Hot and fresh: Pervasive climate stressors of seagrass in a large Gulf coast estuary

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## Abstract

## 1 Introduction

* Importance of seagrass as foundation species
* Tampa Bay context
* Climate change stressors
* Goals/objectives
  + Describe

Background: SST trends in Tampa Bay w/ EPC data, WFS, and deep GOM (Nickerson et al. 2023) showed that 1975 to 2022 trend for EPC was notable, but less so from 1998 to 2022. Also noted the trend was most pronounced in the winter, see Fig 3.

Good CC references for FL: https://www.mdpi.com/2071-1050/15/14/11364

Heat stress combined with highlight accelerates decline of *E. acoroides*, used 36C (Zhang et al. 2023)

Widgeongrass colonization in Chesapeake Bay as an opportunistic, heat-tolerant species that has replaced Eelgrass, although former is sensitive to nutrient pulses. Paper provides an example of implications on climate change and nutrient management on foundation species and system resiliency (Hensel et al. 2023).

Lewis III et al. (1985) review of seagrass in Tampa Bay. Lirman and Cropper (2003) conducted exposure experiments to evaluate seagrass growth in response to a range of salinity conditions. McMillan and Moseley (1967) discusses growth of halodule, syringodium, thalassia, and ruppia in response to salinity increases (up to 75 psu), no info on lower limit. Cites Phillips (1960) for a salinity range of Thalassia in Florida of 33 - 38 psu. Zieman (1975) discusses seasonal variation of thalassia relative to temp and salinity

Focus on two time periods: full record and recent decline

Focus on two physical parameters: temperature and salinity

Temp background for TB: NOAA coastwatch trends

## 2 Methods

### 2.1 Study area

Tampa Bay is the largest open-water estuary in Florida covering 400 mi (1,036 km) and one of the largest in the Gulf of Mexico. The watershed covers an additional 2,200 mi (5,872 km) with the Hillsborough, Alafia, Manatee, and Little Manatee Rivers contributing a majority of freshwater inflow to the bay. Tampa Bay straddles the temperate and tropical boundary of central Florida characterized by warm, humid conditions and a distinct rainy season during the summer months. The watershed is heavily developed and includes over 3 million people with 42% of the land as urban or suburban contributing substantial inputs of wastewater and stormwater runoff that can stress bay resources (M. W. Beck et al. 2023). The geology of the watershed is rich in phosphates and mining activities have greatly altered the landscape, with notable spills and releases of wastewater that have affected water quality and biological resources (Garrett et al. 2011; M. W. Beck, Altieri, et al. 2022). Important subtidal habitats include seagrasses, hard bottom, tidal flats, and oyster reefs, where a majority of management effort has focused on restoring and maintaining seagrass cover (E. T. Sherwood et al. 2017). Additional native habitats include intertidal wetlands (mangroves, salt marshes, salt barriers) and pine forests, oak hammocks, and freshwater wetlands present in upland habitats (Robison et al. 2020). Losses of native uplands and potentially restorable habitats to development in the watershed from 1990 to 2020 have been estimated at 188,429 acres (76,254 ha, M. W. Beck et al. 2023).

Tampa Bay is divided into distinct sub-segments defined by physical and natural boundaries to assist with water quality management activities (Lewis III et al. 1985): Old Tampa Bay (OTB) in the northwest; Hillsborough Bay (HB) in the northeast; Middle Tampa Bay (MTB); and Lower Tampa Bay (LTB) that connects to the Gulf of Mexico. Old Tampa Bay and Hillsborough Bay have historically had the most degraded water quality conditions primarily from nutrient inputs from wastewater and stormwater (H. Greening et al. 2014). Hydrologic conditions vary between the two, such that Hillsborough Bay receives a majority of direct surface water inflow from the Hillsborough and Alafia Rivers, whereas Old Tampa Bay receives much less inflow with a majority from multiple small tributaries (Janicki Environmental, Inc. 2023). Notably, Old Tampa Bay has restricted circulation from multiple land bridges that traverse the bay (E. Sherwood et al. 2015; M.E. Luther, S.D. Meyers 2022). Recurring seasonal harmful algal blooms of the dinoflagellete *Pyrodinium bahamense* have contributed to exceedances of the chlorophyll-a regulatory standard in Old Tampa Bay (Cary B. Lopez et al. 2023). By comparison, water quality conditions in Middle Tampa Bay and Lower Tampa Bay are generally better than the upper two bay segments primarily from more frequent water exchanges with the Gulf of Mexico and lower nutrient loading (Janicki Environmental, Inc. 2023). All bay segments are relatively shallow, with a baywide mean depth of approximately 3 m. Light penetration typically reaches bottom habitats under current conditions, although seagrasses were historically limited by high phytoplankton production that affected light environments prior to wastewater regulation, particularly in Old Tampa Bay and Hillsborough Bay (H. Greening et al. 2014; Johansson and Janicki Environmental, Inc. 2015).

### 2.2 Seagrass change in Tampa Bay

The long-term recovery of seagrass habitats in Tampa Bay is a nationally-recognized success story that demonstrates application a successful management paradigm through the National Estuary Program (Holly Greening and Janicki 2006; H. Greening et al. 2014; E. T. Sherwood et al. 2017). From 1988 to 2016, seagrasses increased 79% to a total areal cover of 41,655 acres (16,857 ha), surpassing the goal of restoring coverage to 95% of that which occurred in 1950 (Figure [1](#fig-seagrasschg)a). Throughout this same period, nitrogen load estimates decreased by about 2/3 from their peak estimated in the mid-1970s as 8.9 x 10 kg/year, largely from advanced wastewater treatment upgrades and in part from the cumulative effects of habitat restoration projects in the watershed (H. Greening et al. 2014; M. W. Beck et al. 2019). These reductions in nutrient loadings resulted in largescale reductions in chlorophyll concentrations and light attenuation in the water column, creating favorable environments for seagrass growth. The most dramatic improvements in seagrass cover were observed in Old Tampa Bay where coverage increased by 122% or 4,465 acres (2,477 ha) to a total of 11,247 acres (4,511 ha) from 1988 estimates. Similar gains were observed in Middle Tampa Bay where seagrass cover increased by 86% or 4,465 acres (1,807 ha) to a total of 9,652 acres (3,906 ha) and Hillsborough Bay where cover increased from nearly zero acres to a total of 2,007 acres (810 ha). Seagrasses have generally been stable over time in Lower Tampa Bay.

From 2016 to present, dramatic losses of seagrasses have been observed in Tampa Bay, despite water quality conditions remaining relatively stable (Figure [1](#fig-seagrasschg)a). Total cover in Tampa Bay has decreased by 28% from the 2016 peak by 11,518 acres (4,661 ha) to ta total of 30,137 acres (12,196 ha). Losses have been most pronounced in Old Tampa Bay (62%; 6,963acres/2,818 ha loss) and Hillsborough Bay (80%; 1,599acres/647 ha loss). The current estimate for Old Tampa Bay of 4,183 acres (1,693 ha) is the lowest ever recorded in that bay segment since mapping efforts began in the 1980s. Similarly, coverage in Middle Tampa Bay decreased by 20% (1,926 acres/779 ha loss). Coverage in Lower Tampa Bay has remained stable, with only a 2% loss that is likely within the mapping error for the coverage estimates.

### 2.3 Seagrass data

Two primary sources of data have been used to track seagrass change over time in Tampa Bay. The Southwest Florida Water Management District (SWFWMD) has estimated areal coverage of seagrasses approximately biennially since the late 1980s (e.g., Southwest Florida Water Management District 2023). These maps are created by photointerpretation of aerial images obtained at the end of the growing season, typically during November-December. The TBEP has used these maps to track progress towards achieving seagrass restoration goals as total cover in Tampa Bay. No species information is provided. A more detailed, but spatially-specific, data source is the Tampa Bay Interagency Seagrass Monitoring Program (Figure [1](#fig-seagrasschg)b, <https://tampabay.wateratlas.usf.edu/seagrass-monitoring/>). Annual transect surveys have been conducted since 1998 at 61 fixed locations in Tampa Bay, many of which were chosen to target seagrass beds of interest (Johansson 2016; E. T. Sherwood et al. 2017). This dataset provides species information, including abundance, cover, frequency occurrence, and condition, collected at fixed meter marks along a transect extending from the shoreline to the deepwater edge of the seagrass bed. Although the areal maps provide the standard for assessment of restoration goals, the transect data allow for inter-annual comparison at greater temporal resolution, particularly for the recent period of interest when seagrasses have declined. As such, the transect data were used below for comparison with temperature and salinity changes for the major bay segments. Other sources of seagrass data are described in the next section.

### 2.4 Water quality data

Several datasets with distinct sample designs are available to assess long-term changes in water temperature and salinity in Tampa Bay. These datasets were evaluated individually to assess trends and relationships with seagrass change to provide a weight-of-evidence approach for potential causal relationships driving the recent decline. First, the Environmental Protection Commission (EPC) of Hillsborough County has collected discrete water quality measurements monthly at 45 stations in the major bay segments since the late 1970s. These data provide the basis for regulatory assessments and compliance reporting for the nutrient TMDL in Tampa Bay. Water quality samples are collected at each station from surface water grabs (e.g., nutrients) or *in situ* measurements for physical parameters (e.g., salinity, temperature), where the latter includes measurements at the surface, mid-depth, and bottom. Time of sampling can vary, although most samples are collected from mid-morning to early afternoon. All surface and bottom salinity and temperature measurements for each of the 45 monitoring stations were evaluated herein. Trends were assessed for both surface and bottom samples, as described below, whereas only the bottom measurements were used for comparison to seagrass trends. The data were obtained using the tbeptools R package that imports the data directly from a stable web address provided by the EPC (M. Beck et al. 2021).

The second dataset used to evaluate water quality trends was available from the Florida Fish and Wildlife Conservation Commission (FWC). The Fisheries Independent Monitoring (FIM) program administered by FWC provides monthly surveys of the entire nekton community in Tampa Bay, including species richness and abundance, using multiple survey gear types that target different habitats. A stratified sample design is used to target multiple habitats where unique sites are sampled each month. We used data from the 21.3 meter center-bag nearshore seine that specifically targets shallow habitats where seagrasses are predominantly found in Tampa Bay and includes the longest consistent sampling protocol (1996 to present). These data are collected by pulling the seine adjacent to the shore to sample approximately 140 m of bay bottom (Schrandt et al. 2021). In addition to collecting fish and selected invertebrates, *in situ* physical measurements for water temperature and salinity are collected at the beginning and end of the seine haul, and typically at the surface and bottom. Only measurements at the beginning of the seine haul and from the bottom were used. Seagrass data are also provided for each site, with information on species and cover. Sites with greater than 50% cover of seagrass were identified as “seagrass” sites and those less than 50% or bare sediment were identified as “no seagrass” sites for comparison with temperature and salinity measurements. Sites exclusively with macroalgae were not included in the analysis. All FIM data from FWC staff by request.

The third and final dataset evaluated was from the Pinellas County Department of Environmental Management (PCDEM). Surface waters in Pinellas county have been monitored since the 1990s, although a consistent stratified random sampling designed has been used in Tampa Bay since 2003 primarily to support robust statistical assessments for NPDES reporting. Data were obtained by recquest to PCDEM staff for the western portion of Old Tampa Bay where sampling occurs from 2003 to present (also available at <https://wateratlas.usf.edu/>). We focused primarily on OTB for the analysis of the PCDEM data given the length of record, consistency of sampling, and relative loss of seagrass compared to the other bay segments. Four distinct spatial zones in OTB are used to stratify the random selection of sample points that typically include 4 sample points per month in each zone. Water quality samples at each site are similar to those collected by EPC, where only bottom measurements for salinity and temperature were retained. Seagrass presence/absence is also recored at each site and all sites were defined as “seagrass” if only seagrass species were identified (any with macroalgae were excluded) and “no seagrass” if bare sediment was observed.

All of the organizations that provided water quality datasets participate in the Southwest Florida Regional Ambient Monitoring Program. This *ad hoc* group has routinely met quarterly to ensure similar standards and methods are used for the collection and processing of surface water quality monitoring data. Split-samples evaluated by each organization are also compared to assess precision between different laboratories. As such, the water quality measurements used herein are considered comparable, relative to the different sampling designs used by each program.

### 2.5 Trend analysis

The first goal of the analysis was to describe spatial and temporal trends in water temperature and salinity using the three water quality datasets described above. This assessment provided an indication of the extent of change in Tampa Bay as context for understanding potential relationships with seagrass change. An assumption was that any changes in physical characteristics in Tampa Bay were driven by interannual changes in weather conditions related to long-term (multi-decadal) climate change. Meteorological data describing air temperature and precipitation were obtained from Tampa International Airport where daily measurements have been collected since 1939. The *rnoaa* R package (Chamberlain and Hocking 2023) was used to retrieve these data starting from 1975 when EPC monitoring began in Tampa Bay. Annual averages for air temperature and cumulative annual precipitation were calculated. Additionally, the Standardized Precipitation Index (SPI, Beguería et al. 2013) was estimated from the daily rainfall data to identify periods of time when rainfall significantly deviated from the long-term average (using the *spei* R package, Beguería and Vicente-Serrano 2023). Annual hydrologic loading data to Tampa Bay beginning in 1985 were also obtained for comparison to annual precipitation (Janicki Environmental, Inc. 2023). All climate and loading data were evaluated annually with simple linear regression trends to assess change over time. Water temperature and salinity trends using the EPC, FIM, and PDEM data were similarly evaluated by averaging the monthly data by year for each bay segment.

Formal trend tests were used to assess station-level changes in water temperature and salinity in the EPC data. These analyses also provided a detailed spatial assessment of trends because the EPC data is the only dataset of the three where the same sites have been sampled over time. Seasonal Kendall trend tests were used to evaluate the monotonic change for temperature and salinity from 1975 to present at each water quality station (Hirsch, Slack, and Smith 1982; **millard13?**). The change per year was also evaluated for each parameter based on the slope estimates returned by each test. Kendall tests were also used to evaluate changes over time for each month to determine when the trends more most pronounced seasonally, e.g., all January estimates across years, all February estimates, etc. The percentage of stations in each bay segment with significant increasing temperature or decreasing salinity trends were evaluated for each month. All tests evaluated both surface and bottom measurements to assess potential differences by water depth.

### 2.6 Links to seagrass

The second goal of the analysis was to evaluate if seagrass changes were linked to long-term changes in water temparerature and salinity, with particular attention on the time periods before and after 2016 (pre/post recovery). The conceptual model for evaluating these changes follows simple niche space theory where seagrass growth and reproduction is hypothesized to be greatest within optimal ranges for forcing factors that are present in the environment (Hutchinson 1957; Vandermeer 1972). In the simplest form, this can conceptualized as a bell curve with optimal conditions defined with a range of values (e.g., minimum and maximum temperatures where a species is typically observed), where reduced growth or mortality is observed outside of these ranges. Because both water temperature and salinity are evaluated herein, the same model can be conceptualized in two-dimensional space [Figure 3](#fig-concept). Seagrass growth can be limited when temperature is below or above the optimum range, when salinity is below or above the optimum, or when both temperature and salinity conditions are outside of the optimum range. Using this conceptual model, we can evaluate whether seagrasses are individually stressed by either temperature or salinity, or whether seagrasses are jointly stressed by both temperature and salinity. Based on the results of the trend tests, we hypothesized that seagrasses are likely stressed by both high temperature and low salinity. The statistical models described below were constructed specifically to assess these hypotheses following the conceptual model in [Figure 3](#fig-concept). Of course, the optimal niche space can be defined in n-dimensions, but we focus on water temperature and salinity given that other dominant forcing factors, i.e., light availability, have generally not been limiting for growth in recent years.

A fundamental challenge in describing niche space is identifying the boundaries within which the space occurs. In reality, the boundaries are typically not discrete and organisms can be found outside of these ranges, although at reduced abundances. Regardless, discrete limits are often used and substantial efforts have been made to identify these boundaries for seagrasses. In Tampa Bay, three dominant seagrass species are observed that include *Halodule wrightii* (shoal grass), *Syringodium filiforme* (manatee grass), and *Thalassia testudinium* (turtle grass) (Lewis III et al. 1985). These species co-occur often in mixed beds, although differences in abundance are observed across salinity ranges. Shoal grass is tolerant of a wide range of salinity (Lirman and Cropper 2003), but is more abundant in oligohaline portions of Tampa Bay. Conversely, turtle grass is most often found in more euryhaline conditions near the mouth of Tampa Bay. Reported salinity ranges for each of these species varies depending on location, season, and other co-occurring factors like temperature (Phillips 1960; McMillan and Moseley 1967; Zieman 1975; Lewis III et al. 1985), although most studies place lower limits of salinity in the range of 15-25 ppt. Optimal temperature ranges are similar between species, with reduced growth observed at temperatures above 30 C (Zieman 1975; Lewis III et al. 1985).

Because of the uncertainty in defining thresholds for optimal temperature and salinity ranges, multiple thresholds were evaluated to both describe the potential for stress and how it may be related to changes in seagrass. Distinctions were not made between species, primarily due to lack of consensus between studies and the likely site-specific ranges that affect seagrass growth in Tampa Bay. First, we developed metrics of potential temperature and salinity stress by quantifying the maximum number of continuous days each year when temperature was above or salinity was below a given threshold. This approach assumed that stress could be observed based on duration of exposure (i.e., maximum number of continuous days each year) relative to a threshold that may are may not be outside of the optimum range for seagrasses. These metrics were quantified from the monthly long-term observations in the EPC data. To quantify daily counts each year, a continuous prediction of temperature and salinity over time at each of 45 stations was estimated using Generalized Additive Models (GAMs) fit to the response variable and a single predictor for decimal year (Wood 2017). The smoothing spline for decimal year had sufficient knots to capture the seasonal signal within each year and the long-term inter-annual trends (M. W. Beck, Valpine, et al. 2022). Model fit for each station was sufficient to calculate daily predictions for assessing the stressor metrics (see supplement, R ranged from 0.85 to 0.95 for temperature models, 0.71 to 0.96 for salinity models).

Counts of the maximum number of days each year that temperature was above or salinity was below a thresold were obtained from the daily GAM predictions. This was done at each of 45 stations in the EPC data using temperature thresholds of 29, 30, and 31 C and salinity thresholds of 15, 20, and 25 ppt. The number of days when both temperature was above and salinity was below the thresholds was also estimated as a combined potential stress measure. Stressor metrics were further aggregated across stations in each bay segment using a mixed-effects regression model where the annual stressor counts for stations in a bay segment were fit against year (1975 to 2022) using a random intercept for station (Zuur et al. 2009; Bates et al. 2015). This produced an overall assessment of how the stressor metrics have changed over time by bay segment, including counts for temperature, salinity, and both relative to the thresholds.

For comparison to seagrass, the annual metrics were also referenced to approximate periods of time between the annual seagrass transect surveys, as opposed to the calendar year as above. Transect surveys are typically done in the fall at the end of the growing season. The average date for each year across the subset of transects in each bay segment was estimated and the stressor metrics were referenced accordingly. For example, if the average transect date for a bay segment was September 15th in 2009 and Oct 1st in 2010, the 2010 stressor counts were based on all days between the two average dates. This was repeated for all years from 1998 to 2022 when transect data were available. Bay segment stressor metrics were calculated as the average counts in each “transect year” from all stations in each segment. The stressor metrics were compared to various response measures at the bay segments-scale from the transect data, including frequency occurrence each year (all species), percent change in frequency occurrence between years, and increase/decrease in frequency occurrence between years as a binary (1/0) variable. Generalized linear regression models were used to evaluate each of the seagrass response variables relative to the stressor metrics. Interaction factors were also included for bay segment and time period (recovery pre-2016, decline post-2016). The most parsimonious models were identified by forward and backward variable selection and comparison with AIC values (Sakamoto, Ishiguro, and Kitagawa 1986; Venables and Ripley 2002). All models excluded Lower Tampa Bay because of minimal seagrass change over time.

Additional models were constructed for the FIM and PDEM datasets to evaluate seagrass change relative to temperature and salinity. These models did not include stressor metrics, but rather used direct measurements of salinity and temperature as independent variables. Models of temperature or salinity to predict daily measurements are not possible with the FIM and PDEM monitoring designs because random locations were used for each sample date. However, the sample sizes for the FIM and PDEM models were much larger than those for the EPC data, where the latter was limited by the annual timestep of the transect surveys. This allowed for more flexible models that are less limited by degrees of freedom. GAMs were used to evaluate seagrass percent cover for the FIM data using continuous temperature and salinity as independent variables. Interactions with factor variables for bay segment and time period as above were also included. The GAMs also evaluated interactions between salinity and temperature to determine if both factors had an additive effect on seagrass change. The GAMs for the PDEM data were constructed similarly, except only Old Tampa Bay was evaluated (no bay segment interaction) and the seagrass response variable was presence/absence (i.e., a binomial distribution was used).

## 3 Results

For [Figure 2](#fig-meteowqraw), salinity shows much higher inter-annual variability, but lower intra-year variation among stations. Trends are similar for bottom vs top, temperature shows stronger trend than salinity.

Kendall test results are shown in [Figure 4](#fig-kendall).

An example of mixed effects models for two selected thresholds is shown in [Figure 5](#fig-mixeff).

NMS results in [Figure 6](#fig-nms) and seagrass decline results in [Figure 7](#fig-sgmod)

## 4 Discussion

Other areas showing seagrass loss - Florida Bay is different, less water flowing out of everglades and compounding SLR has elevated salinity and likely stress in other direction. Also, Biscayne Bay and IRL is a lot like OTB, poor flushing for example.

Viral/disease effects on seagrass change, (Van Bogaert et al. 2018) for TB and (Duffin et al. 2021) for Florida Bay.

Role of additional stressors and why they weren’t considered in the model - they’re important but we can also demonstrate that light attenuation has been relatively stable over time, so may not be important to include in the models.

Issues with thresholds, not definitive and those likely to stress seagrass are potentially not occurring at frequencies sufficient enough to model. Those used herein were chosen statistically. Point is that the temp and sal trends are in a direction such that thresholds that are more likely to stress seagrass are more likely to occur in the future.

Case for continuous monitoring

Are we just showing seagrasses are not present because of temperature limitations or if temperature indeed caused a reduction?

Manamagement implication - we can’t do much to control temp and salinity, but we can temper expectations for how to manage the bay. Adaptive capacity/resiliency is likely decreasing because of these “co-morbidities”, so we have to temper expectations for other stressors that we can control.

OTB is likely at a tipping point…

Alternative nutrient pathways and role of macroalgae, competition for resources, shading, etc.

OTB seagrass loss and role of pyrodinium, optimal temperatures from 28-31 (Cary B. Lopez et al. 2021). For salinity, highest growth rates at 24 psu and above, with decline in growth rates at 20 psu (Usup, Kulis, and Anderson 1994), in Florida growth has occurred in psu 10-45, bloom concentrations observed above 15 psu (Phlips et al. 2006; Cary B. Lopez et al. 2021). Optimal salinity range between 20-32 psu (Cary B. Lopez et al. 2021).

## 5 Acknowledgments

## Figures

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| Figure 1: Seagrass changes over time in Tampa Bay for (a) areal coverage and (b) frequency occurrence of major species. Changes are shown for major bay segments. Note the different time scale between (a) and (b); coverage maps in (a) began in 1988 and seagrass transect monitoring in (b) began in 1998. Red lines in (a) show approximate capacity of seagrass coverage based on the baywide target of 40,000 acres. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay. |

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| Figure 2: Long-term air temperature, precipitation, hydrologic load, Standard Precipitation Index (SPI), water temperature, and salinity trends from 1975 to 2022. Points for salinity and water temperature are colored by sampling location in the water column and show the average (95% confidence interval) across all stations and sampling months for each year in each bay segment. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay. |

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| Figure 3: Conceptual stressor diagram |

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| Figure 4: Trends from 1975 to 2022 for temperature and salinity measurements at long-term monitoring stations in Tampa Bay. Results for (a) seasonal Kendall tests by station and monitoring location (top or bottom of the water column) are shown in (a) with color and shape corresponding to the estimated annual slope as change per year (yr-1). Summarized seasonal trends by month are shown for (b) top and (c) bottom measurements as the percent of stations in each bay segment with significant increasing (temperature) or decreasing (salinity) trends. Bay segment outlines are shown in (a); OTB (northwest): Old Tampa Bay, HB (northeast): Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay. |

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| Figure 5: Example of mixed effects models for the estimated number of days by year that temperature (red) or salinity (blue) were above or below thresholds of 30 degrees C or 25 psu, respectively. The bottom row (black) shows the number of days when both temperature and salinity were above or below the thresholds. The models included station as a random effect for each bay segment, with grey lines indicating individual station trends and thicker lines indicating the overall model fit. Slopes for significant models are shown in the bottom right of each facet. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay. |

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| Figure 6: Ordination plots of Nonmetric Multidimensional Scaling results for annual estimates in each bay segment for total seagrass frequency occurrence (Freq. Occ.), counts of days above the temperature threshold (Temp.), counts of days below the salinity threshold (Sal.), and counts of days above or below for both, respectively. The left plot excludes counts of days with both temperature and salinity above and below the threshods, whereas the right plot excludes the separate temperature and salinity metrics. Points are sized by seagrass frequency occurrence. 95% confidence ellipses are shown for observations in each major bay segment OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay. |

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| Figure 7: Predicted results from generalized linear models evaluating seagrass decline as (a) percent change between years and (b) probability of decline versus number of days when salinity was below the threshold. Models evaluated the response variable on the y-axes as a function of salinity days and interactions with year category (seagrass recovery prior to 2016, seagrass decline 2016 to present) and bay segment. Shaded areas are 95% confidence intervals. Colored lines in (b) as rug plots show instances of seagrass increase (blue) or decrease (red) by year. |

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| Figure 8: Relationship between water temperature and discharge in the Hillsborough River (USGS gage 02303000) over time. The relationship is shown for February (dry month) and August (wet month) grouped by historic (1975-1999) and contemporary (2000-2022) time periods. Regression lines with 95% confidence intervals for each temporal grouping are shown. |

## Tables

Summary of mixed-effects models evaluating increases in the number of days each year from 1975 to 2022 when temperature was above 30C, salinity was below 25ppt, or both temperature and salinity were above/below the thresholds. The start and end columns show the estimated number of days at the beginning and end of the period of record when temperature or salinity were above or below the thresholds. Values are the estimated mean number of days (plus standard error) from 1975 and 2022. \*\* p < 0.005, \* p < 0.05, - no model. OTB (northwest): Old Tampa Bay, HB (northeast): Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

| Bay Segment | Threshold | Slope | Start | End |
| --- | --- | --- | --- | --- |
| OTB |  |  |  |  |
|  | Temperature > 30 | 1.04\*\* | 8 (3.3) | 56 (3.3) |
|  | Salinity < 25 | 1.96\*\* | 128 (13.2) | 219 (13.2) |
|  | Both | 0.81\*\* | 0 (2.8) | 37 (2.7) |
| HB |  |  |  |  |
|  | Temperature > 30 | 1.01\*\* | 8 (4.9) | 55 (4.8) |
|  | Salinity < 25 | 0.86\* | 131 (12.5) | 171 (11.8) |
|  | Both | 0.8\*\* | -2 (2.8) | 35 (2.7) |
| MTB |  |  |  |  |
|  | Temperature > 30 | 1.06\*\* | 9 (3.1) | 57 (3.1) |
|  | Salinity < 25 | 0.67\*\* | 51 (11) | 82 (11) |
|  | Both | 0.37\*\* | -1 (1.7) | 16 (1.7) |
| LTB |  |  |  |  |
|  | Temperature > 30 | 0.92\*\* | 21 (3.5) | 63 (3.5) |
|  | Salinity < 25 | -0.06 | 4 (1.3) | 1 (1.3) |
|  | Both | 0 | 0 (0) | 0 (0) |

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