)	Affected (N=54)
Dieback[a]	6.96 (0.11)	---	7.02 (0.13)
Horse[b]	7.68 (0.07)	---	7.89 (0.03)
Snails[a]	6.84 (0.06)	---	7.06 (0.05)
Wrack[a]	6.91 (0.09)	---	7.11 (0.04)
Zone mean	**7.11 (0.06)**[a]		**7.29 (0.06)**[b]
	Redox (mV)		
	Healthy (N=54)	Edge (N=0)	Affected (N=54)
Dieback	-196 (20)	---	-108 (31)
Horse	-307 (18)	---	-291 (16)
Snail	-175 (47)	---	-107 (45)
Wrack	-273 (11)	---	-286 (22)
Zone mean	**-234 (17)**[a]		**-189 (20)**[b]
	Macro-organic Matter (g 100 cm^{-2})		
	Healthy (N=60)	Edge (N=60)	Affected (N=60)
Dieback	15.3 (2.1)	12.4 (2.4)	14.9 (1.8)
Horse	12.5 (1.9)	12.5 (2.2)	9.0 (1.6)
Snail	19.6 (1.8)	20.5 (1.6)	16.8 (1.2)
Wrack	25.4 (3.6)	18.8 (2.9)	14.7 (1.8)
Zone mean	**18.2 (2.1)**	**16.0 (1.9)**	**13.9 (1.4)**

Table 4.3. Statistical summary of split-plot ANOVAs for testing the effects of disturbance type, zone, and zone x disturbance type on epifaunal and infaunal invertebrates in the 20 survey sites. Values in bold are significant (p<0.05).

Source of Variation	Epifauna Variables			Macroinfauna Variables		
	d.f.	*F*	*P*	d.f.	*F*	*P*
	Periwinkle Snail Density			*Macroinfauna Density*		
Disturbance Type	3	13.37	**<0.0001**	3	4.88	**0.0135**
Sites within disturbance type (whole-plot error term)	16			16		
Zone	2	15.22	**<0.0001**	2	5.16	**0.0114**
Zone x Disturbance Type	6	3.47	**0.0093**	6	0.49	0.8112
Residual (Split-plot error term)	32			32		
	Fiddler Crab Density			*Macroinfauna Taxon Richness*		
Disturbance Type	3	5.11	**0.0074**	3	5.97	**0.0062**
Sites within disturbance type (whole-plot error term)	16			16		
Zone	2	1.97	0.1284	2	5.04	**0.0125**
Zone x Disturbance Type	6	0.52	0.7777	6	0.19	0.9781
Residual (Split-plot error term)	32			32		
	Mussel Density			*Macroinfauna Diversity*		
Disturbance Type	3	3.16	0.5193	3	3.16	0.0534
Sites within disturbance type (whole-plot error term)	16			16		
Zone	2	5.59	0.1676	2	5.59	**0.0083**
Zone x Disturbance Type	6	0.16	0.4112	6	0.16	0.9860
Residual (Split-plot error term)	32			32		

Table 4.4. Macroinfauna mean, taxon percent composition, sum of individuals and taxa collected across cores, and percent presence within plots in the healthy, edge, and affected zones for each disturbance site type. Feeding classification is indicated by SSF= subsurface feeder, SF= surface feeder, and C= carnivore.

	Percent Composition		
	Healthy	Edge	Affected
Dieback Sites			
Macroinfauna (mean, # 100 cm^{-2})	**32.5 ± 27.6**	**57.0 ± 47.9**	**6.1 ± 5.6**
Oligochaeta, SSF	39.1%	93.5%	23.0%
Capitella capitata, SSF	11.5%	---	7.7%
Steblospio benedicti, SF	44.9%	4.6%	69.2%
Neanthes succinea, SF	1.4%	1.8%	---
Manyunkia speciosa, SF	---	---	---
Ceratopogonidae, SF	1.4%	---	---
Nematoda, SSF	1.4%	---	---
Tanaidaceae, SF	---	---	---
Arichnida, C	---	---	---
Sum of individuals by zone (across 10 cores)	69	109	13
Sum of Taxa by zone (across 10 cores)	6	3	0
Percent presence within plots (SE)	30.0% (15.3)	22% (14.7)	20% (13.3)
Horse Sites			
Macroinfauna (mean, # 100 cm^{-2})	**44.1 ± 11.6**	**23.4 ± 7.8**	**11.7 ± 3.3**
Oligochaeta, SSF	88.8%	68.3%	66.1%
Capitella capitata, SSF	1.1%	7.5%	---
Steblospio benedicti, SF	1.1%	---	---
Neanthes succinea, SF	5.3%	4.0%	33.8%
Manyunkia speciosa, SF	---	---	---
Ceratopogonidae, SF	---	---	---
Nematoda, SSF	2.4%	18.1%	---
Tanaidaceae, SF	---	2.0%	---
Arichnida, C	1.3%	---	---
Sum of individuals by zone (across 10 cores)	94	50	25
Sum of Taxa by zone (across 10 cores)	6	5	2
Percent presence within plots (SE)	90.0% (10.0)	80.0% (13.3)	70.0% (15.3)
Snail Sites			
Macroinfauna (mean, # 100 cm^{-2})	**14.1 ± 9.1**	**1.9 ± 1.4**	**1.0 ± 1.0**
Oligochaeta, SSF	33.3%	---	---
Capitella capitata, SSF	40.0%	75.0%	---
Steblospio benedicti, SF	6.7%	---	---
Neanthes succinea, SF	3.3%	---	100.0%
Manyunkia speciosa, SF	3.3%	---	---
Ceratopogonidae, SF	---	25.0%	---
Nematoda, SSF	13.3%	---	---
Tanaidaceae, SF	---	---	---
Arichnida, C	---	---	---
Sum of individuals by zone (across 10 cores)	30	4	2
Sum of Taxa by zone (across 10 cores)	6	2	1
Percent presence within plots (SE)	30.0% (15.3)	20.0% (13.3)	10.0% (10.0)

Table 4.4. (continued)The percent composition of taxa, mean taxon richness, mean diversity, and percent chance of presence of macroinfauna collected in healthy, edge, and affected zones for each disturbance site type. Feeding classification is indicated by SSF= subsurface feeder, SF= surface feeder, and C= carnivore.

	Percent Composition		
	Healthy	Edge	Affected
Wrack Sites			
Macroinfauna (mean, # 100 cm^{-2})	**1.2 ± 0.9**	**0.6 ± 0.5**	**0 ± 0**
Oligochaeta, SSF	50.0%	---	---
Capitella capitata, SSF	50.0%	---	---
Steblospio benedicti, SF	---	---	---
Neanthes succinea, SF	---	---	---
Manyunkia speciosa, SF	---	---	---
Ceratopogonidae, SF	---	---	---
Nematoda, SSF	---	---	---
Tanaidaceae, SF	---	100.0%	---
Arichnida, C	---	---	---
Sum of individuals by zone (across 10 cores)	2	1	0
Sum of Taxa by zone (across 10 cores)	2	1	0
Percent presence within plots (SE)	12.5% (12.5)	14.3% (14.3)	0.0% (0)

Table 4.5. Summary of results of multiple regression models describing variation in invertebrates as predicted by stem density and soil conditions (ph, salinity, redox value, and macro-organic matter) across plots in each state. Asterisks (*, ** and *** indicate p <0.05, <0.01, <0.001 respectively, and none for 0.15> p >0.05) indicate the significance of individual terms in the model. Model adjusted R^2, overall R^2, P-values, and sample size (N) are given. Acronyms and symbols: Std.Sp.= standing dead *S. alterniflora* density, Sp.= live *S. alterniflora* density, Ht.Sp. = height of live *S. alterniflora*, MOM = macro-organic matter, Sal. = salinity. The "+" and "-" signs indicate the relationship of variables in the model to the dependent variable.

Dependent Variables	N	Model	R^2	Adj. R^2	P-value
Epifauna					
Snails	36	+StdSp.**, -pH**, +Sp.*, +Ht.Sp.	0.43	0.36	0.001
Crabs	60	-Sp.***, +Ht.Sp.***	0.45	0.44	<0.0001
Mussels	36	+MOM*, -Sal.*	0.24	0.19	0.01
Macroinfauna					
Total	57	+Sp.***, -Std.Sp.*	0.34	0.32	<0.0001
Shannon H'	36	+Sp.**, -pH	0.23	0.19	0.01
Taxon Richness	57	+Sp.**, -Std.Sp.	0.40	0.33	<0.0001

Table 4.6. Statistical summary of split-plot ANOVAs for testing the main effects of zone, date, and zone x date on *S. alterniflora* and epifauna in the 6 routinely monitored disturbance sites (2 each in dieback, snail, and wrack sites). Values in bold are significant ($p<0.05$).

Source of Variation	Dieback Sites			Snail Sites			Wrack Sites		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
		Spartina density			*Spartina density*			*Spartina density*	
Zone	2	24.63	**0.0002**	2	63.46	**<0.0001**	2	8.63	**0.0101**
Site	1	2.74	0.1363	1	0.88	0.3753	1	7.63	**0.0246**
Plots within zone x site (whole-plot error term)	8			8			8		
Date (within plots)	6	6.01	**0.0001**	6	1.86	0.1078	6	4.68	**0.0008**
Site x date	6	3.91	**0.0029**	6	0.48	0.8199	6	1.15	0.3496
Zone x date	12	2.6	**0.0096**	12	0.63	0.8053	12	1.34	0.2305
Residual (Split-plot error term)	48			48			48		
		Snail density			*Snail density*			*Snail density*	
Zone	2	19.53	**0.0008**	2	101.09	**<0.0001**	2	6.19	**0.0237**
Site	1	14.4	**0.0053**	1	5.5	**0.0471**	1	16.53	**0.0036**
Plots within zone x site (whole-plot error term)	8			8			8		
Date (within plots)	6	0.16	0.9857	6	9.5	**<0.0001**	6	0.73	0.6271
Site x date	6	0.15	0.989	6	1.46	0.2124	6	1.14	0.353
Zone x date	12	0.87	0.5797	12	7.54	**<0.0001**	12	1.33	0.2318
Residual (Split-plot error term)	48			48			48		
		Crab density			*Crab density*			*Crab density*	
Zone	2	8.04	**0.0122**	2	1.1	0.379	2	0.27	0.7693
Site	1	0.01	0.9445	1	0.41	0.5394	1	7.75	**0.0238**
Plots within zone x site (whole-plot error term)	8			8			8		
Date (within plots)	6	3.62	**0.0048**	6	24.08	**<0.0001**	6	11.51	**<0.0001**
Site x date	6	6.09	**0.0001**	6	0.95	0.4691	6	3.72	**0.0042**
Zone x date	12	1.23	0.291	12	1.3	0.2499	12	0.96	0.5028
Residual (Split-plot error term)	48			47			47		

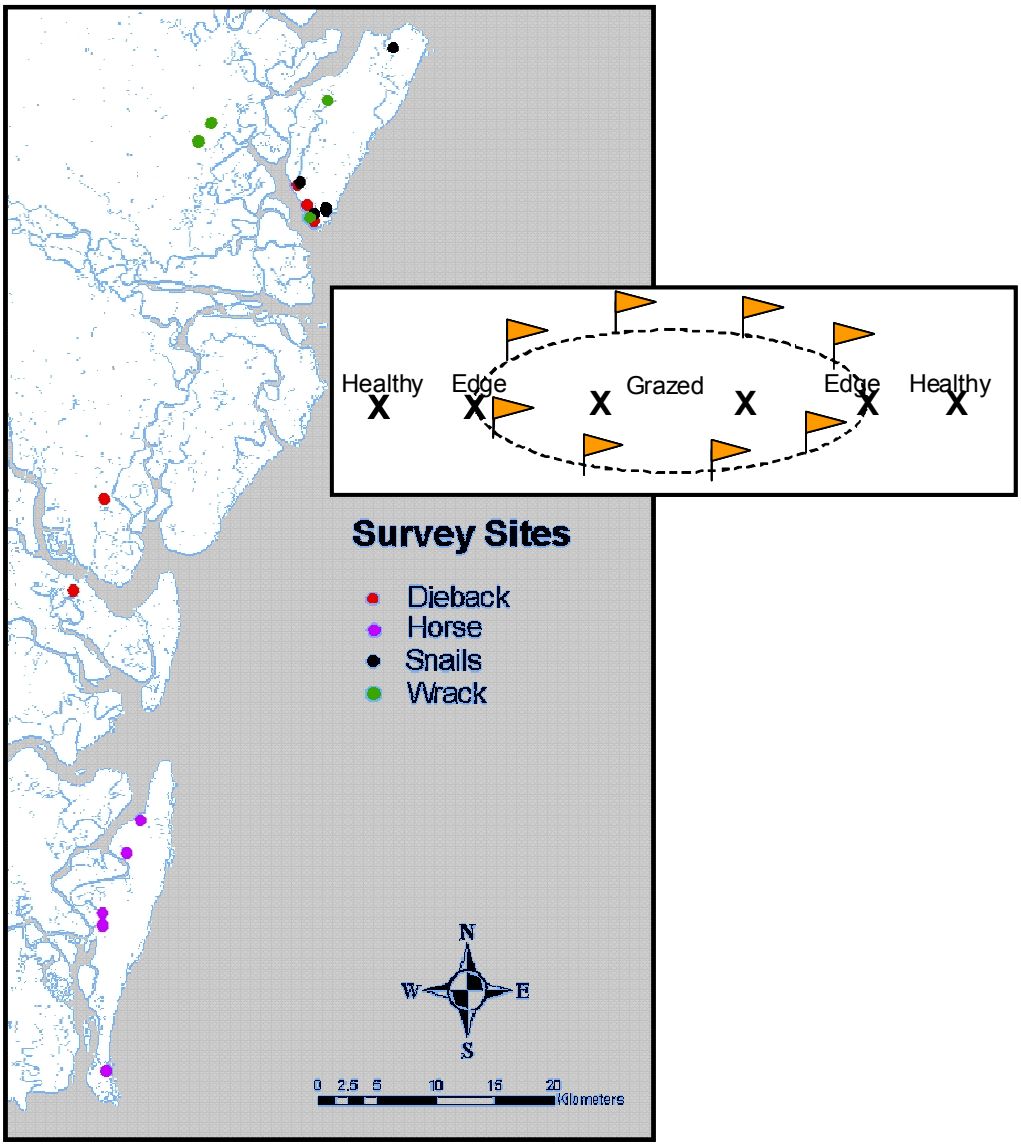


Figure 4.1. Location of dieback, horse, snail, and wrack survey sites along the Georgia Coast. Inset shows how an individual "survey" site was set up. Sites were marked along the edge of the disturbed area with a GPS (and flags at permanent sites), and a transect of 6 plots (0.25 m^2 each) was arranged to encompass healthy, edge, and affected marsh.

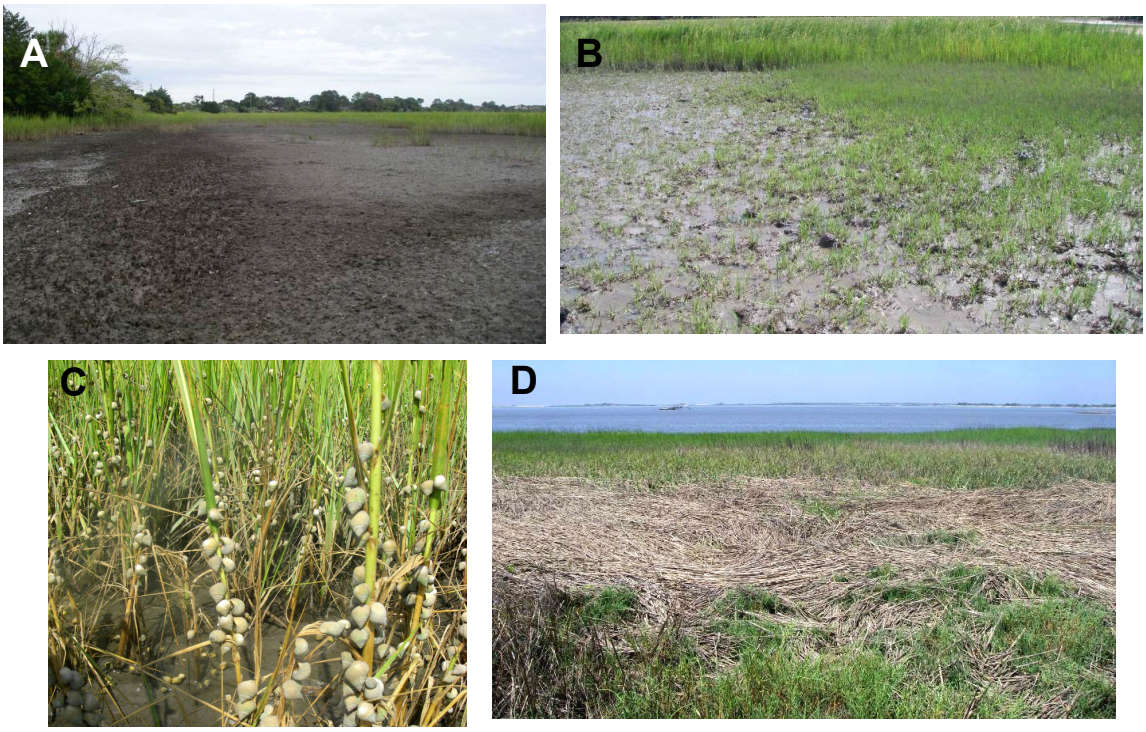


Figure 4.2. Example of disturbance types compared in this study: A) a sudden dieback site in Brunswick, GA, B) a horse-grazed site on Cumberland Island, GA, and C) a snail-grazed and D) wrack-disturbed site, both on Sapelo Island, GA.

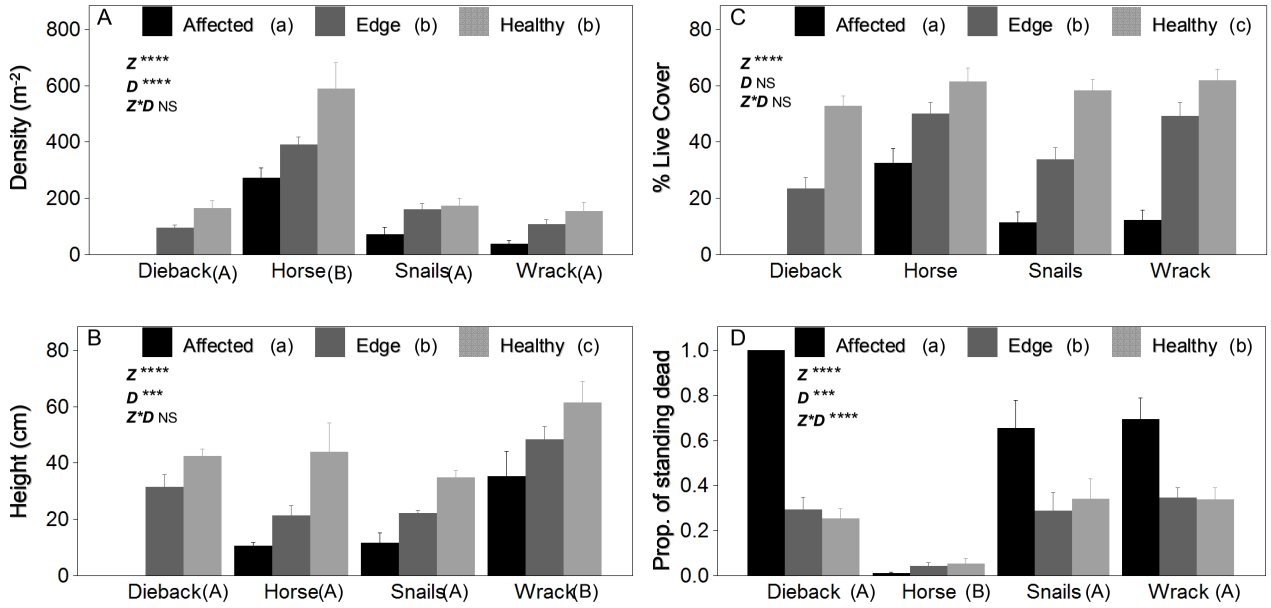


Figure 4.3. Mean (SE) of *S. alterniflora* measurements: A) density, B) height, C) percent live cover, and D) proportion of standing dead stems across affected, edge, and healthy zones. Each bar per zone represents the average of 5 sites for dieback, horse, snail, and wrack disturbances. The significance (*p*-value) of the split –plot ANOVA factors zone (Z), disturbance type (D), and zone x disturbance type (Z*D) are indicated by asterisks, where *<0.5, **<0.01, ***<0.001, ****<0.0001, and NS=not significant, and different letters indicate pairwise differences among zone and disturbance type (Tukey's multiple comparison test).

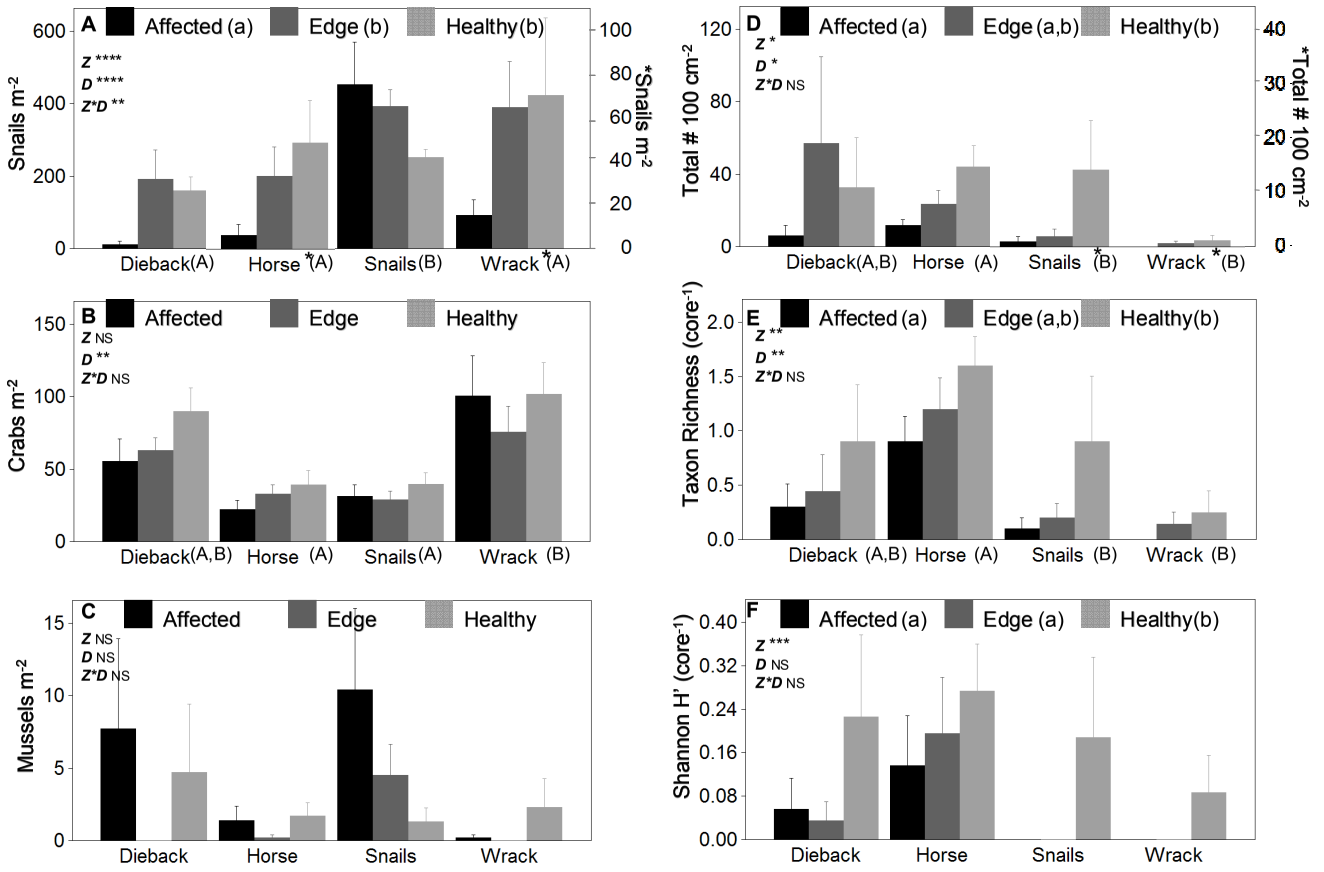


Figure 4.4. Mean (SE) of faunal measurements: A) snails, B) crabs, C) mussels, D) total macroinfauna, E) taxon richness, and F) Shanon H' diversity across affected, edge, and healthy zones. Each bar per zone represents the average of 5 sites for dieback, horse, snail, and wrack disturbances. The significance (*p*-value) of the split –plot ANOVA factors zone (Z), disturbance type (D), and zone x disturbance type (Z*D) are indicated by asterisks, where *<0.5, **<0.01, ***<0.001, ****<0.0001, and NS=not significant, and different letters indicate pairwise differences among zone and disturbance type (Tukey's multiple comparison test).

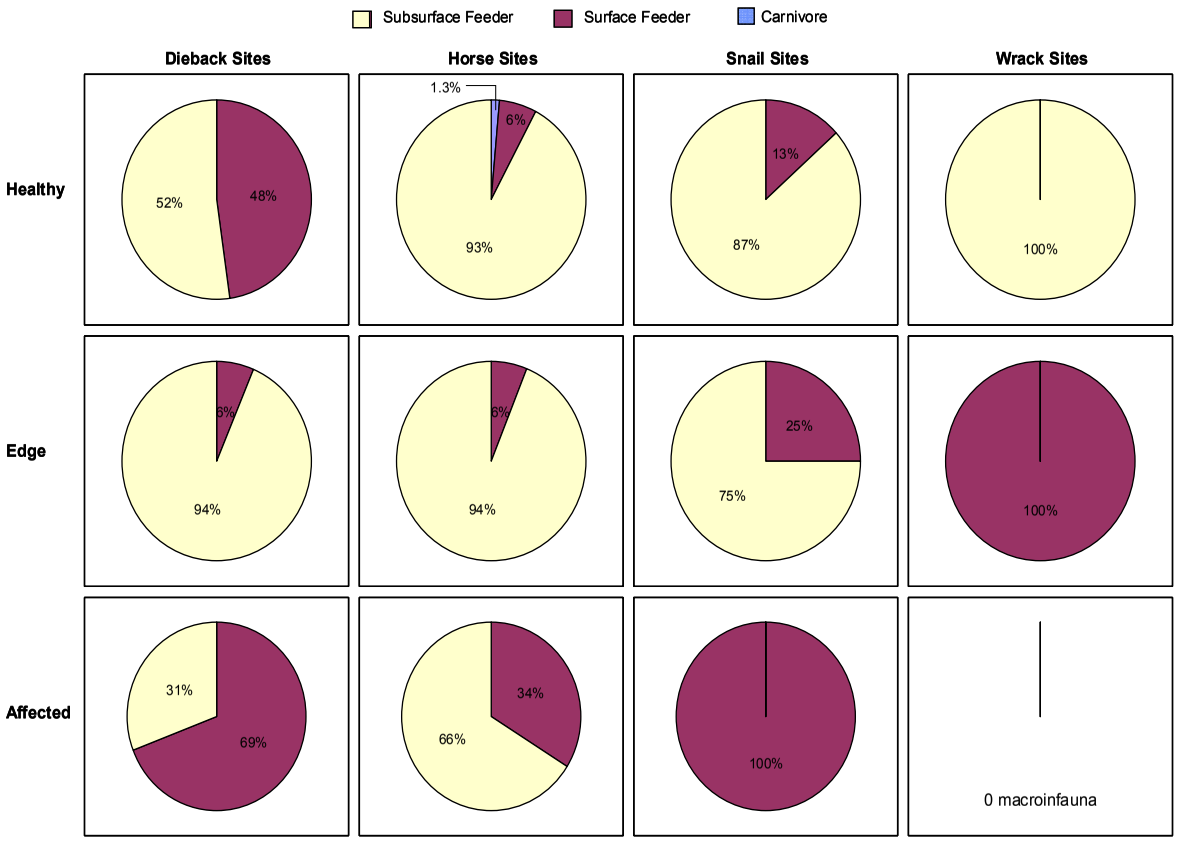


Figure 4.5. Percent frequency of macroinfauna classified as subsurface feeders, surface feeders, and carnivores within the healthy, edge, and affected zones (as indicated on the vertical axis) at dieback sites, horse-affected sites, snail-affected sites, and wrack-affected sites (as indicated on the horizontal axis). The feeding classification used in this analysis is listed in Table 3.1.

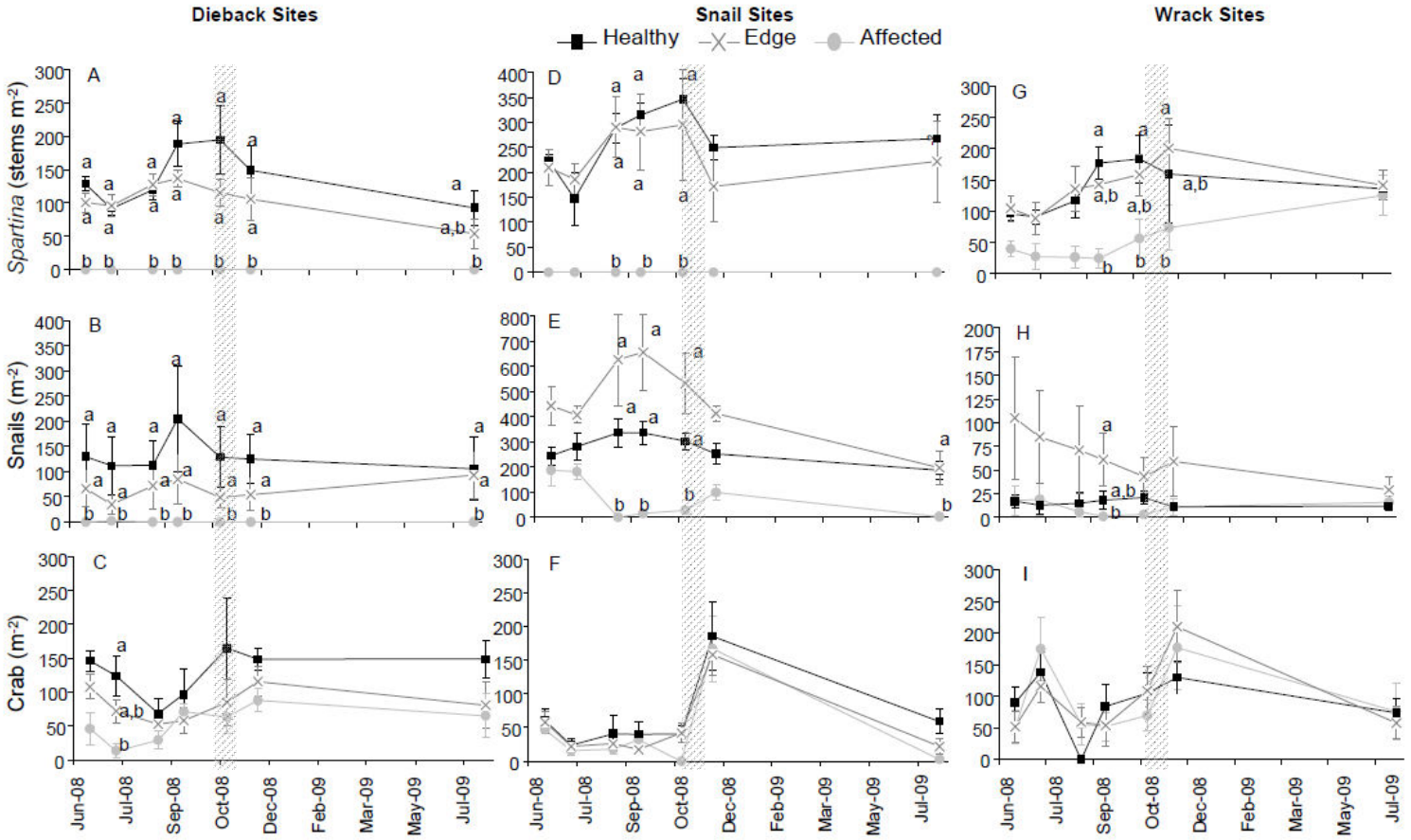


Figure 4.6. Mean (SE) of *S. alterniflora*, snail, and fiddler crab densities over time at the routinely monitored dieback (A-C), snail (D-F), and wrack sites (G-I) (each point represents n=4). Different letters indicate the pairwise differences (Tukey's multiple comparison test) between zones on each date (no letter = no significant difference), as determined following the split-plot ANOVA. Shading indicates the date used in the analysis of the 20 sites.

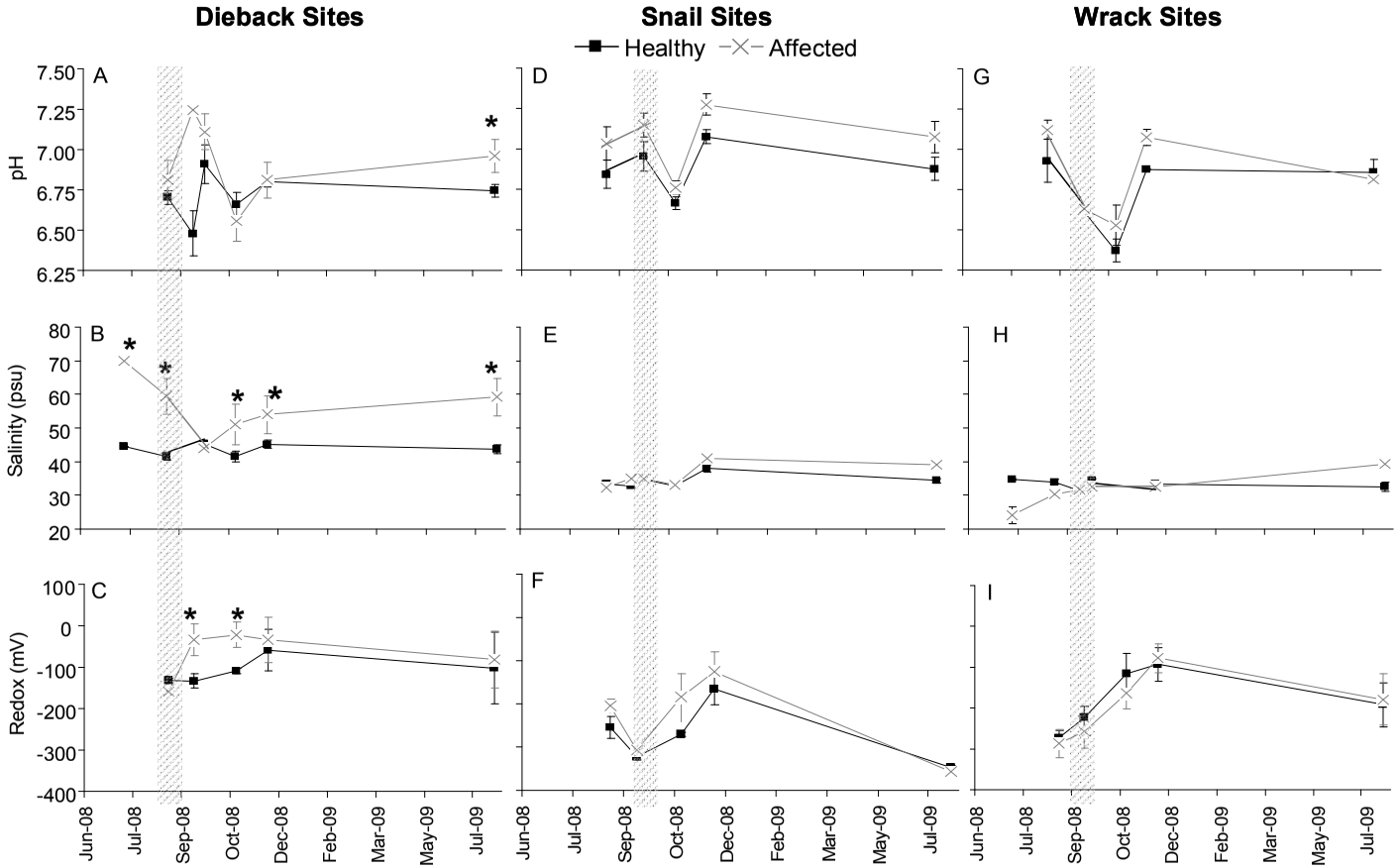


Figure 4.7. Mean (SE) of pH, salinity, and redox values within the healthy and affected zones over time at the routinely monitored dieback (A-C), snail (D-F), and wrack sites (G-I) (each point represents n=6). The results of the 2-way ANOVA are presented where * indicates a significant difference between the healthy and affected zone. The family-wise error rate of the multiple comparisons was controlled for by using a Bonferroni correction where significance at the $\alpha<0.05$ level was indicated when $p<0.008$. Shading indicates the date used in the analysis of the 20 sites.

CHAPTER 5

SALT MARSH DIEBACK IN GA: SEVEN YEARS OF OBSERVATIONS[6]

[6] McFarlin, C.R., and M. Alber. To be submitted to *Estuaries and Coasts*.

Abstract

In 2001 and 2002, Georgia experienced the largest dieback of salt marsh vegetation ever recorded in the state, with ≥ 800 ha affected. Two of these sites were monitored from 2003-2009 to track their recovery from the sudden dieback event (one site with extensive *Spartina alterniflora* dieback and a second with extensive *Juncus roemerianus* dieback). Marsh vegetation (stem density, height), epifauna (density of snails, crabs, mussels), and soil conditions (soil temperature, salinity, pH, and redox potential) were monitored within 18 plots at each site: 9 located in the dieback area, and 9 in the adjacent healthy area. Just after the dieback occurred, there were decreases in the height and density of both *S. alterniflora* and *J. roemerianus* in the dieback areas as compared to the healthy areas. Dieback areas in both sites had begun to recover in September 2004 in plots closest to the healthy marsh, but vegetation characteristics in the healthy and dieback marsh were still significantly different in 2009. *S. alterniflora* began to grow into the *J. roemerianus* dieback areas later in 2007, and increasing in overall density through 2009. The response of the epifauna to the dieback varied: snail densities were close to 0 in the dieback area within <1 year after the onset, and mussels density fell to 0 in 2 years of the onset, whereas there were no difference in fiddler crab densities over the 7 years. At the *S. alterniflora* site, snail and mussel densities in the dieback areas began to recover (in 2005 and 2007, respectively), following the increase in plant density although snail density had not reached that found in the healthy areas by 2009. At the *J. roemerianus* site, only fiddler crabs were present, which were unaffected by the dieback but varied seasonally. Soil conditions did not differ between healthy and dieback areas over time, but fluctuated temporally. The pattern of vegetation recovery suggests that

proximity to healthy marsh is important for regrowth (via rhizomes) in the dieback areas, and that the process occurs slowly (on the order of a decade).

5.1. Introduction

Beginning in spring 2001 and continuing into 2002, Georgia experienced the largest dieback of salt marsh vegetation ever recorded in the state, with ≥ 800 ha affected at least 40 sites. The affected areas were most frequently less than 1 ha but ranged up to 240 ha at the largest site on the Jerico River. Dieback sites were widely distributed along the coastline, with sites on both inland and barrier island marshes (Ogburn 2004). Various parts of the marsh were affected, including along creekbanks, the interior of creekbanks (behind a band of low marsh vegetation), in the mid- and high-marsh, and along raised berms (Ogburn 2004). Although the ultimate cause of the dieback was never established, the event was linked to a severe drought (Alber et al. 2008). Between 1999 and 2002, a decrease in rainfall across the state of GA led to 30-80% reductions in average streamflow of major rivers, with years 2000 and 2002 ranking 77th and 79th in terms of streamflow (m^3 sec^{-1}), respectively, out of 79 years of data (USGS 2011). The decrease in rainfall could have also resulted in diminished delivery of groundwater to marshes. As a result, the marsh surface in many of the affected areas had desiccated and visibly cracked surfaces (Ogburn and Alber 2006, McFarlin pers. obs.).

In June 2003, the Georgia Coastal Research Council (GCRC) initiated a long-term collaborative project to monitor several dieback sites (http://www.marsci.uga.edu/coastal council). I collected data from 2 of those sites from 2004-2009. In one of the sites, *Spartina alterniflora* was the affected vegetation, whereas in the second site, *Juncus roemerianus* was affected. This paper describes the differences in vegetation and invertebrates between healthy and affected areas at these two sites over time, with a goal of determining how long recovery from a sudden dieback event takes.

5.2. Methods

5.2.1. Site setup

Two dieback sites, both located in Liberty County, GA, were included in this study (Figure 5.1). At the Melon Bluff site (MB), *S. alterniflora* was affected, and at the Isle of Wight Road site (IW), *J. roemerianus* was affected by dieback. Permanent sampling plots were established at each site by Matt Ogburn (Ogburn 2004) along 3 transect lines each in the dieback and healthy areas (Figure 5.2). Transects were arranged perpendicular to the transition zone, which was defined as the mid-line between dieback and unaffected (healthy) areas. Three plots (each 0.25 m^2) were established 10 m apart along each transect for a total of 9 dieback and 9 healthy plots per site. PVC poles were used to mark the corners of each plot, and locations were recorded with a handheld GPS.

5.2.2. Sample collection

Sampling at Melon Bluff began in June 2003 and at Isle of Wight Road in September 2003. Both sites were monitored quarterly through September 2005 (with the exception of December 2004), and then annually in the fall through 2009 for a total of 12 sampling dates at IW and 13 at MB. At each sampling time vegetation density and height, epifaunal density, and porewater pH and salinity were measured in each plot. When possible, soil temperature and redox potential were also monitored. All density data were scaled up to 1 m^2 for analysis.

5.2.2.1. Vegetation

Stem densities of live and standing dead plants were generally counted from within the entire plot (0.25 m^2), although in cases where vegetation was extremely dense (i.e. *J. roemerianus*), I used a smaller quadrat area (0.0625 m^2) for sampling. The densities of live plants were further separated into two size categories: tillers > 15 cm and those <15 cm. Additionally, the heights of the five tallest plants from within the plot were recorded.

5.2.2.2. Epifauna

Epifauna were also generally counted from within the entire plot (0.25m^2), although in cases where there were a dense number of individuals (i.e. crab holes), I used a smaller quadrat area for sampling. Observations of dead epifauna were recorded as well. The dominant mollusc species encountered were the periwinkle snail, *Littoraria irrorata* and the bivalve *Geukensia demissa*, but other molluscs (*Melampus bidentatus, Illyanasa sp.*) were also observed on a few occasions (see Table 5.1.). Crab holes (>5 mm) were counted, as a proxy for the number of crabs. Holes from crabs may have represented several species. The dominant species encountered was *Uca spp.*, but *Armases cinereum* and *Eurytium limosum* were seen at some locations, as well. The percent occurrence of live and dead epifauna present within plots was calculated for each date.

5.2.2.3. Porewater and Soil

Salinity and pH and were measured in interstitial porewater during low tide. A handheld refractometer was used to measure the salinity (psu) and a pH probe calibrated at 3 points was used to measure the pH. Soil redox measurements (mV) were collected by inserting a handheld redox probe (Pt electrode, Ag-AgCl reference solution) into the soil to a depth slightly less than the probe length, ~7 cm.

5.2.3. Statistical Analysis

Vegetation, epifauna, and porewater measurements were compared between healthy and dieback areas and among sampling dates at each site. Prior to statistical analysis, variables were either natural log (x+1) or square-root (x+1) transformed to improve normality as needed.

Vegetation (heights and densities) and epifauna (densities) at each site were analyzed using a split-plot 2-way ANOVA with repeated measures which included the fixed factors marsh zone (i.e. healthy vs. dieback area, as the between-subjects factor) and sampling date (as the within-subjects factor), and the interaction term of zone x date. Individual plots were the unit of repeated measures. A significant zone effect meant that there was an overall mean difference between the healthy and dieback areas (irrespective of the date) and a significant date effect meant that there were differences in the overall mean of the measured variables over time (irrespective of marsh zone). However, in this study, I focused on the significance of the interaction term, zone x date, which assessed whether the effect of zone (healthy vs. dieback areas) differed over time. This effect, in addition to pairwise differences (analyzed by Tukey's post-hoc comparison test) between

zone on each date was used to specifically evaluate whether differences between healthy and dieback areas varied over time and to determine on which dates these zones were significantly different from each other. Porewater measurements were further compared using the coefficient of variation to compare the variability within healthy and dieback marsh.

I also used proximity to the unaffected (healthy) marsh to evaluate recovery. Dieback plots were assigned into distance categories based on quadrat number (#1, 2, and 3) to represent distances of 10, 20, and 30 m respectively, from the healthy marsh. There were 3 plots for each distance category (1 per transect). Data were analyzed by the between-subjects factor distance, and the within subject factors date and distance *date using a repeated measures ANOVA. I focused on the effects of distance (to healthy marsh) and date. A significant distance effect meant that there was a difference in the recovery of dieback areas based on distance to the healthy marsh (irrespective of the date), and a significant date effect meant that there were differences in the overall mean recovery over time (irrespective of distance). Analyses were considered significant where $p<0.05$.

5.3. Results

5.3.1. Melon Bluff: the *Spartina*-dieback site

5.3.1.1. Initial Conditions

The initial observations at the Melon Bluff marsh (June 2003) showed a significant decrease in the density and height of living *S. alterniflora* in the dieback marsh (density: 6.7 ± 6.2 m^{-2}; height: 7.4 ± 5.0 cm) as compared to healthy areas

(density: 143 ± 15 m^{-2}; height: 69 ± 3 cm) (Figure 5.3). The density of new ramets of *S. alterniflora* (plants <15 cm) were also significantly decreased in dieback areas (0.9 ± 0.9 m^{-2}) as compared to healthy areas (64 ± 9 m^{-2}). During this time, only one of the dieback plots (2-1), which was located nearest to the healthy area had live *S. alterniflora*. Standing dead *S. alterniflora* densities were very high on the initial sampling date in both the healthy and dieback areas (as compared to later dates). Dieback plots had significantly greater standing dead densities (354 ± 41 m^{-2}) than the healthy areas (226 ± 47 m^{-2}). In contrast, there were no differences in initial soil salinity, pH, redox potential, and temperature between healthy and dieback areas (Table 5.4).

The initial densities (June 2003) of periwinkle snails (healthy: 7 ± 3 m^{-2}; dieback: 14 ± 13 m^{-2}), fiddler crabs (healthy: 28 ± 4 m^{-2}; dieback: 49 ± 7 m^{-2}), and mussels (healthy: 10 ± 6 m^{-2}; dieback: 15 ± 5 m^{-2}) were greater in the dieback than in the healthy areas, although these did not differ(Figure 5.5). Periwinkle snails were primarily concentrated within a single dieback plot (3-1), located 10 m from the healthy area, which had 116 snails m^{-2}; the one other plot with snails present (3-2) was located along the same transect and had only 8 snails m^{-2}. Fiddler crabs and mussels were better distributed among plots in the marsh, but there was a gradient across the marsh with the lowest densities occurring nearest the healthy marsh (1-1, 2-1, 3-1) and the highest densities occurring furthest away from the healthy marsh (1-3, 2-3, 3-3).

5.3.1.2. Temporal Patterns

Vegetation-

S. alterniflora density in dieback areas remained low from June 2003-March 2005. After that point, there was a slow increase in *S. alterniflora* density in the dieback area over the next four years from 12 ± 7 in March 2005 to 92 ± 26 plants per m^2 in September 2007 (Table 5.2, Figure 5.3). After 2007, plant densities in the dieback areas remained relatively constant. The interaction term zone x date was significant, as the magnitude of difference between the dieback and healthy areas decreased over time as the plants in the dieback areas began to grow back. Despite the regrowth, however, densities in the dieback area remained significantly below that of healthy areas on each sampling date over 7 years. On the latest sampling date in 2009, *S. alterniflora* density was still approximately 125 m^{-2} lower in the dieback area than in the healthy area.

S. alterniflora height in the dieback area also began to increase steadily starting in March 2005, from a minimum of 1.5 ± 1.5 to 75 ± 20 cm in September 2006 (Table 5.2, Figure 5.3). *S. alterniflora* height was significantly lower in the dieback areas as compared to healthy areas during the first 3 years. In September 2006, plant height in the dieback areas reached that of the healthy areas, and the two areas were statistically similar from 2006-2008. In 2009, however, the height of plants in the dieback areas was again significantly lower than that of healthy areas, and this may have been the result of a second drought that occurred in 2008. The interaction term zone x date was also significant for the height of *S. alterniflora*, as the magnitude of height difference between dieback and healthy areas varied over time.

The dieback areas had higher numbers of standing dead stems in June and September 2003. There was then a sharp decline in the standing dead densities in both healthy and dieback areas (by ≥150 stems m^{-2} each) in early 2004. From 2004-2009, the standing dead densities then fluctuated together and were not statistically different. There was no significant difference between the two areas from 2004-2009 because of the differences between the early and later part of the time series.

Epifauna-

There were no differences in periwinkle densities in the healthy and dieback areas between June 2003 and March 2004, as densities in both areas remained low (<12 m^{-2}). In the healthy area, snails increased to 24.4 ± 7.2 m^{-2} in June 2004 and continued to increase over time to 39 ± 14 m^{-2} by December 2009, despite some annual fluctuation. Snails in the dieback areas began to increase in October 2006 from 10 ± 6 m^{-2} to a maximum of 20 ± 8 m^{-2} in 2008. There was, however, a decrease in the snail density to 12 ± 4 m^{-2} in the dieback plots in 2009, which coincided with a decrease in the *S. alterniflora* density in those plots. In contrast, the healthy marsh did **not** experience a similar decline in periwinkle density (or *S. alterniflora* density). Snail densities were significantly decreased in the dieback as compared to the healthy areas from September 2004-September 2007, but were statistically similar to that of healthy areas in 2008 and 2009, thus there was a significant zone x date interaction.

The densities of fiddler crabs observed initially were comparable to other dates. Although there were higher densities of crabs in the dieback area on all dates, this was only statistically significant at one time point (September 2004). Fiddler crab densities in

both zones varied seasonally and interannually. There were no obvious trends in the crab densities over time (peak density occurred in 2007 in both the healthy and dieback marsh). Date was the only significant factor in the ANOVA.

Mussel densities at the dieback site ranged between 11.1-26.2 m^{-2} for the first 2 years of sampling. However, they declined sharply to an average 0.0-0.4 m^{-2} in the dieback area between October 2005 and September 2007, after which they again increased. In contrast, mussel densities in the in the nearby healthy marsh ranged from 1.4-14.2 m^{-2} over all observations. Because there was significant variation over time in the dieback area, both date and the interaction term zone x date were significant. There were, however, no significant differences between the healthy and dieback zone on any individual date, possibly due to the large variability in mean mussel density.

When epifauna were considered in terms of their presence and absence rather than by density (Table 5.1.), the trends were similar to those reported above. Periwinkle snails were present in ~60% fewer plots in dieback as compared to healthy areas. The occurrence of dead snails was 10% higher in the dieback as compared to healthy areas. There was little difference in the percent occurrence of fiddler crabs and mussels (either live or dead) between healthy and dieback areas.

5.3.1.3. Spatial Patterns

Vegetation-

The density and height of *S. alterniflora* in the dieback areas increased much faster in plots located closer (10 m away) to the healthy edge as compared to those located further away (20 and 30 m) (Figure 5.4.). In an ANOVA, the factors distance (to

the healthy zone), date, and the interaction term of date x distance were each significant sources of variation of *S. alterniflora* density and height in dieback plots (Table 5.2.). The pattern of regrowth over time varied among the plots. For the first 3 years following the dieback (2003-3005), all new growth occurred in plots nearest to the healthy marsh, i.e. 10 m away (Figure 5.4.). October 2006 was the first date that living *S. alterniflora* was recorded in plots 20 and 30 meters away from the healthy area (Figure 5.4), all of which occurred within transect 2. Transects 1 and 3 did not have living *S. alterniflora* in plots located 20 m from the healthy zone (i.e. 1-2, 3-2) until September 2007 and December 2008, respectively, and plots located 30 meters from the healthy zone (i.e. 1-3, 3-3) in these transects were still bare in December 2009.

Epifauna-

When evaluated in terms of their distance from the healthy area, snail densities in the dieback area increased significantly faster in plots closest to the healthy marsh, in keeping with the pattern of plant regrowth (Table 5.3., Figure 5.6.). Periwinkles averaged 15.6 ± 4 m^{-2} in plots 10 meters away, 3.2 ± 1.4 m^{-2} in plots 20 meters away, and 0 in plots 30 m away. In contrast, distance to the healthy marsh was not a significant factor in the number of fiddler crabs or mussels in the dieback area.

5.3.1.4. Soil conditions

Salinity, pH, and redox values did not differ by zone (healthy vs. dieback areas) at Melon Bluff across all sampling dates (Table 5.4.). Soil temperatures were significantly higher overall in the healthy as compared to dieback area (although this was by less than

1/3rd of a degree), but when individual sampling dates were compared only March and June 2004 were statistically different between the zones. Soil conditions fluctuated over time, however, and both the date and the interaction term zone x date were significant factors for all measurements (except for redox, which was only measured twice). Salinities varied with rainfall and increased after 2006. Lowest soil temperatures were recorded during cooler months (Dec-Mar). The reason for the variation in pH with date, however, is less clear.

There were no obvious changes in the soil conditions as dieback areas began to increase in *S. alterniflora* density. Although the interaction term zone x date was significant the soil variables, soil conditions were either higher or lower in dieback as compared to healthy areas, depending on date. Despite the high variability in soil conditions over time, the coefficient of variation indicated that the dieback area was no more variable than the healthy marsh in terms of salinity, pH, and soil temperature (there were too few samples to judge the trend in redox potential).

5.3.2. Isle of Wight Road: the *J. roemerianus*-dieback site

5.3.2.1. Initial conditions

The initial density and height of *J. roemerianus* was measured on September 2003 at the Isle of Wight Road site. At that time *J. roemerianus* density (41 ± 21 m^{-2}) and height (33 ± 14 cm) in the dieback area was much lower than those in the nearby healthy areas (density: 342 ±24 m^{-2}; height: 103 ± 2 cm) (Figure 5.7). There was also a lower density of short *J. roemerianus* (<15 cm tall) in the dieback area (dieback: 6 ± 3 m^{-2}; healthy: 24 ± 4 m^{-2}). Within the dieback area, no viable *J. roemerianus* was present in

plots located furthest away (30 m) from the healthy marsh (i.e. 1-3, 2-3, 3-3). Standing dead *J. roemerianus* was present within all plots at the site, but was significantly greater in the dieback (376 ± 66 m^{-2}) as compared to healthy area (267 ± 52 m^{-2}). Soil conditions (salinity, pH, soil temperature) were not different between the healthy and dieback marsh despite the change in vegetation (Table5.5).

Fiddler crabs were the only epifaunal organism consistently observed in the Isle of Wight Road marsh (Table 5.1.). The mean density of fiddler crabs was slightly higher in the dieback (138 ± 23 m^{-2}) than in the healthy area (100 ± 12 m-2) in September 2003, although this was not statistically different (Figure 5.10.). The densities of the fiddler crab on that date were nearly average for the Isle of Wight Road marsh (see Table 5.1.), and the crabs were also well distributed among plots within the healthy and dieback areas. Although no live mussels were present, shells observed on the initial sampling date indicated that they had previously been in both the healthy and dieback marsh; it is unclear whether the dieback was associated with this.

5.3.2.2. Temporal Patterns

Vegetation-

J. roemerianus density was significantly lower in the dieback as compared to the healthy areas from September 2003 until March 2004. Beginning in September 2004, the densities in both the healthy and dieback areas showed similar fluctuations, increasing to an overall peak density in June 2005 (healthy: 962 ± 100 m^{-2}; dieback: 668 ± 203 m^{-2}) (Figure 5.7). This was followed by a subsequent decline until 2006, and then a steady increase again through 2009. *J. roemerianus* densities in the dieback areas did not reach

those in the healthy marsh over the 7 years of observations. The interaction term zone x date was not significant (Table 5.2.), which may have been due to high variability in the data. However, the dieback areas were significantly decreased compared to the healthy marsh on the initial sampling dates in June 2003-March 2004, and again later in 2007-2009. It should be noted that *S. alterniflora*, likely from the nearby creekbank stands, began to grow into some of the dieback plots in 2007 and increased to a density of ~54 ± 36 stems m^{-2} by 2009 (Figure 5.8.). *Atriplex patula* was also observed growing into dieback areas, although it did not occur in any of the dieback plots.

The height of *J. roemerianus* was significantly lower in the dieback (by ≥ 30 cm) as compared to the healthy areas on each sampling date over 7 years (Figure 5.7.). Both the dieback and healthy areas showed a similar seasonal variation with peak height occurring in 2006. The interaction term zone x date was significant, as the height in the dieback area began to approach that of the healthy areas, but in 2009 was still 20 cm lower.

Standing dead densities declined from the initially observed densities in 2003, and remained low in both the healthy and dieback areas through June 2004. Beginning in March 2005, standing dead densities in the healthy marsh increased, and surpassed the densities observed following the dieback event, with a peak of standing dead density of 981 ± 108 m^{-2} in 2006 (a year after the peak in the live *J. roemerianus* density). In contrast, standing dead densities in the dieback area remained lower than the initially observed densities, and did not exhibit a similar peak in 2006.

Epifauna-

Fiddler crabs were present in >95% of plots in both the dieback and healthy marsh (Table 5.1.). The densities of crabs observed initially after the dieback were comparable to those observed on other dates. Densities were significantly higher overall in the dieback areas (although differences on individual dates were not significant). Date and the interaction term zone x date were also significant factors in fiddler crab variation, as fiddler crabs densities varied seasonally in both the healthy and dieback marsh. In general, the coldest sampling dates (January 2004, and December 2008, 2009) tended to have the greatest number of crab holes, regardless of marsh status (Figure 5.10.). This may be due to the colder soil surface maintaining more persistent crab holes, rather than increased densities or burrowing activity of fiddler crabs (crabs are less active when temperatures fall below 20° C; Powers and Cole 1976).

Periwinkle snails and coffee-bean snails, which were observed during a few sampling dates, occurred more frequently in healthy plots, whereas the percent occurrence of dead fauna (snails, crabs, and mussels) was slightly greater in dieback plots. Live mussels were not observed over the 7 years of sampling, although dead shells during the initial sampling indicated they had previously been present at that site (Table 5.1.).

5.3.2.3. Spatial Patterns

Vegetation-

When *J. roemerianus* density in the dieback area was analyzed based on distance from the healthy marsh edge, there was a striking pattern with density increasing much

faster in plots located closer (10 m away) to the healthy edge as compared to those located further away (20 and 30 m away) (Figure 5.9.). In an ANOVA, the factors distance (to the healthy zone), date, and the interaction term of date x distance were each significant sources of variation of *J. roemerianus* density in dieback plots (Table 5.3.). *J. roemerianus* was present in all plots 10 and 20 meters from the healthy zone over all sampling dates, although September 2004 was the first date that *J. roemerianus* was present in plots located 30 meters from the healthy marsh[7]. In addition, for the first 3 years following the dieback (2003-3005), all new growth (plants <15 cm) occurred only in plots closest to the healthy marsh (i.e. 10 m away). *J. roemerianus* height in the dieback area also showed a similar pattern as the density, with taller plants in plots 10 meters from the healthy zone as compared to those located 20 and 30 meters away, however this was not significant (Table 5.3., Figure 5.9.)

In 2007, *S. alterniflora* at the Isle of Wight Road site was first recorded in a single plot (1-3) located 30 meters away from the healthy area, and nearest to the creekbank. *S. alterniflora* also grew within a second plot (3-3) by 2008. The *S. alterniflora* density increased in these 2 plots over time from a mean density of 17 ± 17 m^{-2} to 54 ± 36 m^{-2} in 2009.

Epifauna -

There were no significant differences in the density of crabs in the dieback area based on distance to the healthy marsh (Table 5.3., Figure 5.11.).

[7] It should be noted that there was a single small plant (3.7 cm tall) recorded in these plots in March 2004, but there were no plants recorded 3 months later in June 2004.

5.3.2.4. Soil conditions

Salinity, redox potential, and soil temperature did not differ by zone (healthy vs. dieback areas) at Isle of Wight Road across all sampling dates (Table 5.5.). The pH was significantly higher in the healthy as compared to dieback areas when averaged over all dates, however when compared among individual dates, pH differed by zone only twice (and the direction of change between zones varied). Soil conditions fluctuated over time, and thus date was a significant factor for all measurements[8] for reasons similar to those found at Melon Bluff: Soil temperature tended to be cooler in winter; salinity varied with rainfall. The reasons for the variation in pH with date was again not clear, but the minimum pH occurred in June 2004 on the date with the highest soil temperature recorded at this site. The interaction term zone x date was significant only in the case of pH. Overall, the coefficient of variation indicated that dieback sites were no more variable than healthy sites in terms of salinity, pH, and soil temperature.

5.4. Discussion

The sudden dieback phenomenon that occurred in the Southeast beginning in 2001 was associated with a severe drought (Alber et al. 2008). In Georgia, rhizomes in *S. alterniflora* dieback areas were not viable following the dieback, and there were also observations of dry, desiccated soils (Ogburn and Alber 2006). Transplants of both *J. roemerianus* and *S. alterniflora* followed for a period of 6 months in 2003 survived and grew vigorously within dieback areas, which suggested that the causative agent was no longer present at that time (2 years post-dieback) and that recovery would be possible (Ogburn and Alber 2006). Since that time, there have been some reports of recovery in

[8] Redox potential could not be compared because it was only measured on one date.

sudden dieback areas (GCRC 2008 report). Ogburn and Alber (2006) observed small patches of *S. alterniflora* in the dieback area at Melon Bluff ~3 years after the dieback occurred (in 2004), and suggested that this was the result of rhizome extension from healthy areas. However, the pattern of regrowth and the time frame for a full recovery have not been previously described for the sudden dieback disturbance.

Previous studies offer insight into how bare patches in marshes might recover. Bare patches are sites of secondary succession of marsh vegetation, which involves both competitive and facilitative interactions (Penning and Bertness 2001). The rate and trajectory of recovery back to the original species depends upon where the patch is located (high or low marsh) and the environmental conditions (see Figure 12-10 in Valiela 1995). Typically if a patch is located lower in the marsh (below mean tide level), there is reinvasion by the zonal dominant *S. alterniflora*, as anoxic soils prohibit establishment of other species. When patches are located at higher elevations, where species richness is higher, invasion can be more complicated (Pennings and Bertness 2001). If bare patches in the high marsh have relatively benign conditions (i.e. lower salinity), fugitives plants quickly colonize the area, whereas if conditions are harsher (i.e. higher salinity), salt-tolerant species such as *Sarcocornia* spp. or *Distichilis spicata* will invade the bare patch (in extremely salty conditions, only the succulent *Sarcocornia* spp. can become established) (Pennings and Bertness 2001). *Sarcocornia* and many of the fugitive plants (*Atriplex patula*, *Aster tenuifolius*, *Limonium nashii, Solidago sempervirens*) colonize the area by seed (Rand 2000), whereas invasion by *Distichlis spicata* is generally clonal (Bertness and Shumway 1993). The fugitive species can help to ameliorate more saline patches, but are poor competitors, and are eventually out-

competed by the zonal dominant (often within 2-3 years; Bertness and Ellison 1987). The rate of replacement by the zonal dominant may depend on the level of nitrogen in a patch, with higher nitrogen often stimulating faster growth of vegetation and thus, replacement (Valiela 1995).

Recovery times that have been reported for *Spartina* and *Juncus* spp. due to other disturbances provide a useful comparison to the results reported here. In a study in New England, wrack-disturbed bare patches were colonized relatively quickly (≤1 y) by fugitives and *Distichilis spicata* (Bertness and Ellison 1987), with full recoveries (back to zonal dominants) of *Spartina patens* and *Juncus gerardi* within about 3 years (Bertness and Ellison 1987, Bertness and Shumway 1993). Winter ice scouring events in northern latitude marshes are harsher, as the ice initially smoothers grasses, and then is rafted away, removing the top few cm of peat, sediments, and rhizomes (Pennings and Bertness 2001). Recoveries in these areas have been reported to take much longer than those affected by wrack. After 4 years of observations, *Spartina patens* and *Juncus gerardii* had not recovered fully in ice scoured patches in New England (Ewanchuk and Bertness 2003). Very little has been published about recovery from dieback, but natural recovery in dieback sites (~1 ha) that occurred in the Florida Panhandle in the early 1990's has been reported to be slow (Carlson et al. 2001). It may be that the larger patch size of bare areas from sudden dieback events leads to longer recovery times as compared to smaller patches, especially since recovery by zonal dominants typically occurs through rhizome expansion.

In this study, bare patches (approx. 2-5 ha) initially following the dieback event were large at both the Melon Bluff (*S. alterniflora* dieback) and Isle of Wight Road (*J.*

roemerianus dieback) sites, and were adjacent to otherwise healthy marsh (pers. obs.). At the Melon Bluff site, bare patches are being recolonized by *S. alterniflora*, the zonal dominant, which increased in density over time from September 2004 through December 2009. However, densities of *S. alterniflora* were still <50% that of the healthy areas on the final sampling date 8 years after the dieback occurred. *S. alterniflora* height in dieback areas, on the other hand, reached that of the healthy marsh in 2006, 2 years after the regrowth began. At the Isle of Wight Road site, bare patches are being recolonized by the zonal dominant (*J. roemerianus*), but also by other early colonizers *S. alterniflora* and the fugitive *Atriplex patula* (first observed outside of plots in 2005), which are contributing to a more rapid patch closure. Recovery by *J. roemerianus* was also initiated faster than that of *S. alterniflora* at the Melon Bluff site, with *J. roemerianus* initially increasing in June 2004. In June 2005, 4 years after the dieback occurred, densities in the dieback areas were equivalent to those previously observed in healthy areas. However, the densities in healthy areas had also increased in 2005, and thus remained greater than those in the dieback areas. Over the 7 years of observations, the densities and heights of *J. roemerianus* in the dieback areas were significantly lower than those in the healthy areas on a majority of sample dates, including the final sampling in 2009.

The spatial patterns of recovery observed here suggested that both sites were being recolonized via rhizomes. At the *S. alterniflora* dieback site, plots located 20 and 30 meters from the healthy area did not begin to regrow until 2006, whereas those located at 10 meters had some *S. alterniflora* present on all dates. *S. alterniflora* tended to grow along a single transect (rather than in patches). This suggests that *S. alterniflora* regrowth was occurring through rhizome expansion from nearby healthy plants, which is similar to

what was seen in LA dieback sites (GCRC 2002 report, McKee et al. 2004). This is also similar to reports in wrack and ice disturbed areas in New England that recovery of zonal dominants tended to occur from the edge of the disturbed area and/or from intact belowground rhizomes (Bertness and Ellison 1987, Ewanchuk and Bertness 2003). At the *J. roemerianus* dieback site, an average density of 116 ± 37 m^{-2} was observed in plots 10 meters from the healthy area, which may have allowed for a quicker initial recolonization of these plots. In studies of the reinvasion of bare patches from wrack and ice scour, growth from seeds was rare, but tended to occur more often for *Juncus* spp. and was postulated as a reason that *Juncus* was sometimes able to reinvade more quickly than *Spartina* (Bertness and Ellison 1987, Rand 2000).

S. alterniflora began to encroach at the Isle of Wight Road site beginning in 2007, in plots located furthest from the healthy areas. These plots were likely being invaded by rhizomes from nearby stands of *S. alterniflora* located along the creekbank and levee (~10-20 m away, depending on plot). In the two plots where *S. alterniflora* was present (1-3, 3-3), its densities greatly surpassed those of *J. roemerianus*. The fact that *S. alterniflora* has begun to encroach former *J. roemerianus* marsh is not surprising, as this has also occurred in wrack and ice disturbed areas in New England (Bertness and Ellison 1987, Ewanchuk and Bertness 2003), and in other disturbed areas in GA (Pennings, *pers. comm.*). *S. alterniflora* tends to have a faster growth and expansion rate than *J. roemerianus* as it invests more in aboveground growth and has longer adventitious rhizomes (Bertness and Ellison 1987), so it may continue to expand in the plots where *J. roemerianus* is not present or numerically dominant. Over time, however, *J. roemerianus* is a better competitor and is typically able to outcompete early invaders, such as *S.*

alterniflora (Bertness 1991, Pennings et al. 2005). Studies in New England report that reinvasion of *J. roemerianus* into areas colonized after disturbances by *S. alterniflora* can take about 3-4 years (Bertness 1991), so this may also occur at the Isle of Wight Road site once *J. roemerianus* expands to meet *S. alterniflora*. On the other hand, when characteristics such as elevation or nutrients change, the species that finally recolonize an area can differ from the initial species (Valiela 1995, Courtemanche et al. 1999, Pennings et al. 2002). For instance, *S. alterniflora* can often outcompete *J. roemerianus* when soil nitrogen is high (Pennings et al. 2002, McFarlin et al. 2008). Also, in a study in LA, species covered by sediment deposition from Hurricane Andrew (*Avicennia germinans* and *S. alterniflora*) were eventually replaced by high marsh species due to the increased the elevation of the area (Courtemanche et al. 1999). At the Isle of Wight Road site, there is some evidence that the elevation is lower where the *S. alterniflora* has grown into bare areas (see flooded areas in Figure 5.8.), but it is too soon to tell whether *J. roemerianus* will eventually regrow in these areas.

The only epifaunal organism that showed significant differences between healthy and dieback areas were periwinkle snails, which were lower in the initial observations at the *S. alterniflora* site (there were few snails in either dieback or healthy areas in the *J. roemerianus* site). Periwinkle snail densities in the *S. alterniflora* dieback area remained low for 6 years. Although they began to increase in 2006, overall densities in the dieback area were still below that in healthy areas as of 2009. Periwinkle recovery followed that of *S. alterniflora*, beginning when stem densities reached more than 50 m^{-2}. Recovery of snails also occurred fastest in plots located closest to the healthy area: in 2009 there was still no recovery in plots located 30 m away from the healthy area. These observations

support the notion that periwinkle snails are dependent upon the establishment of *S. alterniflora*, but that recolonization is not immediate. *Littoraria* has also been slow to colonize newly constructed marshes and those recovering from disturbance (Havens et al. 1995, Levin et al. 1996, Knott et al. 1997). On the other hand, there were no significant differences of fiddler crabs or mussels over time between the healthy and dieback areas.

There were no significant differences in the pH, redox value, or salinity between healthy and dieback areas. Although these variables fluctuated over time, there were no consistent differences between healthy and dieback areas over 7 years of observations. In GA dieback areas, Oburn and Alber (2006) also did not see any differences among these characteristics, although they did observe elevated NH_4 in dieback areas. In LA dieback areas, there was also little difference among these soil conditions at most sites, except for one site that had an extremely low pH (McKee et al. 2004). Although these authors suggested that low pH may have contributed to the original dieback, this condition was apparently short-lived in LA and was not observed in GA.

5.5. Conclusions

Observations at *S. alterniflora* and *J. roemerianus* dieback sites suggest that recovery of large patches from sudden dieback takes longer than 8 years for both species. This time frame is ≥2-3x longer than that of recovery following wrack, and may be similar to or longer than that of ice scouring events in New England (these areas have not been followed until complete reestablishment of the zonal dominant has taken place). The overall patterns of succession appear to be similar to studies of bare patches elsewhere, in that *S. alterniflora* reinvades the lower, more flooded marsh (i.e. Melon

Bluff), whereas *S. alterniflora* and fugitive species contribute to patch closure in the more benign environments in the higher marsh (i.e. Isle of Wight Road). It is likely that the larger size of the dieback areas associated with sudden dieback is responsible for the slow time to recovery, as both species appear to be recovering via vegetative spreading of rhizomes from healthy areas. Perwinkle snails, which are dependent on *S. alterniflora* for habitat and as a source of their organic matter (Hamilton 1976, Haines and Montague1979, Hutchens and Walters 2006), have begun to recolonize in the *S. alterniflora* site, but their recovery lags that of the plants by several years.

5.6. Acknowledgments

This study was supported by the Georgia Coastal Management Program (NOAA Award NA170Z2331), the Georgia Coastal Ecosystems Long Term Ecological Project (NSF Award OCE 99-82133), the Georgia College Sea Grant Program (NOAA Award NA06RG0029), and the EPA (EPA Star Grant). We thank Matt Ogburn for the initial setup and measurements at the sites, and thank Kristen Anstead, Jenny Fenton, Janice Flory, Christine Hladik, Ben Maher, Chip McFarlin, Steven O'Connell, Ashley Rich-Robertson, Christine Tilburg, Monica Watkins, Susan White, and Catilin Yeager for help in the field. We would also like to express our appreciation to the Devendorf family for hosting us at Dunham Farms and allowing access to field sites.

References

Alber, M., and J.E. Flory. 2003. Georgia Coastal Research Council: A forum for scientists and managers. In: K.J. Hatcher (ed.), *Proceedings of the 2003 Georgia Water Resources Conference*, held April 23-24, 2003, at the University of Georgia.

Alber, M., E.M. Swenson, S.C. Adamowicz, and I.A. Mendelssohn. 2008. Salt marsh dieback: An overview of recent events in the US. *Estuarine, Coastal and Shelf Science* 80: 1-11. Alber et al. 2008.

Bertness, M.D. 1991a. Interspecific interactions among high marsh perennials in a New England salt marsh. *Ecology* 72(1): 125-137.

Bertness, M.D. and A.M. Ellison. 1987. Determinants of pattern in a New England marsh plant community. *Ecological Monographs* 57: 129-147.

Bertness, M.D., and S.W. Shumway. 1993. Competition and facilitation in marsh plants. *The American Naturalist* 142 (4): 718-724.

Carlson, P.R., L.A. Yarbro, F.X. Courtney, H. Arnold, D. Leslie, J. Hughes and N. Craft. 2001. Panhandle salt marsh mortality: a prelude to Louisiana brown marsh? Abstract. Coastal Marsh Dieback Conference, January 11-12, Baton Rouge, LA.

Courtemanche, R.P., Jr., M.W. Hester, and I.A. Mendelssohn. 1999. Recovery of a Louisiana barrier island marsh plant community following extensive hurricane-induced overwash. *Journal of Coastal Research* 15 (4): 872-883.

Ewanchuk, P.J., and M.D. Bertness. 2003. Recovery of a northern New England salt marsh plant community from winter icing. *Oecologia* 136 (4): 616-626.

GCRC Website of the Georgia Coastal Research Council: http://www.marsci.uga.edu/coastalcouncil

Georgia Coastal Research Council (GCRC). 2004. Proceedings of the Marsh Dieback Workshop, held February 3-4, 2004, Savannah, GA.

Georgia Coastal Research Council (GCRC). 2011. *Focus Areas: Marsh Dieback*. URL: http://www.gcrc.uga.edu/FocusAreas/marsh_dieback.htm. Accessed September 12, 2011.

Georgia Coastal Research Council (GCRC). 2002. Dead marsh information, 2002 report.

Georgia Coastal Research Council (GCRC). 2008. Update on Coastal Marsh Dieback, Prepared for GA DNR – Coastal Resources Division. http://www.gcrc.uga.edu/PDFs/GCRC%20Dieback%20update_08.pdf

Haines, E.B., and C.L. Montague. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. *Ecology* 60(1): 48-56.

Hamilton, P.V. 1976. Predation on *Littorina irrorata* (Mollusca: Gastropoda) by *Callinectes sapidus* (Crustacea: Portunidae). *Bulletin of Marine Science* 26:403-409.

Havens, K.J., L.M. Varnell, and J.G. Bradshaw. 1995. An assessment of ecological conditions in a constructed tidal marsh and two natural reference tidal marshes in coastal Virginia. *Ecological Engineering* 4: 117-141.

Hutchens, JJ, and K. Walters. 2006. Gastropod abundance and biomass relationships with salt marsh vegetation within ocean-dominated South Carolina, USA estuaries. *Journal of Shellfish Research* 25 (3): 947-953.

Knott, D.M., E.L. Wenner, and P.H. Wendt. 1997. Effects of pipeline construction on the vegetation and macrofauna of two South Carolina, USA salt marshes. *Wetlands* 17 (1): 66-81.

Levin, L.A., D. Talley and G. Thayer. 1996. Succession of macrobenthos in a created salt marsh. *Marine Ecology Progress Series* 141: 67-82.

McFarlin, C.R., J.S. Brewer, T.L. Buck, and S.C. Pennings, S.C. 2008. Impact of fertilization on a salt marsh food web in Georgia. *Estuaries and Coasts*. 31: 313-325.

McKee, K.L., I.A. Mendelssohn, and M.D. Materne. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecology and Biogeography* 13 (1): 65-73.

Ogburn 2004. Salt marsh dieback in Georgia: field survey and transplant experiments. M.S. Thesis, University of Georgia, Athens, GA.

Ogburn, M.B., Alber, M., 2006. An investigation of salt marsh dieback in Georgia using field transplants. *Estuaries and Coasts* 29: 54–62.

Ogburn, M.B., Alber, M., 2006. An investigation of salt marsh dieback in Georgia using field transplants. *Estuaries and Coasts* 29: 54–62.

Pennings, S.C., and M.D. Bertness. 2001. Salt marsh communities. In: M.D. Bertness and M.E. Hay (eds.), *Marine community ecology*. Sunderland, Massachusetts: Sinauer.

Pennings, S.C., L.E. Stanton, and J.S. Brewer. 2002. Nutrient effects on the composition of salt marsh plant communities along the Southern Atlantic and Gulf Coasts of the United States. *Estuaries* 25 (6): 1164-1173.

Pennings, S.C., M.B. Grant, and M.D. Bertness. 2005. Plant zonation in low-latitude salt marshes: disentangling the roles of flooding, salinity and competition. *Journal of Ecology* 93 (1): 159-167.

Powers, L.W., and J.F. Cole. 1976. Temperature variation in fiddler crab microhabitats. *Journal of Experimental Marine Biology and Ecology* 21: 141-157.

Rand, T.A. 2000. Seed dispersal, habitat suitability and the distribution of halophytes across a salt marsh tidal gradient. *Journal of Ecology* 88: 608-621.

USGS (U.S. Geological Survey). 2011. National Water Information System. Surface-Water Annual Statistics for the Nation: USGS 02226000, Altamaha River at Doctortown, GA. http://waterdata.usgs.gov/nwis/annual/

Valiela, I. 1995. *Marine Ecological Processes*. Second edition. Springer, New York.

Table 5.1. The mean number (± SE) of epifaunal invertebrates (per m^2), the number of dates on which epifauna were observed, and the percent occurrence of live and dead epifauna within healthy and dieback marsh over 7 years (2003-2009) at the Melon Bluff and Isle of Wight Road sites in GA.

	Mean		***# of dates epifauna observed***[a]		***% occurrence of epifauna***		***% occurrence of dead epifauna***	
	- Healthy -	- Dieback -	- Healthy -	- Dieback -	- Healthy -	- Dieback -	- Healthy -	- Dieback -
Melon Bluff								
Epifaunal crabs	28.1 ± 1.7	50.9 ± 4.1	13	13	95 ± 1%	96 ± 1%	---	1.7 ± 0.3%
Periwinkle snails	21.7 ± 2.0	6.3 ± 1.6	13	9	85 ± 1%	22 ± 2%	4.3 ± 0.6%	14.5 ± 1.0%
Mussels	5.9 ± 1.2	10.3 ± 1.5	13	12	36 ± 1%	46 ± 2%	36 ± 4%	40.2 ± 2.1%
Coffeebean snails	<1	<1	1	---	<1%	---	---	---
Mudsnails	<1	<1	1	1[b]	<1%	<1%	---	---
Isle of Wight Road								
Epifaunal crabs	92.4 ± 8.0	114.5 ± 6.8	12	12	100 ± 0%	98 ± 1%	---	0.9 ± 0.2%
Periwinkle snails	<1	<1	6	2	7 ± 1%	3 ± 1%	---	2.8 ± 0.4%[d]
Mussels	---	---	---	---	---	---	16.7 ± 1.2%[c]	24.7 ± 1.9%[c]
Coffeebean snails	2.3 ± 0.7	<1	5	2	19 ± 1%	6 ± 1%	---	---
Mudsnails	<1	<1	1[b]	1[b]	<1%	<1%	---	---

[a]Out of at total of 13 sampling dates at Melon Bluff and 12 sampling dates at Isle of Wight Road.

[b]December 2008 sampling date.

[c]All of the dead mussels were noted on the first sampling date in September 2003 and remained in plots through 2006.

[d]2003 sample date only.

Table 5.2. Statistical comparison of healthy and dieback zones across sampling dates at the Melon Bluff (*S. alterniflora*) dieback site and the Isle of Wight Road (*J. roemerianus*) dieback site. The significance (*p*-value) of the factors zone (healthy vs. dieback), date, and zone*date is shown for the 2-way repeated measures ANOVA model applied to each of the various vegetation measurements and faunal densities. NS=not significant; ---=densities too low (or zero) for statistical analysis.

Melon Bluff				
	Vegetation	**Live *Spartina***	**Standing dead *Spartina***	**Height *Spartina***
	zone	<0.0001	NS	<0.0001
	date	<0.0001	<0.0001	<0.0001
	zone*date	<0.0001	<0.0002	<0.0003
	Fauna	**Periwinkle snails**	**Fiddler crabs**	**Mussels**
	zone	<0.0001	NS	NS
	date	<0.0001	<0.0001	0.0005
	zone*date	0.008	NS	0.0004
Isle of Wight Road				
	Vegetation	**Live *Juncus***	**Standing dead *Juncus***	**Height *Juncus***
	zone	0.0022	0.0002	0.004
	date	<0.0001	<0.0001	<0.0001
	zone*date	NS	<0.0001	0.04
	Fauna	**Periwinkle snails**	**Fiddler crabs**	**Mussels**
	zone	---	0.04	---
	date	---	<0.0001	---
	zone*date	---	0.002	---

Table 5.3. Statistical comparison of dieback plots based on "distance" to the healthy zone over sampling dates at the Melon Bluff (*S. alterniflora*) dieback site and the Isle of Wight Road (*J. roemerianus*) dieback site. The significance (*p*-value) of the factors distance, date, and distance*date is shown for the 2-way repeated measures ANOVA model applied to each of the various vegetation measurements and faunal densities. NS=not significant; ---=densities too low (or zero) for statistical analysis.

Melon Bluff				
	Vegetation	**Live *Spartina***	**Standing dead *Spartina***	**Height *Spartina***
	distance	0.0027	NS	0.0036
	date	<0.0001	<0.0001	<0.0001
	distance*date	0.0004	NS	NS
	Fauna	**Periwinkle snails**	**Fiddler crabs**	**Mussels**
	distance	0.002	NS	NS
	date	0.002	<0.0001	0.0008
	distance*date	NS	NS	NS
Isle of Wight Road				
	Vegetation	**Live *Juncus***	**Standing dead *Juncus***	**Height *Juncus***
	distance	0.02	0.03	NS
	date	<0.0001	0.0005	<0.0001
	distance*date	<0.0002	NS	NS
	Fauna	**Periwinkle snails**	**Fiddler crabs**	**Mussels**
	distance	---	NS	---
	date	---	<0.0001	---
	distance*date	---	NS	---

Table 5.4. Mean ± SE (n=9 plots) for salinity, pH, redox, and soil temperature in healthy and dieback zones among dates at the Melon Bluff (*S. alterniflora*) dieback site. The overall mean and coefficient of variation for the healthy and dieback zones were calculated for comparison across all dates and plots. The significance (p-value) of the factors zone (i.e. healthy vs. dieback), date, and zone*date in a 2-way repeated measures ANOVA with interaction is shown below each parameter, with significant terms in bold. Different letters indicate that the healthy and dieback zones on a sampling date were significantly different based on a Tukey's post-hoc comparison of means.

Melon Bluff	Salinity (psu)		pH		Redox (mV)		Soil Temp (°C)	
	Healthy	Dieback	Healthy	Dieback	Healthy	Dieback	Healthy	Dieback
Jun-03	21.3 ± 0.9	21.7 ± 1.1	6.63 ± 0.07	6.59 ± 0.04	---	---	26.2 ± 0.1	26 ± 0
Sep-03	17.7 ± 1.3	15.2 ± 1.4	6.64 ± 0.03	6.71 ± 0.05	-206 ± 10	-223 ± 11	24.2 ± 0.1	24 ± 0
Jan-04	24.9 ± 0.3	24.4 ± 0.6	6.75 ± 0.04	6.88 ± 0.04	---	---	12.3 ± 0.2	13.1 ± 0.2
Mar-04	23.9 ± 0.3	24 ± 0.5	6.85 ± 0.02	7.02 ± 0.05	---	---	17.9 ± 0.1 a	16.8 ± 0.1b
Jun-04	28.7 ± 0.3	29.1 ± 0.8	6.45 ± 0.05	6.66 ± 0.06	---	---	28.2 ± 0.2 a	26.2 ± 0.2b
Sep-04	15.9 ± 0.8	16.3 ± 0.8	6.62 ± 0.05	6.86 ± 0.08	---	---	27.6 ± 0.6	28.2 ± 0.2
Mar-05	10.3 ± 0.2** a	15.3 ± 2.1**b	---	---	---	---	15 ± 0	15 ± 0
Jun-05	12.0 ± 0.5**	14.7 ± 1.2**	6.67 ± 0.02	6.66 ± 0.03	---	---	26.6 ± 0.4	25.6 ± 0.3
Oct-05	16.4 ± 0.5**	15.8 ± 0.8**	---	6.65 ± 0.05	---	---	26.2 ± 0.1	26.7 ± 0.2
Oct-06	29.9 ± 0.4	28.4 ± 0.7	---	---	---	---	19.8 ± 0.2	19.9 ± 0.1
Sep-07	32.6 ± 0.8	30.3 ± 0.9	6.48 ± 0.09	6.61 ± 0.14	---	---	27 ± 0.1	26 ± 0
Dec-08	36.2 ± 0.6	32.2 ± 0.7	6.59 ± 0.05	6.56 ± 0.03	---	---	12.2 ± 0.1	12 ± 0.2
Dec-09	29.8 ± 0.3	29.6 ± 0.3	6.57 ± 0.04	6.47 ± 0.1	-248 ± 14	-219 ± 28	---	---
Overall Mean	***23.1 ± 0.7***	***22.8 ± 0.7***	***6.63 ± 0.02***	***6.70 ± 0.03***	***-227 ± 10***	***-221 ± 15***	***21.9 ± 0.5***	***21.6 ± 0.6***
C.V.	*35.2*	*31.0*	*2.58*	*3.78*	*18*	*28*	*27.1*	*26.3*
Repeated measures ANOVA (p-values)	Zone=0.71, **Date<0.0001**, **Zone*Date<0.0001**		Zone=0.32, **Date<0.0001**, **Zone*Date=0.015**		Zone=0.54, Date=0.31, Zone*Date=0.22		**Zone<0.0001**, **Date<0.0001**, **Zone*Date<0.0001**	

*Soil was too dry to take measurements with handheld probes.
**Rain during sampling likely lowered salinity readings.

Table 5.5. Mean ± SE (n=9 plots) for salinity, pH, redox, and soil temperature in healthy and dieback zones among dates at the Isle of Wight Road (*J. roemerianus*) dieback site. The overall mean and coefficient of variation for the healthy and dieback zones were calculated for comparison across all dates and plots. The significance (p-value) of the factors zone (i.e. healthy vs. dieback), date, and zone*date in a 2-way repeated measures ANOVA with interaction is shown below each parameter, with significant terms in bold. Different letters indicate that the healthy and dieback zones on a sampling date were significantly different based on a Tukey's post-hoc comparison of means.

Isle of Wight Road	Salinity (psu)		pH		Redox (mV)		Soil Temp (°C)	
	Healthy	Dieback	Healthy	Dieback	Healthy	Dieback	Healthy	Dieback
Jun-03	---	---	---	---	---	---	---	---
Sep-03	16.7 ± 1.2	17.1 ± 1.6	6.5 ± 0.09	6.04 ± 0.07	---	---	24.9 ± 0.1	24.6 ± 0.2
Jan-04	---	---	---	---	---	---	13.3 ± 0.2	14.1 ± 0.1
Mar-04	24.8 ± 0.6	*	6.44 ± 0.06	*	---	---	17.1 ± 0.1	17.1 ± 0.3
Jun-04	35.6 ± 0.6	36.2 ± 0.2	5.26 ± 0.06 a	5.72 ± 0.03 b	---	---	33.8 ± 0.4	33.1 ± 0.8
Sep-04	20.6 ± 0.3	20.4 ± 0.5	6.49 ± 0.13 a	6.45 ± 0.12 b	---	---	24.8 ± 0.8	25.4 ± 0.9
Mar-05	35.9 ± 0.4	38.2 ± 0.8	6.29 ± 0.04	6.21 ± 0.12	---	---	16.1 ± 0.4	15.4 ± 0.2
Jun-05	16.4 ± 0.7**	18.3 ± 2.6**	6.36 ± 0.05	6.35 ± 0.07	---	---	25.1 ± 0.2	24.2 ± 1.2
Oct-05	16.7 ± 0.5**	17.1 ± 0.5**	6.97 ± 0.11	6.89 ± 0.15	---	---	25.4 ± 0.2	25.4 ± 0.2
Oct-06	38.8 ± 0.5	37.6 ± 1.1	---	---	---	---	18.8 ± 0.4	18.3 ± 0.3
Sep-07	20.9 ± 3.3	28.2 ± 2.4	6.35 ± 0.05	6.18 ± 0.05	---	---	24.3 ± 0.2	24.8 ± 0.2
Dec-08	41.8 ± 1.0	41.6 ± 0.4	6.44 ± 0.06	6.33 ± 0.07	---	---	10.7 ± 0.2	11.4 ± 0.2
Dec-09	30.7 ± 0.6	29.7 ± 0.5	6.5 ± 0.05	6.26 ± 0.05	-85 ± 9	-109 ± 16	---	---
Overall Mean	***27.2 ± 1.0***	***28.4 ± 1.0***	***6.36 ± 0.05***	***6.27 ± 0.04***	***-85 ± 9***	***-109 ± 16***	***21.2± 0.6***	***21.3 ± 0.6***
C.V.	*36.2*	*35.0*	*7.29*	*6.29*	*33*	*43*	*30.5*	*30.0*
Repeated measures ANOVA (p-values)	Zone=0.10, **Date<0.0001**, Zone*Date=0.07		Zone=0.04, **Date<0.0001**, **Zone*Date=0.0002**		Zone=0.07***		Zone=0.85, **Date<0.0001**, Zone*Date=0.47	

*Soil was too dry to take measurements with handheld probes.
**Rain during sampling likely lowered salinity readings.
***Samples taken on one date only.

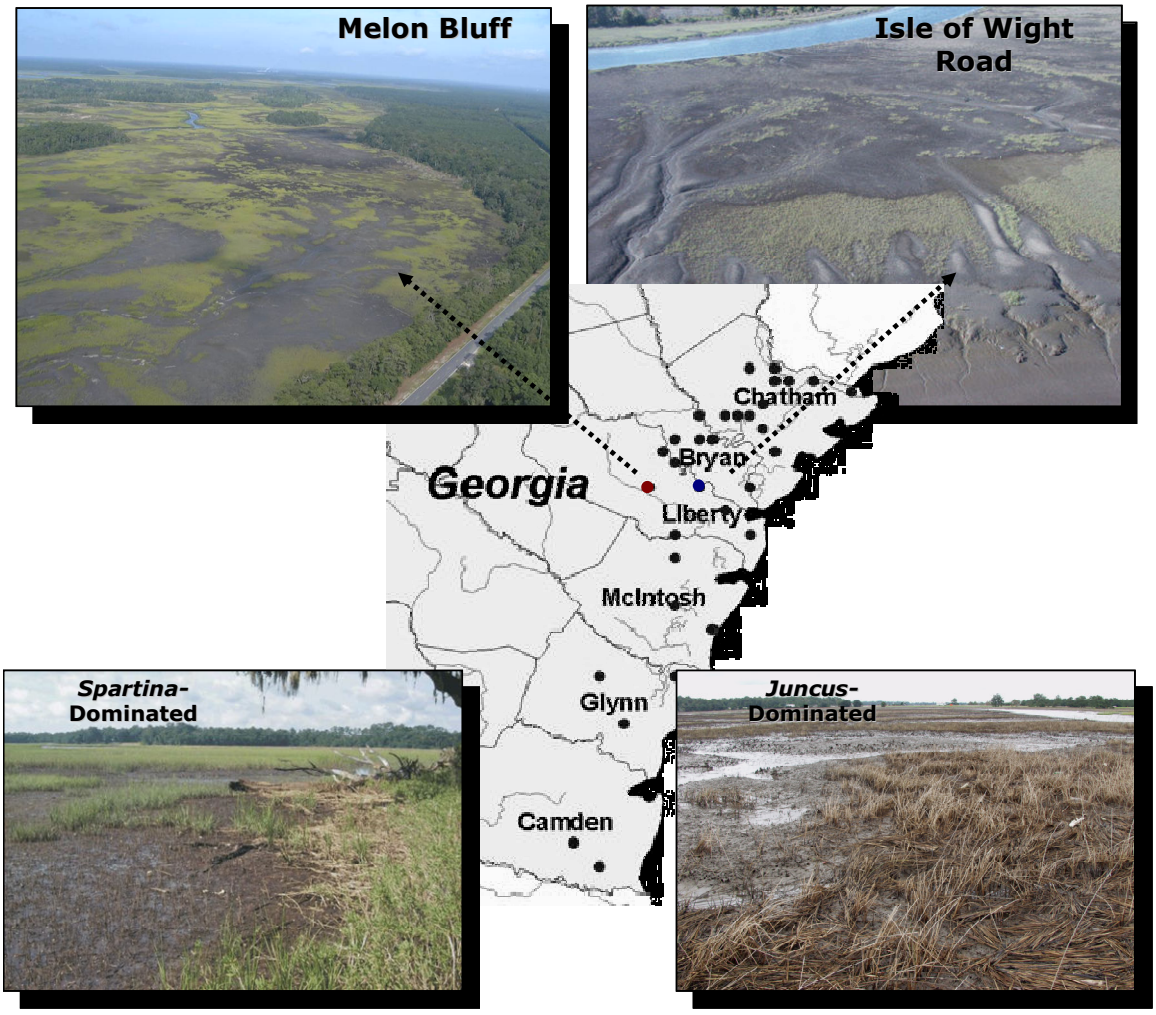


Figure 5.1. Map of the sudden dieback salt marsh sites distributed in GA coastal counties in 2000-2002. Arrows point to 2 sites in Liberty County -- Melon Bluff (*S. alterniflora*-affected) and Isle of Wight Road (*J. roemerianus*-affected) -- that I monitored during 2003-2009 as a part of the Georgia Coastal Research Center's salt marsh dieback monitoring program. Upper photographs were taken as a part of an aerial survey of dieback sites by GA-DNR Coastal Resources Division (J. McKinnon) and lower photographs are close-ups of each of the two sites taken by UGA.

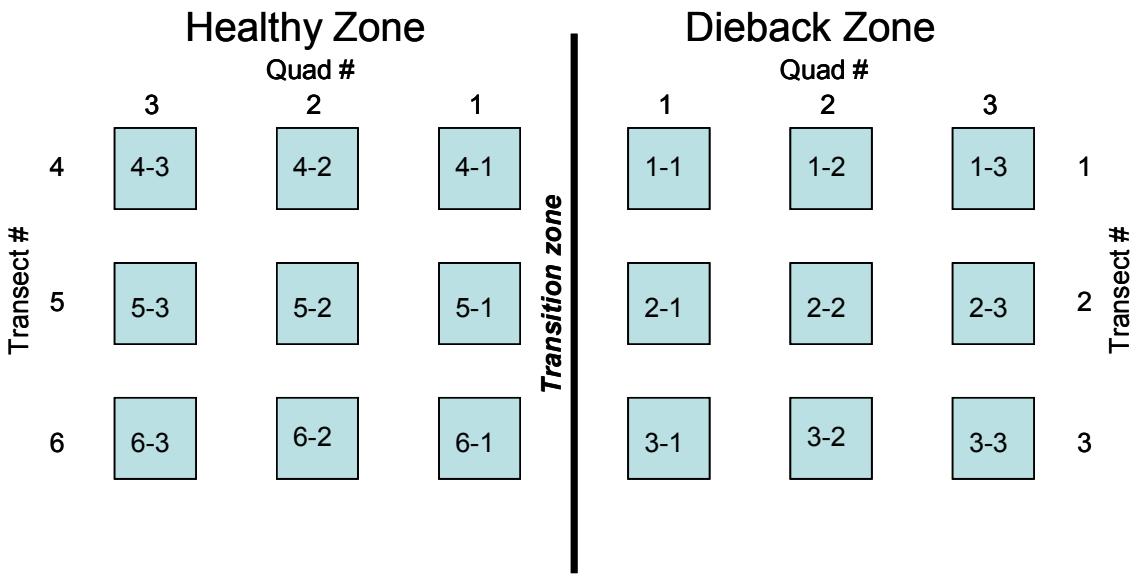


Figure 5.2. A diagram of the plot layout at the Melon Bluff and Isle of Wight Road dieback sites in GA. The shaded squares represent permanent plots (each 0.25 m^2) and are 10 m apart from one another. Each plot is coded with a unique label consisting of the transect and quadrat numbers. The transition zone represents the line of demarcation between apparently healthy marsh and affected (dieback) marsh that was physically marked with tall PVC in 2003.

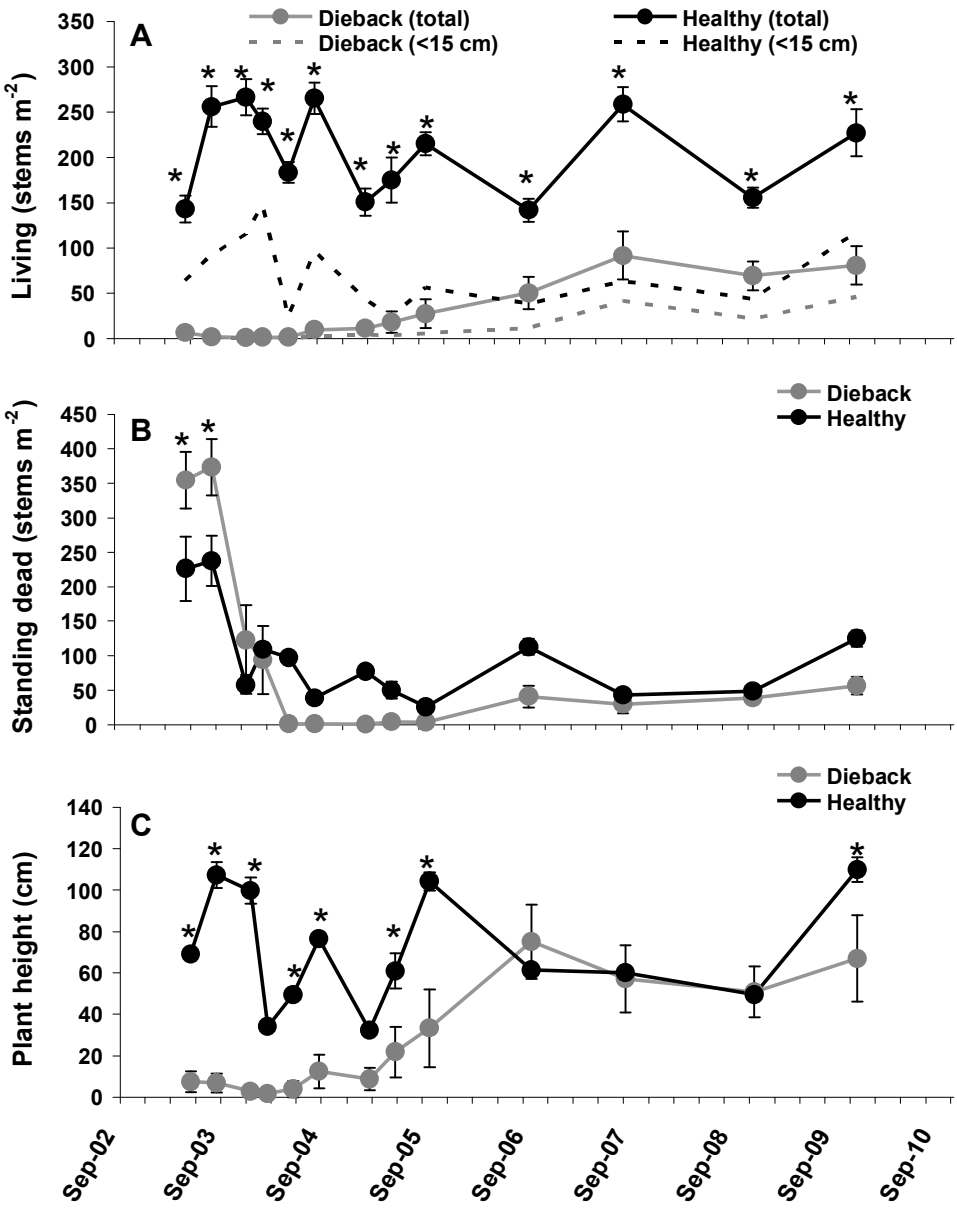


Figure 5.3. The variation in A) living (total and those <15 cm tall) and B) standing dead *S. alterniflora* density (per 1 m^{-2}) and the C) height of the 5 tallest *S. alterniflora* plants within plots of the healthy and dieback marsh zones at the Melon Bluff site over time. Each point represents the mean of 9 plots ± SE. Asterisks indicate that the healthy and dieback zones on sampling dates were significantly different based on a Tukey's post-hoc comparison of means.

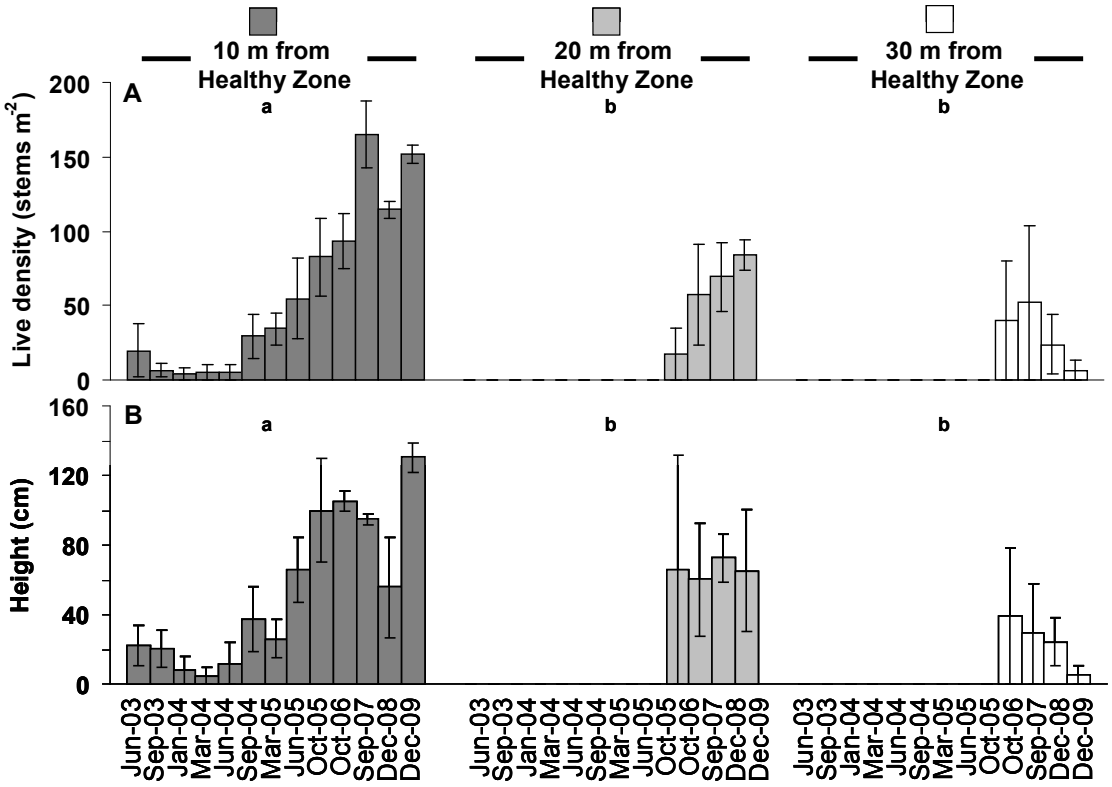


Figure 5.4. . The variation in A) *S. alterniflora* density (per 1 m^{-2}) and B) *S. alterniflora* height in plots of the dieback zone located 10, 20, and 30 meters from the healthy marsh at the Melon Bluff site over time. Each bar represents the mean of 3 plots ± SE. Letters indicate significant differences among dieback plots with distance to the healthy zone based on a Tukey's post-hoc comparison of means.

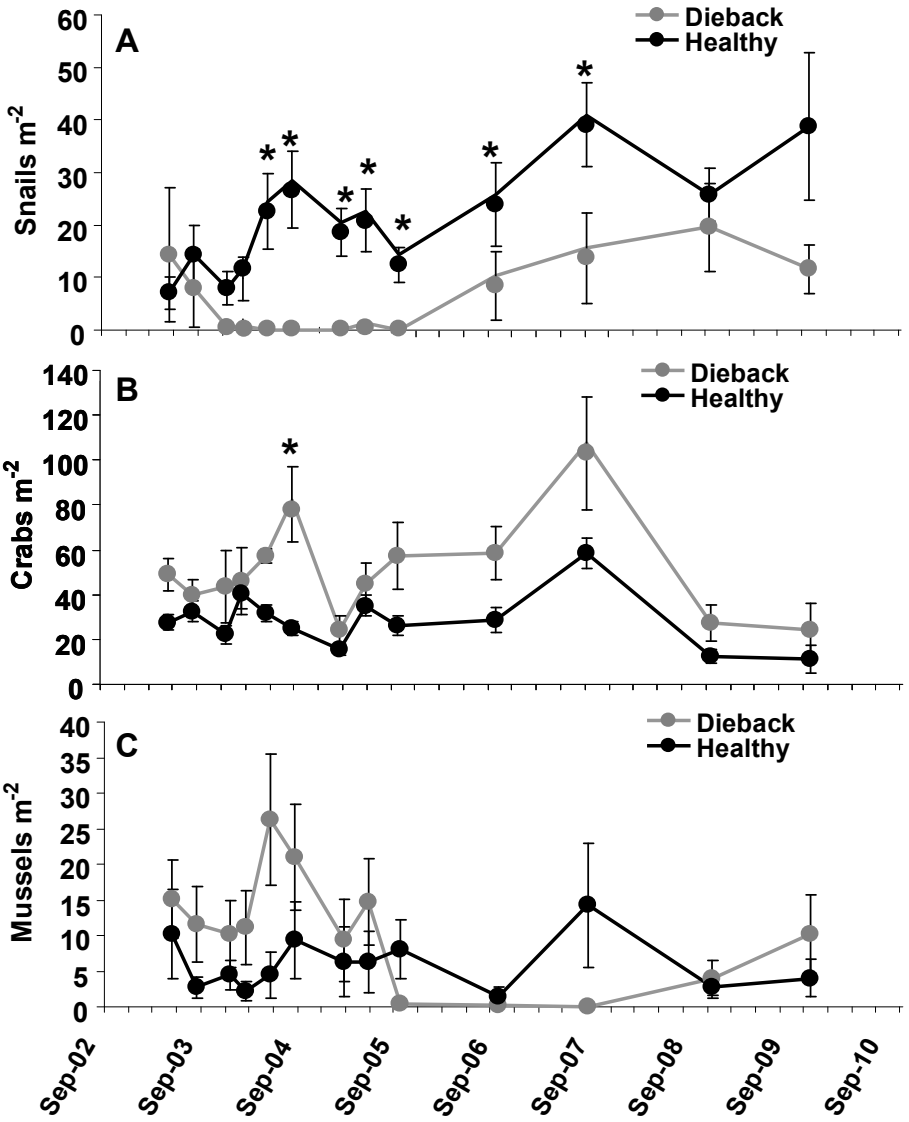


Figure 5.5. The variation in the density (per 1 m^{-2}) of A) periwinkle snails, B) fiddler crabs, and C) ribbed mussels in healthy and dieback zones at the Melon Bluff site over time. Each point represents the mean of 9 plots ± SE. Asterisks indicate that the healthy and dieback zones on sampling dates were significantly different based on a Tukey's post-hoc comparison of means.

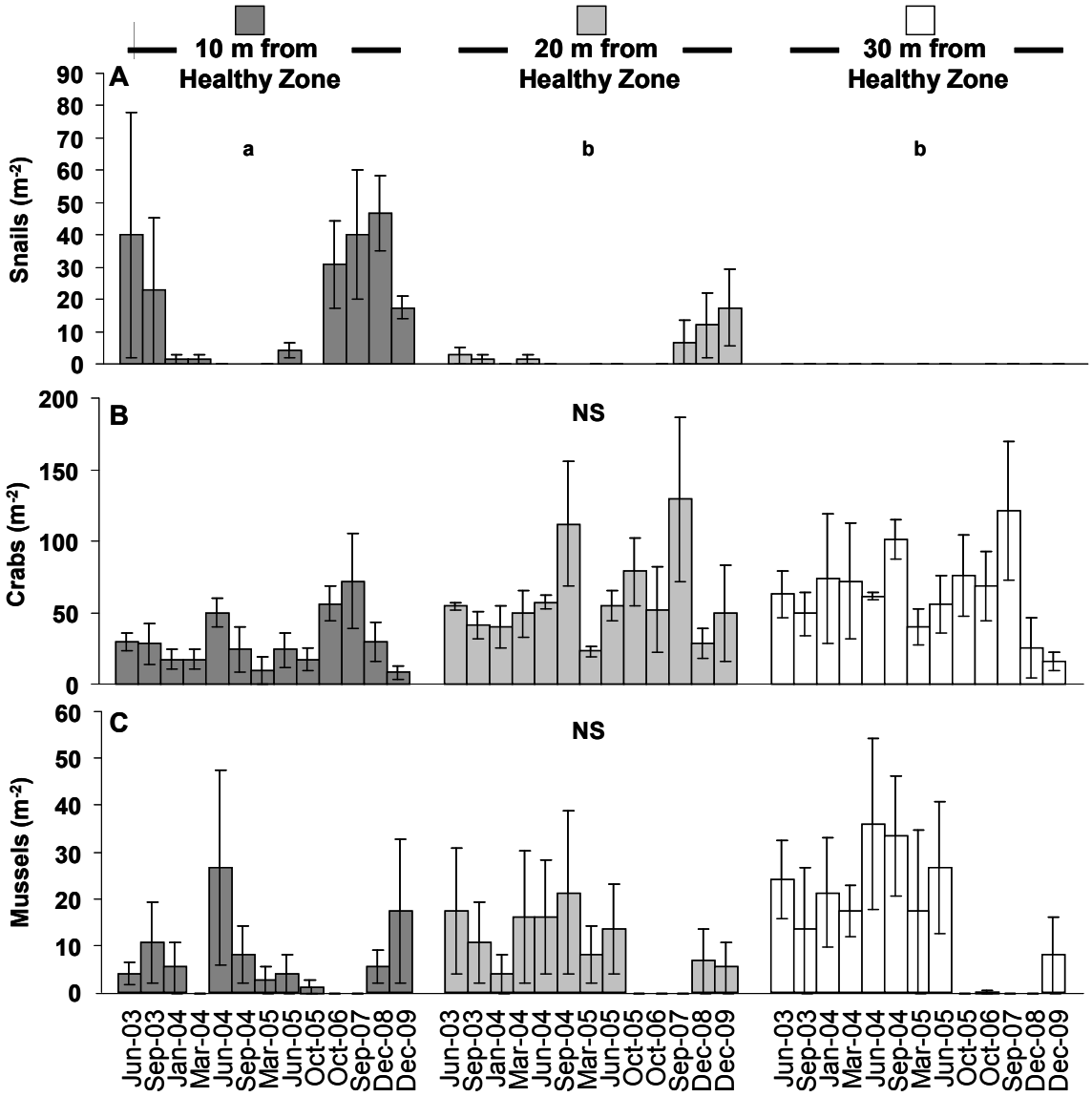


Figure 5.6. The variation in A) periwinkle snail, B) fiddler crab, and C) mussel densities (per 1 m^{-2}) in plots of the dieback zone located 10, 20, and 30 meters from the healthy marsh at the Melon Bluff site over time. Each bar represents the mean of 3 plots ± SE. Letters indicate significant differences among dieback plots with distance to the healthy zone based on a Tukey's post-hoc comparison of means.

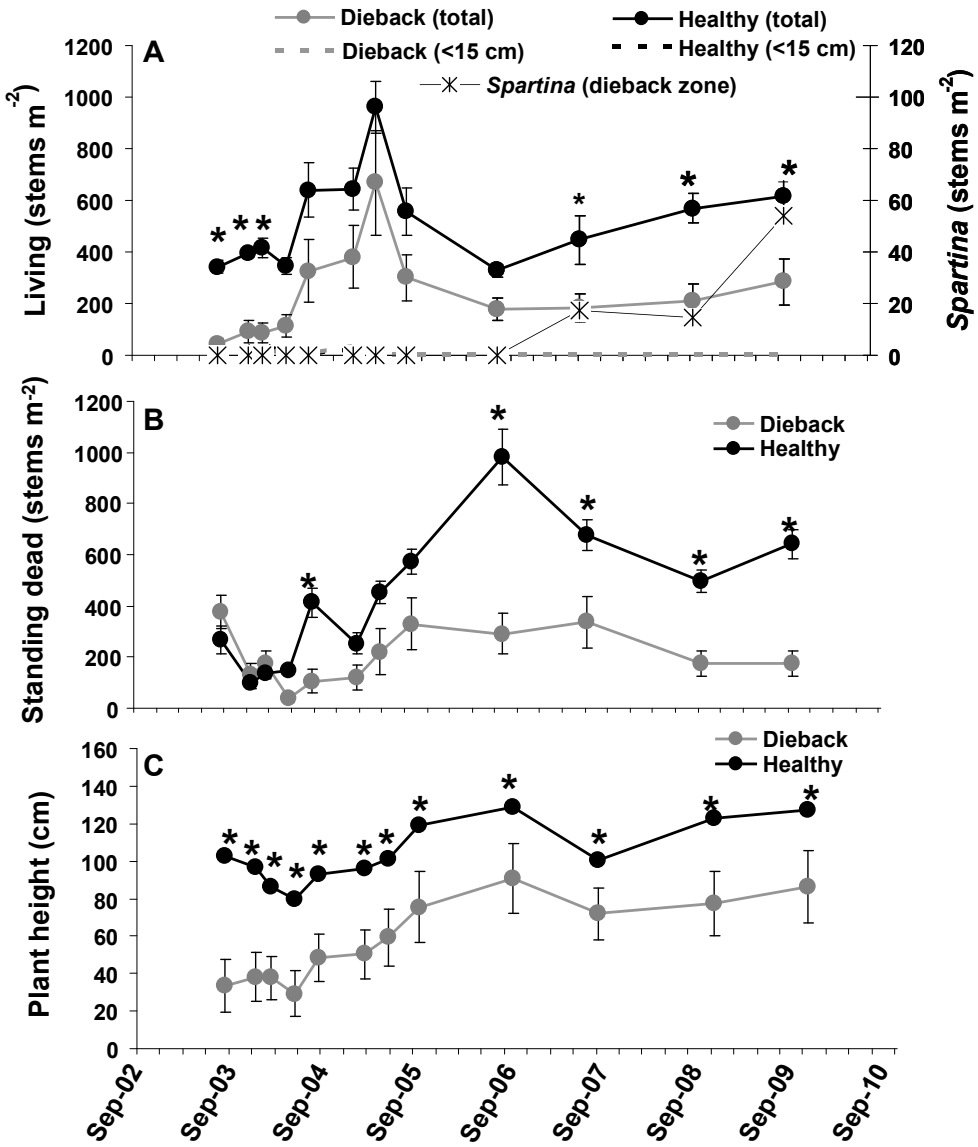


Figure 5.7. The variation in A) living (total and those <15 cm tall) and B) standing dead *J. roemerianus* density (per 1 m^{-2}) and the height of the 5 tallest *J. roemerianus* plants within plots of the healthy and dieback marsh zones at the Isle of Wight Road site over time. Each point represents the mean of 9 plots ± SE. Asterisks indicate that the healthy and dieback zones on sampling dates were significantly different based on a Tukey's post-hoc comparison of means.

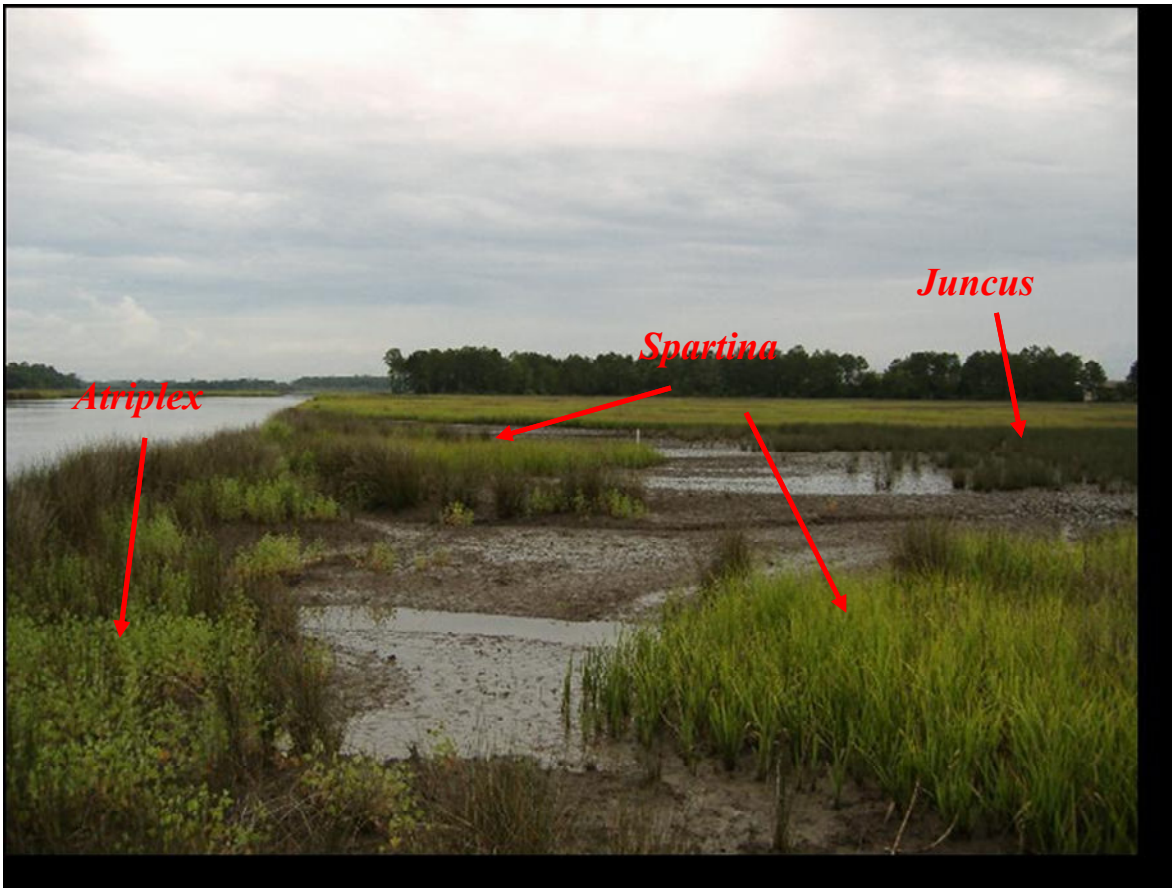


Figure 5.8. Photo of the Isle of Wight Road site in September 2005, showing regrowth by *J. roemerianus* (on right), and the invasion of *S. alterniflora* and *Atriplex patula* (on left and lower right) into the bare patch left by dieback.

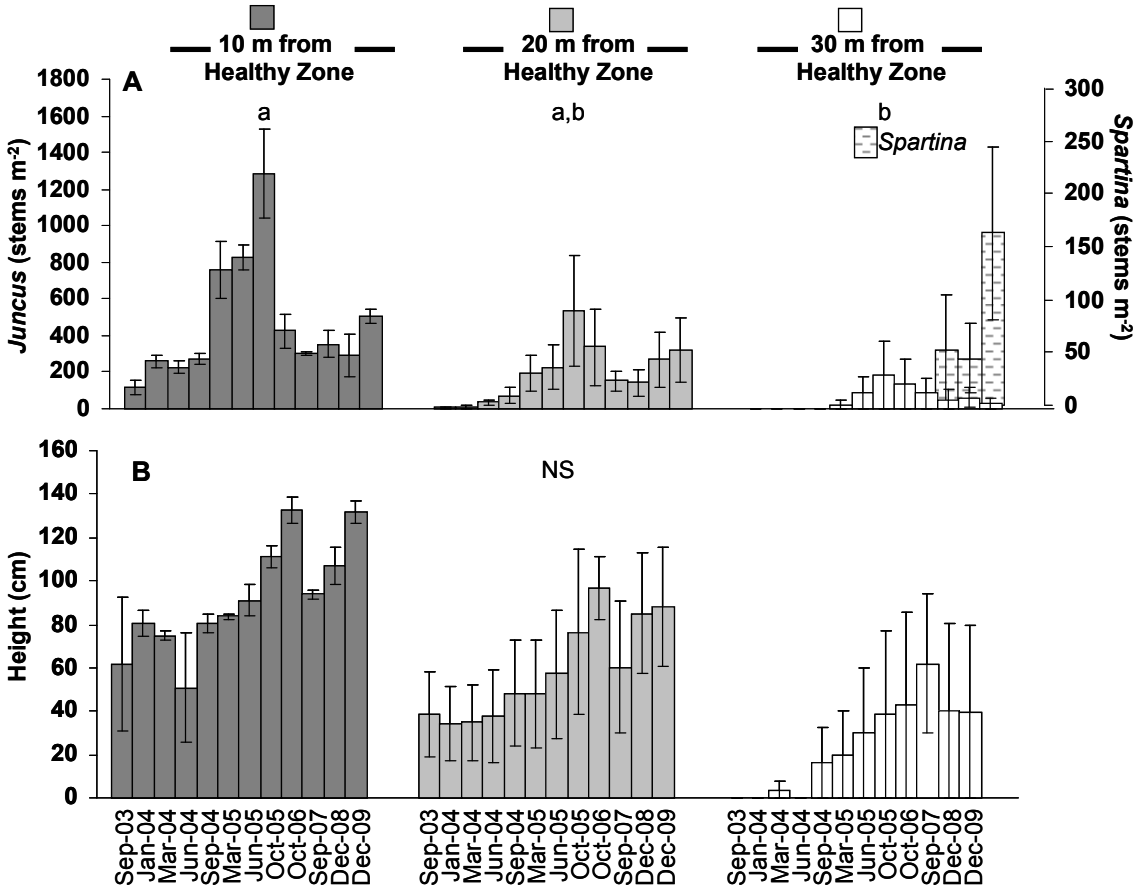


Figure 5.9. The variation in A) *J. roemerianus* (and *S. alterniflora*) density (per 1 m^{-2}) and B) *J. roemerianus* height in plots of the dieback zone located 10, 20, and 30 meters from the healthy marsh at the Isle of Wight Road site over time. Each bar represents the mean of 3 plots ± SE. Letters indicate significant differences among dieback plots with distance to the healthy zone based on a Tukey's post-hoc comparison of means.

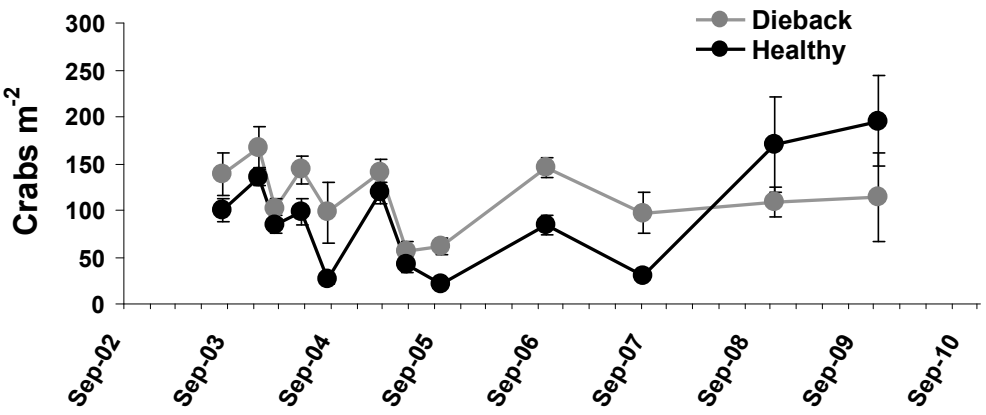


Figure 5.10. The variation in the density (per 1 m^{-2}) of fiddler crabs in healthy and dieback zones at the Isle of Wight Road site over time (there were too few other epifauna for a time-course analysis at this site). Each point represents the mean of 9 plots ± SE. There were no significant differences between the healthy and dieback zones on sampling dates based on a Tukey's post-hoc comparison of means.

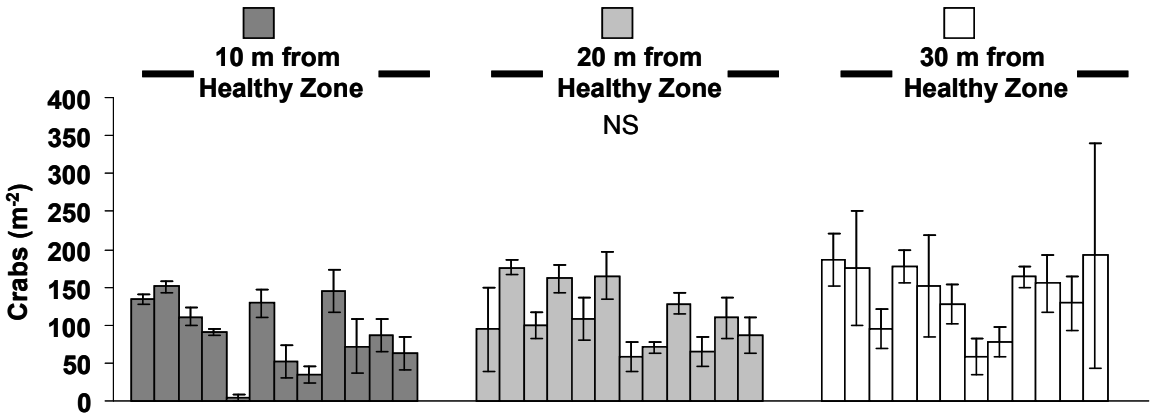


Figure 5.11. The variation in fiddler crab density (per 1 m^{-2}) in plots of the dieback zone located 10, 20, and 30 meters from the healthy marsh at the Isle of Wight Road site over time. Each bar represents the mean of 3 plots ± SE. There were no significant differences among dieback plots with distance to the healthy zone based on a Tukey's post-hoc comparison of means.

CHAPTER 6

CONCLUSIONS

Spartina alterniflora serves as an important foundation species in salt marsh environments, providing habitat complexity and stabilizing abiotic conditions (Bruno and Bertness 2001). A central principle across ecological studies has been that foundation species promote the presence and biological diversity of associated species in an ecosystem (Ellison et al. 2005). Few studies, however, have explicitly compared the effects of salt marsh loss across larger geographical regions (that differ greatly in environmental conditions) or among various disturbance types, nor has recovery been tracked for large bare patches (such as those left by sudden dieback). In addition, because the effects of many disturbances in the salt marsh (pollution, increases in flooding) may be gradual and difficult to detect (Mendelssohn and McKee 1992, Bertness et al. 2002, Weilhoefer 2011), understanding early physiological signs of stress may help to identify areas that are at risk for *S. alterniflora* loss.

This thesis addressed these issues by 1) describing the effect of sudden marsh dieback on the invertebrate communities in two different geographic regions (Georgia and Louisiana), 2) evaluating the physiological response of *Spartina* to four disturbance types (sudden dieback, snail grazing, horse grazing, and wrack deposition), 3) comparing the invertebrate response to the same four disturbances, and 4) documenting the patterns of recovery of both *S. alterniflora* and *Juncus roemerianus* from a sudden dieback event.

In the evaluation of invertebrate responses to the sudden dieback of *Spartina* in GA and LA (Chapter 2), I found that there was a similar decrease in the density of epifaunal snails in bare areas of both states, which was expected as *Spartina* provides an obligate habitat and source of organic matter. However, I found a contrasting response of benthic infauna between the states: in GA, infaunal density and diversity was greatly reduced in bare areas, whereas in LA infauna abundances did not differ (macroinfauna) or were increased (meiofauna) in bare areas as compared to reference (vegetated) areas. These differences were likely due to the fact that the LA sites were at a lower elevation and plots tended to remain wet, whereas the GA sites were at a higher elevation and bare plots often dried out between tides. Thus, the presence of *Spartina* helped to ameliorate soil conditions in bare areas in GA by providing shade, which was not necessary in LA. These findings suggest that hydrogeomorphic context is important for evaluating disturbance.

A second climatic disturbance in each state (a drought in GA and a hurricane in LA) provided an opportunity to evaluate the resilience of bare versus reference areas. In LA, it also allowed me to examine an additional function that *Spartina* provides (as a buffer against storm erosion). In both states, benthic infaunal density and diversity decreased following the disturbances in both bare and reference plots, but these decreases were <u>much</u> greater (by 15-100%) in the bare plots. Take together, these results support the notion that healthy densities of *Spartina* are critical to the resiliency of invertebrates to multiple disturbances, and that the role of foundation species is also dependent upon the function provided (amelioration of habitat vs. physical buffer).

In Chapter 3, I found that the physiological response of *Spartina* was similar among 4 different disturbance types (sudden dieback, horse grazing, snail grazing, and wrack deposition). In all cases, the DMSO:DMSP ratio and metal concentrations were increased in the leaves of plants in disturbed areas. Differences in both of these metrics were independent of the leaf chlorophyll concentrations, suggesting that each may serve as a potential early warning signal of stress. These responses should be tested further in controlled studies that alter stress to *Spartina* in order to corroborate the field results observed here. It would also be interesting to collect samples over time, in order to observe at what point DMSO:DMSP ratios and metal concentrations become significantly different in stressed versus healthy areas. Finally, it would be useful to analyze soil samples for metal content as a comparison to leaf uptake and to examine foliar excretion of metals from the salt glands under stress (as in Burke et al. 2000), to evaluate the mechanism by which stressed plants obtain higher concentrations of foliar metals.

In Chapter 4, I found that the response of the invertebrates to *Spartina* loss was similar across the same 4 disturbance types evaluated in Chapter 3, with significant decreases in periwinkle snail densities and benthic macroinfauna density, taxon richness, and diversity. These results contrast with the results of a manipulative study in which I observed no effect on benthic invertebrates (see Appendix D). The difference between these may be due to the size of the affected areas: in the survey all bare areas >40 m^2, whereas in the manipulation experimental patches were 2.5 m x 1.0 m. It would therefore be interesting to manipulate the size of bare patches in order to find out what minimum patch size can cause an effect on invertebrates.

All of these study sites were in GA, and the decrease of infauna in disturbed areas is in keeping with what was observed in GA in Chapter 2. Thus, the cause of the loss of *Spartina* within a particular region does not seem important in terms of invertebrate response, whereas the effect of *Spartina* loss across regions (e.g. GA vs. LA) can lead to differing responses.

In Chapter 5, I found that recovery times for vegetation in some sudden dieback sites were longer than 8 years. Both the *Spartina* and *J. roemerianus* sites monitored here began to show signs of recovery in September 2004 (2 years after the dieback occurred), but vegetation densities in these areas were still significantly below those of healthy areas in 2009. Both sites exhibited the greatest regrowth nearest the healthy marsh, and thus appear to be recovering primarily through rhizome expansion of the zonal dominants. The recovery times observed here were much slower than those reported for other common disturbances (i.e. wrack). The differences may be because the bare patches in sudden dieback areas were much larger. In addition, the loss of elevation as a result of erosion in the dieback areas at the *J. roemerianus* site may lead to a situation where the zonal dominant does not recolonize the area. Epifaunal snails were especially affected by the dieback at the *Spartina* site, decreasing to 0 in bare areas, and exhibiting a lagged response in recolonizing the area (i.e. 2 years after the recolonization of plants).

Given that climate change and anthropogenic effects will likely increase disturbances to the salt marsh, it is essential to understand how disturbances will affect the foundation species in the marsh, and in turn how the loss of these species will affect the invertebrate community. My studies in GA suggest that the loss of *Spartina* has a

similarly strong negative effect on the invertebrate community, and that the cause of the disturbance is not important. However, in LA I saw a decrease in epifauna but not infauna in dieback areas. The difference between these results may be that in GA *Spartina* functions as habitat and to ameliorate abiotic conditions, whereas hydrogeomorphic differences make these functions less important to infauna in LA. Therefore, a study that spans multiple geographical regions (latitudinally and longitudinally) with various tidal and climate regimes would be useful in order follow up on the idea of context-dependency in terms of the function that is being provided by marsh plants.

References

Bertness, M.D., P.J. Ewanchuk, and B.R. Silliman. 2002. Anthropogenic modification of New England salt marsh landscapes. *Proceedings of the National Academy of Science* 99(3): 1395-1398.

Bruno, J.F. and M.D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. In: M.D. Bertness and S.D. Gaines (eds.), *Marine Community Ecology*, pp. 201–218. Sinauer Associates, Sunderland, Massachusetts.

Burke DJ, Weis JS, Weis P. 2000. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuarine and Coastal Shelf Science* 51: 153–159.

Burke, D.J., J.S. Weis, and P. Weis. 2002. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuaries, Coastal, and Shelf Science* 51: 153-159.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliott, C.R. Ford, D.R. Foster, B.D. Kloeppel, J.D. Knoepp, G.M. Lovett, J. Mohan, D.A. Orwig, N.L. Rodenhouse, W.V. Sobczak, K.A. Stinson, J.K. Stone, C.M. Swan, J. Thompson, B. Von Holle, and J.R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3 (9): 479-486.

Mendelssohn, I.A., and K.L. McKee. 1992. Indicators of environmental stress in wetland plants. In: D.H. McKenzie, D.E. Hyatt, and V.J. McDonald (eds.), *Ecological Indicators*, Vol. 1, pp. 603-624. Elseiver, New York.

Weilhoefer, C.L. 2011. A review of indicators of estuarine tidal wetland condition. *Ecological Indicators* 11: 514-525

APPENDIX A.

ISOTOPIC ANALYSIS OF BENTHIC INVERTEBRATES IN BARE AND REFERENCE MARSH IN GA AND LA

To analyze the benthic food web for reference and bare plots in each state, isotopic carbon and sulfur ratios were examined among various producers and consumers. Sulfur is particularly useful in the interpretation of the food web structure in the estuarine environment to resolve primary producer signatures, as it distinguishes those that utilize seawater vs. sediment sulfur or a combination (Deegan and Garritt 1997).

Aboveground *Spartina*, benthic microalgae, dominant resident (fiddler crabs, periwinkle snails) and transient (blue crabs) epifaunal taxa, and infaunal taxa (oligochaetes) and were collected from the bare and reference (vegetated) treatments for $\delta^{13}C$ and $\delta^{34}S$ (where possible) isotope analysis. Subsamples collected from 3 sites each in GA and LA were homogenized and pooled by treatment for each state such that there were 4 isotopic samples per species (i.e. GA bare, GA reference, LA bare, LA reference) (Table A.1.).

Representative *Spartina* plants were clipped at the marsh surface, washed to remove mud, dried to a consistent weight, and ground using a Wiley Mill (#60). Benthic microalgae (BMA) were collected using the net technique, which takes advantage of the vertical migration of benthic diatoms at low water (Darley et al. 1979). Briefly, 150 µm

mesh Nitex was placed on the sediment surface with a smaller piece of 75 μm mesh Nitex on top, and the Nitex nets were sprayed with filtered seawater to adhere to the sediment surface. Nets were left exposed on the marsh surface during low tide until maximum low water. At that point, the upper sheet was removed and rinsed with twice-filtered (0.7 μm) seawater into an acid-washed plastic bottle. apparatus fitted with a pre-combusted, 47 mm, GF/F filter to collect the benthic microalgae. The rinse water was later filtered onto a pre-combusted GF/F filter (47 mm), and dried at 50 º C for 3 days.

Muscle tissue collected from live *Callinectes sapidus*, *Uca pugnax*, *U. rapax,* and *Littoraria irrorata* was removed and washed with deionized water, acid-treated with 10% HCl to remove non-organically bound carbonates, dried, and ground using a Wig-L-Bug or mortar and pestle. *L. irrorata* was allowed to purge gut contents for 12-24 hrs prior to removal of tissue. Oligochaetes were preserved in 100% ethanol, stained with Rose Bengal (this method of preservation and stain was determined to have the least affect on the $\delta^{13}C$ isotopic signature, Serrano et al. 2008) picked from marsh organic matter, rinsed in deionized water, acid-treated, and dried but not ground (due to small size).

In GA, there were a total of 5 samples (producer and consumer) analyzed for isotopic composition in the reference plots and 2 in bare plots. In LA, there were a total of 6 samples (producer and consumer) analyzed in the reference plots and 3 in bare plots. In each state, subsamples were collected from 3 sites and pooled by plot. The total number of subsamples collected and pooled by plot varied among producers and consumers (Table A.1.). All isotope samples were sent to Coastal Sciences Laboratory, Austin, TX for determination of stable isotopes of C and S (using a VG mass spectrometer). In a few cases, organisms were either absent (*Spartina* and *Littoraria* in bare plots of each state) or too few (oligochaetes in GA, *Uca rapax* in LA) to allow for a

bare plot isotope comparison. Additionally, obtaining sufficient biomass for sulfur ratios was difficult as it requires more tissue than do carbon ratios.

The results (Table A.2) show a clear separation between primary producers, and similarities in the organic matter source among same or analogous consumers in each state. In each state, the $\delta^{13}C$ signal of *Spartina* and BMA were distinguishable within reference (vegetated) areas, and there was a slight (GA) to strong (LA) shift in $\delta^{34}S$ in the BMA in bare as opposed to reference areas. In general, there was little indication of isotopic shifts between consumers collected in bare versus reference plots in either state, except for a shift in the carbon ratio of oligochaetes in LA. In vegetated plots in LA and GA, the oligochaete signal was generally closest to benthic microalgae whereas that of *Littoraria* was closer to *Spartina*. However, in bare plots in LA, the oligochaetes were slightly more enriched in ^{13}C, a signal intermediate between that of *Spartina* and BMA. In GA, the $\delta^{13}C$ and $\delta^{34}S$ data for fiddler crabs suggest that they were relying on a combination of *Spartina* and BMA in both bare and vegetated areas. In LA, the fiddler crab signal was more difficult to interpret. The values may have reflected input from mangroves because the crabs were collected along a berm in an area where mangroves were abundant (fiddler crab burrows were noted in the experimental plots but no crabs were ever collected there). Nevertheless, the results were similar to those of fiddler crabs in GA, in that the crabs appeared to show a reliance on both vegetation and BMA sources. The blue crabs collected in LA in both bare and vegetated areas also provided clear evidence for a combination of *Spartina* and BMA as the source of their organic matter.

The isotopic analysis, though limited, suggest that the food webs are behaving similarly in both states and treatment areas, with benthic microalgae an important source of material for infauna; *Spartina* a source for snails; and more mobile organisms (fiddler crabs, blue crabs) relying on a combination of BMA and *Spartina* regardless of plant density. There was no indication for a shift in food sources utilized when *Spartina* was lost.

References

Darley, W.M., C. T. Ohlman, and B. P. Wimpee. 1979. Utilization of dissolved organic carbon by natural populations of epibenthic salt marsh diatoms. *Journal of Phycology* 15: 1-5.

Deegan, L., and R. Garrett. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147: 31-47.

Serrano, O., L. Serrano, and M.A. Mateo. 2008. Effects of sample pre-treatment on the δ13C and δ18O values of living benthic foraminifera. *Chemical Geology* 257: 218-220.

Table A.1. The number of subsamples collected, homogenized, and pooled across sites to represent bare and reference plots for each isotopic sample (primary producers, epifauna, and macroinfauna) in GA and LA.

Isotope Samples	No. subsamples per pooled per plot
Primary Producers	
Aboveground *Spartina*	~10 plants
Benthic Microalgae	3-4 GF/F filters
Epifauna	
C. sapidus	5-6 claws
Uca spp.	15-20 claws
L. irrorata	15-20 animals
Macroinfauna	
Oligochaetes	25-60 animals

Table A.2. δ^{13}Carbon and δ^{34}Sulfur isotopic composition (‰) of primary producers and consumers in reference and bare marsh in GA and LA.

	Reference		Bare	
	^{13}C	^{34}S	^{13}C	^{34}S
Georgia				
Producers				
Spatina alterniflora	-13.8	14		
Benthic microalgae	-18.6	16	-20.7	13.9
Consumers				
Oligochaetes	-20.3			
Littoraria irroata	-13.65	15.3		
Uca pugnax	-16.2	12.5	-15.5	10.8
Louisiana				
Producers				
Spatina alterniflora	-13.4	-0.2		
Benthic microalgae	-20.4	-0.3	-19.7	6.6
Consumers				
Oligochaetes	-19.9		-15.9	
Littoraria irroata	-11.8	15.6		
*Uca rapax**	-17.4	3.4		
Callinectes sapidus	-16.4	5.7	-16.6	6.6

*Collected along a vegetated mangrove berm.

APPENDIX B.

THE EFFECT OF *SPARTINA* DIEBACK ON THE SIZE OF PERIWINKLE SNAILS AND MEIOFAUNA IN GA AND LA SALT MARSHES

Molluscs –

Snails (*Littoraria irrorata*) were collected from within 3 replicate (2500 or 5000 cm^2) quadrats in bare and reference plots at all 6 sites in GA and LA each, and opportunistically from within the intermediate density (transplanted) plots during the fall of 2006-2008, as described in Chapter 2, Section 2.2.1. The 2 intermediate density plots per site were transplanted with *Spartina* to represent "low density" (i.e. 1 planting unit every 1 m on center) and "high density" (i.e. 1 planting unit every 30 cm on center). Following collection, snails were preserved and later measured in the lab with calipers to the nearest 0.1 mm. I analyzed the variation in *Littoraria* length by treatment and year for each state using a split-plot 2-way ANOVA (with significance tested at α=0.05). In order to analyze how *Littoraria* length was distributed by size class within treatment and year, I constructed histograms at 2-mm length increments of all snails measured.

In GA, there was a significant treatment effect on the length of snails, while there was no effect of treatment on the length of snails in LA (Figure B.1.). In GA, snails were significantly larger in the reference plots as compared to the low density and high density treatment plots. Though there was no effect of year on snail length in GA, there was a significant effect of the interaction of treatment*year, indicating that the difference in

snail length among treatments varied by year. In LA, there was a significant of effect of year only, with snails becoming significantly larger each sampling year from an average shell height in LA in 2006 (9.1 ± 0.34 mm) to 2008 (14.7 ± 0.26 mm).

Using size frequency distributions, I was able to observe distinct cohorts over time in both states (Figures B.2. and B.3.). In GA, there were 2 distinct cohorts observed (modal peaks), while in LA, there were 3 distinct cohorts observed. In each state, these cohorts tended to occur regardless of treatment, with the exception of the low density treatments in LA in 2006 which had a sample size of 1. Also in both states, these cohorts can be seen shifting to the right over time as the snails grew. In 2008, it was interesting that very few snails were recruited into the populations in GA and LA, indicated by the low frequency of snails in the 2-4 and 4-6 mm range. It is also interesting to note that the maximum shell length regularly obtained by snails in LA is about 4 mm larger than those in GA.

Meiofauna –

Meiofauna (>63 μm) were collected with a corer (diam. 5.2 cm) to a depth of 5 cm from within bare and reference plots from 2 sites per state in 2006. In each case, meiofauna were preserved in 10% formalin and stained with rose bengal dye. From each site, a total of 5 nematodes and 5 copepods each were haphazardly chosen from the bare and reference treatments and measured to the nearest 0.01 mm using an ocular micrometer. Differences in meiofaunal length between bare and reference plots in each state were compared using a two-sample t-test.

In GA, nematodes were significantly smaller in bare plots, but there was no difference in copepod length among treatments (Figure B.4.). In LA, there were no differences in either nematode or copepod length taken from bare and reference plots.

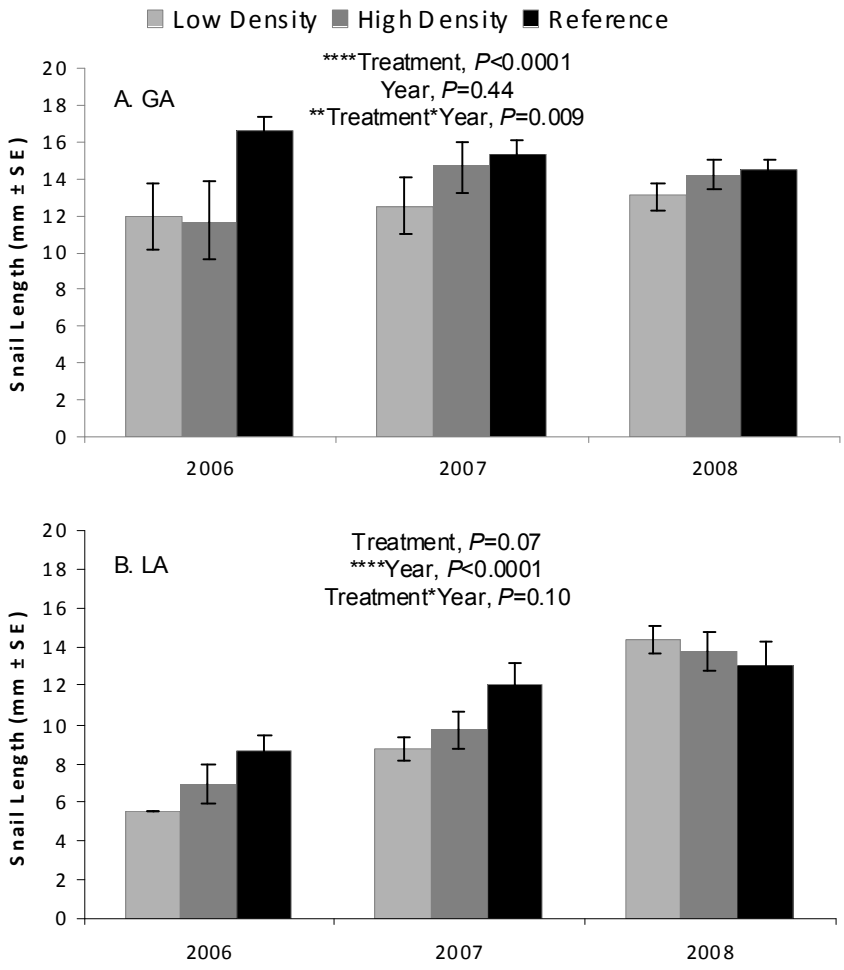


Figure B.1. Shell length of periwinkle snails (*Littoraria irrorata)* by treatment and year in A) GA and B) LA. The significance of treatment, year and treatment*year in a 2-way ANOVA is shown for each state.

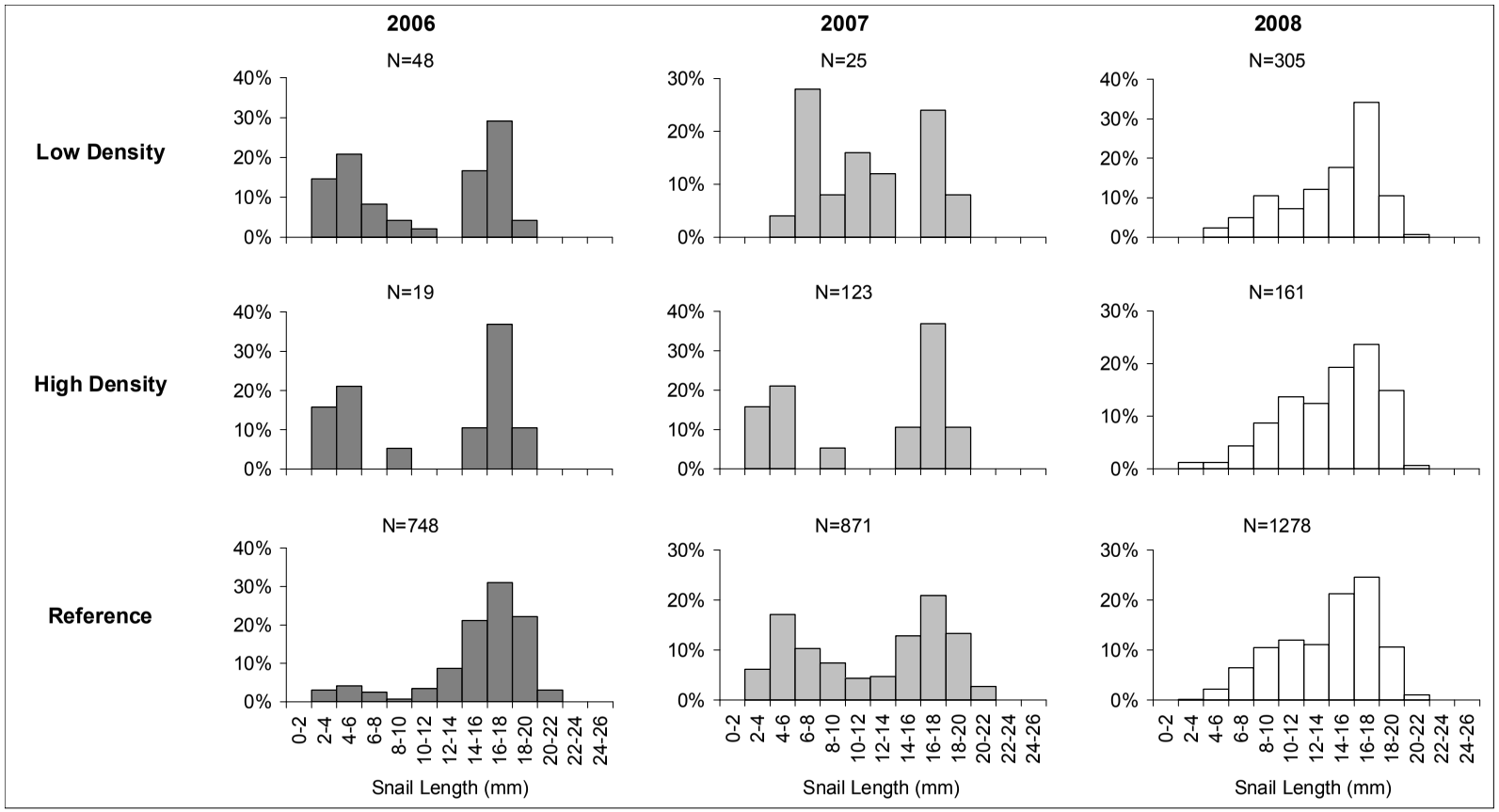


Figure B.2. The frequency of *Littoraria irrorata* snail length measured in GA in 2006-2008 in low density, high density, and reference plots (no snails were present in bare plots). For each graph, N represents the number of snails measured.

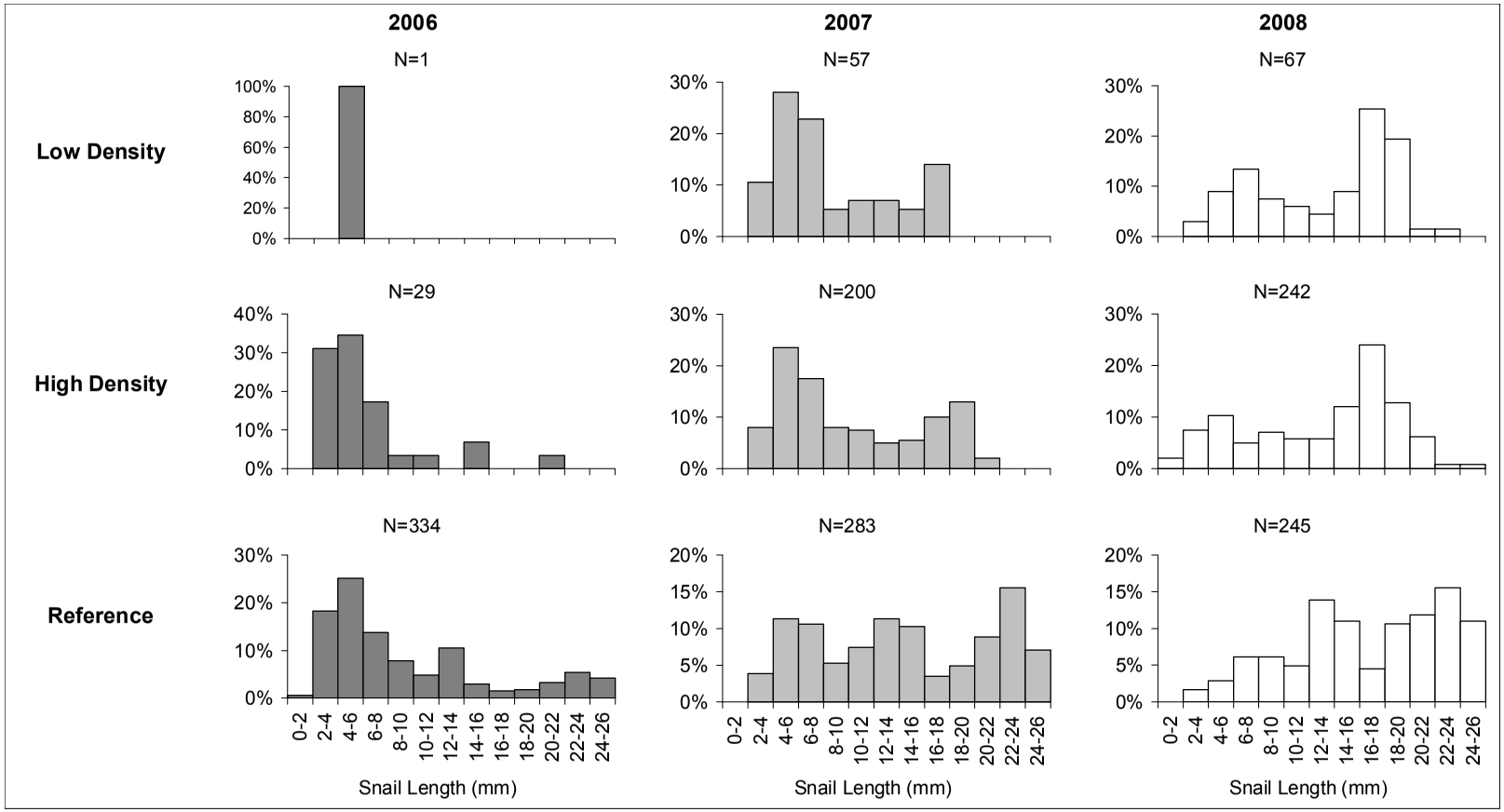


Figure B.3. The frequency of *Littoraria irrorata* snail length measured in LA in 2006-2008 in low density, high density, and reference plots (no snails were present in bare plots). For each graph, *N* represents the number of snails measured.

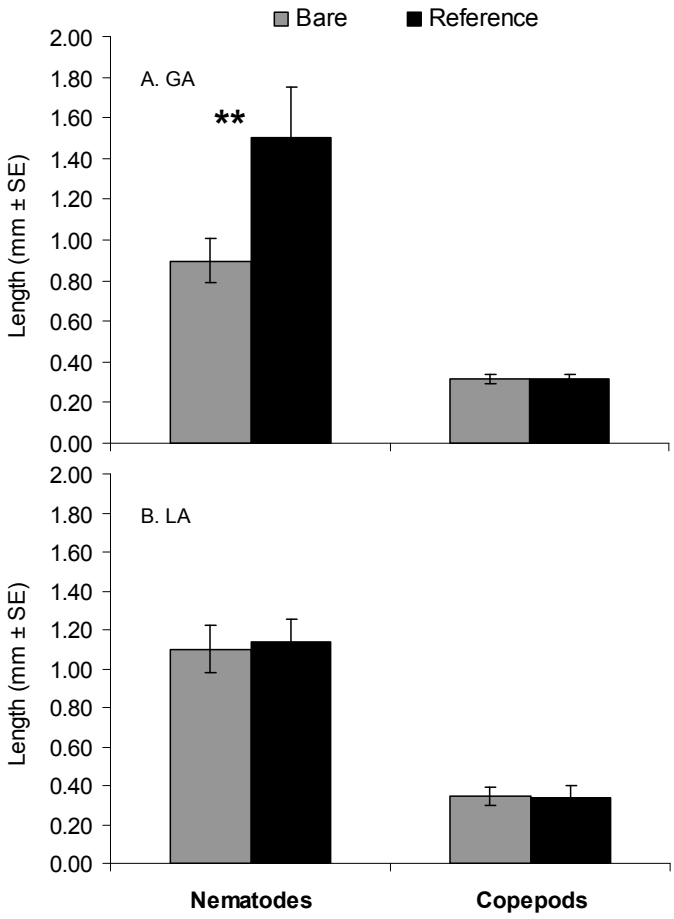


Figure B.4. Nematode and copepod length in bare and reference plots in 2006 in A) GA and B) LA (*N*=10 per bar). Significant differences between bare and reference plots for nematode and copepod lengths is indicated by asterisks (two-sample t-test, p-value: *<0.05, **<0.01).

APPENDIX C

SUPPLEMENTAL MATERIAL FOR CHAPTER 3

Appendix C.1.

The relationship of DMS area to DMS gas is linear on a log-log scale and the following equations were used to back-calculate foliar DMSP and DMSO from standard curves:

1) **DMSP --**

Solve for x (DMS gas) in 1 mL:

[y= 1.8984 x + 2.7912], where y= log [DMS area] (in 1 mL of headspace gas); x= log [nmol DMS] (in 1 mL of headspace gas)

Determine gas total in serum vial headspace volume:

[10^x * serum vial headspace volume]

Determine gas total dissolved in liquid volume of serum vial:

[10^x * liquid volume in mL * 0.0907 (solubility coefficient)]

Determine DMSP per gram of leaf:

[(DMS gas in headspace) + (DMS gas in liquid)]/[leaf weight (g)]

2) **DMSO --**

[y = 1.8225x + 2.224], where y= log [DMS area] (in 1 mL of headspace gas); x= log [nmol DMS] (in 1 mL of headspace gas)

Determine gas total in serum vial headspace volume:

[10^x * serum vial headspace volume]

Determine gas total dissolved in liquid volume of serum vial:

[10^x * liquid volume in mL * 0.0907 (solubility coefficient)]

Determine DMSO per gram of leaf:

[(DMS gas in headspace) + (DMS gas in liquid)]/[leaf weight (g)]

Appendix C.2. Mean (SE) based on N samples of root DMSP, DMSO, and the DMSO:DMSP ratio in roots of *Spartina alterniflora* collected in healthy, edge, and affected zones at dieback, horse, and snail sites. Roots from wrack sites were unavailable for analysis. The highest mean concentrations per zone is shown in bold, in order to highlight trends.

Site-type	Zone	DMSP		DMSO		DMSO:DMSP	
		Mean (SE)	N	Mean (SE)	N	Mean (SE)	N
dieback	Affected	0.09 (0.012)	6	0.01 (0.01)	6	**0.93 (0.02)**	6
	Edge	0.91 (0.15)	8	0.22 (0.06)	8	0.66 (0.05)	8
	Healthy	**1.55 (0.32)**	8	**0.22 (0.11)**	8	0.51 (0.05)	8
horse	Affected	0.69 (0.12)	10	0.21 (0.06)	10	**0.43 (0.15)**	10
	Edge	0.83 (0.17)	10	0.09 (0.03)	10	0.11 (0.02)	10
	Healthy	**1.15 (0.19)**	10	**0.34 (0.07)**	10	0.39 (0.11)	10
snails	Affected	0.74 (0.31)	8	0.14 (0.04)	8	0.78 (0.10)	8
	Edge	0.96 (0.21)	8	**0.90 (0.64)**	8	**0.96 (0.29)**	8
	Healthy	**1.10 (0.19)**	8	0.74 (0.41)	8	0.87 (0.21)	8

Appendix C.3. Certified and non-certified elemental concentrations of the NIST Standard Reference Material (SRM) 1515, apple leaves.

Certified elemental constituents --

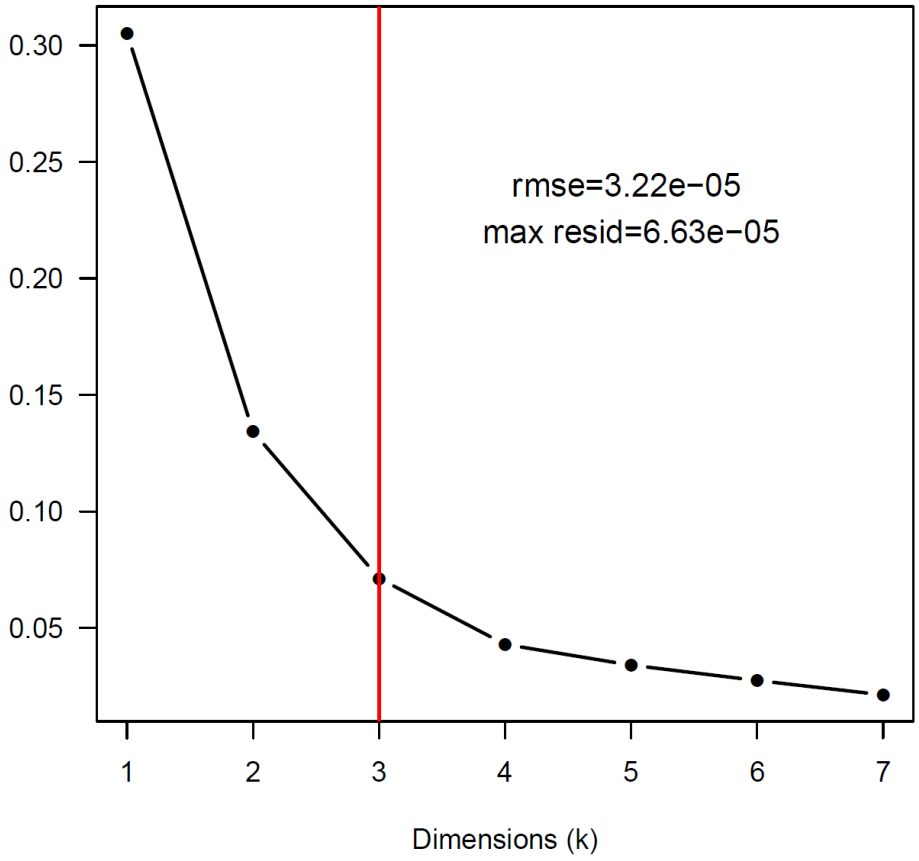
Element	Concentration, wt. percent
Calcium	1.526 ± 0.015
Magnesium	0.271 ± 0.008
Nitrogen (Total)	2.25 ± 0.19
Phosphorus	0.159 ± 0.011
Potassium	1.61 ± 0.02

Element	Concentration, µg/g	Element	Concentration, µg/g
Aluminum	286 ± 9	Mercury	0.044 ± 0.004
Arsenic	0.038 ± 0.007	Molybdenum	0.094 ± 0.013
Barium	49 ± 2	Nickel	0.91 ± 0.12
Boron	27 ± 2	Rubidium	10.2 ± 1.5
Cadmium	0.013 ± 0.002	Selenium	0.050 ± 0.009
Chlorine	579 ± 23	Sodium	24.4 ± 1.2
Copper	5.64 ± 0.24	Strontium	25 ± 2
Iron	83 ± 5	Vanadium	0.26 ± 0.03
Lead	0.470 ± 0.024	Zinc	12.5 ± 0.3
Manganese	54 ± 3		

Non-certified elemental constituents --

Element	Concentration, µg/g	Element	Concentration, µg/g
Antimony	(0.013)	Neodymium	(17)
Bromine	(1.8)	Samarium	(3)
Cerium	(3)	Scandium	(0.03)
Chromium	(0.3)	Terbium	(0.4)
Cobalt	(0.09)	Thorium	(0.03)
Europium	(0.2)	Tin	(<0.2)
Gadolinium	(3)	Tungsten	(0.007)
Gold	(0.001)	Uranium	(0.006)
Iodine	(0.3)	Ytterbium	(0.3)
Lanthanum	(20)		

Appendix C.4. Scree plot showing stress versus the number of dimensions in a non-metric ordination. Based on this figure, 3 dimensions were chosen to best represent the MDS ordination of elemental constituents in *Spartina* leaves in Figure 5.1.



Appendix C.5. Pearson's correlation matrix of *Spartina* elemental constituents in the healthy marsh zone. The degree of association is represented by the product moment correlation (r) and asterisks which indicate the significance of the relationship among constituents in a linear regression (*p*-value: *<0.05, **<0.01, ***<0.001, ****<0.0001).

	AL	AS	B	BA	CA	CD	CO	CR	CU	FE	K	MG	MN	NA	NI	P	PB	SI	SR
AS	0.95 ****																		
B	0.20	0.10																	
BA	0.13	0.34	-0.27																
CA	0.49	0.41	-0.04	-0.25															
CD	0.34	0.49	0.01	0.08	0.51														
CO	0.64 *	0.77 **	0.10	0.35	0.01	0.54													
CR	0.20	0.22	-0.48	0.15	-0.39	-0.29	0.35												
CU	0.58	0.58	0.55	0.08	-0.04	0.14	0.61	0.01											
FE	0.86 **	0.90 ***	0.32	0.21	0.17	0.39	0.79 **	0.24	0.83 **										
K	0.48	0.45	0.29	0.42	-0.09	-0.21	0.03	0.08	0.28	0.44									
MG	0.21	0.34	-0.16	0.27	0.67 *	0.77 **	0.10	-0.53	-0.12	0.10	0.02								
MN	-0.02	-0.08	0.33	-0.58	0.29	0.19	-0.17	-0.36	0.27	0.10	-0.27	-0.02							
NA	0.50	0.54	-0.25	0.63	0.20	-0.01	0.13	0.12	-0.05	0.24	0.65 *	0.31	-0.45						
NI	0.42	0.33	0.58	-0.40	0.22	0.29	0.38	-0.02	0.56	0.56	0.02	-0.03	0.28	-0.50					
P	-0.09	-0.28	0.15	-0.81 **	0.51	-0.02	-0.55	-0.39	-0.07	-0.20	-0.25	0.09	0.66 *	-0.44	0.33				
PB	0.93 ****	0.96 ****	0.07	0.23	0.37	0.48	0.81 **	0.32	0.57	0.89 ***	0.36	0.28	-0.17	0.41	0.45	-0.24			
SI	0.68 *	0.81 **	0.18	0.67 *	-0.03	0.30	0.67 *	0.14	0.66 *	0.82 **	0.65	0.22	-0.13	0.59	0.08	-0.54	0.70 *		
SR	0.53	0.46	-0.12	-0.22	0.98 ****	0.48	0.00	-0.28	-0.06	0.21	0.00	0.66 *	0.26	0.28	0.20	0.51	0.42	0.02	
ZN	0.00	0.08	0.52	0.15	-0.34	0.11	0.11	-0.39	0.53	0.30	0.35	0.04	0.27	0.04	-0.02	-0.10	-0.01	0.48	-0.36

Appendix C.6. Pearson's correlation matrix of *Spartina* elemental constituents in the edge marsh zone. The degree of association is represented by the product moment correlation (r) and asterisks which indicate the significance of the relationship among constituents in a linear regression (*p*-value: *<0.05, **<0.01, ***<0.001, ****<0.0001).

	AL	AS	B	BA	CA	CD	CO	CR	CU	FE	K	MG	MN	NA	NI	P	PB	SI	SR
AS	0.95 ****																		
B	-0.03	0.20																	
BA	0.39	0.53	0.06																
CA	0.22	0.14	-0.12	-0.40															
CD	0.36	0.56	0.67 *	0.24	0.31														
CO	0.37	0.61	0.72 *	0.47	-0.13	0.76 *													
CR	0.41	0.66 *	0.72 *	0.52	-0.12	0.79 **	0.99 ****												
CU	0.26	0.51	0.81 **	0.52	-0.23	0.75 *	0.93 ****	0.92 ****											
FE	0.80 **	0.94 ****	0.42	0.63	-0.08	0.67 *	0.79 **	0.84 **	0.72 *										
K	0.03	0.05	-0.31	0.63 *	-0.53	-0.44	0.06	0.04	0.06	0.07									
MG	0.20	0.25	0.19	-0.02	0.67 *	0.70 *	0.13	0.17	0.21	0.18	-0.51								
MN	0.08	0.12	0.39	-0.40	0.69 *	0.43	0.19	0.19	0.15	0.09	-0.61	0.37							
NA	-0.18	-0.20	-0.45	0.33	-0.31	-0.38	-0.09	-0.14	-0.06	-0.18	0.52	-0.37	-0.40						
NI	-0.26	-0.31	-0.34	-0.03	-0.19	-0.25	-0.11	-0.19	-0.05	-0.31	0.22	-0.13	-0.39	0.82 **					
P	0.27	0.42	0.74 *	-0.08	0.16	0.63	0.67 *	0.68 *	0.59	0.53	-0.52	0.08	0.63	-0.34	-0.34				
PB	0.71 *	0.86 **	0.44	0.60	-0.07	0.67 *	0.88 ***	0.89 ***	0.80 **	0.92 ***	0.16	0.17	0.02	0.02	0.00	0.50			
SI	0.48	0.68 *	0.42	0.77 **	-0.13	0.72 *	0.68 *	0.76 *	0.71 *	0.84 **	0.08	0.34	0.05	-0.13	-0.35	0.35	0.69 *		
SR	0.28	0.18	-0.19	-0.38	0.99 ****	0.25	-0.17	-0.16	-0.27	-0.07	-0.49	0.64 *	0.65	-0.30	-0.19	0.11	-0.07	-0.15	
ZN	0.19	0.39	0.86 **	0.22	0.01	0.72 *	0.69 *	0.70 *	0.83 **	0.56	-0.21	0.36	0.51	-0.41	-0.38	0.60	0.51	0.59	-0.03

Appendix C.7. Pearson's correlation matrix of *Spartina* elemental constituents in the affected marsh zone. The degree of association is represented by the product moment correlation (r) and asterisks which indicate the significance of the relationship among constituents in a linear regression (*p*-value: *<0.05, **<0.01, ***<0.001, ****<0.0001).

	AL	AS	B	BA	CA	CD	CO	CR	CU	FE	K	MG	MN	NA	NI	P	PB	SI	SR
AS	0.98 ****																		
B	0.43	0.41																	
BA	-0.02	-0.02	0.02																
CA	-0.08	-0.14	-0.49	-0.19															
CD	0.27	0.37	0.06	-0.43	0.32														
CO	0.23	0.31	0.21	-0.69	0.17	0.79 *													
CR	-0.07	0.04	0.21	-0.48	-0.18	0.44	0.83 *												
CU	-0.04	0.02	0.52	0.26	-0.42	-0.05	0.28	0.65											
FE	0.31	0.43	0.56	0.16	-0.74	0.19	0.34	0.59	0.74										
K	-0.94 **	-0.93 **	-0.17	0.20	-0.17	-0.44	-0.34	0.07	0.27	-0.11									
MG	0.65	0.68	0.07	-0.13	0.50	0.80 *	0.51	0.04	-0.19	0.02	-0.78 *								
MN	0.19	0.08	0.43	-0.65	0.32	0.17	0.38	0.08	-0.15	-0.34	-0.22	0.17							
NA	0.53	0.45	0.38	-0.70	0.34	0.38	0.58	0.20	-0.17	-0.17	-0.60	0.47	0.89 **						
NI	-0.08	0.06	0.47	-0.12	-0.64	0.35	0.50	0.75	0.68	0.85 *	0.21	-0.10	-0.19	-0.17					
P	-0.45	-0.50	0.22	-0.52	-0.31	-0.37	0.10	0.35	0.19	-0.04	0.51	-0.71	0.47	0.21	0.23				
PB	0.82 *	0.89 **	0.16	-0.21	-0.14	0.48	0.53	0.36	0.10	0.53	-0.84 *	0.62	-0.08	0.36	0.23	-0.41			
SI	-0.28	-0.20	0.52	0.39	-0.63	-0.11	0.02	0.43	0.85 *	0.73	0.53	-0.38	-0.32	-0.46	0.78 *	0.21	-0.18		
SR	0.09	0.04	-0.41	-0.07	0.97 ***	0.39	0.14	-0.28	-0.46	-0.67	-0.32	0.65	0.27	0.33	-0.65	-0.50	-0.03	-0.64	
ZN	0.18	0.16	0.72	0.33	-0.75	-0.52	-0.19	0.16	0.70	0.62	0.15	-0.46	-0.01	-0.09	0.40	0.37	0.03	0.67	-0.74

APPENDIX D

AN EXPERIMENTAL MANIPULATION OF FOUR DISTURBANCES IN THE SALT MARSH

Sampling sites and design -

I manipulated four of common disturbances that occur in southern marshes (wrack deposition, sudden dieback, and mammalian and snail overgrazing) at 3 sites located on Sapelo Island, GA (Timber Dock, Marine Institute, and Lighthouse Marsh). This experiment was designed to allow observation of how various disturbances affect *S. alterniflora* and its associated fauna in a controlled manner. Sites were at different elevations and flooded by different tidal creeks: the Timber Dock site is flooded by the Duplin River and consists of short-form *S. alterniflora* (~30-35 cm), the Marine Institute site is flooded by South End Creek and is medium *S. alterniflora* (~40-45 cm), and the Lighthouse site is flooded by Dean Creek and is also medium *S. alterniflora* (~45-50 cm). Each location was monitored for initial site conditions in July 2008, then bimonthly for 2 months, and monthly for 4 months through January 2009 while the disturbances were maintained. Disturbance treatments were removed in January 2009, and recovery of *Spartina* and invertebrates was monitored 5 and 10 months later, in June and November 2009, respectively.

At each site, 2 blocks were set up using a complete randomized block design for snail, wrack, clipped (herbivore overgrazing), and herbicide (sudden dieback) treatments,

and controls. Blocks were approximately 8 m x 11 m and the treatment areas were each 2.5 m x 2.5 m, divided in half by a 0.5 m walkway with 3 permanent 0.25 m^2 plots randomly assigned between the 2 sides of the treatment area (Figure D.1.). Below is a description of each treatment:

Snails- For the snail treatment, 1 m x 2.5 m cages on each side of the walkway were constructed of fiberglass screening stapled to wooden stakes, with the bottom of the screening staked into the ground to minimize snail escape. A total of 1500 average-sized snails (~10 mm), representing a density of 600 snails/m^2, were maintained in each cage for the duration of the experiment. The snail density chosen was based on that which was observed to affect *S. alterniflora* during routine sampling at snail disturbance survey sites.

Wrack- For the wrack treatment, deer netting was used to cover a 2-5 cm thickness of natural wrack collected from other marshes on each 1 m x 2.5 m side of the treatment area. The netting was staked down with garden staples and wrack was maintained within plots over the duration of the experiment.

Herbivory- To mimic herbivory, *S. alterniflora* was clipped nearest the surface as possible bimonthly for 2 months and then monthly for 3 months in each 1 m x 2.5 m side of the treatment area.

Sudden Dieback- To mimic sudden dieback, *S. alterniflora* was sprayed with the

herbicide Roundup® bimonthly for 2 months and then monthly at each site until plants were dead in each 1 m x 2.5 m side of the treatment area. Herbicide was used to mimic dieback in that it kills above and belowground portions of the plant.

Controls- For the control group, unmanipulated healthy *S. alterniflora* was left alone in each block. The cage control consisted of a 1 m x 2.5 m 3-sided cage, open on one end to allow ambient snails to go in and out and the mesh control consisted of deer netting without wrack underneath covering a 1 m x 2.5 m area.

Sample collection-

Vegetation and Fauna

Within each of the 3 plots, variables were collected and processed similarly to the survey project described in Chapter 4 (section 4.2. methods), and included vegetation density and height, epifaunal density, and porewater pH, salinity, and reduction-oxidation potential. Two samples each of infaunal invertebrates (meiofauna and macrofauna) were collected from within each treatment (N=30 samples, i.e. 5 treatments x 3 sites x 2 cores) using a PVC corer (21.2 cm^{-2}) to a depth of 5 cm while the disturbance was in place (September 2008 and January 2009), and then after the disturbance was removed (June 2009 and November 2009). The invertebrate cores were separated by size class using nested 500 µm and 63 µm sieves, and then each portion was preserved (10% Formalin, i.e. 3.7% formaldehyde) and stained with Rose Bengal dye. Soil cores and macroinfauna (# per 100 cm^2) were processed for all 4 dates in a similar manner to the survey project,

and were averaged across sites and blocks for an overall treatment mean by date.

The meiofauna were processed on the January 2009 date only. Because meiofauna samples typically contained >1000 individuals, a subsample was taken from the core sediments by sampling from a known slurry volume with a goal of attaining ~150-200 animals from the dominant group (copepods or nematodes) and then adjusted to core volume. Density centrifugation (Ludox HS40) was used to aid in separating meiofauna from the sediment, in a ratio of approximately 1:10 of sediment: Ludox volume. In each case, a dissecting microscope was used to identify taxa and the total number of meiofauna was scaled to # per 10 cm^{-2}.

Below ground biomass was additionally collected from each treatment/block/site on the final dates of the disturbance (January 2009). A 30-cm deep core was taken and sectioned into 0-5, 5-10, 10-20, and 20-30 cm increments. In the lab, the material collected was washed free of sediment, separated into live and dead biomass, dried at 60° C to a constant weight, and weighed to the nearest 100^{th} of a gram.

Foliar metals

A single green leaf of *Spartina* was collected from an area adjacent to the 3 sampling plots where possible from each treatment on the final date of the disturbance in January 2009. Leaves were pooled across treatments and blocks at each site, such that there were N=15 samples (3 sites x 5 treatments). Samples were dried at 60°C, ground using a Wiley mill (mesh #40), and then burned in a muffle furnace at 500°C for 4 hours. Samples were analyzed for a suite of 20 elemental constituents (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si, Sr, Zn) with an ICP spectrometer (Jarrell-

Ash 965 Inductively Coupled Plasma-Optical Emission Spectrograph) at the University of Georgia's Chemical Analysis Lab (as in Chapter 3, section 3.2.5. of methods)

Results –

Live *Spartina* density was significantly different between each treatment beginning in late September 2008-January 2009 (Figure D.2.). Healthy areas had the highest live *Spartina* densities during this time period, followed by snails, wrack, clipped ("herbivory"), and roundup ("dieback"), which had the lowest densities. Live belowground biomass of *Spartina* collected on the last date of the disturbance period differed especially in the 0-5 cm portion, with roundup having the lowest live belowground biomass, followed by snails, clipped, wrack, and then healthy (control) areas (Figure D.3.). However, there was no difference in the total belowground biomass in any treatment, and each had nearly 2000 g m^{-2} of biomass within the 0-5 cm portion. There was also no significant difference in the soil pH, redox potential, or salinity among the treatments.

Snail densities differed among treatments over time (Figure D.4.). Snails decreased quickly and remained low in clipped plots, which had 0 live aboveground *Spartina* on most dates. There was a slower decrease in snail densities over time in the wrack and roundup treatments, whereas there was no decrease in the healthy plots (snail treatments were not compared, as snails were the disturbance and were artificially high in these plots). The slower decrease in snail densities in the wrack and roundup treatments was because of the availability of standing dead plants initially, which snails could utilize as habitat (McFarlin *pers obs*.). After the standing dead plants decreased, the snail

densities decreased in the plots as well. These finding support other literature that the snails require *Spartina* for habitat and a source of organic matter.

I found no difference in the densities of fiddler crabs, or the benthic infauna (meiofauna and macroinfauna) among treatments (not shown). That fiddler crabs did not differ in response to the *Spartina* loss was not surprising because this was also observed in my other studies (Chapter 2, 4, 5). It is likely that their requirement for *Spartina* is based on a more loose facultative relationship, as they do not require *Spartina* directly for habitat *per se*. That the benthic infauna did not differ in response to the *Spartina* loss was, however, surprising. In my other studies (Chapter 2, 4), I saw decreases in the density and diversity of the benthic infauna in disturbed areas. There are several reasons that I may not have seen a change in the benthic infauna in this case: 1) the belowground biomass, which serves as a source of organic matter and holds soil moisture, did not differ across treatments and was much higher than the cited threshold of 500 g m^{-2} in the 0-5 cm portion (Craft and Sacco 2003), below which infauna have been reported to decline, 2) the treatment plots (which were 1 m x 2.5 m) may not have been large enough to show an effect, as the surrounding *Spartina* may have been substantial enough to shade and ameliorate the area, and 3) the surrounding healthy *Spartina* areas may have been close enough to supply the treatment areas with larval infauna (both dispersing polychaetes and non-dispersing oligochaetes).

Foliar metals differed among the treatments, but were not always consistently highest in a particularly treatment, nor were the healthy areas consistently lowest in the concentration of various metals (not shown). This is again different from my other observations (Chapter 3). This may again be because the treatment plots were too small,

and the proximity to and connection to healthy plants (via rhizomes) could potentially have allowed for distribution of metals via clonal integration. Because there were no treatment differences, metals were grouped and the relationships between metals were explored using a Pearson's correlation matrix (Table D.1.). Interestingly, many of the same correlations that were highly significant in Chapter 3 were also seen here, i.e. see the relationship of Al with As (r=0.99), Al with Pb (r=0.95), and As with Pb (r=0.95) and Sr with Ca (r=0.91). It may simply be that these metals are found, in particular, bound to the clay fraction of soils (National Research Council 1977, Walsh et al. 1977, ATSDR 2007), and that the simultaneous uptake by the plants occurred through an oxidized rhizosphere surrounding the roots, which makes these fractions more bioavailable to the plant (Sunby et al. 1998, Taggert et al. 2009).

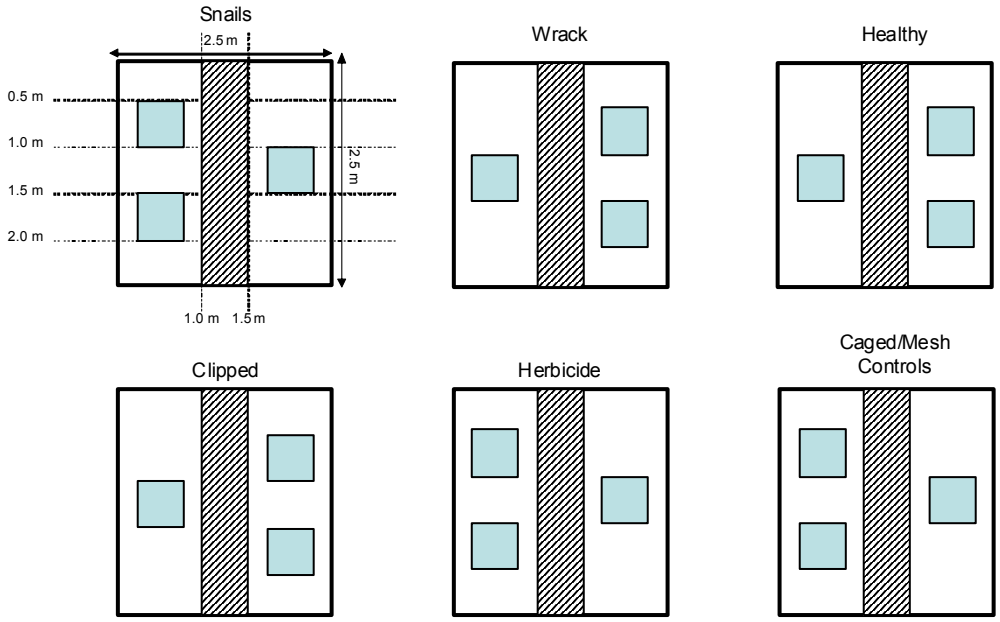


Figure D.1. Design of a single disturbance manipulation experiment block. Diagonally-shaded areas are walkways and blue-shaded areas are the permanent plots. In each block, treatments were randomly assigned to each of the larger 2.5 m x 2.5 m square plots, and permanent 0.5 m x 0.5 m sampling plots were randomly laid out as to which side of the treatment plot received 1 or 2 plots; this figure is an example of treatment and plot layout. Cage and mesh controls were set up at the Lighthouse site only.

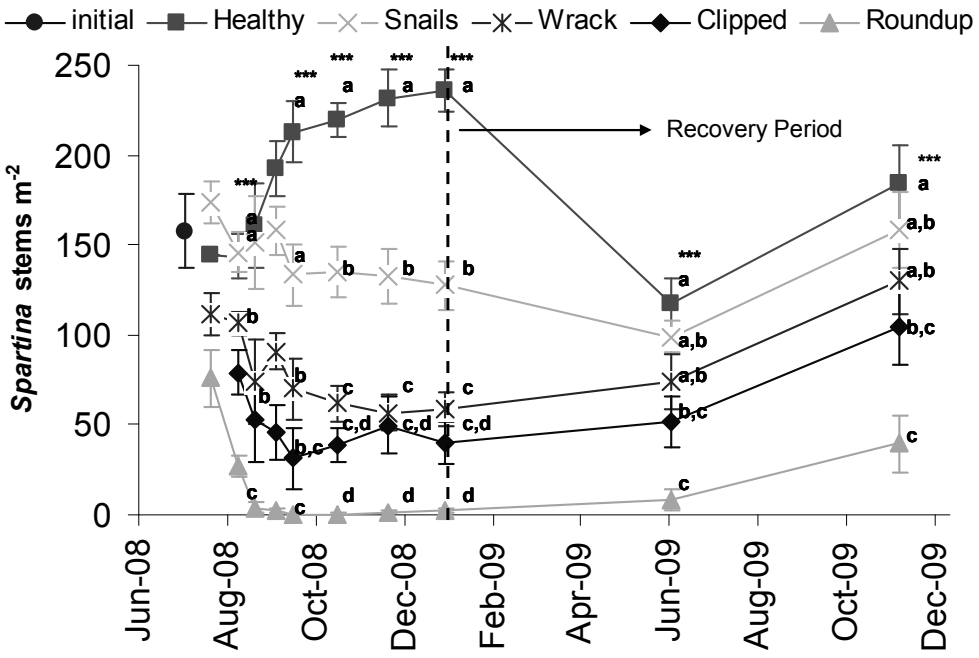


Figure D.2. The variation in *Spartina* density (per m^2) in healthy (control), snail, wrack, clipped, and roundup treatments over time. Each point represents the mean of 18 plots ± SE (3 plots x 2 blocks x 3 sites). Letters indicate which treatments on each sampling date were significantly different based on a Tukey's post-hoc comparison of means. Asterisks indicate the overall level of significance of treatment differences on each date (based on a 1-way ANOVA with a Bonferroni correction).

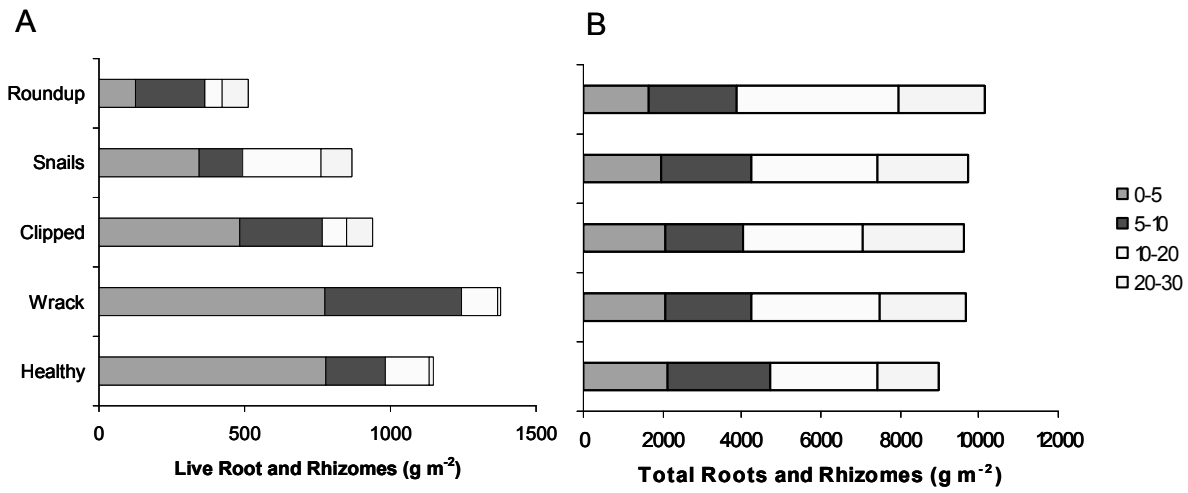


Figure D.3. The portion of live (A) and total (B) root and rhizome biomass collected on January 2009 from a 0-5, 5-20, 20-20, and 20-30 cm depth from the roundup, snail, clipped, wrack, and healthy treatments.

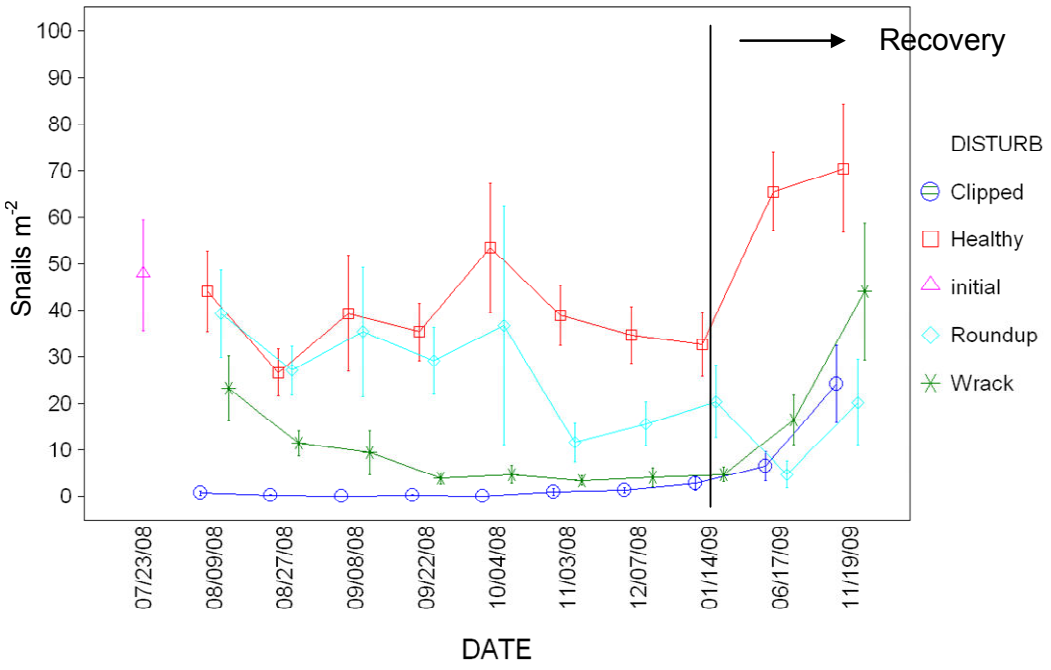


Figure D.4. The variation in snail density (per m^2) in healthy (control), snail, wrack, clipped, and roundup treatments over time. Each point represents the mean of 18 plots ± SE (3 plots x 2 blocks x 3 sites).

Table D.1. Pearson's correlation matrix of *Spartina* elemental constituents in plants collected as a part of the manipulation project. The degree of association is represented by the product moment correlation (r) and the p-value below that indicates the significance of the relationship among constituents in a linear regression.

	AL	AS	B	BA	CA	CD	CO	CR	CU	FE	K	MG	MN	NA	NI	P	PB	SI	SR
AS	**0.9989**																		
P-VALUE	****																		
B	**0.615**	**0.6222**																	
	0.0253	**0.0232**																	
BA	**0.6876**	**0.6737**	0.2299																
	0.0094	**0.0116**	0.45																
CA	-0.0155	0.0191	-0.0955	-0.198															
	0.9599	0.9505	0.7562	0.5168															
CD	0.5006	0.5325	**0.6223**	-0.1091	0.5688														
	0.0815	0.061	**0.0231**	0.7227	0.0425														
CO	**0.9333**	**0.9424**	**0.5878**	**0.6201**	0.1337	**0.5652**													
	0	**0**	**0.0346**	**0.0238**	0.6632	**0.0441**													
CR	**0.7954**	**0.8124**	**0.6572**	0.2081	0.295	**0.7952**	**0.8573**												
	0.0011	**0.0007**	**0.0146**	0.4952	0.3278	**0.0012**	**0.0002**												
CU	0.1999	0.1901	0.1351	-0.0387	-0.3184	0.0384	0.1638	0.2442											
	0.5126	0.534	0.6599	0.9	0.289	0.9009	0.5929	0.4213											
FE	**0.8779**	**0.8753**	0.3738	0.5176	-0.0242	0.395	**0.8895**	**0.8213**	0.3077										
	0.0001	**0.0001**	0.2083	0.07	0.9375	0.1817	**0**	**0.0006**	0.3064										
K	**-0.5878**	**-0.5816**	-0.5359	**-0.7712**	0.2708	-0.062	**-0.5574**	-0.2485	-0.1117	-0.3255									
	0.0346	**0.0371**	0.059	**0.002**	0.3708	0.8405	**0.0478**	0.4129	0.7164	0.2778									
MG	0.3298	0.3684	0.385	-0.0863	**0.7563**	**0.9054**	0.4022	**0.5625**	-0.1239	0.16	-0.0345								
	0.2711	0.2155	0.194	0.7792	**0.0028**	**0**	0.173	**0.0454**	0.6868	0.6015	0.9109								
MN	-0.0746	-0.0901	-0.0557	0.3314	-0.0161	-0.3609	-0.0538	-0.3084	**-0.5587**	-0.1444	-0.1097	-0.3273							
	0.8086	0.7698	0.8566	0.2687	0.9583	0.2256	0.8615	0.3052	**0.0472**	0.6379	0.7214	0.275							
NA	**0.6344**	**0.6359**	0.2903	0.2388	-0.0434	0.3885	0.4892	0.424	0.0975	0.4531	-0.2416	0.3412	-0.3725						
	0.0199	**0.0195**	0.3359	0.4321	0.8881	0.1895	0.0898	0.1488	0.7513	0.1199	0.4264	0.2539	0.2101						
NI	0.0069	0.0286	0.1125	-0.074	0.5193	0.4888	0.0815	0.1968	0.1541	-0.0314	-0.1446	**0.5982**	-0.1958	-0.2395					
	0.9821	0.9262	0.7143	0.8102	0.0689	0.0901	0.7914	0.5193	0.6153	0.9188	0.6374	**0.0308**	0.5215	0.4306					
P	-0.4501	-0.4382	-0.2716	**-0.7157**	0.2563	0.0644	-0.4262	-0.1482	-0.0346	-0.3317	**0.7287**	0.0882	-0.0244	-0.0991	-0.0729				
	0.1228	0.1342	0.3694	**0.0059**	0.398	0.8345	0.1464	0.6289	0.9107	0.2682	**0.0047**	0.7745	0.9369	0.7474	0.8129				
PB	**0.9462**	**0.9498**	**0.6448**	0.5407	0.0472	**0.633**	**0.9047**	**0.8562**	0.3162	**0.8534**	-0.5069	0.4352	-0.2729	**0.5981**	0.1306	-0.4859			
	0	**0**	**0.0173**	0.0564	0.8783	**0.0202**	**0**	**0.0002**	0.2925	**0.0002**	0.0771	0.1372	0.367	**0.0308**	0.6706	0.0923			
SI	0.1692	0.1757	-0.4004	-0.1447	0.2795	0.1761	0.2852	0.3688	0.215	0.5428	0.3983	0.1004	-0.3363	0.203	0.0312	0.1505	0.2425		
	0.5805	0.5658	0.1752	0.6372	0.355	0.565	0.3449	0.2149	0.4805	0.0553	0.1776	0.7441	0.2613	0.506	0.9193	0.6237	0.4247		
SR	0.2607	0.2921	0.1028	0.1983	**0.905**	**0.5861**	0.3846	0.382	-0.3549	0.1298	-0.0987	**0.775**	0.1301	0.0426	0.5249	-0.0335	0.2767	0.1044	
	0.3896	0.3329	0.7383	0.516	**0**	**0.0353**	0.1944	0.1977	0.234	0.6725	0.7483	**0.0019**	0.6719	0.8902	0.0655	0.9136	0.3602	0.7344	
ZN	0.2118	0.2018	0.4303	0.1991	-0.5295	-0.1538	0.2138	0.0602	0.3057	0.1274	-0.4687	-0.2795	0.1484	0.0952	-0.1951	-0.002	0.0468	-0.366	-0.419
	0.4872	0.5086	0.1422	0.5142	0.0627	0.616	0.4831	0.845	0.3098	0.6784	0.1062	0.355	0.6285	0.757	0.523	0.9948	0.8794	0.2187	0.1541