APPENDIX 3

Wetland characteristics based on diatom inferences: a comparison of TLP (thin layer placement) and control sites located in Cape May peninsula and Delaware Bay

Summary

Surface sediment samples were collected from wetlands located on the northern shore of Delaware Bay and Atlantic coast of Cape May peninsula and analyzed for diatom species composition. A total of twenty (20) samples were collected from sites located in the vicinity of existing surface elevation tables (SETs) from Fortescue, Avalon, Ring Island and Dias Headwaters salt marshes. Based on the New Jersey wetland diatom flora published on NJDEP website, more than 200 species were identified in the twenty study sites. Diatom-based models have been previously developed for inferring salinity, nitrogen sediment content, and tidal exposure in New Jersey (NJ) salt marshes by Desianti *et coll*. Based on the species composition and relative abundances, these models were used to infer nutrient (TN) sediment concentration, water salinity and wetland tidal exposure indices. The tidal exposure is represented by the Tidal Exposure and Standardized Water Level Indices – TEI and SWLI. TEI represents the portion of time a site is above the tide level where supratidal samples have a zero TEI value, the mean tidal level has a value of 50, and submerged sites have a TEI value of 100. SWLI estimates salt marsh elevation relative to tidal level; SWLI with the value 0 represents salt marsh elevation at mean lower low water and 1 at mean higher high water. Based upon these diatom-based inferences, a comparison was conducted to examine differences in wetland characteristics between sites that were located inside the thin layer placement (TLP) and outside the TLP (control) areas for the Fortescue, Avalon, and Ring Island salt marshes. The Dias Headwaters marshes didn't undergo a TLP. Fortescue sites were represented by both high and low marsh sites while Avalon and Ring Island sites were all located within low marsh areas.

Fortescue high marsh sites were represented by one (1) TLP and one control site only. Inferences for these sites revealed that the TLP site is characterized by higher sediment TN and SWLI, but lower salinity and TEI, particularly TEI was 50% lower than the control site. A slightly lower sediment TN, TEI and SWLI were found for the low marsh sites and higher salinity. Both TLP and control areas were represented by three (3) sites within each category. These inferences suggest that the TLP high marsh site is exhibiting increased marsh elevation and less impacts from tide activity compared to the control site, while the low marsh TLP sites did not produce a similar positive result.

Avalon sites were all low marsh with three (3) TLP and three control sites. The diatom-based inferences revealed slight increase in salinity and TEI, while SWLI and TN recorded slight decreases in TLP sites. These results appear to suggest the Avalon TLP sites are under higher tidal influence than control sites, and likely the TLP is not yet producing the expected protection from sea level rise (SLR) for these sites.

The Ring Island sites were also represented by three TLP and three control low marsh sites. The diatom-based inferences revealed even more pronounced differences between TLP and control sites, with the TLP sites experiencing increased SLR effects concretized in increased TEI and salinity and with lower TN and SWLI than the control sites. The differences observed in Ring Island were statistically significant suggesting higher exposure of TLP sites to tides and SLR, while the differences in inferred values for TLP and control sites in Fortescue and Avalon were not statistically significant. However, more sampling sites would be necessary for drawing a more accurate assessment of differences between the TLP and control marsh characteristics.

The Dias Headwater sites did not have a thin layer placement and exhibited similar species composition with variations in relative abundances between the two sites and also slight differences in the inferred TN, TEI, and salinity.

This investigation concludes that diatoms can provide an additional, independent tool with multiple possible applications in marsh condition assessment and monitoring.

Sampling sites and Sample collection

Samples comprising 10-cm square soil portions with a ~1-cm thickness were collected from wetland surface sediments located in the vicinity of existing surface elevation tables (SETs) (Figure 1). List of sampling sites is provided in Table 1. Fortescue and Dias Headwaters sites are located on the northern shore of Delaware Bay, while Avalon and Ring Island sites were located on the Atlantic Coast of Cape May peninsula. All samples were collected by The Nature Conservancy (TNC).

Table 1. List of sampling sites, geographic coordinates and habitat characteristics.

Sample code	Abbreviation	Lat	Long	Habitat	Vegetation
Fortescue FT-C-A-1	FT-C-A-1	39.24394	-75.17239	high marsh	S. alt, S. pat, Salicornia
Fortescue FT-C-B-17	FT-C-B-17	39.24797	-75.18142	low marsh	S. alterniflora
Fortescue FT-C-B-19	FT-C-B-19	39.24845	-75.18042	low marsh	S. alterniflora
Fortescue FT-P-1-44	FT-P-1-44	39.24693	-75.17788	low marsh	S. alterniflora
Fortescue FT-P-1-48	FT-P-1-48	39.24619	-75.17913	high marsh	Salicornia, S. alt (tall and short), Distichlis
Fortescue FT-P-2-37	FT-P-2-37	39.24864	-75.17554	low marsh	Salicornia, S. alt (tall and short)
Avalon AV-C-A-9	AV-C-A-9	39.07389	-74.75834	low marsh	S. alterniflora
Avalon AV-C-A-27	AV-C-A-27	39.07404	-74.76135	low marsh	S. alterniflora
Avalon AV-C-G-95	AV-C-G-95	39.08474	-74.7545	low marsh	S. alterniflora
Avalon AV-P-D-53	AV-P-D-53	39.08207	-74.7609	low marsh	Salicornia, Spa Alt
Avalon AV-P-E-72	AV-P-E-72	39.08143	-74.75674	low marsh	S. alterniflora
Avalon AV-P-F-88	AV-P-F-88	39.07886	-74.75484	low marsh	S. alterniflora
Ring Island RI-C-TLPC-4	RI-C-TLPC-4	39.05104	-74.77634	low marsh	S. alterniflora
Ring Island RI-C-TLPC-7	RI-C-TLPC-7	39.05076	-74.77654	low marsh	S. alterniflora
Ring Island RI-C-TLPC-15	RI-C-TLPC-15	39.05032	-74.7767	low marsh	S. alterniflora
Ring Island RI-P-TLP1-18	RI-P-TLP1-18	39.05362	-74.77312	low marsh	S. alterniflora
Ring Island RI-P-TLP2-6	RI-P-TLP2-6	39.05201	-74.77489	low marsh	S. alterniflora
Ring Island RI-P-TLP2-7	RI-P-TLP2-7	39.05186	-74.77515	low marsh	S. alterniflora
Dias Headwaters 2	DH-2	39.08100	-74.88400	low marsh	S.alt. , S.patens
Dias Headwaters 3	DH-3	39.07200	-74.88000	low marsh	S. alterniflora tall form

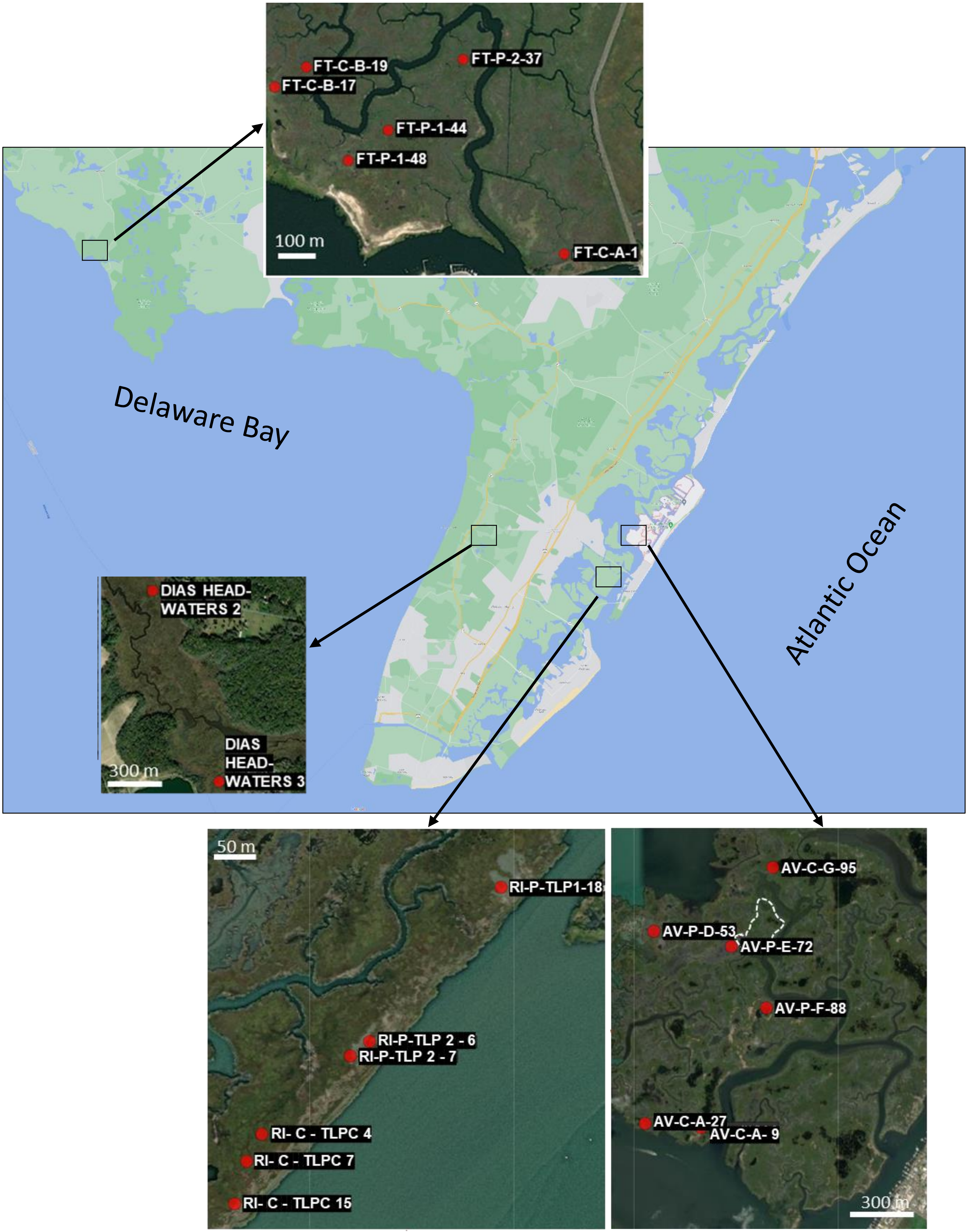

Figure 1. Location of sampling sites. Sites full names (codes) are provided in Table 1.

Diatom assemblages and inferences

Sediment samples were subsampled for ~1g wet sediment and digested in 70% nitric acid to remove organic matter content. Samples were then repeatedly rinsed with reverse osmosis (RO) water until near-neutral pH. Approximately 20 to 1000 µL of the cleaned sample containing diatom frustules were dripped onto coverslips and air-dried. Coverslips were then mounted on microscope slides using Naphrax™ mounting medium (Brunel Microscopes, U.K.). All diatom taxa identifications were made to species/variety level whenever possible using primarily the "Diatom Flora of the New Jersey Coastal Wetlands" identification guidebook (Desianti and Potapova 2019). Some small-celled taxa within the genera of *Chaetoceros* and *Minidiscus* could not be identified to species level under light microscopy and they were lumped in the "*Chaetoceros* spp.", and "*Minidiscus* spp." categories.

Diatom transfer functions, also named inference models were previously developed by Desianti et al. (2019) and were used in this study to calculate total nitrogen sediment content (TN, %dry weight), salinity, Standardized Water Level Index (SWLI), and Tidal Exposure Index (TEI) values. The values of salinity in the training dataset range from 0 to 32.1 practical salinity unit (psu), SWLI range from 0 to 1.6, TEI range from 1 to 100, and TN range from 0 to 2.32 (% DW) (Desianti et al. 2019). The SWLI model is used to estimate salt marsh elevation relative to tidal level. SWLI with a value of 0 represents salt marsh elevation at mean lower low water and 1 at mean higher high water (Kemp et al. 2013). TEI represents the portion of time a site is above the tide level where supratidal samples have a zero TEI value, samples at mean tidal level have a TEI value of 50 (Sawai et al. 2016) and samples submerged permanently have a value of 100.

More than 250 diatom species were identified in the 20 study sites (APPENDIX I). However, most of these species were present in low abundances or very rare. Only 35 species reached abundances more than 5% in at least one site. Across all sites, highest abundances were reached by *Navicula* sp. 63 (44%), *Denticula* subtilis (28%) and *Navicula* sp. 41 (26%). *Navicula* sp. 63, a species characteristic of high marsh habitat reached highest abundance in Fortescue FT-P-1-48, a high marsh TLP site. *Denticula subtilis*, a species related to lower tidal exposure reached highest abundances in Ring Island control sites RI-C-TLPC-4 and RI-C-TLPC-7. *Navicula* sp. 41 reached highest abundances in Ring Island TLP sites RI-P-TLP2-6 and RI-P-TLP2-7, associated with *Navicula salinicola* and other species attesting of lower elevation and/or higher tidal impacts. However, the Ring Island TLP sites had lower diatom abundances overall, likely due to higher tide/wave activity, possibly coarser substrates with poorer diatom population establishment after thin layer placement.

Diatom species composition and the inferred sediment TN, Salinity, TEI and SWLI in Fortescue TLP and control sites

Fortescue high marsh TLP and control sites were represented by one (1) sampling site each: Fortescue FT-C-A-1 and FT-P-1-48 (Fig. 1). The control site is characterized by a diverse diatom species composition, with highest abundances for *Navicula salinicola*, *Navicula* sp. 63 and *Planothidium* cf. *frequentissimum,* species that are typical of high marsh diatom associations previously found in Delaware Bay (Desianti et al. 2019). With lower abundances were also present *Denticula subtilis*, *Minidiscus proshkinae*, *Chamaepinnularia* aff. *begeri* (see APPENDIX 1 for full count results; Fig. 2). The TLP high marsh site displays changes in species composition with highest increase in *Navicula* sp. 63 relative abundance from ~12% (in control site) to 44%. Navicula salinicola strongly declines, from ~12% to 2% relative abundance while *Halamphora aponina*, *Nitzschia scalpelliformis*, *Nitzschia sp. 25* reach moderate or higher abundances. These species composition changes, especially the high increase in *Navicula* sp. 63 abundance, are implicitly reflecting different conditions existing in the TLP high marsh site.

The diatom inferences for these sites revealed that the high marsh TLP site is characterized by higher sediment TN and SWLI, but lower salinity and TEI, particularly TEI was 50% lower than in the control site (Fig. 2). These changes could be related either to the pre-existing difference in the location and tide exposure of the two sites, or due to the effect of the TLP protection.

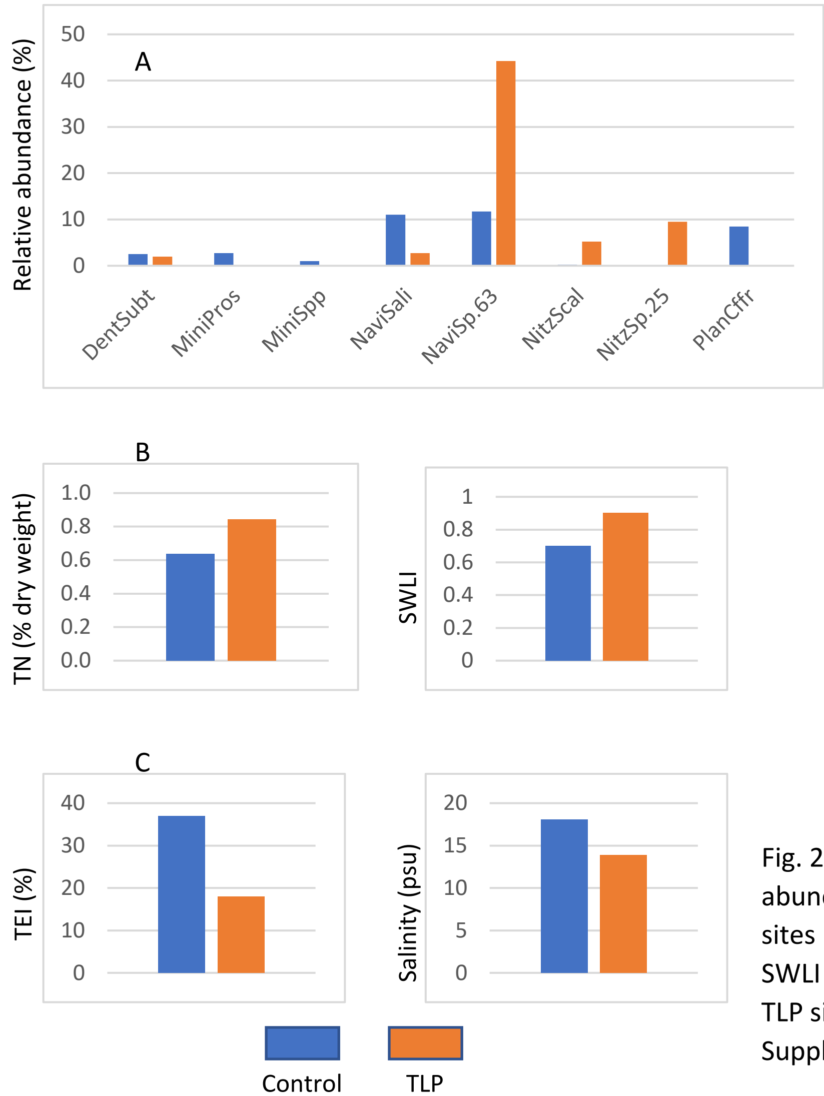

Fig. 2. Diatom species with relative abundances >5% in Fortescue high marsh sites (A); inferred TN sediment content and SWLI (B); TEI and salinity (C) in control and TLP sites. Full species names are found in Supplementary material I.

The Fortescue low marsh TLP and control sites were represented by 2 sites each: Fortescue FT-C-B-17, FT-C-B-19 for control sites and FT-P-1-44, FT-P-2-37 for the TLP sites (Fig. 1). The two control sites were represented by same species, although their abundances varied between the two sites. For example, most abundant species were *Denticula subtilis*, *Navicula* sp. 63, and *Planothidium* cf. *frequentissimum* (FT-C-B-19), and *Minidiscus proschkinae*, *Minidiscus* spp in FT-C-B-17; the above-mentioned FT-C-B-19 species are also present in FT-C-B-17 but with lower abundances. These differences in species relative abundance suggest that FT-C-B-17 receives more tidal input than the amont FT-C-B-19 site, although the two sites are close to each other (Fig. 1). These differences are also attesting the ability of New Jersey coastal diatoms to discriminate between microtidal marshes and their utility as indicators of relative sea level, as found in the previous study of New Jersey coastal diatoms by Desianti et al. (2019).

The TLP sites were represented by similar species composition, with *Navicula* sp. 63 and *Nitzschia* sp. 25 reaching highest abundances. Diatom-based inferences found a slightly lower sediment TN, TEI and SWLI suggesting that the TLP did not produce significant improvement in marsh elevation. However, the inferred differences between TLP and control sites were not statistically significant at $p<0.05$.

The averaged diatom species abundances, sediment TN, SWLI, salinity and TEI in Fortescue control and TLP low marsh sites are shown in Fig. 3.

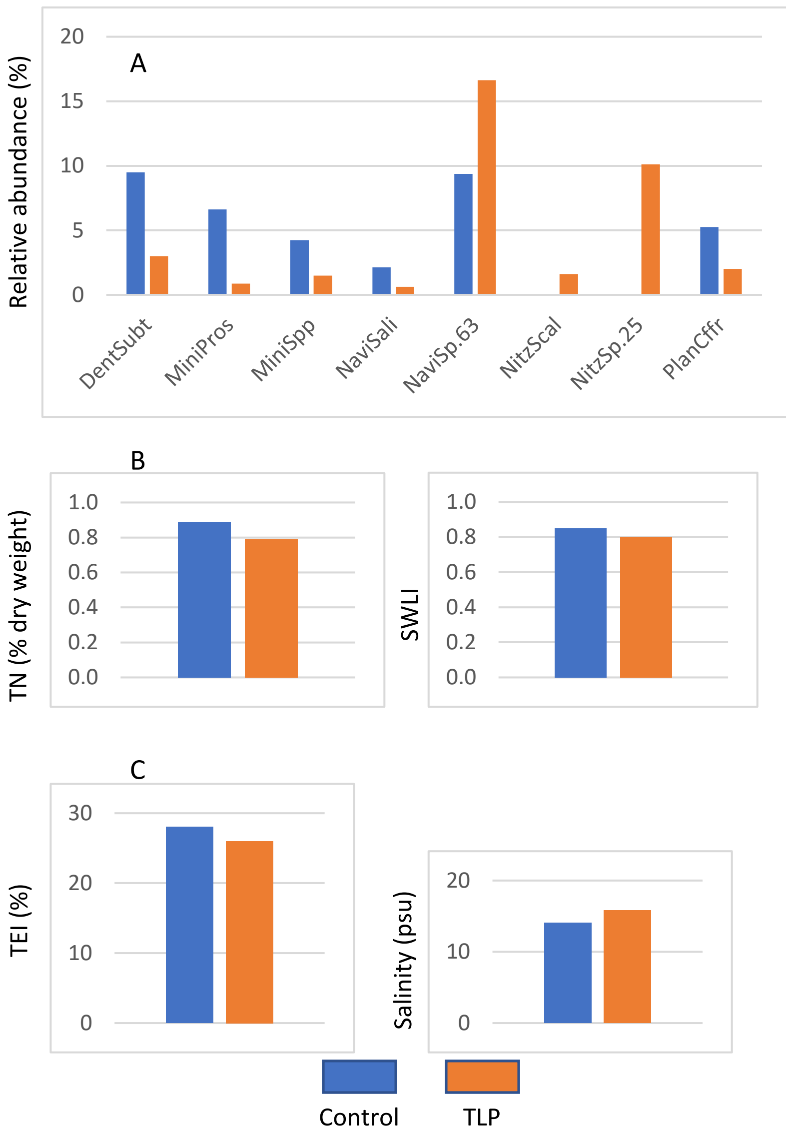

Fig. 3. Diatom species with relative abundances >5% in Fortescue low marsh sites (A); inferred TN sediment content and SWLI (B); TEI and salinity (C) in control and TLP sites. Full species names are found in Supplementary material I.

Diatom species composition and the inferred sediment TN, Salinity, TEI and SWLI in Avalon TLP and control sites

Avalon TLP and control sites were represented by low marsh sites only, three sites were sampled for each category: Avalon AV-C-A-9, AV-C-A-27, AV-C-G-95 (control sites) and Avalon AV-P-D-53, AV-P-E-72, AV-P-F-88 (TLP sites) (Fig. 1). The Avalon AV-C-A-9, AV-C-A-27 control sites had similar species composition with *Denticula subtilis* and *Navicula* sp. 63 dominance. AV-P-F-95 was represented by species attesting of higher tidal exposure, such as *Nitzschia scalpelliformis* and *Halamphora tenerrima*.

The Avalon TLP sites AV-P-E-72 and AV-P-F-88 shared similar abundances for *Cocconeis stauroneiformis* but with differences in the relative abundances of other species, such as *Pseudostaurosira perminuta*, found abundant only in AV-P-E-72. The Avalon AV-P-D-53 site has a species composition with higher abundances for *Denticula subtilis, Navicula* sp. 63, and *Planothidium* cf. *frequentissimum*, species which are attesting of a higher elevation and lower impact from tides.

Diatom inferences showed a lower inferred TN sediment content and SWLI, higher salinity and TEI for the TLP sites in average. These inferences suggest that the TLP sites receive higher impacts from tides activity and SLR in comparison to the control sites. However, these differences were not statistically significant at $p<0.05$.

The averaged diatom species abundances, sediment TN, SWLI, salinity and TEI in Avalon control and TLP low marsh sites are shown in Fig. 4.

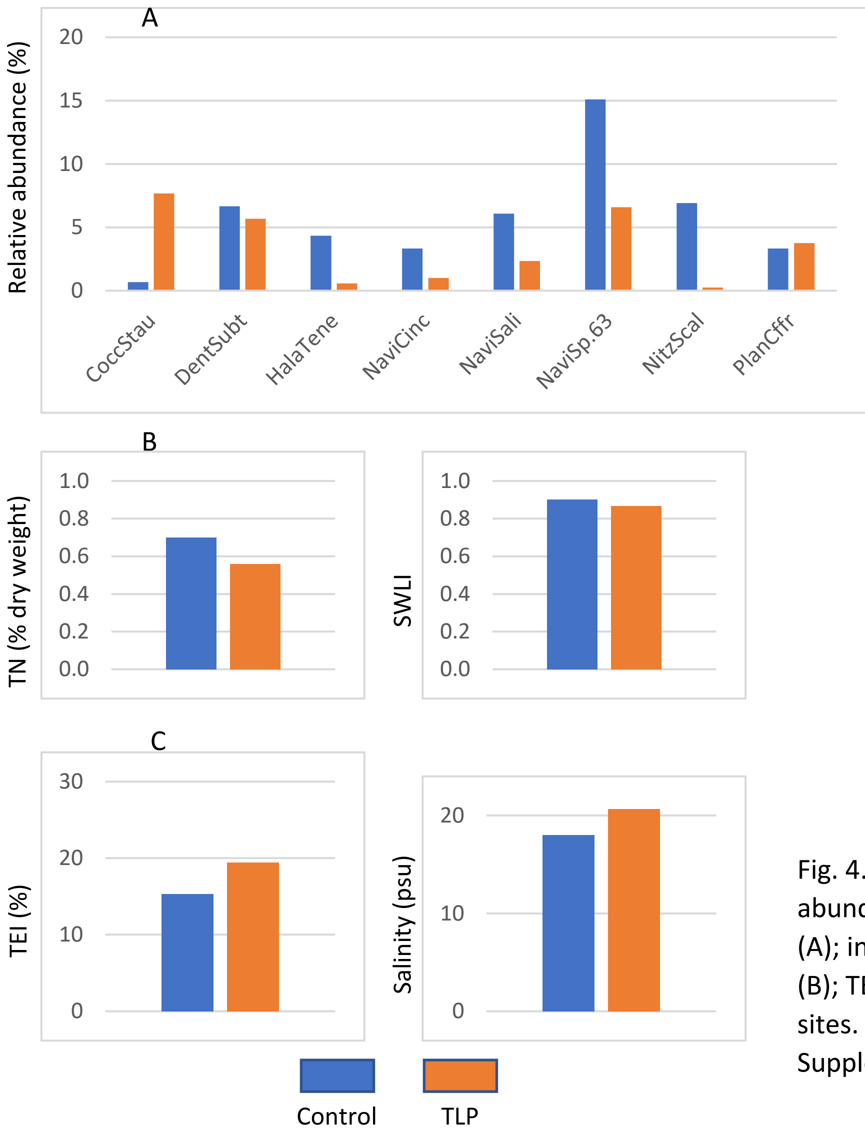

Fig. 4. Diatom species with relative abundances >5% in Avalon low marsh sites (A); inferred TN sediment content and SWLI (B); TEI and salinity (C) in control and TLP sites. Full species names are found in Supplementary material I.

Diatom species composition and the inferred sediment TN, Salinity, TEI and SWLI in Ring Island TLP and control sites

Ring Island TLP and control sites were represented by low marsh sites with three sites sampled for each category: Ring Island RI-C-TLPC-4, RI-C-TLPC-7, RI-C-TLPC-15 (control sites) and Ring Island RI-P-TLP1-18, RI-P-TLP2-6, RI-P-TLP2-7 (TLP sites) (Fig. 1). All Ring Island control sites had similar species composition with *Denticula subtilis* and *Navicula* sp. 63 dominant species, associated to lower abundances of *Planothidium* sp. 11 and *Planothidium delicatulum*. The TLP sites RI-P-TLP2-6 and RI-P-TLP2-7 had very similar species composition with *Navicula* sp. 41 the dominant species (up to 27%). The RI-P-TLP1-18 site had higher species diversity with *Navicula* sp. 63 and *Paralia sulcata* reaching slightly above 5% relative abundance (APPENDIX I).

Diatom inferences showed that TLP sites have on average significantly lower TN sediment content, lower SWLI, significantly higher salinity and TEI at $p<0.05$. These inferences suggest that the TLP sites receive higher impacts from tides activity and SLR in comparison to the control sites.

The averaged diatom species abundances, sediment TN, SWLI, salinity and TEI in Ring Island for control and TLP low marsh sites are shown in Fig. 5.

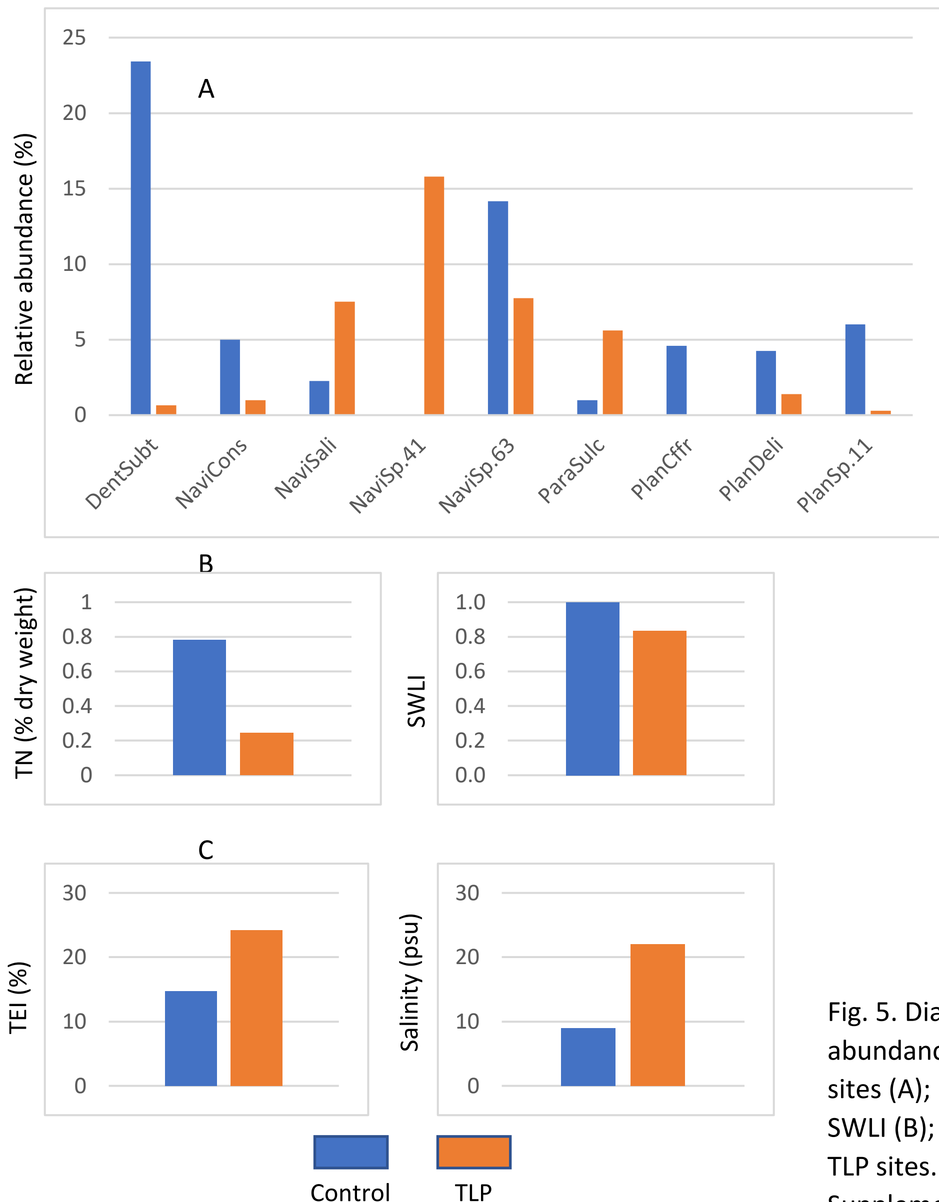

Fig. 5. Diatom species with relative abundances > ~5% in Ring Island low marsh sites (A); inferred TN sediment content and SWLI (B); TEI and salinity (C) in control and TLP sites. Full species names are found in Supplementary material I.

Diatom species composition and the inferred sediment TN, Salinity, TEI and SWLI in Dias Headwaters

Two sites (DH-2 and DH-3) were sampled from Dias Headwaters wetlands, situated approximately 1 mile distance from each other. Although with very similar species composition, the relative abundances of many of these species are different between the two sites (Fig. 6). Notably, DH-3 site has higher abundances of *Navicula* sp.63, *Denticula subtilis*, *Triblionella debilis*, etc. Although inferred SWLI is similar for both sites, DH-3 has slightly higher TN and lower salinity and TEI suggesting that this site is receiving lower influence from tides and SLR.

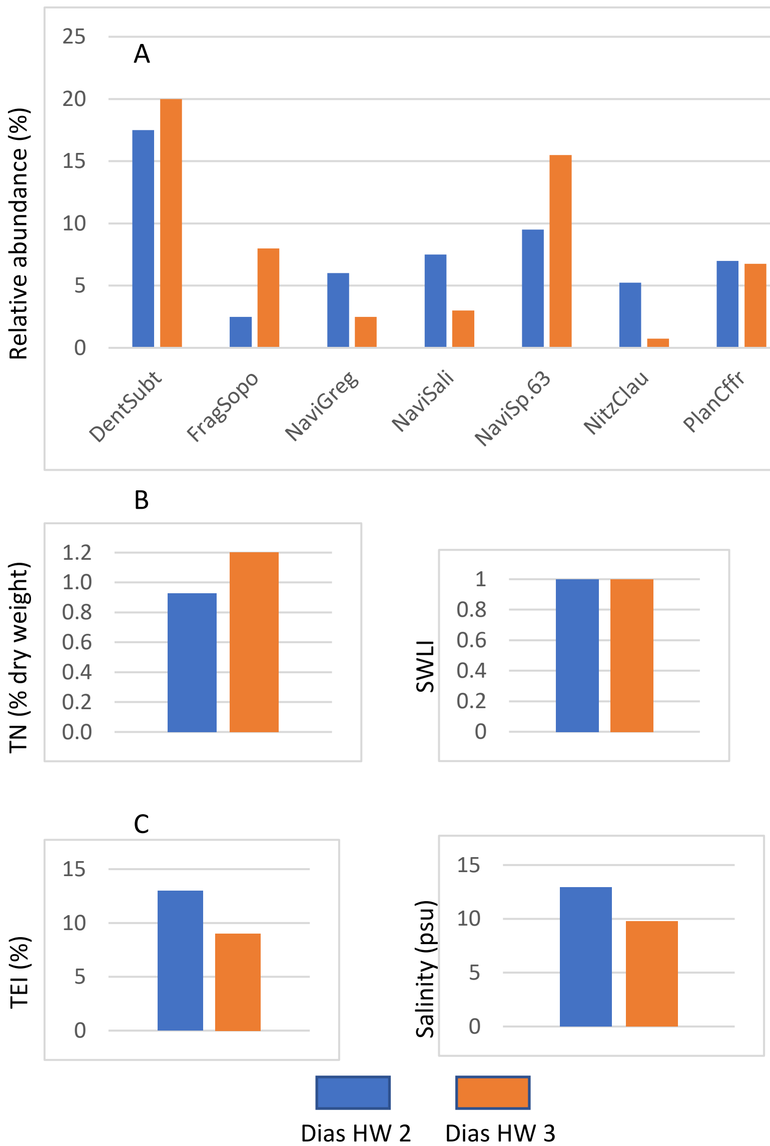

Fig. 6. Diatom species with relative abundances > 5% in Dias Headwaters sites (A); inferred TN sediment content and SWLI (B); TEI and salinity (C) in DH-2 and DH-3 sites. Full species names are found in Supplementary material I.

Conclusion

Diatom species identified in the 20 sampling sites from Delaware Bay and Cape May peninsula were used to compute inferences for wetlands TN sediment content, TEI, SWLI, and salinity. The diatom species composition and computed inferences were used to compare the characteristics of TLP and control wetland sites. This comparison revealed that the Fortescue TLP site is less impacted by tidal activity, while Avalon and especially Ring Island TLP sites appeared more disturbed by tidal activity than control site. Ring Island TLP sites had lower diatom abundances while presence of species attesting of increased tidal activity and SLR impacts compared to the control sites was found in both Avalon and Ring Island TLP sites. The differences found between TLP and control sites were significant ($p<0.05$) in Ring Island, but not in Fortescue and Avalon wetlands. However, wetland conditions prior to thin layer placement in control and TLP sites are unknown and the extent to which these inferred differences may be related to pre-TLP wetland conditions are not known. This investigation was based on a small number of sites. More sampling sites would be necessary for drawing a more accurate assessment of differences between TLP and control wetland condition.

The diatom-based inferences for tidal exposure, salinity and TN can provide an assessment tool to wetland managers for the identification of most vulnerable sites in need of protection. Due to their high sensitivity to New Jersey wetlands microtidal differences, diatom species can also be useful in evaluating and monitoring the rate of success of wetland restoration projects.

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