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Mesohaline Submerged Aquatic Vegetation Survey Along the U.S. Gulf of Mexico Coast, 2000: A Stratified Random Approach

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Estimates of submerged aquatic vegetative (SAV) along the U.S. Gulf of Mexico (Gulf) generally focus on seagrasses. In 2000, we attempted a synoptic survey of SAV in the mesohaline (5–20 ppt) zone of estuarine and nearshore areas of the northeastern Gulf. Areas with SAV were identified from existing aerial 1992 photography, and a literature review was used to select those areas that were likely to experience mesohaline conditions during the growing season. In 2000, a drought year, we visited 217 randomly selected SAV beds and collected data on species composition and environmental conditions. In general, sites were either clearly polyhaline (≥ 20 ppt) or oligohaline (≤ 5 ppt), with only five sites measuring between 5 and 20 ppt. *Ruppia maritima* L. (13–35 ppt, n = 28) was the only species that occurred in mesohaline salinities. *Halodule wrightii* Asch. occurred in 73% of the beds. The nonindigenous *Myriophyllum spicatum* L. was present in four locations with salinities below 3 ppt. No nonindigenous macroalgae were identified, and no nonindigenous angiosperms occurred in salinities above 3 ppt. Selecting sample locations based on historical salinity data was not a successful strategy for surveying SAV in mesohaline systems, particularly during a drought year. Our ability to locate SAV beds within 50 m of their aerially located position 8 yr later demonstrates some SAV stability in the highly variable conditions of the study area.

INTRODUCTION

As a primary producer, submerged aquatic vegetation (SAV) serves several functions within the Gulf: it functions as a faunal nursery, it provides high biomass production and export, and it functions as a food source for herbivores (Zieman and Zieman, 1989). Although SAV distributions in some regions of the Gulf have been reported (e.g., Iverson and Bittaker, 1986; Handley et al., 2007; Mills et al., 2008), to date (Appendix) there has been no comprehensive Gulf-wide survey of mesohaline (5–20 ppt) SAV distribution. Most SAV studies in the Gulf have focused on marine SAV (seagrasses and macroalgae) that occur in large beds in clear waters near beaches, barrier islands, and open bays (Continental Shelf Associates, Inc., and Martel Laboratories, Inc., 1985; Lores et al., 2000). These seagrass beds are the SAV habitat reported in estimates of the nation's natural resources (e.g., Duke and Kruczynski, 1992; Mattson, 1999). While these estimates are used to determine impacts on those resources as a result of human actions, such as in the National Environmental Protection Act analyses required by all Federal actions (Public Law 91-190, as amended), these estimates are based on marine SAV and may underestimate the diversity and quantity of the SAV resource.

While native SAV serve an important role in estuarine communities, for example, as food and habitat for a variety of vertebrate and invertebrate species (e.g., Carpenter and Lodge, 1986; Kantrud, 1991), researchers are also interested in better understanding the distribution, abundance, interactions, and functions of nonindigenous and native SAV species. Several invasive (i.e., harmful) SAV species, such as *Hydrilla verticillata* (L. f.) Royle, *Myriophyllum spicatum* L., and the floating *Eichhornia crassipes* (Mart.) Solms, have caused water quality problems and habitat loss and have hindered navigation in freshwater systems throughout the United States (e.g., Penfound and Earle, 1948; Smith and Barko, 1990; Langeland, 1996), including the northern Gulf of Mexico [U.S. Geological Survey (USGS), 2009]. Other potential invaders, such as *Caulerpa taxifolia* (M. Vahl) C. Agardh, could cause ecological disruptions if they become introduced (Meinesz et al., 2001; Williams and Grosholz, 2002).

Our goals for this survey were twofold. We wanted to (1) document species composition and distribution for SAV occurring in mesohaline waters (5–20 ppt) of estuaries while determining the environmental conditions in which these SAV occur and (2) assess the extent to which nonindigenous species have become established in mesohaline SAV beds. Macroalgae were also documented when found. We used two different approaches to conduct a region-wide synoptic survey: a stratified random sampling approach and a gradient analysis approach. This report describes the stratified random approach and its limitations, whereas Merino et al. (2009) report on the latter topic.

Materials and Methods

An aerial survey was conducted in 1992 to determine the location, size, and patchiness of SAV beds. The study area ranged from the Chandeleur Islands, LA (30°N, 88.8°W) east to Anclote Keys, FL (28.2°N, 82.8°W). These SAV data represent 1:24,000 scale natural-color aerial photography, with SAV beds identified according to a National Wetlands Research Center (NWRC) classification scheme and then digitized (USGS NWRC, 1998). Because the data set was based strictly on aerial photography with little ground truthing, it does not include data on species composition or environmental characteristics. However, because it does have the locations of SAV beds, we used it as the basis for conducting a survey of mesohaline SAV beds.

We used a stratified random design to select our survey sites from the USGS NWRC (1998) database and to identify locations at which SAV were reported to occur. The database includes over 11,000 SAV bed locations. Using salinity maps of estuaries (Orlando et al., 1993) we created a subset of points with a moderate to high probability of having salinity in the range of 5 to 20 ppt during the growing season (May–Sep.). Seventy percent of the SAV sites selected met these salinity criteria. Additionally, 30% of the SAV sites selected were from areas with no salinity data. We included SAV sites within 0.5 km of submarine springs and freshwater rivers, as areas of unknown salinity presumed to have mesohaline salinities. In order to provide a representative sample of the coastal habitats from the 1992 database, 300 locations were randomly selected from the larger set of sites. These sites were stratified using the protocols established by the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program–Estuaries (EMAP-E; Summers et al., 1992) program. EMAP-E uses a statistical process that ensures a probabilistic sampling network representative of geographic (latitude and longitude) variability. This results in a set of sampling points representing a statistically valid probability sample (Summers et al., 1992). We began systematically sampling these locations, moving from west to east. Sampling began on 6 June 2000 and ended 1 July of 2000.

We traveled to each site by boat guided by a handheld consumer-grade global positioning satellite unit. If no SAV bed was found at the given latitude and longitude we searched a radius of 50 m from that point until we either encountered an SAV bed or had searched the entire area around the given point. If vegetation was present, we took a grab sample from the middle of the bed, recorded all macrophytes and macroalgae present, and collected water quality data. Species were identified in the field using the method of Godfrey and Wooten (1979) or that of Stutzenbaker (1999). Species that could not be identified in the field were saved for later identification. The U.S. Department of Agriculture (USDA) PLANTS database naming standards were used (USDA, 2006). At each site containing SAV, we recorded location (estuarine system, county, state, and latitude/longitude), date and time, substrate type, water depth, dissolved oxygen, temperature, conductivity, salinity, pH, and water turbidity. Measurements were taken using handheld meters (e.g., YSI meters). Water samples for nutrient content (nitrate, nitrite, ammonium, and phosphate), chlorophyll *a*, and total inorganic carbon were collected. A sediment sample was also collected for particle size and organic matter content analyses. Voucher specimens were collected and deposited in the USGS NWRC herbarium.

We used the Canonical Correspondence Analysis (CCA) module of PC-ORD v3 (McCune and Mefford, 1997) software to examine the relationship between species assemblages and environmental variables. In CCA, the ordination of the main matrix (species by site) is constrained by a multiple regression on variables included in the second matrix (environmental variables by site). The ordination of CCA was conducted on the complete data set, including all species and 11 environmental variables.

Results

We visited or attempted to visit 277 of the 300 predetermined locations along the northern Gulf, starting at Horn Island, MS, and ending at Shired Island, FL. We found 217 SAV beds. Nine beds were in Mississippi, 10 were in Alabama, and 198 were in Florida. SAV was absent in 52 of the sites visited, and eight sites we attempted to visit were inaccessible. A total of 10 SAV species were collected. Five species were from sites with measured mean salinities at or below 6 ppt: *Vallisneria americana* Michx., *Myriophyllum spicatum* L., *Potamogeton pectinatus* L., *Heteranthera dubia* (Jacq.) MacMillan, and *Potamogeton pusillus* L. (Table 1). Another five species were found on sites with measured mean salinities greater than 25 ppt: *Ruppia maritima*, *Halodule wrightii* Asch., *Halophila englemannii*

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TABLE 1. Summary of submerged aquatic vegetation surveyed in 2000 with environmental data (mean [range]) by species.[a]

Species	DO (g/liter)	Salinity (ppt)	Depth (cm)	pH	TIC (mM)	Turbidity (NTU)	Chlorophyll (μg/liter)	Nitrate (μM)	NH4 (μM)	PO4 (μM)	N
Halodule wrightii	6.4 [0.6–11]	31.2 [16.3–40]	91 [10–200]	7.2	1.9 [0.99–2.8]	71.1 [0–1,000]	460.8 [60.8–5,469]	0.572 [0.144–3.018]	0.328 [0.004–3.16]	0.12 [0.026–0.632]	158
Macroalgae	6.2 [0.5–9.7]	29.3 [0.6–38.9]	83 [20–170]	7.4	2.0 [0.5–2.9]	68 [0–282]	598.3 [75–2,631]	0.884 [.0154–9.078]	0.328 [0.004–3.41]	0.19 [0.029–3.99]	83
Ruppia maritima	6.6 [4.3–9.3]	26.5 [13–35]	63 [25–130]	7.1	2.0 [1–2.9]	72 [0–283]	671.9 [97–2,563]	0.704 [0.25–1.9]	0.394 [0.007–2.98]	0.068 [0.026–0.16]	28
Thalassia testudinum	6.8 [0.5–11.2]	32.3 [21.2–43]	100 [20–180]	7.2	1.8 [1.35–2.5]	72 [0–282]	293.7 [69–1,104]	0.690 [0.141–3.72]	0.176 [0.004–1.85]	0.151 [0.038–0.641]	26
Syringodium filiforme	6.7 [4.6–9.1]	33.1 [21.2–39]	110 [60–150]	6.8	1.97 [1.7–2.3]	158 [0–1,000]	435.6 [123–870]	0.882 [0.228–3.72]	0.135 [0.005–1.04]	0.328 [0.038–0.641	17
Halophyla englemanni	6.2 [4.9–8.4]	29.6 [25.5–39]	96 [35–180]	7.2	2.1 [1.7–2.5]	85 [0–159]	754.2 [77–2,631]	1.013 [0.228–2.29]	0.684 [0.009–3.41]	0.20 [0.009–3.41]	15
Vallisneria americana	8.3 [7.0–8.8]	3.9 [0.6–11.5]	64 [40–90]	6.8	0.8 [0.5–1.2]	6.5 [0.7–13.1]	1,411.1 [1,059–1,590]	1.098 [0.399–1.85]	0.657 [0.01–1.11]	0.043 [0.027–0.056]	4
Myriophyllum spicatum	8.0 [6.5–8.8]	1.4 [0.6–2.8]	83 [55–110]	6.8	0.5 [0.3–0.8]	6.7 [0.7–12.9]	1,095 [524–1,466]	1.052 [0.137–1.85]	1.075 [0.85–1.38]	0.063 [0.045–0.105]	4
Potamogeton pusillus	7.6 [6.5–8.8]	0.7 [0.6–0.7]	65 [55–75]	6.6	0.57 [0.32–0.82]	6.8 [12.9–0.65]	1,399 [1,333–1,466]	1.84 [1.82–1.85]	1.12 [0.85–1.38]	0.076 [0.047–0.105]	2
Heteranthera dubia	8.79 [8.8–8.8]	6.1 [11.5–0.7]	63 [50–75]	6.8	1.02 [1.22–0.82]	2.2 [3.83–0.65]	1,527.9 [1,590–1,466]	1.45 [1.04–1.85]	0.430 [0.01–0.85]	0.037 [0.027–0.047]	2
Potamogeton pectinatus	8.6	2.8	90	6.8	0.64	8.44	1,058.6	0.399	1.110	0.056	1

[a] DO = dissolved oxygen; TIC = total inorganic carbon; N = number of sites.

TABLE 2. Axis summary statistics of Canonical Correspondence Analysis on species (A) and environmental variable correlation (B) from a submerged aquatic vegetation survey in 2000.

	Axis 1	Axis 2	Axis 3
(A) Species			
Eigen value	0.799	0.248	0.171
Variance in species data			
% of variance explained	16.8	5.2	3.6
Cumulative % explained	16.8	22.0	25.6
Pearson correlation, Spp-Envt*	0.902	0.611	0.630
Kendall (Rank) correlation, Spp-Envt	0.323	0.325	0.254
(B) Environmental variable correlation			
Dissolved oxygen	0.244	0.005	−0.154
Temperature	−0.130	−0.250	0.054
Salinity	−0.861	0.023	0.005
Depth	−0.183	0.336	0.142
pH	−0.266	0.127	−0.027
Total inorganic carbon	−0.569	−0.206	−0.165
Chlorophyll	0.689	−0.060	−0.277
Nitrogen	0.183	0.121	−0.270
Ammonium	0.571	0.042	0.178
Phosphate	−0.103	0.135	0.036
Turbidity	−0.175	−0.083	0.058

Asch., *Syringodium filiforme* Kuetz., and *Thalassia testudinum* Banks & Sol. Ex Koening. Among the macroalgae we found *Caulerpa* spp., *Acetabularia crenulata* J.V.Lamouroux, and at least five other species of macroalgae. No nonnative macroalgae were discovered.

Vallisneria americana, *M. spicatum*, *P. pusillus*, *P. pectinatus*, and *H. dubia* were found in the upper portion of Mobile Bay, AL. *Vallisneria americana* and *H. dubia* were also found in Escambia Bay, FL. *Thalassia testudinum* was found at several locations along the Florida coast between Santa Rosa Sound and the Suwannee River, but was absent in Choctawhatchee Bay sites. *Halophila englemannii* was found in 15 sites, located between St. Marks National Wildlife Refuge (NWR) and Horseshoe Beach, FL. *Halodule wrightii* was the most abundant SAV in our survey, being found in more than half of the sites visited. *Syringodium filiforme* was found in sites between Santa Rosa Sound and St. Joe Bay and between Carrabelle River and St. Marks NWR in Florida. The only nonindigenous SAV found in the survey was *M. spicatum*, a well-documented invasive species (USGS, cited 23 Feb 2008). The salinities of the four *M. spicatum* occurrences ranged from 0.6 ppt to 2.8 ppt.

Salinity at sites with SAV ranged from a low of 0.6 ppt in upper Mobile Bay, AL, to 42.7 ppt in St. Joseph Bay, FL. Five survey sites had salinities of less than 3 ppt, five locations had salinities between 3 and 20 ppt, and all other locations (207) had salinities greater than 20 ppt. Because of drought conditions along the U.S. Gulf during the survey year (2000), most sites were at the higher end of their reported salinity range (Orlando et al., 1993).

The CCA was conducted on the 217 sites with SAV present. Results of CCA indicate that a total of 25.6% of the variance in the data is explained by three axes (Table 2A). The variables most strongly associated with Axis 1 are (negative) salinity, chlorophyll *a*, and ammonia. Variables associated with Axis 2 are depth and (negative) temperature, and variables associated with Axis 3 are (negative) chlorophyll *a* and (negative) nitrogen (Table 2B). Plots of these results show a clear separation of freshwater from marine–mesohaline species along Axis 1 (Fig. 1). Two marine–mesohaline species, *R. maritima* and *Caulerpa*, are separated from the rest of species in this group along the second axis (temperature and depth). The plot of Axes 2 and 3 presents a better separation of the freshwater species (10 of 217 sites) along the third axis, with *H. dubia* and *V. americana* toward high nitrogen and chlorophyll *a* and *P. pectinatus* and *M. spicatum* on low nitrogen and chlorophyll *a* sites (Fig. 2).

DISCUSSION

There are currently more than eight invasive nonindigenous species reported in freshwater systems in the survey area (USGS, cited 23 February 2008). The only nonindigenous SAV found in the survey was *M. spicatum*. *Caulerpa*

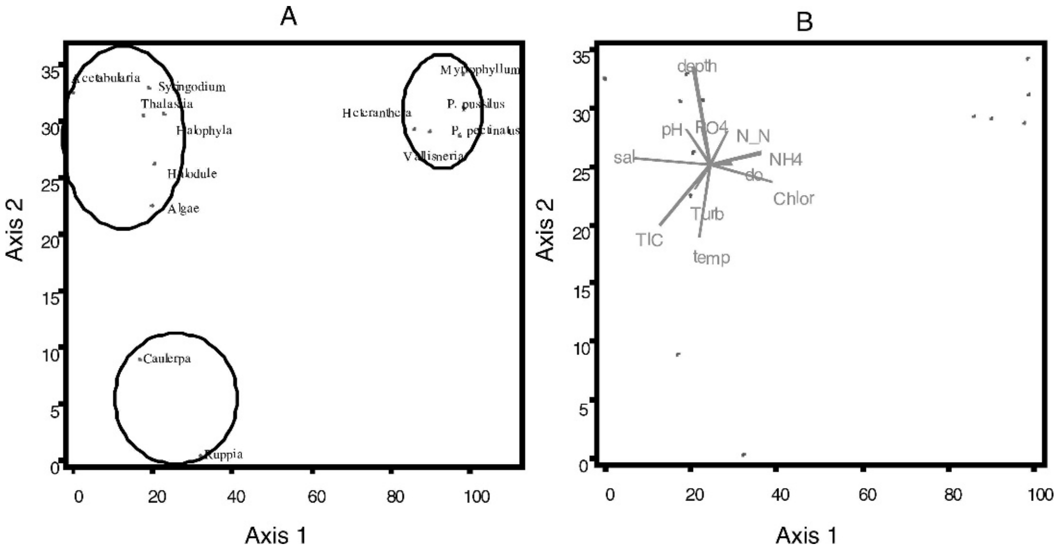

Fig. 1. Canonical Correspondence Analysis for all species in a summer survey of submerged aquatic vegetation. (A) The relationship of the species to the two dominate axes. (B) The relationship of the environmental variables to the dominant axes.

taxifolia is a new and aggressive macroalgae known to be invading coastlines of the world (Meinesz et al., 2001; Williams and Grosholz, 2002). Although we collected algae in the *Caulerpa* genus, *C. taxifolia* was not one of them. No nonindigenous macroalgae were discovered.

Ruppia maritima was the only SAV species that occurred in the mesohaline salinities of our survey. *Halodule wrightii* occurred in 73% of the beds. Nine other species occurred in no more than 13% of the beds. Even though 8 yr had elapsed between the aerial and ground surveys, 80.7% of the locations previously identified as having SAV beds still had them within 50 m of the reported location. This shows that the location of SAV beds can be stable in the highly variable conditions of their estuarine and near-shore environments.

We also find it interesting that the majority of the SAV beds sampled had plant communities

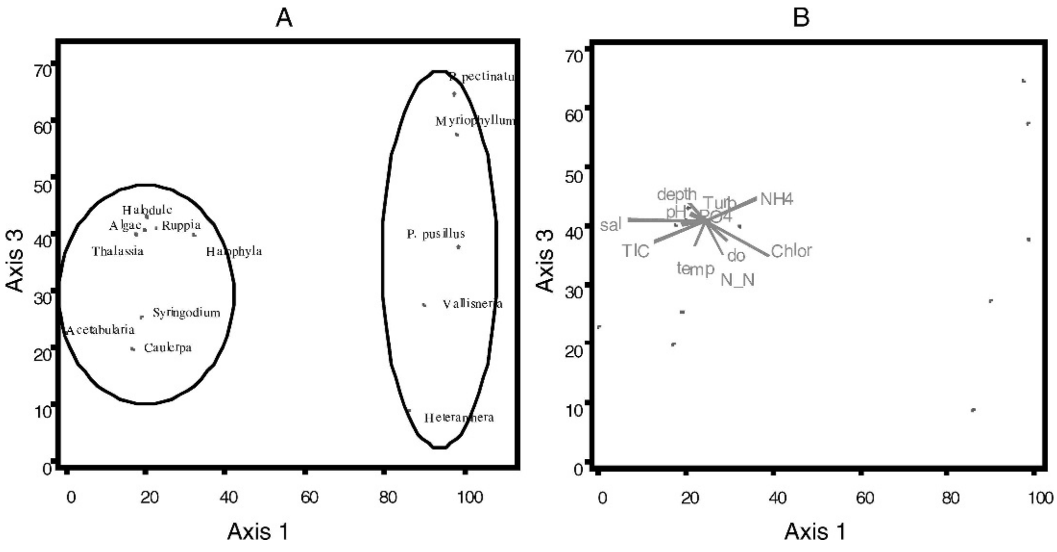

Fig. 2. Canonical Correspondence Analysis results for all species collected in a summer 2000 survey. (A) The relationship of species to the first and third axes. (B) The relationships of the environmental variables to those axes.

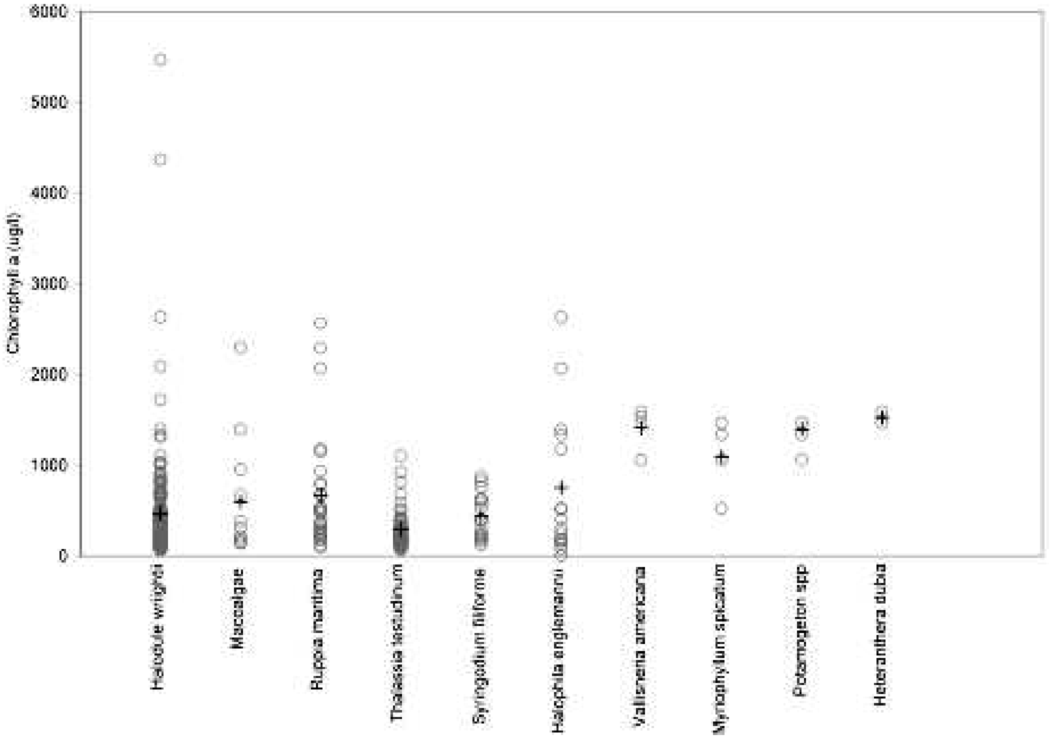

Fig. 3. Chlorophyll *a* concentrations for species of SAV in μg/liter. Crosses represent mean chlorophyll *a* concentrations. Mean chlorophyll *a* concentrations were higher for the four freshwater species on the right side of the figure.

typical of marine systems, although we tried to concentrate our samples in areas that were reportedly mesohaline during the growing season. There could be several explanations for this. First, marine species dominate areas that have mesohaline salinities during the growing season because these locations have higher salinities during other times of the year. Second, marine-adapted species replaced the mesohaline species in formally mesohaline areas that had become saltier. Third, the method used to identify SAV beds using aerial photography was inherently biased toward finding marine SAV beds, perhaps as a result of water clarity considerations. Fourth, the work by Orlando et al. (1993) was not reflective of the salinity conditions of the year during which the aerial photography was taken (1992) and of subsequent years. Any one or some combination of the above reasons might explain the discrepancy between measured and reported salinities and the presence of primarily seagrasses. Our measurements of mean chlorophyll *a* support the third explanation, that water quality inherently biased aerial photography toward finding seagrasses. High chlorophyll *a* content was measured for the few sites that contained fresher species (Fig. 3). While sample numbers were low for freshwater species, the CCA balances species frequency and also shows this by division of fresh and marine species along the axis with a strong association with chlorophyll *a* (Axis 1, Fig. 2). In contrast, the turbidities were highest at locations with seagrasses (Table 1).

Although our goal was to sample mesohaline sites, the high salinity of the majority of our sample sites resulted in few locations of the targeted salinity zone in our survey. Also, our survey was restricted to the western half of the northern Gulf, partly because that was the only area for which a complete and uniform aerial assessment of SAV was available at the beginning of this study. These facts highlighted the need to take a different approach for a synoptic survey of mesohaline SAV for the northern Gulf. Merino et al. (2009) describes that approach.

Using a method independent of aerial photography and historic salinities, we (Merino et al., 2009) sampled locations that have some overlap but incorporate more sites in the upper reaches of the estuaries. The geographic extent of these surveys prevents illustration of all sites in this paper. Mobile Bay, AL, and Apalachicola Bay, FL, are representative of the variability in

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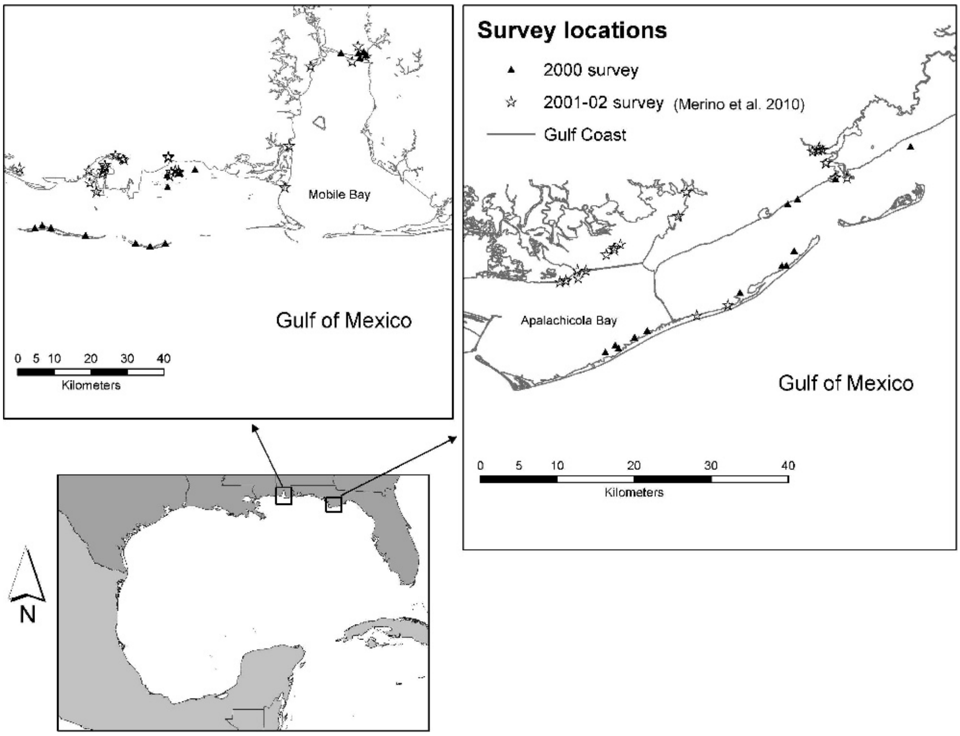

Fig. 4. Mobile Bay, AL, and Apalachicola Bay, FL, with sites of two surveys using different methods for site selection. 2000 = Survey in June and July of 2000 using stratified random site selection that relied on aerial photography and historic average salinity. 2001–02 = survey of June and July of 2001 and 2002 using a salinity gradient method of site selection.

site location (Fig. 4). In northern Mobile Bay, sample sites were similar in both surveys. However, this survey's traditional methodology in site selection produced more locations along barrier islands and open bays than did our subsequent survey (Merino et al., 2009), which was independent of aerial photography and historical salinity (Fig. 4).

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Appendix

We commissioned the librarian of the USGS National Wetlands Research Center to conduct a search of relevant databases using the search terms 'survey,' 'Gulf of Mexico,' 'seagrass,' 'submerged aquatic vegetation,' 'submersed aquatic vegetation,' and 'SAV.' The search did not turn up surveys of SAV in mesohaline systems in the northern Gulf of Mexico during the last 20 yr (1989–2009).

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