Estuaries and Coasts

Hot and fresh: evidence of climate-related suboptimal conditions for seagrass in a large Gulf coast estuary --Manuscript Draft--

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Corresponding Author:	Marcus W Beck Tampa Bay Estuary Program UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Tampa Bay Estuary Program
Corresponding Author's Secondary Institution:	
First Author:	Marcus W Beck
First Author Secondary Information:	
Order of Authors:	Marcus W Beck
	Kerry Flaherty-Walia
	Sheila Scolaro
	Maya C Burke
	Bradley T Furman
	David J Karlen
	Christopher Pratt
	Christopher J Anastasiou
	Edward T Sherwood
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Seagrasses have long been a focal point for management efforts aimed at restoring ecosystem health in estuaries worldwide. In Tampa Bay, Florida (USA), seagrass coverage has declined since 2016 by nearly a third (11,518 acres), despite sustained reductions of nitrogen loads supportive of light environments for growth. Changing physical water quality conditions related to climate change may be stressing seagrasses beyond their optimal growth ranges, requiring an assessment to determine if this decline can be linked to climate stress. Three ambient water quality datasets of varying sampling design and coverage were evaluated to characterize physicochemical environments in Tampa Bay and the potential relationships with seagrass change. Tampa Bay has become hotter and fresher with water temperature increasing by 0.03 - 0.04 [[EQUATION]]C per year and salinity decreasing by 0.04 - 0.06 ppt per year, translating to an increase of 1.3 to 1.7 [[EQUATION]]C and a decrease of 1.6 to 2.6 ppt over the last fifty years. These changes varied spatially and seasonally, with the most dramatic changes observed in the upper bay. Simple linear models provided a weight-of-evidence that recent seagrass declines are somewhat associated with hotter and fresher conditions. Trends in warming and increased precipitation in the region are likely to continue, further creating suboptimal conditions for seagrasses in Tampa Bay. These results should compel resource managers to

	consider the likelihood that reduced resilience of estuarine resources due to shifting ecological baselines driven by additional climate change drivers will complicate long-standing management paradigms. While conventional management approaches that focus on limiting nutrient loads should be continued, their future effectiveness may be confounded by climate change drivers and warrant additional, complementary interventions to maintain ecosystem health into the future.
Suggested Reviewers:	Lauren Hall Ihall@sjrwmd.com
	Randall Parkinson rparkins@fiu.edu
	Kimberly Yates kyates@usgs.gov
	Luis Lizcano-Sandoval luislizcanos@usf.edu
	Gerold Morrison gerold.morrison@gmail.com

February 9, 2024

Dr. Paul Montagna, Dr. Linda Deegan, Editors-in-Chief Estuaries and Coasts

Dear Dr. Montagna and Dr. Deegan,

Enclosed please find our manuscript, titled 'Hot and fresh: evidence of climate-related suboptimal conditions for seagrass in a large Gulf coast estuary', to be considered as an original article in Estuaries and Coasts.

The long-term recovery of Tampa Bay is a nationally recognized success story that demonstrates how the USEPA National Estuary Program is a valuable model for collaborative management. Through large-scale reductions in external nutrient loading, seagrasses have recovered in the bay, reaching an all-time high in 2016. Since that time, seagrasses have declined by nearly 1/3 and questions remain regarding the role of climate-related stressors in contributing to this decline. We leverage multiple data sources to describe changing water temperature and salinity in the bay and relate these trends to the recent seagrass loss from 2016 to present. No such comprehensive assessment has previously been conducted for Tampa Bay and our results provide context for how climate stressors can confound existing management models, particularly related to nutrient management.

This work has not been published elsewhere, nor is it under consideration for publication anywhere else. Each author has contributed substantially to this work. All authors have contributed to the writing and review of the original draft. Analysis was conducted by the first author, with interpretation of results supported by all other co-authors. All authors have approved the final draft and the submitted product was prepared according to instructions for authors provided on the journal's website.

We greatly appreciate the opportunity to publish this work in Estuaries and Coasts and are confident readers will consider it a valuable contribution. Please feel free to contact us directly should additional information be needed.

Regards,

Marcus W. Beck, Tampa Bay Estuary Program, St. Petersburg, Florida, USA, mbeck@tbep.org

Hot and fresh: evidence of climate-related suboptimal conditions for seagrass in a large Gulf coast estuary

- 3 Marcus W Beck^{1,⊠}, Kerry Flaherty-Walia¹, Sheila Scolaro¹, Maya C Burke¹, Bradley T Furman²,
- David J Karlen³, Christopher Pratt³, Christopher J Anastasiou⁴, and Edward T Sherwood¹
- ¹ Tampa Bay Estuary Program, 263 13th Ave S., Suite 350, St. Petersburg, Florida 33701 USA
- 6 ² Florida Fish and Wildlife Conservation Commission, Florida Fish and Wildlife Research
- 7 Institute, 100 8th Avenue SE, St. Petersburg, FL 33701, USA
- 8 ³ Environmental Protection Commission of Hillsborough County, 3629 Queen Palm Drive,
- 9 Tampa, FL 33619, USA
- ⁴ Southwest Florida Water Management District, 7601 US Highway 301 North, Tampa, FL
- 11 33637, USA

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12 [™]Correspondence: Marcus W Beck <mbeck@tbep.org>

Abstract

- 14 Seagrasses have long been a focal point for management efforts aimed at restoring ecosystem
- 15 health in estuaries worldwide. In Tampa Bay, Florida (USA), seagrass coverage has declined
- since 2016 by nearly a third (11,518 acres), despite sustained reductions of nitrogen loads
- supportive of light environments for growth. Changing physical water quality conditions related
- 18 to climate change may be stressing seagrasses beyond their optimal growth ranges, requiring an
- 19 assessment to determine if this decline can be linked to climate stress. Three ambient water
- 20 quality datasets of varying sampling design and coverage were evaluated to characterize
- 21 physicochemical environments in Tampa Bay and the potential relationships with seagrass
- change. Tampa Bay has become hotter and fresher with water temperature increasing by 0.03 -
- 23 0.04 °C per year and salinity decreasing by 0.04 0.06 ppt per year, translating to an increase of

1.3 to 1.7 °C and a decrease of 1.6 to 2.6 ppt over the last fifty years. These changes varied spatially and seasonally, with the most dramatic changes observed in the upper bay. Simple linear models provided a weight-of-evidence that recent seagrass declines are somewhat associated with hotter and fresher conditions. Trends in warming and increased precipitation in the region are likely to continue, further creating suboptimal conditions for seagrasses in Tampa Bay. These results should compel resource managers to consider the likelihood that reduced resilience of estuarine resources due to shifting ecological baselines driven by additional climate change drivers will complicate long-standing management paradigms. While conventional management approaches that focus on limiting nutrient loads should be continued, their future effectiveness may be confounded by climate change drivers and warrant additional, complementary interventions to maintain ecosystem health into the future.

35 Key words: climate change; seagrass; salinity; temperature; Tampa Bay

Statements and Declarations

37 The authors have no competing interests to declare that are relevant to the content of this article.

1 Introduction

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40 The monitoring and management of seagrasses in coastal environments has received substantial 41 attention on a global scale. Seagrasses are fundamental indicators of coastal ecosystem health 42 (Roca et al. 2016; Orth et al. 2017), while also serving as foundational species that provide 43 numerous ecosystem services (Fourqurean et al. 2012; Orth et al. 2020; Orth and Heck 2023). 44 Seagrasses have been in global decline with rapid development of coastal environments, 45 particularly in the latter half of the 20th century with accelerating losses estimated at a rate of 110 km² vr⁻¹ since the 1980s (Waycott et al. 2009; Dunic et al. 2021). These losses are 46 47 comparable to, if not more significant than, other interconnected critical coastal environments 48 such as mangroves and salt marshes (Duarte et al. 2008), which threatens the natural and 49 economic services that these habitats provide. Losses have been attributed to numerous stressors 50 including decline in light environments with nutrient enrichment, sedimentation, and physical 51 disturbance primarily from human activities (Duarte 1995; Margaret O. Hall et al. 1999; Orth et 52 al. 2006; Burkholder et al. 2007). Furthermore, natural disturbances such as storm events and 53 disease have also been implicated (Robblee et al. 1991; Tomasko et al. 2020). Contemporary 54 management actions aimed at mitigating loss and ultimately supporting restoration require 55 adaptive approaches to address the effects of multiple stressors that have contributed to seagrass 56 decline (Dunic and Côté 2023). 57 The sustained coverage or restoration of seagrasses in coastal environments requires 58 environmental conditions that support vegetative growth, reproduction, and coverage expansion. 59 A long-standing approach adopted by numerous management entities has been the control of 60 external nutrient inputs in systems where excessive algal growth has created poor light environments for seagrasses (Boesch et al. 2001; Greening and Janicki 2006; Greening et al. 61

2014; Han and Liu 2014). There are limited examples of successful recovery of seagrass through control of nutrient inputs alone, primarily because of the difficulty in identifying and regulating both point and diffuse non-point sources. Notable exceptions include Tampa Bay on the west coast of Florida (Greening et al. 2014) and the much larger Chesapeake Bay on the east Atlantic US coast (Lefcheck et al. 2018), where both showed significant increase in seagrass areal coverage through sustained and long-term reductions in external nutrient loads. Cooperation among management, regulatory, public, and private sectors were critical aspects of both examples (Sherwood et al. 2016; Tango and Batiuk 2016). In other cases, the reversal of seagrass losses through nutrient reductions alone may not be possible because of system hysteresis, where the path to recovery is not the same as the path to decline (Maxwell et al. 2016). Complementary management actions, in addition to nutrient reductions, are needed in these situations. Climate change has complicated the understanding of ecosystem response to conventional stressors, presenting new challenges and expectations for how ecological resources will respond to management actions (Statham 2012; Sherwood and Greening 2013). In addition to sea-level rise, the most anticipated effects of climate change in coastal environments are increased temperature and altered precipitation patterns. These changes will profoundly alter physicochemical habitats, creating suboptimal or uninhabitable conditions for many species (Madeira et al. 2012; Lefcheck et al. 2017; Hammer et al. 2018; Hensel et al. 2023). Lefcheck et al. (2017) evaluated interactive effects of water clarity and rising temperatures on seagrasses with over 30 years of data in Chesapeake Bay. The environmental stress on seagrasses from acute warming related to climate change was compounded by stress from poor light environments in shallow waters, demonstrating a concerning synergy of stressors most likely to affect Tampa Bay. Moreno-Marín et al. (2018) produced similar results using a multifactorial

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experiment that considered temperature, light, and nutrient (nitrogen) availability for North Sea eelgrass (Zostera marina). Species shifts are also expected to occur as changing physical conditions decrease the competitive advantages of historically abundant species. In Chesapeake Bay, the abundance of widgeongrass (Ruppia maritima) has responded positively to nutrient reduction and is replacing the formerly dominant but now heat-stressed (Z. marina, Hensel et al. 2023). Changing frequency and severity of precipitation patterns may further alter salinity regimes and with it the distribution and abundance of seagrasses throughout the estuary (Rasheed and Unsworth 2011; Webster et al. 2021). These changes may produce hypersaline conditions under periods of prolonged drought or more freshwater conditions with increased storm events. Seagrass species may respond differently under individual or multiple stressors and each watershed will respond differently to climate change (Lefcheck et al. 2017; Zhang et al. 2023), suggesting place-based empirical assessments will be needed to properly inform management decisions. In Tampa Bay, Florida, seagrasses are a primary indicator of bay health and have been the focus of management efforts for the last thirty years (Sherwood et al. 2017). Through successful reduction of external nitrogen loads, seagrasses have recovered from a low, system-wide coverage in the 1980s to an all-time high in 2016 of 41,655 acres (16,857 ha, Greening and Janicki 2006; Greening et al. 2014). Seagrass distribution and abundance has been dynamic throughout this period, overall responding positively to increases in water clarity with nutrient load reductions, while also demonstrating more short-term variability in response to regional climate events (Greening and Janicki 2006). Since 2016, seagrass areal cover has decreased by nearly 1/3 despite relatively stable water quality (Janicki and Wade 1996; Beck 2020a). Factors that have influenced this recent decline are unknown and the effects of climate change drivers on

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physical water quality conditions have been implicated as potential stressors. Following global trends, recent work has demonstrated a broad long-term trend of increasing water temperature in Tampa Bay, although at a relatively coarse scale (Nickerson et al. 2023). Changing salinity has not been well-described, nor have potential links of changing salinity and temperature with recent seagrass change. Tampa Bay is rich with historical data that can be used to evaluate longterm trends. This information can fill a critical knowledge gap that can inform regional management activities, while also demonstrating the confounding effects of climate change with ecosystem response to conventional stressors. This paper describes a comprehensive assessment of long-term trends in water temperature and salinity in the Tampa Bay estuary over the last fifty years. Three datasets of varying sampling designs and temporal coverage were used to assess the primary hypothesis that Tampa Bay is trending towards hotter and fresher conditions that are likely stressing seagrasses beyond their optimal tolerance ranges, particularly in upper bay segments where marginal light environments exist. This hypothesis was generated from preliminary assessments of datasets used herein and discussions with the regional scientific and management community. While other studies have suggested negative effects from climate change in the form of increased salinity (e.g., Costa et al. 2023), lower salinity may be a stressor for seagrass in Tampa Bay. The analysis was supported by 30-year seagrass datasets including aerial surveys of total seagrass distribution, annual transect monitoring describing species-specific percent cover, and synoptic seagrass data

collected with routine biotic and water quality surveys. Specific focus was on the periods prior to

and after 2016, when seagrasses recovered baywide and then declined despite water quality

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conditions remaining relatively stable.

2 Methods

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2.1 Study area

Tampa Bay is the largest open-water estuary in Florida covering 400 mi² (1,036 km²) and the second largest in the Gulf of Mexico. The watershed covers an additional 2,200 mi² (5,872 km²) with the Hillsborough, Alafia, Manatee, and Little Manatee rivers that contribute to a majority of freshwater inflow to the bay. The climate of Tampa Bay is subtropical with warm humid summers and cool less humid winters (Garcia et al. 2023). Unique to Tampa Bay and the entire central Florida peninsula is that this region is within a transition zone from more temperate weather to the north, similar to the rest of the southeastern United States, and more tropical weather to the south (Morrison et al. 2006). El Niño weather events have also been associated with prolonged periods of heavy rainfall contributing to seagrass reductions through increased stormwater nutrient loads (Schmidt and Luther 2002; Greening and Janicki 2006; Morrison et al. 2006). The watershed is heavily developed and includes over 3 million people (Todd et al. 2023) with 42% of the land as urban or suburban contributing substantial inputs of wastewater and stormwater runoff that can stress bay resources (Beck, Robison, 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; Beck et al. 2022). 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 (Figure 1a). Old Tampa Bay and Hillsborough Bay have historically had the most degraded water quality primarily from direct

external nutrient inputs from wastewater and stormwater (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, channelized tributaries and manmade flood control conveyances (Janicki Environmental, Inc. 2023). Notably, Old Tampa Bay has restricted circulation from multiple land bridges associated with causeways that traverse the bay, causing relatively longer residence times and accumulation of legacy pollutant loads compared to the other bay segments (Sherwood et al. 2015; Luther and 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 (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 (Greening et al. 2014; Johansson and Janicki Environmental, Inc. 2015).

2.2 Seagrass change in Tampa Bay

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The long-term recovery of seagrass habitats in Tampa Bay since the 1980s is a nationally recognized success story that demonstrates application of a successful management paradigm through the EPA-administered National Estuary Program (Greening and Janicki 2006; Greening et al. 2014; Sherwood et al. 2017). From 1988 to 2016, seagrasses increased 79% to 41,655 acres (16,857 ha), surpassing the regional management goal of restoring coverage to 95% of a

benchmark 1950s coverage. Though Tampa Bay was far from pristine in the 1950s, aerial imagery was sufficient to estimate a relatively unimpacted condition for seagrass coverage. Since that time, the greatest areal coverage expansions were observed in the upper bay segments (OTB, HB, and MTB; Figure 2a). External nitrogen load estimates decreased by about 2/3 by the 1980s from a peak 1970s estimate of 8.9 x 10⁶ kg/year, largely from advanced wastewater treatment upgrades and in part from the cumulative effects of habitat restoration and additional stormwater control projects implemented in the watershed (Greening et al. 2014; Beck et al. 2019). From 2016 to present, dramatic seagrass loss has been observed in Tampa Bay, despite nutrient loading and light attenuation remaining relatively stable (Figure 2a). Total cover in Tampa Bay has decreased by 28% (11,518 acres/4,661 ha) from the 2016 peak to a total baywide coverage of 30,137 acres (12,196 ha) in 2022. Losses have been most pronounced in Old Tampa Bay (62%; 6,963 acres/2,818 ha loss) and Hillsborough Bay (80%; 1,599 acres/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. Coverage in Middle Tampa Bay decreased by 20% (1,926 acres/779 ha loss), whereas coverage in Lower Tampa Bay has remained stable, with only a 2% loss which is close to the mapping error.

2.3 Seagrass data

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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 (Figure 2a, available at https://data-swfwmd.opendata.arcgis.com/). These maps are created from aerial images collected specifically to map seagrass and are acquired during a flight window from December and February. The maps are created by photointerpretation of image signatures coupled with a robust field

verification and accuracy assessment. The maps provide a spatial estimate of seagrass cover at the landscape scale and is irrespective of species. Complementary to the SWFWMD seagrass mapping program is the Tampa Bay Interagency Seagrass Monitoring Program (Figure 2b, https://tampabay.wateratlas.usf.edu/seagrass-monitoring/). Annual transect surveys have been conducted since 1998 at 62 fixed locations in Tampa Bay, many of which were chosen to target seagrass beds of interest (Johansson 2016; Sherwood et al. 2017). This dataset provides species information on cover-abundance, 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 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. Additional 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 fixed stations in the major bay segments since the early 1970s (Figure 1b). The 45 stations with the longest and most complete temporal record from 1975 to present were used herein. Water quality samples are collected at each station from surface water grabs (e.g., nutrients, biological, and chemical constituents) or *in situ* measurements of physical parameters

(e.g., salinity, temperature) collected at the surface, mid-depth, and bottom. Most analyses herein used only bottom water measurements given the shallow depth and mixed water column of most of Tampa Bay (Weisberg and Zheng 2006), although 1975 bottom salinity used middle water column sampling since the former was not available until the following year. Most samples are collected from mid-morning to early afternoon. Compared to the additional datasets described below, the monitoring stations are generally in deeper water beyond where seagrasses occur along the shallow margins of the bay. The data were obtained using the theptools R package that imports the data directly from a stable web address provided by the EPC (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 gear types that target different habitats (Schrandt et al. 2021). A stratified sampling design is used to select sites for 21.3 meter center-bag seines that target shallow habitats (<1.5 m) where seagrasses are predominantly found in Tampa Bay and includes the longest consistent sampling protocol (1996 to present, Figure 1c). In addition to collecting fish and selected invertebrates, in situ physical measurements for water temperature and salinity are collected at the bag, and at the surface and at 1 m intervals to the bottom. Only measurements from the bottom were used. Seagrass data are also provided for each site, with information on species and cover. Total percent cover for all species at a site was used for comparison with temperature and salinity measurements. Sites exclusively with macroalgae were not included in the analysis. All FIM data were provided from FWC staff upon request.

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The third and final dataset evaluated was from the Pinellas County Department of Environmental Management (PDEM). Data were obtained by request to PDEM staff for the western portion of Old Tampa Bay where sampling occurred from 2003 to present (Figure 1d, also available at https://wateratlas.usf.edu/). We focused primarily on OTB for the analysis of the PDEM data given the length of record, consistency of sampling, and relative loss of seagrass compared to the other bay segments. Water quality samples at each site are similar to those collected by EPC but can occur in shallower locations. Seagrass presence/absence is also recorded 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 (Sherwood et al. 2016). This *ad hoc* group meets quarterly to ensure similar standards and methods are used for the collection and processing of surface water quality monitoring data in the region. Split-samples evaluated by each organization are also compared to assess precision between different laboratories. As such, the water quality

2.5 Trend analysis

used by each program.

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 water quality 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 drivers. For comparison to water quality conditions,

measurements used herein are considered comparable, relative to the different sampling designs

daily air temperature (Tampa International Airport [TIA] National Weather Service site) and precipitation (SWFWMD area-weighted watershed summaries) were used to characterize regional conditions for the most consistent period of record covered by the water quality samples (i.e., 1975 to present for the EPC data). The rnoaa R package (Chamberlain and Hocking 2023) was used to obtain the TIA temperature data. Regional precipitation summaries were obtained directly from the SWFWMD (https://www.swfwmd.state.fl.us/resources/data-maps/rainfallsummary-data-region). Only rainfall data for the wet season (June to September) were evaluated for trends, whereas the complete record was used to calculate the Standardized Precipitation Index (SPI, Beguería et al. 2013) to identify periods of time when rainfall significantly deviated from the long-term average (using the *spei* R package, Beguería and Vicente-Serrano 2023). All climate 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 each 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 et al. 1982; Millard 2013). Kendall tests were also used to evaluate changes over time for each month across years to determine when the trends were most pronounced seasonally (e.g., all January estimates across years, all February estimates, etc.). The percentage of stations in each bay segment with significantly increasing temperature or decreasing salinity trends were evaluated for each month.

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2.6 Quantifying potential stress

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The second goal of the analysis was to evaluate if seagrass changes were linked to long-term changes in water temperature and salinity. The conceptual model for evaluating these changes describes the fundamental niche space 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 be conceptualized as a bell curve with optimal conditions defined within a range of values for a single parameter, where reduced growth or mortality is observed outside of these ranges. Because both water temperature and salinity were evaluated, the same model can be conceptualized in two-dimensional space (Figure 3). Seagrass growth can be limited when temperature is below or above the optimum range, when salinity is below or above the optimum range, or when both temperature and salinity conditions are outside of the optimum range. Based on the results of the trend tests, we hypothesized that seagrasses are likely stressed by both high temperature and low salinity (bottom right, Figure 3). Although the fundamental niche space can be defined in multiple dimensions for many parameters, we focus on water temperature and salinity given that other dominant forcing factors, i.e., light availability, have been relatively stable in recent years (Beck, Burke, et al. 2023). A fundamental challenge describing niche space is identifying the boundaries for optimal conditions. In Tampa Bay, three dominant seagrass species occur: Halodule wrightii (shoal grass), Syringodium filiforme (manatee grass), and Thalassia testudinum (turtle grass) (Lewis III et al. 1985; R. Phillips and Meñez 1988). Other less common species include Ruppia maritima (widgeon grass) and Halophila engelmanii (star grass), where the former is often mapped during wet years in the upper bay segments from the aerial surveys. These species cooccur often in mixed beds throughout the bay, although some 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 oligo/mesohaline portions of Tampa Bay. Conversely, turtle grass is less tolerant of low salinity and is more abundant 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 (R. C. 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 these temperate-tropical species, with reduced growth observed at temperatures above 30° C (Zieman 1975; Lewis III et al. 1985). Because of the uncertainty in defining *in situ* thresholds for optimal temperature and salinity ranges, multiple thresholds were evaluated to 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 likely site-specific ranges that affect seagrass growth in Tampa Bay, as well as challenges of modeling fundamental and realized niche spaces between competing species (Araújo and Guisan 2006). 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 or 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

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(GAMs) fit to temperature or salinity with a single predictor for decimal year (Wood 2017). Model fit for each station was considered sufficient to calculate daily predictions to assess potential stressor metrics (Figure S5, R² ranged from 0.85 to 0.95 for temperature models, 0.66 to 0.95 for salinity models, Tables S1, S2).

Counts of the maximum continuous number of days each year that temperature was above or salinity was below a threshold were obtained from the daily GAM predictions. This was done at each of the 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

2.7 Links to seagrass

by bay segment.

For comparison to seagrass, the annual metrics calculated from the EPC data were referenced to approximate periods of time between the annual seagrass transect surveys, as opposed to the calendar year for describing trends above. Bay segment stressor metrics were calculated as the average counts in each "transect year" from all stations in each segment from 1998 to 2022. The stressor metrics were compared to frequency occurrence (all species) each year by bay segment. Generalized Linear regression Models (GLMs) were used to evaluate frequency occurrence in response to the independent variables, where the latter were the stressor metrics for temperature, salinity, both, bay segment, and time period (recovery pre 2016, decline post 2016). Two models

were evaluated, one with the temperature and salinity metrics together and another with the both metric. Interactions were included between temperature, salinity, and time period or the both metric and time period, depending on the model. Bay segment was included as a main effect without interactions. The most parsimonious models were identified by forward and backward selection of the independent variables and comparison with AIC values (Sakamoto et al. 1986; Venables and Ripley 2002). All models excluded Lower Tampa Bay because of minimal seagrass change over time.

Separate models were constructed to provide an additional weight-of-evidence for the FIM and PDEM temperature and salinity datasets relative to seagrass change. These models used direct

PDEM temperature and salinity datasets relative to seagrass change. These models used direct measurements of salinity and temperature as independent variables because the stressor metrics could not be calculated using the sampling designs from these monitoring programs. GLMs were used for the FIM data to evaluate seagrass percent cover using continuous temperature and salinity as independent variables. Interactions with time period and a main effect for bay segment were also included as above. GLMs for the PDEM data were constructed similarly, except only Old Tampa Bay was evaluated due to spatial limitations of the data. The seagrass response variable was presence/absence (i.e., a binomial distribution was used) to model the probability of observing seagrasses. Model input data were further subset to include only months from July to November to describe seagrasses during the growing season and to reduce potential seasonal effects.

3 Results

3.1 Temperature and salinity trends

Long-term meteorological data showed increasing trends for air temperature and precipitation (Figure 4). Mean annual air temperature has increased by 0.04 °C per year (p < 0.005, R^2 0.51).

Mean annual air temperature in 1975 was 22.1 (+/-0.17 st. err.) °C, whereas current mean annual air temperature in 2022 was 24.1 (+/-0.17 st. err.) °C, showing an overall increase in the period of record of 2 °C. Similarly, total precipitation during the rainy season has increased by 12.16 mm per year, although the model was not significant (p = 0.08). Removing September from the rainy season showed a significantly increasing trend of 11.44 mm per year (p < 0.05, $R^2 = 0.07$; September alone did not have a significant trend, p = 0.87). Using this model, mean precipitation in 1975 was 2829.9 (+/-150.44 st. err.) mm, whereas current mean precipitation in 2022 was 3356 (+/-145.72 st. err.) mm, showing an overall increase in the period of record of 526.1 mm. Notably, trends for the dry season (October through May) were not significant (p = 0.35), except April which had a significantly increasing trend (p < 0.05, $R^2 = 0.08$). The SPI showed notable anomalies in precipitation, with pronounced rainy periods in the early 1980s, late 1990s, 2005, and 2015-2020. Increasing water temperature and decreasing salinity generally followed the meteorological trends for all three in situ datasets (EPC, FIM, and PDEM, Figure 4, Tables 1, 2, Figures S1, S2, S3). Note that for tables 1 and 2, comparable time periods were evaluated between the datasets when possible given the different sample sizes, and therefore power, to detect trends. The strongest trends were observed for the EPC dataset. The top and bottom water temperature or salinity changes were similar across bay segments (Figure 4). Trends in water temperature were similar across bay segments with significant increases for all four bay segments varying from 0.03 to 0.04 °C per year, with a total change from 1.3 (OTB) to 1.7 (HB) °C across the period of record from 1975 to 2022 (Table 1). Salinity trends were also similar between bay segments, although overall salinity was predictably higher for bay segments closer to the Gulf of Mexico. Only Old Tampa Bay and Lower Tampa Bay had significantly decreasing trends, with

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decreases of 0.06 (OTB) and 0.04 (LTB) ppt per year, with a total decrease of 2.6 (OTB) and 1.6 (LTB) ppt from 1975 to present.

Significant increasing temperature trends were also observed for the FIM dataset from 1996 to present in OTB and from 2004 to present in OTB and HB (Table 1). No significant temperature or salinity trends were observed in the other bay segments for the FIM and PDEM datasets from 1996 to present nor from 2004 to present (Figures S2, S3). However, the EPC dataset showed significant increasing temperature across all time periods and bay segments. Salinity trends from the EPC dataset were not significant from 1996 to present, but were significantly decreasing from 2004 to present in all bay segments (Table 2).

The EPC dataset was also used to provide detailed information on station-level trends from 1975 to present (Figure 5, see Figure S4 for 1998 to 2022). All stations had significantly increasing temperature and decreasing salinity from 1975 to present, although several stations in HB did not have significant salinity trends, the exception being the northernmost stations near the mouth of the Hillsborough Rive (Figure 5a). Seasonally, most bay segments had more significant stations during the summer, early fall period for both temperature and salinity (Figure 5b, c), although some variation was observed throughout the bay. Temperature trends were more often observed in the summer or early fall for the upper bay segments (OTB, HB), whereas the lower bay segments (MTB, LTB) had more seasonally homogeneous trends. Seasonal trends in salinity did not vary as much between segments, although OTB and LTB had more stations with significantly decreasing trends than the other bay segments.

3.2 Stressor metrics

Linear mixed-effects models for the EPC data showed similar trends in each bay segment for the number of days when temperature was above different thresholds. All the temperature models for

each of the three thresholds (29, 30, 31 °C) showed increasing trends for each bay segment, with the largest slope of 1.5 days per year in OTB when temperature was above 29 °C (Table S3). The estimated slopes for the number of days when temperature was above 30 °C varied from less than 1 day per year in LTB to 1.1 days per year in MTB. Likewise, the mean number of days when temperature was above 30 °C at the beginning and end of the period of record varied between bay segments, although most had an approximate increase of 50 days each year for the period of record (Table 3, Table S5, see Table S6 for 1998 to 2022). The increase in the number days each year when temperatures were above 29 or 31 °C was similar. The salinity models were less similar between bay segments compared to the temperature models, primarily because of the natural salinity gradient along the bay's longitudinal axis (Tables S3, S4). None of the bay segments had significantly increasing number of days per year when salinity was below 15 ppt. The only significantly increasing model for MTB was the number of days when salinity was below 25 ppt, which was increasing at a rate of 0.5 days per year. Both HB and OTB had significantly increasing number of days per year when salinity was below 20 and 25 ppt, although the slopes varied such that the rates in OTB were more than twice those in HB. Some salinity models had significantly decreasing trends, although the total number of days at the beginning and end of the period of record were neglible (Table S5). Overall, OTB showed the largest increase in the number of days each year when salinity was below a theshold, particularly for 25 ppt, where the change was 86 days per year from 1975 to 2022 (130 days to 216 days per year, Table 3, Table S5, see Table S6 for 1998 to 2022). The number of days when both temperature was above and salinity was below a threshold also varied by bay segment (Table S3). The number of days when temperature was above 29 °C and salinity was below 25 ppt had the largest slopes of 1.4, 1.1, and 0.7 days per year for OTB, HB,

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and MTB, respectively. Likewise, the average number of days when both temperature was above 29 °C and salinity was below 25 ppt from the beginning to the end of the period showed the greatest increase for OTB of 68 days (4 days to 72 days per year from 1975 to 2022).

Figure 6 provides visual examples of the mixed-effects models for the estimated number of days over time for each bay segment from 1975 to present when temperature was above 30°, salinity was below 25 ppt, and when both occurred (see Figure S6 for 1998 to 2022). Temperature trends were similar among segments, whereas the number of days when salinity was below the threshold decreased with proximity to the Gulf of Mexico (Table 3). The number of days when both temperature was above and salinity was below the threshold generally followed the trends for the number of days when salinity was below the threshold. These thresholds were used for comparison to seagrass changes described below, based primarily on the statistical strength of the trends and the variance of counts across stations within each bay segment (points in Figure 6). That is, more restrictive thresholds did not provide sufficient counts of days per year

3.3 Seagrass response

primarily on statistical considerations.

Linear models to assess the potential effects of temperature and salinity on seagrass change provided modest evidence that seagrass loss after 2016 was influenced by climate stressors. The most parsimonious model for the EPC data included the temperature and salinity metrics as potentially important for explaining inter-annual variation in seagrass frequency occurrence (Adj. $R^2 = 0.59$, F = 12.66, df = 9, 65, p < 0.005). An interaction of the metrics with time period was also observed, with notable differences by predictor (Figure 7a, Table 4). For the period prior to 2016, frequency occurrence of seagrass increased as the number of days where

to more rigorously develop seagrass response models and the chosen thresholds were based

temperature was above 30° increased, whereas no relationship was observed after 2016. However, the interaction between time period and temperature was not significant, suggesting no difference between time periods. The opposite trend was observed for the number of days when salinity was below 25 ppt, where no relationship was observed prior to 2016 and a decreasing relationship was observed after 2016 (interaction term between salinity and time period was marginally significant, p < 0.1, Figure 7a, Table 4). The interaction between the temperature and salinity metric was included in the most parsimonious model, although it was not significant. The second model for the EPC data that evaluated the number of days when both temperature was above and salinity was below the threshold was also significant (Adj. $R^2 = 0.6$, F = 29.16, df = 4, 70, p < 0.005), although the interaction term with time period was not included in the most parsimonious model, suggesting no effect of this metric in describing seagrass change after 2016 (Table 4). Note that Figure 7 shows model predictions for OTB only, although the shape of the relationships do not change for other bay segments other than as additive shifts of the curves up or down. The most parsimonious model for the FIM dataset also showed potential temperature and salinity associations with seagrass change after 2016, although explanatory power was lower than the EPC models likely due to larger spread of the data (Adj. $R^2 = 0.12$, F = 32.75, df = 7, 1659, p < 0.005). Increases in temperature and decreases in salinity were both associated with reductions in percent cover after 2016, whereas no relationships were observed prior to 2016 (p < 0.05 and p < 0.1 for the interactions between temperature or salinity with time period, respectively, Figure 7b, Table 4). The interaction between salinity and temperature was not included as a significant term in the final FIM model. Lastly, the most parsimonious model for the PDEM dataset only

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included time period as a marginally significant variable and the overall model fit was not significant (Table 4).

4 Discussion

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Global increases in temperature and altered precipitation patterns related to climate change have had measurable effects on the structure and functioning of a wide range of natural environments (Osland et al. 2015; Oliver et al. 2018). For Tampa Bay, these changes have been demonstrated using long-term trends in water temperature and salinity, which mirrored long-term changes in air temperature and precipitation. Tampa Bay has gotten hotter and fresher; water temperature has increased by 0.03 - 0.04 °C per year and salinity has decreased by 0.04 - 0.06 ppt per year, translating to an increase of 1.3 to 1.7 °C and a decrease of 1.6 to 2.6 ppt over the past fifty years. These changes were demonstrated in three long-term datasets with different sampling methods and periods of record. Understandably, the trends were most clearly observed in the dataset with the longest period of record (EPC), covering nearly fifty years of monthly observations. These long-term changes manifested into consistent trends in known seagrass stressors; the continuous number of days increased each year when temperature, salinity, or both crossed thresholds. Similar regional, long-term changes in coastal waters and estuaries have been observed by others (Carlson et al. 2018; Nickerson et al. 2023; Shi and Hu In review). Nickerson et al. (2023) evaluated sea surface trends at a larger spatial scale for Tampa Bay, the West Florida Continental Shelf, and the adjacent Gulf of Mexico. Temperature trends were similar to those herein for Tampa Bay (the EPC dataset was also used). Nickerson et al. (2023) also noted that temperature increases in Tampa Bay were most pronounced in the winter, although they rightfully acknowledge the sensitivity of their results to conditions at the start and end of the time series.

Our assessment evaluated non-parametric trends (i.e., Kendall tests, less sensitive to outliers) at individual EPC stations and bay segments, whereas Nickerson et al. (2023) evaluated the EPC data as an average for the entire bay for consistency of comparison to their larger spatial area and model domain. Our results showing increases in temperature and decreases in salinity in the summer, early fall, most notably for OTB and northern stations of HB Figure 5, are likely related to hydrodynamic characteristics of these segments relative to MTB and LTB that flush more regularly with the Gulf of Mexico. These upper bay segments are more affected by hydrologic inflows (HB), lack of circulation (OTB), or thermal stress related to more rapid warming with shallower depths. The significant reduction in salinity for LTB is also of note, perhaps related to gravitational circulation patterns that export lower salinity water from upstream in the main shipping channels (Weisberg and Zheng 2006). Additionally, Shi and Hu (In review) provided a recent assessment of a 2023 heatwave in south Florida, supported by a 20-year trend assessment that suggested estuaries were warming at nearly double the rate of the Gulf of Mexico. The upper limit of our warming estimate for Tampa Bay is comparable. Notably, Carlson et al. (2018) suggest a link between historical seagrass losses in Florida Bay and rapid warming in shallow areas with low surface reflectance. Our relatively simple modeling approach provided some evidence that climate-related stressors impart some effect on recent seagrass losses in Tampa Bay. The models did not provide a consistent, nor statistically powerful, explanation that increasing temperature and decreasing salinity were key (or the sole) drivers. However, evaluating all models together as weight-ofevidence suggests there is value in considering multiple datasets and models to interpret noisy patterns and compounding ecological processes. Model results for the EPC and FIM datasets both suggested that increasing temperature and decreasing salinity were associated with potential

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seagrass loss post 2016, described primarily using separate interaction terms of temperature or salinity with time period. For the EPC model, the interaction term was not significant for temperature, whereas the interaction was marginally significant for the salinity metric, such that a negative association between seagrass and salinity stress was observed post 2016 as compared to pre 2016. Likewise, the FIM models had a significant interaction term for the association of temperature with time period and a marginally significant interaction term for the association of salinity with time period. An important distinction between the EPC and FIM models is that the former evaluated the number of days above/below thresholds each year to quantify annual temperature or salinity stress, whereas the latter evaluated observed temperature and salinity values at the time of seagrass sampling. As such, both models attempted to describe the role of these stressors on potential seagrass change, but use different independent variables given the different sampling designs of each monitoring program. These differences highlight challenges describing autecological relationships in long-term datasets, while also demonstrating the utility of our weight-of-evidence approach to describe such relationships. An additional caveat of our models was the use of "thresholds" to define potential stressor metrics for temperature and salinity on an annual time scale. Our choice to use 30 °C and 25 ppt for temperature and salinity was primarily a statistical consideration given a consistent increase over time in the number of days when these thresholds were crossed. That is, sufficient change and variation in the independent variables for the models of seagrass change were needed to statistically describe potential relationships. The reported threshold values in tropical and subtropical environments suggest that the limits of the ecological niche for seagrasses are higher for temperature and lower for salinity (R. C. Phillips 1960; McMillan and Moseley 1967; Zieman 1975; Lirman and Cropper 2003). Because we did not see a dramatic increase in the number of

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days each year when the thresholds were crossed at more stressful values, conditions in Tampa Bay in recent years are generally within the ecological niche for seagrasses. This does not suggest that these factors are unimportant, both currently and in the future. Extreme temperature or precipitation events acting individually or in combination are likely captured by the trends in stressor metrics using these lower thresholds, i.e., an increase in a bay segment median number of days also suggests extremes are increasing given the variation around these summary metrics (Figure 6). Our thresholds may also be indicative of the potential for chronic sublethal effects of stress on seagrasses, reducing their resilience to other stressors. Regardless, our models suggested that temperature and salinity are at least associated with seagrass loss and, if so, longterm trends in both are set to amplify their effect. Additional limitations of our models may relate to an incomplete description of factors influencing seagrass growth, such as the inclusion of additional drivers and an incomplete or overly simplified causal network. For the former, the primary management paradigm in Tampa Bay for the past three decades has relied on the role of external nitrogen inputs in affecting light environments for seagrass growth (Greening et al. 2014; Sherwood et al. 2017). As such, light attenuation or water clarity could have been included in our models to more completely describe factors influencing growth, i.e., the residual differences after accounting for light attenuation could additionally be explained by temperature or salinity. However, our use of time period (pre/post 2016) as a categorical variable indirectly addressed this issue. Modeling seagrass change as related to temperature or salinity for the entire record would have shown a spurious correlation of both with seagrass given the long-term recovery of seagrass, i.e., modeling challenges related to correlated predictors (Fourqurean et al. 2003). Thus, time period was

necessary to control for these confounding relationships. Additionally, light attenuation has been

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relatively consistent since 2016 and within the limits estimated to be supportive of seagrass growth in Tampa Bay, particularly in OTB where the most loss occurred (Janicki and Wade 1996; Beck 2020a). A final consideration for our models relates to how seagrasses may influence their environment, particularly for the PDEM and FIM datasets where temperature and salinity were measured at the same locations as seagrass. For example, temperature may simply be lower in locations where seagrasses are present and can absorb solar radiation, i.e., seagrasses may be influencing their environment rather than the environment influencing seagrasses (Carlson et al. 2018). This explanation cannot be ruled out with the existing datasets, although the trend analyses and models suggest that climate-related stressors are a more likely scenario. This is especially true for water temperature trends captured by the EPC dataset which includes deeper, fixed sites adjacent to shallow seagrass flats. The seagrass loss in Tampa Bay since 2016 is a notable phenomenon that is not limited to our study area (Lizcano-Sandoval et al. 2022). Losses have been observed throughout southwest Florida during this time period, including Sarasota Bay directly south of Tampa Bay and Charlotte Harbor further south (Tomasko et al. 2020). These regional losses suggest that largescale stressors are driving these changes, supporting our initial hypothesis that climate-related stressors could partially explain the change in Tampa Bay. Based on our results, the losses elsewhere may potentially be explained by temperature and salinity and are worth exploring in other southwest Florida coastal regions where long-term datasets exist (Tomasko et al. 2005). Additional factors that could explain these changes are also likely co-occurring with climate stress, some of which are unique to Tampa Bay and others that are more likely pervasive. For Tampa Bay, annual summer/fall blooms of the toxic dinoflagellate *Pyrodinium bahamense* have occurred in OTB since 2008 (Usup et al. 1994; Lopez et al. 2023) and the specific relationships

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of these blooms with seagrass change is unclear, although the expectation is that seagrass growth may be limited by the degradation of the light environment with algal growth. These blooms are exacerbated by the hydrologic conditions in OTB that contribute to relatively longer water residence times (Phlips et al. 2006; Lopez et al. 2021). The effect of warming temperature and decreasing salinity in OTB will further complicate the understanding of how these blooms manifest and persist each year (Koch et al. 2007; Stelling et al. 2023), and ultimately contribute to changes in the light environment affecting seagrass resources in this bay segment. Additional biotic factors could be influencing regional patterns in seagrass growth. In Tampa Bay and elsewhere, enhanced macroalgal production has been a recent concern (L. M. Hall et al. 2022; Janicki Environmental, Inc. 2022; Brewton and Lapointe 2023; Scolaro et al. 2023). Attached macroalgae abundance has increased over time and has been observed to colonize locations where seagrass was formerly present in Tampa Bay (Beck 2020b). Competitive differences between seagrasses and macroalgae are poorly understood in these systems (but see Bell and Hall 1997; Taplin et al. 2005; Brewton and Lapointe 2023), in addition to insufficient macroalgae data in Tampa Bay that cannot clearly describe seasonal growth, distribution patterns, and nutrient cycling. Discrete pollutant loading events in Tampa Bay have been documented to promote both phytoplankton and macroalgae growth (Beck et al. 2022; Scolaro et al. 2023; Tomasko 2023). The role that evolving nutrient loading and changing climatic conditions may have on Tampa Bay's primary producers – particularly algal and seagrass growth and interactions in recent years – is not well understood. Finally, additional research has focused on how diseases and pathogens can influence seagrass growth patterns in Florida (Robblee et al. 1991; Van Bogaert et al. 2018; Duffin et al. 2021). For example, the parasitic slime mold Labryinthula spp. that causes seagrass wasting disease has been known to infect Thalassia

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testudinium in Tampa Bay (Blakesley et al. 2001), although it is unclear if these infections have had large-scale, population-level effects. Existing research has primarily focused on describing spatial patterns, past die-off events, or immunology of these pathogens (Robblee et al. 1991; Duffin et al. 2021). More research should be directed towards the influence of climate stressors on seagrass pathogen vulnerability. Lastly, our result showing that salinity has decreased in Tampa Bay is contrary to expectations for how sea-level rise will affect coastal systems (Costa et al. 2023; Alarcon et al. 2024), as salinity increases with sea-level rise have already caused numerous alterations of subtidal and nearshore habitats (Brinson et al. 1995; White and Kaplan 2017). In southwest Florida, the most common ecological example is the upland expansion of mangroves in response to increased porewater salinity and water levels over the past few decades (Borchert et al. 2018). Alteration of salinity regimes for surface and groundwater resources have been well documented. In Florida Bay, for example, widespread decline of T. testudinium has been attributed to altered hydrology and drought-induced hypersaline conditions, and sea level rise is expected to further modify salinity dynamics in the region (Margaret O. Hall et al. 2016). Dessu et al. (2018) noted that sea level rise is expected to have the largest effect on salinity changes during periods of low freshwater outflow from the Florida Everglades, emphasizing that measured salinity represents the relative contributions of oceanic and freshwater surface waters. In Tampa Bay, the long-term trends of decreasing salinity, especially in the upper bay segments, suggest that the hydrologic loading has had a greater influence on salinity regimes than the effects of sea-level rise. This hypothesis is supported by our assessment of precipitation patterns over time, where the longterm increase is inversely associated with the decrease in salinity.

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4.1 Conclusions

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This study provided a detailed assessment of long-term water temperature and salinity changes in Tampa Bay supported by datasets from three long-term monitoring programs of different length and sampling design. An evaluation of each dataset showed a clear pattern of increasing temperature and decreasing salinity mirrored by long-term changes in air temperature and precipitation, suggesting that Tampa Bay has become hotter and fresher with the trends likely continuing in the future. Simple regression models provided weak, but partially-supporting evidence, that these changes can be linked to recent seagrass losses since 2016. Our models suggested that rising temperatures and decreasing salinities have had additive rather than multiplicative effects on seagrass, as evidenced by lack of significant interactions in models involving both stressors. Future analyses may show more significant associations between physicochemical habitat conditions and seagrass change as the trends are very likely to continue to push seagrasses further outside of their tolerance ranges. These analyses should be supported by additional data collection efforts, particularly high-resolution continuous monitoring data that provide a more precise assessment of diurnal stress across multiple time-scales. Morpohological or physiological measurements at the individual level could also provide early indications of heat and osmotic stress. Natural resource managers should consider how these climate-related stressors may alter the effectiveness of intervention activities aimed at protecting ecological resources in Tampa Bay. Management actions that have historically been effective may not be able to maintain ecosystem resilience to climatic change. For example, nitrogen load reductions have been effective at restoring seagrass in Tampa Bay (Greening and Janicki 2006; Greening et al. 2014). As Tampa Bay becomes hotter and fresher, current nutrient loads may no longer be effectively assimilated

and algal and seagrass ecology dynamics may shift. Strategies that mimic or restore predevelopment hydrology or that further reduce allowable load inputs from regulated entities (e.g.,
additional stormwater controls, hydrological modifications) may be needed to confer additional
resilience and adaptive capacity for seagrass in Tampa Bay. These considerations are especially
critical for upper parts of Tampa Bay where a majority of seagrass loss has occurred and where
temperature and salinity trends appear most pronounced. Reversal of recent trends may be more
likely to occur if aggressive actions and controls are pursued sooner rather than later, given the
challenges of restoring these long-lived foundation species once lost, ongoing development in
the watershed, and the current climate trajectory.

Acknowledgments

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697 Figures

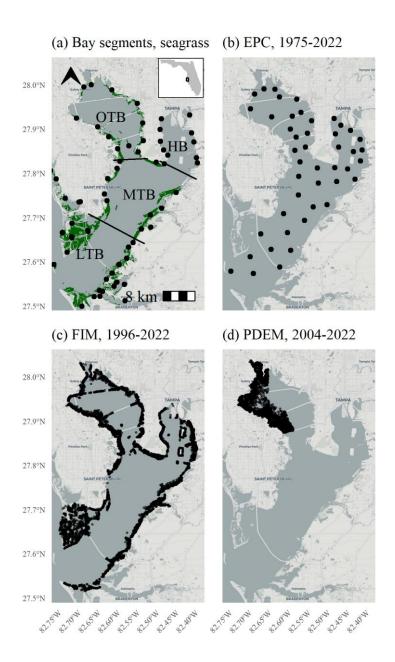
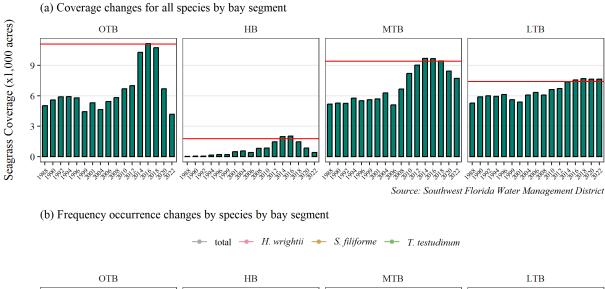


Fig. 1: Map of Tampa Bay and the three datasets used for trend analysis, including (a) bay segments, 2022 seagrass coverage (green), and transect starting points (black), (b) Environmental Protection Commission (EPC) long-term monitoring sites, (c) Fisheries Independent Monitoring (FIM) random sampling for seine hauls, and (d) OTB portion of Pinellas County Department of Environmental Management (PDEM) random sampling. Date ranges for each dataset are shown in the title. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.



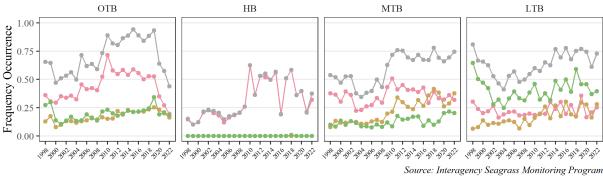


Fig. 2: Seagrass changes over time in Tampa Bay for (a) areal coverage 1988 - 2022 from mapping and (b) frequency occurrence of major species 1998 - 2022 from annual transect monitoring. Changes are shown for major bay segments. Red lines in (a) show approximate capacity of seagrass coverage based on the baywide target of 40,000 acres. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

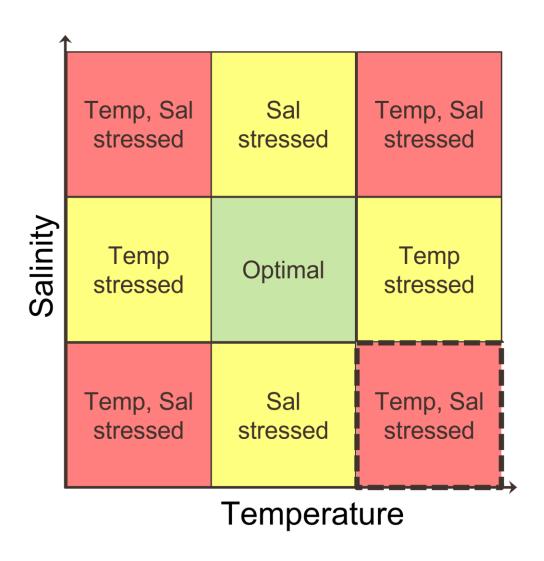


Fig. 3: Conceptual stressor diagram demonstrating a two-dimensional niche space for temperature and salinity. Tampa Bay is trending towards the bottom right.

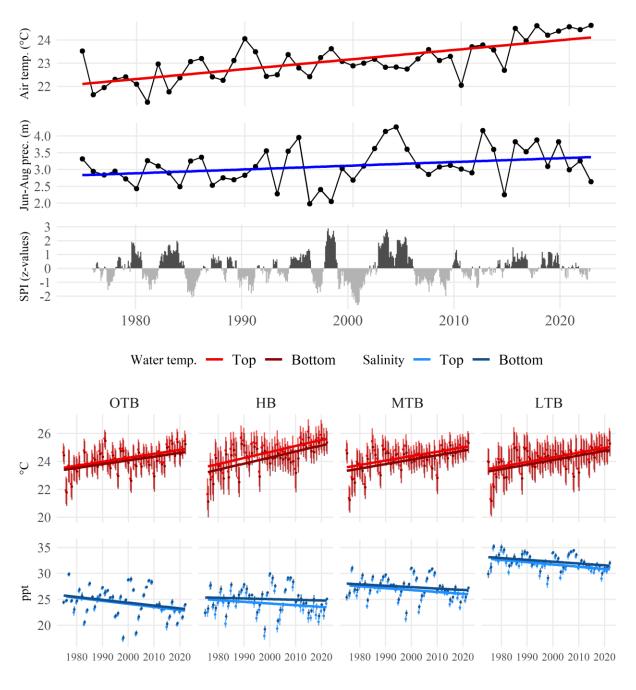


Fig. 4: Long-term air temperature, precipitation (Jun-Aug), Standard Precipitation Index (SPI), water temperature, and salinity trends from 1975 to 2022. The color shades for water temperature and salinity indicate sampling location and values shown are the averages (95% confidence interval) across all Environmental Protection Commission (EPC) stations in each bay segment and sampling months for each year. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

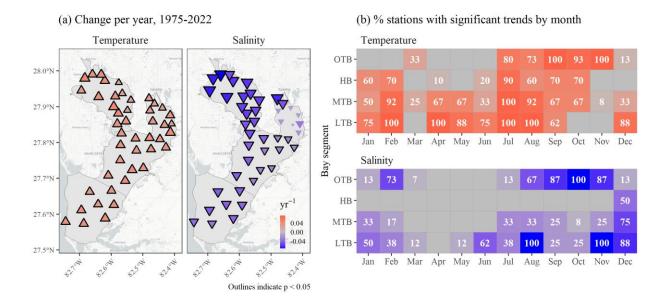


Fig. 5: Trends from 1975 to 2022 for bottom water temperature and salinity measurements at long-term monitoring stations in Tampa Bay. Results for seasonal Kendall tests by station are shown in (a) with color and shape corresponding to the estimated annual slope as change per year (yr⁻¹). Summarized seasonal trends by month are shown as (b) the percent of stations in each bay segment with significant increasing (temperature) or decreasing (salinity) trends. Bay segment outlines are shown in (a); OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

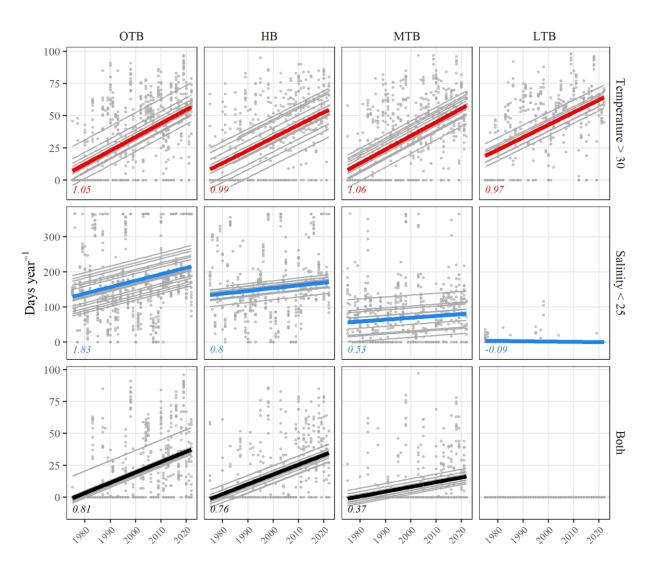


Fig. 6: Example of mixed effects models for the estimated number of days per year that temperature (red) or salinity (blue) were above or below thresholds of 30 °C or 25 ppt, respectively, from the EPC data. The bottom row (black) shows the number of days when both temperature was above and salinity was below the thresholds. The models included station as a random effect for each bay segment, with grey lines indicating individual station trends, grey points as actual number of days, and thicker lines indicating the overall model fit. Slopes for significant models are shown in the bottom left of each facet. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

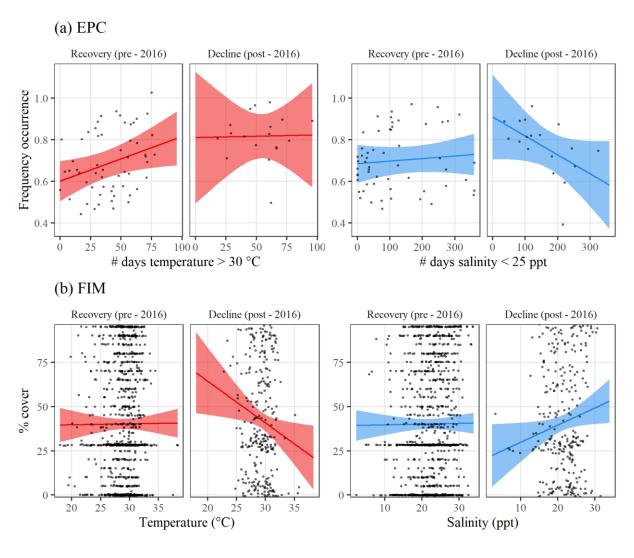


Fig. 7: Predicted results from generalized linear models evaluating seagrass changes versus temperature or salinity predictors for the (a) EPC and (b) FIM datasets. Results are shown only for OTB, although the shape of the relationships do not change between bay segments. The predictors for (a) were the number of days when temperature was above the threshold and the number of days when salinity was below the threshold. The predictors for (b) were measured temperature and salinity at the bottom of the water column. Time periods were seagrass recovery prior to 2016 and seagrass decline after 2016. Shaded areas are 95% confidence intervals. Points are observed data that include aggregated effects of bay segment included in the models.

Tables

Table 1: Bottom temperature trends (°C) by bay segments and datasets. Start year describes the range of the trend test to the present year (2022). The starting value is the estimated temperature at the start year and the end value is the estimated temperature in 2022. Total change is the difference between the two. Datasets evaluated were from the Environmental Protection Commission of Hillsborough County (EPC), Fisheries Independent Monitoring (FIM), and Pinellas County Department of Environmental Management (PDEM). Note that PDEM includes data only for the western portion of Old Tampa Bay from 2004 to present and EPC is the only dataset beginning prior to 1996. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay. ** p < 0.005, * p < 0.05

Start year	Bay segment	Dataset	Change / year	Start value	End value	Total change
1975	OTB	EPC	0.03**	23.4	24.7	1.3
	HB	EPC	0.04**	23.4	25.1	1.7
	MTB	EPC	0.03**	23.3	24.8	1.5
	LTB	EPC	0.03**	23.3	24.8	1.5
1996	OTB	EPC	0.04*	23.6	24.8	1.2
		FIM	0.05*	23.5	24.8	1.3
	HB	EPC	0.06**	23.8	25.3	1.5
		FIM	0.05	24.0	25.3	1.3
	MTB	EPC	0.04**	23.8	24.8	1.0
		FIM	0.03	24.0	24.8	0.8
	LTB	EPC	0.03*	23.9	24.8	0.9
		FIM	0.02	24.1	24.6	0.5
2004	OTB	EPC	0.06*	23.8	25.0	1.2
		FIM	0.06	23.8	24.9	1.1
		PDEM	0.04	24.4	25.2	0.8
	HB	EPC	0.09**	23.9	25.5	1.6
		FIM	0.1*	23.9	25.7	1.8
	MTB	EPC	0.07**	23.8	25.0	1.2
		FIM	0.07	23.8	25.1	1.3
	LTB	EPC	0.06*	23.8	25.0	1.2
		FIM	0.05	23.9	24.8	0.9

Table 2: Bottom salinity trends (ppt) by bay segments and datasets. Start year describes the range of the trend test to the present year (2022). The starting value is the estimated salinity at the start year and the end value is the estimated salinity in 2022. Total change is the difference between the two. Datasets evaluated were from the Environmental Protection Commission of Hillsborough County (EPC), Fisheries Independent Monitoring (FIM), and Pinellas County Department of Environmental Management (PDEM). Note that PDEM includes data only for the western portion of Old Tampa Bay from 2004 to present and EPC is the only dataset beginning prior to 1996. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay. ** p < 0.005, * p < 0.05

Start year	Bay segment	Dataset	Change / year	Start value	End value	Total change
1975	OTB	EPC	-0.06*	25.7	23.1	-2.6
	HB	EPC	-0.01	25.4	24.7	-0.7
	MTB	EPC	-0.03	28.0	26.7	-1.3
	LTB	EPC	-0.04*	33.1	31.5	-1.6
1996	OTB	EPC	-0.08	24.9	22.8	-2.1
		FIM	-0.05	24.1	22.7	-1.4
	HB	EPC	-0.08	26.1	23.9	-2.2
		FIM	0	24.0	23.9	-0.1
	MTB	EPC	-0.07	28.0	26.3	-1.7
		FIM	-0.03	26.6	25.8	-0.8
	LTB	EPC	-0.04	32.5	31.4	-1.1
		FIM	0.01	31.6	32.0	0.4
2004	OTB	EPC	-0.2*	25.5	22.0	-3.5
		FIM	-0.15	24.6	22.0	-2.6
		PDEM	-0.19	24.3	20.9	-3.4
	HB	EPC	-0.19*	26.5	23.1	-3.4
		FIM	-0.08	24.7	23.4	-1.3
	MTB	EPC	-0.17*	28.6	25.6	-3.0
		FIM	-0.11	27.2	25.3	-1.9
	LTB	EPC	-0.12*	33.0	30.9	-2.1
-		FIM	-0.05	32.5	31.5	-1.0

Table 3: Summary of mixed-effects models evaluating increases in the number of days each year from 1975 to 2022 when temperature was above 30 °C, salinity was below 25 ppt, 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. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

Bay Segment	Threshold	Slope	Start	End
OTB	Temperature > 30	1.05**	7 (3.3)	57 (3.2)
	Salinity < 25	1.83**	130 (13)	216 (13)
	Both	0.81**	-1 (2.7)	37 (2.7)
HB	Temperature > 30	0.99**	8 (4.9)	55 (4.8)
	Salinity < 25	0.8*	134 (12.2)	171 (11.5)
	Both	0.76**	-1 (2.9)	35 (2.7)
MTB	Temperature > 30	1.06**	8 (3.1)	58 (3.1)
	Salinity < 25	0.53*	56 (11.8)	81 (11.8)
	Both	0.37**	-1 (1.7)	16 (1.7)
LTB	Temperature > 30	0.97**	19 (3.4)	64 (3.4)
	Salinity < 25	-0.09*	4 (1.2)	0 (1.2)
	Both	-	-	-

Table 4: Summary of the most parsimonious Generalized Linear Models (GLMs) developed to evaluate the effects of temperature and salinity variables on seagrass change between time periods. Models were developed for different datasets described in the model column. The significant intercept term for each model is not shown and described factor conditions not explicitly shown by the remaining terms (e.g., OTB and pre-2016). The model term estimates include the standard error in parentheses. Note that the "Temp" and "Sal" terms for the EPC models describe metrics that measure the number of days when temperature was above or salinity was below a threshold value, whereas the terms in all other models describe observed temperature or salinity (see text for description). ** p < 0.005, * p < 0.05, · p < 0.1. EPC: Environmental Protection Commission, FIM: Fisheries Independent Monitoring, PDEM: Pinellas County Department of Environmental Management. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay.

Model	Term	Estimate	t-statistic
EPC 1	Sal	0.00068 (0.00037)	1.8·
	Temp	0.0034 (0.0013)	2.6*
	Time_post	0.48 (0.29)	1.7
	Baysegment_HB	-0.39 (0.042)	-9.3**
	Baysegment_MTB	-0.12 (0.047)	-2.5*
	Sal:Temp	-1.2e-05 (7.5e-06)	-1.7
	Sal:Time_post	-0.0027 (0.0015)	-1.8·
	Temp:Time_post	-0.0057 (0.0052)	-1.1
	Sal:Temp:Time_post	3.6e-05 (2.7e-05)	1.4
EPC 2	Both	0.0028 (0.00081)	3.4**
	Time_post	0.059 (0.039)	1.5
	Baysegment_HB	-0.38 (0.04)	-9.4**
	Baysegment_MTB	-0.063 (0.042)	-1.5
FIM	Sal	0.043 (0.22)	0.2
	Temp	0.052 (0.42)	0.12
	Time_post	52 (32)	1.6
	Baysegment_MTB	33 (2.5)	13**
	Baysegment_OTB	28 (2.2)	13**
	Sal:Time_post	0.93 (0.5)	1.8·
	Temp:Time_post	-2.4 (1.1)	-2.2*
PDEM	Time_post	-0.45 (0.24)	-1.8·

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Supplement Hot and fresh: evidence of climate-related suboptimal conditions for seagrass in a large Gulf coast estuary

Marcus W Beck^{1,⊠}, Kerry Flaherty-Walia¹, Sheila Scolaro¹, Maya C Burke¹, Bradley T Furman², David J Karlen³, Christopher Pratt³, Christopher J Anastasiou⁴, and Edward T Sherwood¹

¹ Tampa Bay Estuary Program, 263 13th Ave S., Suite 350, St. Petersburg, Florida 33701 USA

² Florida Fish and Wildlife Conservation Commission, Florida Fish and Wildlife Research Institute, 100 8th Avenue SE, St. Petersburg, FL 33701, USA

³ Environmental Protection Commission of Hillsborough County, 3629 Queen Palm Drive, Tampa, FL 33619, USA

⁴ Southwest Florida Water Management District, 7601 US Highway 301 North, Tampa, FL 33637, USA

[™]Correspondence: Marcus W Beck <mbeck@tbep.org>

Figures

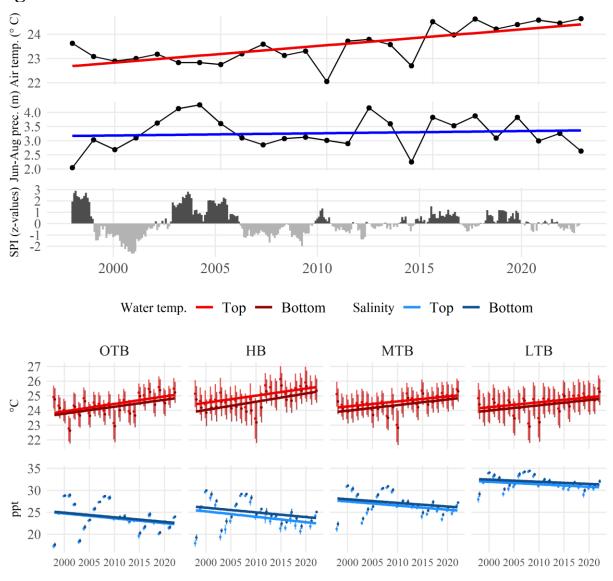


Fig. S1: Air temperature, precipitation (Jun-Aug), Standard Precipitation Index (SPI), water temperature, and salinity trends from 1998 to 2022. The color shades for water temperature and salinity indicate sampling location and values shown are the averages (95% confidence interval) across all Environmental Protection Commission (EPC) stations in each bay segment and sampling months for each year. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

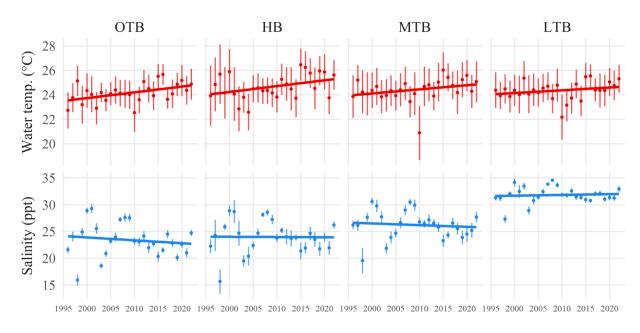


Fig. S2: Bottom water temperature and salinity trends from 1996 to 2021 for stations from the Fisheries Independent Monitoring program. Points show the average (95% confidence interval) across all stations in each bay segment and sampling months for each year. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

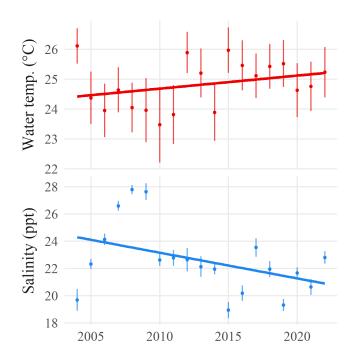


Fig. S3: Bottom water temperature and salinity trends from 2004 to 2022 for stations from the Pinellas County Department of Environmental Management in Old Tampa Bay. Points show the average (95% confidence interval) across all stations in each bay segment and sampling months for each year.

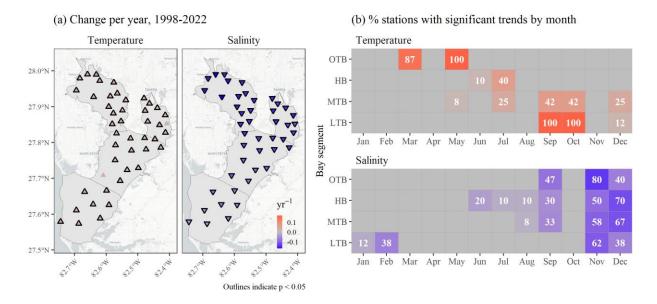


Fig. S4: Trends from 1998 to 2022 for temperature and salinity measurements at long-term monitoring stations in Tampa Bay. Results for seasonal Kendall tests by station and monitoring location (top or bottom of the water column) are shown in (a) with color and shape corresponding to the estimated annual 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: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

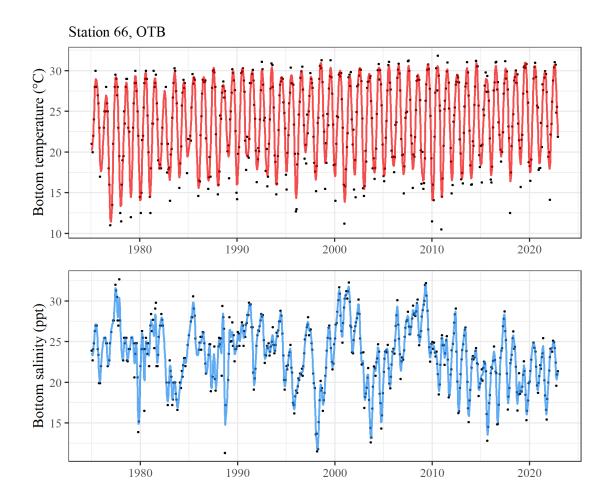


Fig. S5: Examples of Generalized Additive Models of bottom water temperature and salinity for station 66 in Old Tampa Bay. Models were fit using a continuous smoothing spline for decimal year. Monthly observations are shown as points and daily predictions from the models as lines.

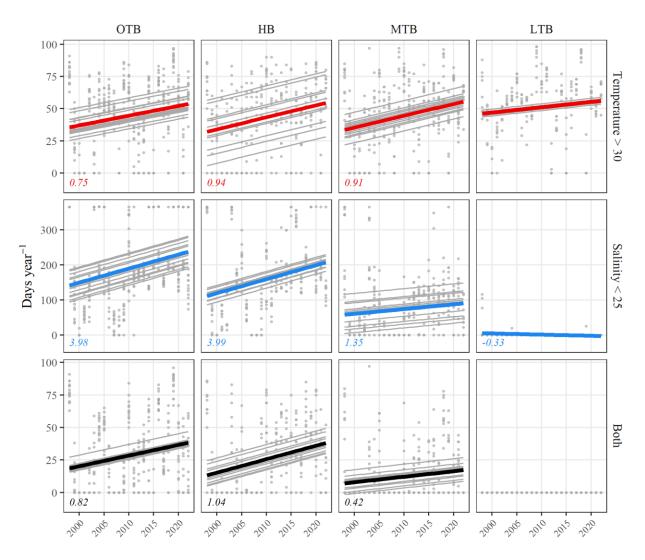


Fig. S6: Example of mixed effects models for the estimated number of days per year that temperature (red) or salinity (blue) were above or below thresholds of 30 °C or 25 psu, respectively, from 1988 to 2022. The bottom row (black) shows the number of days when both temperature was above and salinity was below the thresholds. The models included station as a random effect for each bay segment, with grey lines indicating individual station trends, grey points as actual number of days, and thicker lines indicating the overall model fit. Slopes for significant models are shown in the bottom left of each facet. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

Tables

Table S1: Summary statistics for Generalized Additive Model fits to bottom water temperature data at all stations in Tampa Bay monitored by the Environmental Protection Commission of Hillsborough County. Models were fit on monthly data from 1975 to 2022 using decimal year as the single predictor. Fit statistics are Akaike Information Criterion (AIC), Generalized Cross-Validation (GCV) scores, and R-squared values of observed to predicted. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

Bay Segment	Station	Lon	Lat	AIC	GCV	R^2
OTB	36	-82.553	27.856	2153	3.74	0.92
	38	-82.577	27.882	2206	4.16	0.91
	40	-82.587	27.929	2177	3.83	0.92
	41	-82.565	27.937	2282	4.69	0.90
	46	-82.659	27.990	2298	6.02	0.87
	47	-82.620	27.973	2243	4.28	0.91
	50	-82.538	27.919	2246	4.56	0.90
	51	-82.549	27.890	2073	3.87	0.91
	60	-82.632	27.990	1937	5.51	0.88
	63	-82.576	27.968	2231	5.31	0.89
	64	-82.683	27.979	1110	5.49	0.90
	65	-82.694	27.946	2110	5.03	0.88
	66	-82.640	27.928	2207	4.18	0.91
	67	-82.592	27.900	2274	4.52	0.90
	68	-82.581	27.852	2185	3.98	0.92
HB	6	-82.477	27.889	2348	4.85	0.89
	7	-82.469	27.859	2275	4.30	0.90
	8	-82.409	27.852	1288	4.49	0.90
	44	-82.481	27.924	1520	5.46	0.88
	52	-82.438	27.897	2241	4.13	0.91
	55	-82.431	27.849	2260	4.20	0.91
	70	-82.463	27.909	1545	4.23	0.90
	71	-82.414	27.876	2379	5.06	0.88
	73	-82.413	27.828	2318	5.05	0.89
	80	-82.446	27.810	2229	4.15	0.91
MTB	9	-82.427	27.787	2503	6.22	0.85
	11	-82.479	27.813	2217	4.53	0.90
	13	-82.523	27.812	2032	3.09	0.94
	14	-82.520	27.778	2193	3.74	0.92
	16	-82.534	27.724	2074	3.39	0.94
	19	-82.556	27.693	2039	3.25	0.94
	28	-82.609	27.708	1989	3.25	0.94
	32	-82.571	27.793	2015	3.07	0.94
	33	-82.567	27.826	2091	3.43	0.93
	81	-82.474	27.781	2204	3.85	0.91
	82	-82.572	27.751	1958	3.17	0.94
	84	-82.499	27.729	2080	4.45	0.90

LTB	23	-82.599	27.666	1978	3.04	0.95
	24	-82.619	27.588	2380	5.19	0.89
	25	-82.668	27.663	2188	4.37	0.91
	90	-82.591	27.626	2109	3.49	0.93
	91	-82.642	27.628	2019	3.18	0.94
	92	-82.687	27.574	2065	3.35	0.94
	93	-82.744	27.579	2038	3.29	0.94
	95	-82.695	27.611	2042	3.29	0.94

Table S2: Summary statistics for Generalized Additive Model fits to bottom salinity data at all stations in Tampa Bay monitored by the Environmental Protection Commission of Hillsborough County. Models were fit on monthly data from 1975 to 2022 using decimal year as the single predictor. Fit statistics are Akaike Information Criterion (AIC), Generalized Cross-Validation (GCV) scores, and R-squared values of observed to predicted. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

Day Coment	Station	Lon	Lot	A IC	GCV	R^2
Bay Segment	Station	Lon	Lat	AIC		
OTB	36	-82.553	27.856	1645	2.07	0.92
	38	-82.577	27.882	1602	1.88	0.94
	40	-82.587	27.929	1571	1.75	0.94
	41	-82.565	27.937	1640	1.92	0.93
	46	-82.659	27.990	1590	2.18	0.94
	47	-82.620	27.973	1616	1.81	0.94
	50	-82.538	27.919	1601	1.92	0.93
	51	-82.549	27.890	1526	1.84	0.93
	60	-82.632	27.990	1339	2.05	0.95
	63	-82.576	27.968	1572	2.02	0.94
	64	-82.683	27.979	1114	5.70	0.89
	65	-82.694	27.946	1404	1.75	0.95
	66	-82.640	27.928	1600	2.00	0.94
	67	-82.592	27.900	1550	1.77	0.94
	68	-82.581	27.852	1725	2.22	0.91
HB	6	-82.477	27.889	2091	4.36	0.86
	7	-82.469	27.859	2088	4.03	0.85
	8	-82.409	27.852	1446	6.74	0.82
	44	-82.481	27.924	1482	6.68	0.89
	52	-82.438	27.897	1809	2.66	0.89
	55	-82.431	27.849	2026	3.33	0.82
	70	-82.463	27.909	1567	5.01	0.87
	71	-82.414	27.876	1980	3.54	0.87
	73	-82.413	27.828	1895	3.35	0.87
	80	-82.446	27.810	1847	2.89	0.88
MTB	9	-82.427	27.787	1778	2.50	0.89
	11	-82.479	27.813	2344	5.31	0.68
	13	-82.523	27.812	1702	2.34	0.90
	14	-82.520	27.778	1750	2.25	0.88
	16	-82.534	27.724	1553	2.60	0.90
	19	-82.556	27.693	1954	2.83	0.79
	28	-82.609	27.708	2031	3.61	0.82
	32	-82.571	27.793	1789	2.49	0.87
	33	-82.567	27.826	1630	2.06	0.92
	81	-82.474	27.781	1920	2.93	0.85
	82	-82.572	27.751	1409	2.31	0.94
	84	-82.499	27.729	1568	2.68	0.90
LTB	23	-82.599	27.666	1792	2.43	0.82

24	-82.619	27.588	1885	2.85	0.81
25	-82.668	27.663	1898	2.75	0.78
90	-82.591	27.626	2010	2.91	0.75
91	-82.642	27.628	1933	2.75	0.73
92	-82.687	27.574	1859	2.25	0.73
93	-82.744	27.579	1839	2.04	0.66
95	-82.695	27.611	1757	1.94	0.74

Table S3: Summary of mixed-effects models evaluating increases in the number of days each year from 1975 to 2022 when temperature or salinity were above or below critical thresholds, respectively. The slope estimates in the "Both" column indicate trends in the number of days when both temperature and salinity were above or below critical thresholds. ** p < 0.005, * p < 0.05, - no model. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

	Thresholds		Slopes		
Bay Segment	Temperature	Salinity	Temperature	Salinity	Both
OTB					
	29	15	1.47**	0.07	0.02
	29	20	1.47**	1.01**	0.49**
	29	25	1.47**	1.83**	1.44**
	30	15	1.05**	0.07	-0.01
	30	20	1.05**	1.01**	0.15**
	30	25	1.05**	1.83**	0.81**
	31	15	0.13*	0.07	-0.01
	31	20	0.13*	1.01**	-0.01
	31	25	0.13*	1.83**	0.08
НВ					
	29	15	1.15**	0.05	0.04*
	29	20	1.15**	0.37**	0.43**
	29	25	1.15**	0.8*	1.13**
	30	15	0.99**	0.05	0.02*
	30	20	0.99**	0.37**	0.21**
	30	25	0.99**	0.8*	0.76**
	31	15	0.18**	0.05	-
	31	20	0.18**	0.37**	-
	31	25	0.18**	0.8*	0.09**
MTB					
	29	15	1.33**	-0.01*	-
	29	20	1.33**	-0.01	0.05*
	29	25	1.33**	0.53*	0.71**
	30	15	1.06**	-0.01*	-
	30	20	1.06**	-0.01	0.01
	30	25	1.06**	0.53*	0.37**
	31	15	0.14**	-0.01*	-
	31	20	0.14**	-0.01	-
	31	25	0.14**	0.53*	0.03*
LTB					
	29	15	1.14**	0	-
	29	20	1.14**	-0.01*	-
	29	25	1.14**	-0.09*	0
	30	15	0.97**	0	-
	30	20	0.97**	-0.01*	-
	30	25	0.97**	-0.09*	-
	31	15	0.23**	0	-

	Thresholds		Slopes		
Bay Segment	Temperature	Salinity	Temperature	Salinity	Both
	31	20	0.23**	-0.01*	-
	31	25	0.23**	-0.09*	-

Table S4: Summary of mixed-effects models evaluating increases in the number of days each year from 1998 to 2022 when temperature or salinity were above or below critical thresholds, respectively. The slope estimates in the "Both" column indicate trends in the number of days when both temperature and salinity were above or below critical thresholds. ** p < 0.005, * p < 0.05, - no model. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

	Thresholds		Slopes		
Bay Segment	Temperature	Salinity	Temperature	Salinity	Both
OTB					
	29	15	0.58**	-0.81**	-0.03
	29	20	0.58**	0.69	0.37
	29	25	0.58**	3.98**	1.7**
	30	15	0.75**	-0.81**	-0.01
	30	20	0.75**	0.69	0.1
	30	25	0.75**	3.98**	0.82**
	31	15	0.18	-0.81**	-
	31	20	0.18	0.69	0.09
	31	25	0.18	3.98**	0.13
НВ					
	29	15	0.68**	-0.43**	0.09
	29	20	0.68**	-0.23	0.72**
	29	25	0.68**	3.99**	1.84**
	30	15	0.94**	-0.43**	0.06
	30	20	0.94**	-0.23	0.41**
	30	25	0.94**	3.99**	1.04**
	31	15	0.29*	-0.43**	-
	31	20	0.29*	-0.23	-
	31	25	0.29*	3.99**	0.07
MTB					
	29	15	0.78**	-	-
	29	20	0.78**	-1**	-0.06
	29	25	0.78**	1.35*	0.83**
	30	15	0.91**	-	-
	30	20	0.91**	-1**	-0.01
	30	25	0.91**	1.35*	0.42*
	31	15	-0.03	-	-
	31	20	-0.03	-1**	-
	31	25	-0.03	1.35*	-0.07
LTB					
	29	15	0.83**	-	-
	29	20	0.83**	-	-
	29	25	0.83**	-0.33*	0.02
	30	15	0.42	-	-
	30	20	0.42	-	-
	30	25	0.42	-0.33*	-
	31	15	0.07	-	-

	Thresholds		Slopes		
Bay Segment	Temperature	Salinity	Temperature	Salinity	Both
	31	20	0.07	-	-
	31	25	0.07	-0.33*	-

Table S5: Number of days at the beginning and end of the period of record when temperature or salinity were above or below critical thresholds, respectively. Values are the estimated mean number of days (plus standard error) from 1975 to 2022. Columns for "Both" indicate the estimated number of days when both temperature and salinity were above or below critical thresholds. Note that not all models are significant (see table 3). - no model. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

	Thresholds		Temperature		Salinity		Both	
Bay Segment	Temperature	Salinity	Start	End	Start	End	Start	End
ОТВ								
	29	15	37 (3.4)	106 (3.3)	3 (2.2)	6 (2.2)	0 (0.7)	1 (0.7)
	29	20	37 (3.4)	106 (3.3)	21 (7.8)	68 (7.7)	-1 (2.8)	22 (2.8)
	29	25	37 (3.4)	106 (3.3)	130 (13)	216 (13)	4 (3.8)	72 (3.7)
	30	15	7 (3.3)	57 (3.2)	3 (2.2)	6 (2.2)	1 (0.4)	0 (0.4)
	30	20	7 (3.3)	57 (3.2)	21 (7.8)	68 (7.7)	1 (2.1)	8 (2.1)
	30	25	7 (3.3)	57 (3.2)	130 (13)	216 (13)	-1 (2.7)	37 (2.7)
	31	15	2 (2.3)	8 (2.3)	3 (2.2)	6 (2.2)	0 (0.4)	0 (0.4)
	31	20	2 (2.3)	8 (2.3)	21 (7.8)	68 (7.7)	2 (1.5)	1 (1.5)
	31	25	2 (2.3)	8 (2.3)	130 (13)	216 (13)	2 (1.9)	5 (1.9)
НВ								
	29	15	48 (3.9)	103 (3.8)	2 (1.8)	4 (1.7)	0 (0.7)	2 (0.7)
	29	20	48 (3.9)	103 (3.8)	15 (5.3)	33 (5.1)	-1 (2.5)	19 (2.3)
	29	25	48 (3.9)	103 (3.8)	134 (12.2)	171 (11.5)	14 (3.6)	67 (3.3)
	30	15	8 (4.9)	55 (4.8)	2 (1.8)	4 (1.7)	0 (0.3)	1 (0.2)
	30	20	8 (4.9)	55 (4.8)	15 (5.3)	33 (5.1)	-2 (1.2)	8 (1.2)
	30	25	8 (4.9)	55 (4.8)	134 (12.2)	171 (11.5)	-1 (2.9)	35 (2.7)
	31	15	-1 (1.4)	8 (1.4)	2 (1.8)	4 (1.7)	-	-
	31	20	-1 (1.4)	8 (1.4)	15 (5.3)	33 (5.1)	-	-
	31	25	-1 (1.4)	8 (1.4)	134 (12.2)	171 (11.5)	-1 (0.7)	4 (0.7)
MTВ								
	29	15	45 (2.6)	107 (2.6)	0 (0.1)	0 (0.1)	-	-
	29	20	45 (2.6)	107 (2.6)	5 (1.6)	5 (1.6)	0 (0.6)	2 (0.6)
	29	25	45 (2.6)	107 (2.6)	56 (11.8)	81 (11.8)	1 (3.2)	34 (3.2)
	30	15	8 (3.1)	58 (3.1)	0 (0.1)	0 (0.1)	-	-
	30	20	8 (3.1)	58 (3.1)	5 (1.6)	5 (1.6)	0 (0.3)	1 (0.3)
	30	25	8 (3.1)	58 (3.1)	56 (11.8)	81 (11.8)	-1 (1.7)	16 (1.7)
	31	15	0 (0.9)	6 (0.9)	0 (0.1)	0 (0.1)	-	-
	31	20	0 (0.9)	6 (0.9)	5 (1.6)	5 (1.6)	-	-
	31	25	0 (0.9)	6 (0.9)	56 (11.8)	81 (11.8)	0 (0.5)	2 (0.5)
LTB								
	29	15	55 (3)	109 (3)	0 (0.1)	0 (0.1)	-	-
	29	20	55 (3)	109 (3)	0 (0.1)	0 (0.1)	-	-
	29	25	55 (3)	109 (3)	4 (1.2)	0 (1.2)	0 (0.2)	0 (0.2)
	30	15	19 (3.4)	64 (3.4)	0 (0.1)	0 (0.1)	-	-
	30	20	19 (3.4)	64 (3.4)	0 (0.1)	0 (0.1)	-	-
	30	25	19 (3.4)	64 (3.4)	4 (1.2)	0 (1.2)	_	_

	Thresholds		Temperature		Salinity		Both	
Bay Segment	Temperature	Salinity	Start	End	Start	End	Start	End
	31	15	-1 (1.5)	10 (1.5)	0 (0.1)	0 (0.1)	-	-
	31	20	-1 (1.5)	10 (1.5)	0 (0.1)	0 (0.1)	-	-
	31	25	-1 (1.5)	10 (1.5)	4 (1.2)	0 (1.2)	-	-

Table S6: Number of days at the beginning and end of the period of record when temperature or salinity were above or below critical thresholds, respectively. Values are the estimated mean number of days (plus standard error) from 1998 to 2022. Columns for "Both" indicate the estimated number of days when both temperature and salinity were above or below critical thresholds. Note that not all models are significant (see table 4). - no model. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

	Thresholds		Temperature		Salinity		Both	
Bay Segment	Temperature	Salinity	Start	End	Start	End	Start	End
ОТВ								
	29	15	81 (3.7)	95 (3.7)	17 (2.8)	-2 (2.8)	2 (0.8)	1 (0.8)
	29	20	81 (3.7)	95 (3.7)	49 (9.4)	65 (9.4)	13 (3.7)	22 (3.7)
	29	25	81 (3.7)	95 (3.7)	142 (14.7)	238 (14.7)	34 (4.7)	75 (4.7)
	30	15	36 (3.9)	54 (3.9)	17 (2.8)	-2 (2.8)	0 (0.1)	0 (0.1)
	30	20	36 (3.9)	54 (3.9)	49 (9.4)	65 (9.4)	5 (2.3)	8 (2.3)
	30	25	36 (3.9)	54 (3.9)	142 (14.7)	238 (14.7)	19 (3.4)	38 (3.4)
	31	15	4 (2.3)	9 (2.3)	17 (2.8)	-2 (2.8)	-	-
	31	20	4 (2.3)	9 (2.3)	49 (9.4)	65 (9.4)	0 (1.1)	2 (1.1)
	31	25	4 (2.3)	9 (2.3)	142 (14.7)	238 (14.7)	3 (1.7)	6 (1.7)
НВ								
	29	15	80 (3.8)	97 (3.8)	10 (2.6)	0 (2.6)	0(1)	2(1)
	29	20	80 (3.8)	97 (3.8)	33 (6.7)	27 (6.7)	5 (3.6)	23 (3.6)
	29	25	80 (3.8)	97 (3.8)	112 (15.2)	207 (15.2)	30 (4.7)	74 (4.7)
	30	15	32 (6.1)	55 (6.1)	10 (2.6)	0 (2.6)	0 (0.4)	1 (0.4)
	30	20	32 (6.1)	55 (6.1)	33 (6.7)	27 (6.7)	1 (1.9)	11 (1.9)
	30	25	32 (6.1)	55 (6.1)	112 (15.2)	207 (15.2)	13 (4)	38 (4)
	31	15	2 (2.1)	9 (2.1)	10 (2.6)	0 (2.6)	-	-
	31	20	2 (2.1)	9 (2.1)	33 (6.7)	27 (6.7)	-	-
	31	25	2 (2.1)	9 (2.1)	112 (15.2)	207 (15.2)	2 (1.1)	3 (1.1)
MTB								
	29	15	81 (2)	99 (2)	-	-	-	-
	29	20	81 (2)	99 (2)	19 (2.6)	-5 (2.6)	3 (1)	1 (1)
	29	25	81 (2)	99 (2)	58 (13.6)	91 (13.6)	16 (4.7)	36 (4.7)
	30	15	34 (3.5)	55 (3.5)	-	-	-	-
	30	20	34 (3.5)	55 (3.5)	19 (2.6)	-5 (2.6)	1 (0.5)	0 (0.5)
	30	25	34 (3.5)	55 (3.5)	58 (13.6)	91 (13.6)	7 (2.8)	17 (2.8)
	31	15	5 (1.5)	5 (1.5)	-	-	-	-
	31	20	5 (1.5)	5 (1.5)	19 (2.6)	-5 (2.6)	-	-
	31	25	5 (1.5)	5 (1.5)	58 (13.6)	91 (13.6)	2 (0.9)	1 (0.9)
LTB								
	29	15	82 (2.1)	102 (2.1)	-	-	-	-
	29	20	82 (2.1)	102 (2.1)	-	-	-	-
	29	25	82 (2.1)	102 (2.1)	6 (1.7)	-2 (1.7)	0 (0.3)	0 (0.3)
	30	15	46 (3.6)	56 (3.6)	-	-	-	-
	30	20	46 (3.6)	56 (3.6)	-	-	-	-
	30	25	46 (3.6)	56 (3.6)	6 (1.7)	-2 (1.7)	-	-

	Thresholds		Temperature		Salinity		Both	
Bay Segment	Temperature	Salinity	Start	End	Start	End	Start	End
	31	15	7 (2.4)	9 (2.4)	-	-	-	-
	31	20	7 (2.4)	9 (2.4)	-	-	-	-
	31	25	7 (2.4)	9 (2.4)	6 (1.7)	-2 (1.7)	-	-