Estuaries and Coasts

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

--Manuscript Draft--

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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 °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. Additionally, the number of days when temperature was above 30 °C or salinity was below 25 ppt has increased on average across all bay segments by 48 and 37 days, respectively, since 1975. These changes varied spatially and seasonally, with the most dramatic changes observed in the upper bay. Generalized Additive 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 and continuous monitoring data to support ecosystem health into the future.

May 23, 2024

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

Dear Dr. Montagna and Dr. Deegan,

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

We sincerely appreciate the constructive comments provided by the editors and reviewers and have made every attempt to address these comments in our revision. The largest change to the manuscript was a reanalysis of the potential seagrass response to climate stressors, which included more flexible Generalized Additive Models and inclusion of additional predictors (i.e., time as a continuous variable, light attenuation). Overall, our conclusions remain the same that climate stressors are potentially driving recent seagrass changes, although our more robust modeling approach provides greater weight of evidence for this conclusion. Note that we have also made substantial revisions in response to comments from the co-editor in chief regarding statistical descriptions of results to focus more on effect sizes and magnitudes of change.

Again, 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

We sincerely thank the associate editor and all reviewers for providing useful comments on our manuscript. We have made every effort to address these concerns. In particular, we have provided a more thorough assessment of seagrass response to climate-related stressors using more flexible models (GAMs) and including additional predictors (light attenuation). Please see the responses below (line numbers in responses refer to the revised manuscript).

AE comments:

I enjoyed reading this paper and feel it could be an important contribution, especially given its strength in combining many different datasets and analyses to build a comprehensive story and set of conclusions. However, there are some outstanding issues that need to be addressed prior to publication, mainly related to the analyses. My impression is these can be addressed, and so I encourage the authors to consider my points below and those of the reviewers, as they will result in a stronger contribution.

Response: Thank you for the comments. We have revised the text based on your suggestions. Please see our responses to each comment for more details.

Major comments:

1. Datasets: I appreciate the use of multiple datasets and the effort taken to conduct multiple analyses that draw on the strengths of these datasets that build towards a comprehensive study conclusion. I did, however, have trouble following which datasets were used for which analysis, and why, and what was included and omitted. I realize all that information is included in the text, but I found it was quite buried and when I tried to refer back from figures and tables to the text it was difficult to find the information. I would suggest inserting a table with this information summarized so that the reader can easily refer to this.

Response: A new table (Table 1) was added that includes a description of each dataset, the temporal and spatial coverage, and the analyses used for each.

2. Justification for period of decline: I struggle with the assertion that 2016 represents the beginning of the dramatic seagrass loss across Tampa Bay, as stated on Line 183. This is especially problematic when observing seagrass coverage in Figure 2a, which represents total species coverage. Here, coverage is relatively stable 1988 to 2012, then increases 2014-16, with levels dropping after this. The extent of these patterns differs per bay segment – in OTB, there is a dramatic increase with some decrease, although only the last year (2022) is below the baseline values 1988 to 2012. For other segments, there is a decline but coverage post 2016 is either similar to the baseline or higher. What are the drivers of those large increases in more recent years, and are declines relevant or is seagrass coverage simply returning to baselines levels that were evident 1988-2012? Please comment in the text. Further, the decline in cover is more evident when observing species specific data, particularly for H. wrightii in OTB and MTB, but not other segments. Should the analyses focus on the dominant species driving these patterns? Please revise text and acknowledge these aspects, perhaps reanalyse the data or interpretation if necessary, to present a more convincing justification.

Response: The above interpretation is correct in that the patterns of increase/decrease vary by bay segment and dataset (coverage or frequency occurrence). This suggests that multiple factors *are influencing these changes and that they potentially vary by location. Our development of more robust models (GAMs) that attempted to better describe these patterns is an explicit acknowledgment of these differences, which were not adequately characterized with the earlier models. Please see our description below of these updated models.*

Additional text was also added to clarify these nuanced changes and the focus on total species occurrence.

Line 197: "As such, trajectories of recovery and decline have varied by bay segment in magnitude and timing of the change, although a consistent decline baywide has been observed since 2016. Change by species has been most notable for Halodule wrightii (shoal grass) in OTB and HB, whereas some losses have been observed for Thalassia testudinum (turtle grass) in LTB, likely related to dominance of each species across the salinity gradient and proximity to hydrologic inputs (Lewis et al. 1985)."

Line 372: "Additionally, total seagrass frequency occurrence was used as a response variable as an aggregate measure of community change to potential stressors given the likelihood that species-specific changes may be more difficult to model and that the majority of seagrass loss in recent years was dominated by H. wrightii in the upper bay segments (Figure 2b)."

3. Exclusion of time series and use of pre/post category: Use of pre/post category in GLM analyses with stressors collapses the data across the time series and removes the interannual variability and information contained therein. I don't understand why this was done instead of explicitly including the time series in the analyses. I would recommend conducting initial GAMs that includes the time series for these data. The GAM approach would be particularly useful to show the relationship smoothers with the stressor values and/or the basic temperature salinity time series (ie smoother relationships). As it stands, it seems that an initial key part of the analyses was omitted and it is not justified to bin into categories. This is my biggest concern with the analyses and I'd like to see this addressed.

Response: We have revised our models for describing seagrass change using a GAM approach that uses time as a continuous variable (year) and includes light attenuation as a predictor, when able. For the latter, light attenuation was included only in the EPC model, whereas it was excluded from the FIM and PDEM models due to a majority of Secchi observations (from which light attenuation was derived) being on the bottom. As such, light attenuation could not be derived for these models, nor would it likely explain much variation in seagrass response since light environments are generally not limiting at most of the locations sampled in the FIM and PDEM datasets. Also note the addition of Figure 2c that shows the improvement in light attenuation over time as shown with the EPC data.

Sections 2.7 (links to seagrass) and 3.3 (seagrass response) were revised to describe the updated modeling approach, including the addition of equations 1 to 4 showing the structure for each GAM, Figures 7, 8, and S7 showing partial effects for each model, and Tables S7 to S10 providing summary statistics for each model. Overall, the general conclusions that climate-related stressors are likely contributing factors to seagrass change remains as before, although the revised approach provides a more complete description using more flexible models that can describe non-linear relationships and additional predictors (time, light attenuation).

4. Exclusion of nutrient/light data: A key part of the Tampa Bay story is the success in seagrass recovery through nutrient reduction. I agree with the reviewers that exclusion of these data (or some representative) is perplexing, especially given they are available. I recommend including these in the models, and excluding them only through the model selection process.

Response: This is an excellent suggestion that we have carefully considered. Several revisions were made to address the concern. First, Figure 2 was updated to include a time series of light attenuation by bay segment over the last fifty years. Regression lines in each plot show that light environments have improved for the period of record covered by the transect monitoring data (1998 to present). This plot provides an overview of the history of bay recovery as well as the perplexing issue that seagrass has changed despite light attenuation being low and continuing to improve. It is also noted that light attenuation in recent years has been below the threshold to support seagrass growth. Second, we have improved our models of seagrass response to climate stressors as noted in the response to the previous comment.

Minor comments:

Line 205: Define frequency occurrence.

Response: Text revised as "…cover-abundance, frequency occurrence (number of sample points with seagrass divided by total points on a transect, as in Sherwood et al. 2017), and condition, …"

Line 207: My understanding is that both transects and areal maps were done once a year. Clarify here why transect data provided greater temporal resolution.

Response: As noted on line 206, the areal maps are produced every other year, whereas the transect data are collected every year. The text was revised as follows: "Although the areal maps provide the standard for assessment of restoration goals, the maps are produced every other year. The transect data are collected each year, allowing inter-annual comparison…"

Line 183: Provide reference or data to show that nutrient loading and light attenuation remained stable during 2016 to present. Better yet, include it in your analyses and exclude through model selection.

Response: Please see the response to the general comments above.

Line 368: remind reader why these datasets can't be used to calculate stressor metrics (point samples?)

Response: Sentence was revised as follows: "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 (i.e., each sample was a distinct location)."

Figure 6: grey points are the actual number of days per year per transect (station), correct? Insert text.

Response: Edited as "…grey points as actual number of days for each station…"

Reviewer #1:

This was a very thorough study, with an impressive amount of data. These types of analyses are greatly needed with all of the long-term datasets that are becoming available in different estuarine systems. The authors were extremely detailed in explaining their methods, and using different tools and models to analyze different datasets. The hang-up for me is the extremely weak, and I think really no convincing links to changes in seagrass. They acknowledge that it is weak and do not try to really oversell it though. However, I am unconvinced as to the omission of any kind of water clarity data into the seagrass models.

Response: Thank you for the thorough review of our manuscript. Please see our response to your comments below, as well as those in response to the comments from the associate editor. We have re-evaluated seagrass change over time using a more comprehensive modeling approach that includes GAMs and additional predictors (i.e., light attenuation).

The authors cite Lopez et al. in their site description of Old Tampa Bay. This paper shows that HAB durations have increased from 2011-2020. Since the largest seagrass declines occurred in Old Tampa Bay, could they be more directly related to algal blooms and less directly related to temperature and salinity changes? Based on the 2022 Tampa Bay Water Quality Assessments report that is cited, Old Tampa Bay has not met their chlorophyll criteria for over half the years since seagrass declines started being observed after 2016. In particular, the 3 years in a row from 2019-2021 seem of great interest to this study, and I'm wondering why chlorophyll wasn't included in the models.

Response: We have included light attenuation in our models when able. Additionally, the FIM and PDEM datasets that target more shallow areas of the bay had a majority of Secchi observations on the bottom, indicating that light environments are generally supportive of seagrass growth in recent years. See the revision to section 2.7 for details on the updated methods. Also note the addition of Figure 2c that shows the long-term improvement in light environments over the period of record.

Continuing with this same issue, I find the argument to leave out water clarity data fairly weak and at times based on anectodal evidence. For example, Lines 166-167 "Light penetration typically reaches bottom habitats under current conditions" and lines 183-184 "despite nutrient loading and light attenuation remaining relatively stable". What is the justification for these statements? It seems like there is anecdotal evidence used to try to defend the use of not looking at light conditions in the model, but many times citations are not used that show these statements to be true. If light is going to be excluded from the models, I think a stronger justification is needed.

Response: Please see our response to the previous comment as well as those in response to the general comments from the associate editor.

Lines 303-305 "we focus on water temperature and salinity given that other dominant forcing factors, i.e., light availability, have been relatively stable in recent years." Here a citation is given, however these mentioned conditions appear to be stable for areas except OTB, which is also the area that saw the greatest declines in seagrass. So again, why leave light availability out of the models?

Response: Please see our above responses.

Lines 225-226 "Most samples are collected from mid-morning to early afternoon". That is a major issue with using temperature data that is a snapshot and not continuous. Temperatures can vary greatly between morning to afternoon. Were any attempts made to normalize temp data to time of day? I don't know if it would be possible given the datasets, but something to consider.

Response: This is certainly an issue worth considering using the temperature data from the monthly discrete samples and was the motivation for including a statement on the need for continuous monitoring data in the original draft (lines 679 - 681). Applying a correction or normalization for time of day would be challenging and we would not be confident in the results to accurately describe maximum or minimum temperature values for a given day. Moreover, we feel the trends in the temperature summaries (e.g., increase in number of days above a threshold over time) would likely be similar. As such, we have not attempted this for the current analysis. However, ongoing work in Old Tampa Bay for a separate project is attempting to describe short-term diurnal variation in temperature with continuous data loggers. We suspect this information will provide further insight into acute temperature stressors that could affect seagrass. We have added text describing this general need and a statement with a short description of this work, although these loggers are in very shallow areas that likely show different temperature characteristics than the discrete samples at deeper depths.

Line 582: Without more continuous, diel observations of these metrics over the period of record, we were heavily reliant on these model outputs to determine relevant thresholds for Tampa Bay seagrass. This further highlights a long-standing data gap and need for the estuary."

Line 681: "Ongoing work in OTB using continuous data loggers in shallow areas where seagrass has been gained or lost will provide insights into short-term diurnal changes as potential acute temperature stress (see https://tbep-tech.github.io/otb-temp/tempeval)."

Lines 313-314 "Shoal grass is tolerant of a wide range of salinity…" Shoal grass accounted for the largest seagrass declines, according to Figure 2b, so why would decreases in salinity negatively impact this species? Wouldn't a decrease of 0.04-0.06 per year be well within the tolerance range for this species?

Response: Our previous statement on lines 571-574 suggests this may be a plausible explanation for the weak association of seagrass change with the stressors. The statement was further revised to specifically mention that shoal grass accounted for the largest seagrass decline: "This is especially true for H. wrightii that had the greatest changes over the period of record and is tolerant of a wide range of salinity."

Lines 350-352: Did you experiment with different lagged responses?

Response: Yes, preliminary analyses evaluated lagged associations between the salinity and temperature stressor metrics and seagrass change. Specifically, we evaluated the number of days in 30 day periods from one month to one year when temperature was above or salinity was below a threshold in relation to seagrass change (see code here, https://github.com/tbep-tech/temp-manu/blob/9044bb07cd81532a7fc83c02dc035f7d8ff16b2c/R/dat_proc.R#L865, lines 865 to 925 in the file). No compelling differences were observed for any of the lags compared to *the entire year, which was the justification for using the latter in the current analysis. Additional text was added to this effect:*

Line 360: "Preliminary analyses evaluated different lagged associations between the stressor metrics and seagrass change, although initial results suggested no additional insight could be gained using lagged assessments compared to the transect year summaries. As such, the stressor metrics…"

Line 384: what was the justification for removing September?

Response: The assessment of inter-annual changes in precipitation during the rainy season showed a weak increase over time, whereas removing the last month (September) showed a more statistically powerful increase over time. The removal of September was meant to show that the trend was driven by an increase in the first three months of the rainy season (Jun - Aug). Text was added to line 413: "…suggesting precipitation increases were driven by the earlier months (June - August)." Also note that our previous estimates of rainfall total were based on an incorrect sum across overlapping areas. We have fixed the issue, although the results and trends remain the same.

Line 399: "for all" is repeated twice

Response: Fixed.

Line 407: Table 1 shows only HB significant for FIM from 2004 to present, not OTB.

Response: This was corrected.

Reviewer #2:

Overall, I found this manuscript to be interesting and useful. It utilizes existing data from various existing, governmental water quality monitoring programs of varying design and coverage to assess if recent declines in seagrass populations in portions of the subtropical Tampa Bay coastal system can be associated with increased water temperatures or lower salinities which might be a result of changing climatic conditions.

Although the results of their conservative, linear modeling analyses determined that the bay waters were only marginally warmer and fresher during the recent period of seagrass declines beginning in 2016, the study provides a good example of how existing monitoring data might be used for similar questions in other areas, as well as what new monitoring or assessment approaches, including evaluation of shorter-term periods of stress might be needed. It reinforces the need for updated coastal water quality management approaches and seagrass restoration criteria. Most existing coastal management restoration paradigms, programs and restoration criteria were developed and first implemented 20-40 years ago or earlier when declines in seagrass populations were first quantified. Consequently, they do not address many of the interactive and potentially negative factors associated with a rapidly changing climate.

The thorough discussion section addresses many of the caveats and limitations of their approach to assessing temporal trends in the Tampa Bay physical environment during the recent period of seagrass decline. Additionally, the authors discuss some of the potentially important unmeasured interactive biotic and abiotic factors which could be important in explaining recent seagrass declines.

Before acceptance I recommend that several points should be addressed to improved the manuscript's clarity and usefulness.

Response: Thank you for providing useful feedback on our manuscript. Please see our detailed responses below.

First, although light availability is one of the most principal water quality factors affecting seagrass performance, stability and resilience, data is not presented here to characterize the light environment either spatially or temporally, other than to suggest it has been relatively stable and consistent across the system. However, on L. 120 it is highlighted that "… upper bay segments where marginal light environments exist".

Response: Line 125 was revised to describe that the upper bay segments are more shallow and receive a majority of hydrologic inflow. The previous statement was inaccurate. Also, please see the addition of Figure 2c that shows the long-term improvement in the light environment over time. Of particular note is that all bay segments are currently below the threshold supportive for seagrass growth.

Additionally, on L. 183-184 the authors state, "…dramatic seagrass loss has been observed in Tampa Bay, despite nutrient loading and light attenuation remaining relatively stable (Figure 2a)". However, Figure 2a only shows seagrass abundance changes and Figures S1, S2, S3 only show 2000 to 2020 air and water temperatures, precipitation, and salinity levels and trends. If nutrients and light are to be considered constants and excluded from the analysis (as discussed L. 574-597) the data should be similarly presented in figures so that their exclusion can be better supported. One possibility would be to add to the supplementary materials in a format such as Fig. S1 or S2.

Response: We have added light attenuation trends in Figure 2 and have included it as a predictor in the seagrass models when able. As noted above, a majority of Secchi observations in the FIM and PDEM datasets have been recorded on the bottom, indicating sufficient light environments for seagrass growth in recent years.

L. 535-537 states that, "The models did not provide a consistent, nor statistically powerful, explanation that increasing temperature and decreasing salinity were key (or the sole) drivers." Therefore, a weight-of-evidence approach from all the models is used to conclude their effects. This is OK provided that the other potentially important interactive factors can be better supported as being constant. Monitoring data for chlorophyll concentrations nitrogen loadings and other parameters as they relate to light availability to the bottom do exist and should be included in the figures for each of the four regions.

Response: Please see our above response regarding the addition of these data in Figure 2 and our use of more robust modeling approaches to describe these potential changes in response to comments from the editor and other reviewer (i.e., GAMs)

Another important component excluded from analyses or discussion here is identification of the individual seagrass species distributions and their potential effects on seagrass responses or resilience to climate related stresses. Thalassia, which is typically dominant in the lower, higher salinity areas in this system can have greater persistence and resilience to environmental stresses than Halodule and other common seagrass species, however its recovery after loss can also be slow. Since it can grow to greater depths Halodule, its water clarity requirements can be higher. Additionally, at greater depths the effects of temporary air temperature increases on water temperatures can be buffered compared to shoal environments. If the seagrass community consists only of Thalassia then it should be so stated.

Response: We agree there are likely important distinctions between species that could be considered when evaluating the response to climate-related stressors. As correctly noted by the reviewer, Thalassia is dominant in Lower Tampa Bay with coverage decreasing towards the upper bay segments. We did not evaluate lower Tampa Bay in our analysis of seagrass response to climate stressors given that coverage has generally been stable in recent years and salinity has not been changing dramatically. For the remainder of the bay, most of the change in cover has been dominated by Halodule, especially in the upper bay segments where most of the seagrass loss has been observed. An evaluation of individual species response would likely not be more informative than the current evaluation of total frequency occurrence. Also please see our response to a similar comment from the associate editor.

The definition of "hotter and fresher" is as used here summarized as the average rates of annual increases in water temperature and decreases in salinity. Yet, more importantly, this study presents estimates of the number of days annually when defined thresholds for water temperature above 30C and salinities below 25 occur. While these thresholds which were used for comparisons, were set, in part, on their statistical strength, they do have merit as indexes of episodic stress. These metrics perhaps more important than averages when related to evaluating climate change stressors as referenced here and elsewhere. I suggest these metrics be highlighted and included in the abstract, as they are included and emphasized in the discussion.

Response: We agree these metrics are useful to highlight in the abstract. We have added this sentence to the abstract, with similar clarification added in section 3.2 and the beginning of the discussion: "Additionally, the number of days when temperature was above 30 ˚C or salinity was below 25 ppt has increased on average across all bay segments by 48 and 37 days, respectively, since 1975."

Additionally, recommendations for future management efforts highlighted in the last sentence of the Abstract (L. 31-34) do not mention recommendations for additional monitoring approaches needed to evaluate future climate stressors as highlighted in the Conclusions. I suggest adding something such as, "…and high-resolution data collection efforts", after "additional, complementary interventions…" to emphasize the need for more precise, spatial and temporal sampling in monitoring programs both here and elsewhere.

Response: The final sentence of the abstract was revised as follows: "…and warrant additional, complementary interventions and continuous monitoring data to support ecosystem health into the future."

Co-Editor in Chief:

1) Maybe change the title to "Hot and fresh water:…"

Response: We have retained the title preface. All co-authors preferred this option. However, we have added "water" later in the title to describe "suboptimal water conditions" to make the focus of the manuscript clear.

2) ESCO now requires a "Declarations" section as described in the submission guidelines https://link.springer.com/journal/12237/submission-guidelines. Include declarations on: funding sources; financial or non-financial competing interests, or conflicts of interest; ethics compliance for research involving humans, animals, or plants; data availability and repository DOI; author contributions; pre-print location if applicable; etc.

Response: The declaration section that was previously after the abstract was added to the end and relevant sections were added (data availability, author contributions, ethics, and funding).

3) Lines 384-390 and other places, and Tables 1-4: ESCO has a policy discouraging the use of an arbitrary rejection rate and recommends describing trends and reporting the actual p-values, see Smith (2020) https://doi.org/10.1007/s12237-019-00679-y. This means that you should avoid the phrases "significant," "$p < 0.05$," or "*"and replace these phrases with effect size values that represent the trend, the actual p-value, and the sample size. For example: X was 100% higher than Y, $p = 0.0001$, $n=20$; or X was 10 units, which is greater than Y at 5 units, $p = 0.0001$, $n=20$. Use the same number of significant digits in all your reporting, meaning values < 0.0001 should be reported as < 0.0001. Don't forget n. More examples and suggestions are provided in the Smith citation. So, please revise your results section, and any other parts of the manuscript, to address this issue.

Response: We have removed or revised all instances in the text, tables, and figures to focus on trends, effect sizes, and magnitudes. Any use of "significant" regarding statistical tests was also removed. Sample sizes were reported where missing.

4) Figure 2: I think the font on X-axis is too small to read. Consult submission guidelines on figures.

Response: Font size was increased.

5) Fig. 6, 7: There is a charge to print color figures.

Response: We intend on publishing as open access.

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

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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 °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. Additionally, the number of days when temperature was above 30 °C or salinity was below 25 ppt has increased on average across all bay segments by 48 and 37 days, respectively, since 1975. These changes varied spatially and seasonally, with the most dramatic changes observed in the upper bay. Generalized Additive 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 and continuous monitoring data to support ecosystem health into the future.

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

1 Introduction

The monitoring and management of seagrasses in coastal environments has received substantial attention on a global scale. Seagrasses are fundamental indicators of coastal ecosystem health (Roca et al. 2016; Orth et al. 2017), while also serving as foundation species that provide numerous ecosystem services (Fourqurean et al. 2012; Orth et al. 2020; Orth and Heck 2023). Seagrasses have been in global decline with rapid development of coastal environments, particularly in the latter half of the 20th century with accelerating losses estimated at a rate of 110 km^2 yr^{-1} since the 1980s (Waycott et al. 2009; Dunic et al. 2021). These losses are comparable to, if not more significant than, other interconnected critical coastal environments such as mangroves and salt marshes (Duarte et al. 2008). Losses have been attributed to numerous stressors including decline in light environments with nutrient enrichment, sedimentation, and physical disturbance primarily from human activities (Duarte 1995; Hall et al. 1999; Orth et al. 2006; Burkholder et al. 2007). Furthermore, natural disturbances such as storm events and disease have also been implicated (Robblee et al. 1991; Tomasko et al. 2020). Contemporary management actions aimed at mitigating loss and ultimately supporting restoration require adaptive approaches to address the effects of multiple stressors that have contributed to seagrass decline (Dunic and Côté 2023).

The sustained coverage or restoration of seagrasses in coastal environments requires environmental conditions that support vegetative growth, reproduction, and coverage expansion. A long-standing approach adopted by numerous management entities has been the control of 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. 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). Top-down effects and associated trophic cascades can also complicate the sustained growth or recovery of seagrass, particularly where herbivores are abundant (e.g., sea turtles, manatees, Heck and Valentine 2007; Fourqurean et al. 2010). 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 also affect Tampa Bay. Moreno-Marín et al. (2018) produced similar results using a multifactorial 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; also see Bartenfelder et al. 2022). 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 (Hall et al. 2016; 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 physical water quality conditions independent of light environments 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 long-term 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. The improvement of bio-optical models that can describe light requirements for seagrasses may also result from this information.

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 that are more shallow and receive a majority of hydrologic inflow. 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.

2 Methods

2.1 Study area

Tampa Bay is the largest open-water estuary in Florida covering 400 mi^2 (1,036 km^2) and the second largest in the Gulf of Mexico. The watershed covers an additional 2,200 mi^2 (5,872 km^2) with the Hillsborough, Alafia, Manatee, and Little Manatee rivers contributing the 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 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 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 shallow, with a baywide mean depth of approximately 3 m. Light penetration typically reaches bottom habitats under current conditions (Figure 2c), 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

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 what was present in the 1950s. Though Tampa Bay was far from pristine at the time, aerial imagery was sufficient to estimate a relatively unimpacted condition for seagrass coverage. Since then, the greatest areal coverage expansions were observed in the upper bay segments (OTB, HB, and MTB; Figure 2a). Light environments have improved (Figure 2c) through a 2/3 reduction of external nitrogen loadings from a peak 1970s estimate of 8.9 x 10^6 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 light environments remaining supportive of growth (Figure 2c) as defined by historical relationships between nitrogen, chlorophyll-a, and water clarity (Janicki and Wade 1996; Greening et al. 2011). 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. As such, trajectories of recovery and decline have varied by bay segment in magnitude and timing of the change, although a consistent decline baywide has been observed since 2016. Change by species has been most notable for *Halodule wrightii* (shoal grass) in OTB and HB, whereas some losses have been observed for *Thalassia testudinum* (turtle grass) in LTB, likely related to dominance of each species across the salinity gradient and proximity to hydrologic inputs (Lewis III et al. 1985).

2.3 Seagrass data

Two primary sources of data have been used to track seagrass change in Tampa Bay (Table 1). 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, 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 (number of sample points with seagrass divided by total points on a transect, as in Sherwood et al. 2017), 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 maps are produced every other year. The transect data are collected each year, allowing 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 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 (Table 1). 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 *tbeptools* 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.

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. Only bottom temperature and salinity were used for analysis. 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.

2.5 Trend analysis

The first goal of the analysis was to describe spatial and temporal trends in water temperature and salinity using the three water quality datasets described above. This assessment provided an indication of the extent of 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, 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, Table 1). 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/rainfall-summary-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 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.).

2.6 Quantifying potential stress

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 sufficient for growth in recent years (Figure 2c).

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; Phillips and Meñez 1988). Other less common species include *Ruppia maritima* (widgeon grass) and *Halophila engelmannii* (star grass), where the former is often mapped during wet years in the upper bay segments from the aerial surveys. These species co-occur 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 (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 (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, R^2 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 by bay segment.

2.7 Links to seagrass

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. Preliminary analyses evaluated different lagged associations between the stressor metrics and seagrass change, although initial results suggested no additional insight could be gained using lagged assessments compared to the transect year summaries. As such, the stressor metrics were compared to frequency occurrence (all species) each year by bay segment. GAMs were used to evaluate frequency occurrence in response to the independent variables, where the latter were the stressor metrics for temperature, salinity, or both. Additional predictors included year and light attenuation as estimated from Secchi depth (Janicki and Wade 1996). For 1998 to 2022, 17% of the Secchi observations were recorded on the bottom. A single smooth term was used for each predictor using a thin plate regression spline, including a tensor product interaction term with a cubic regression spline that evaluated the potential interacting effects of each predictor with year. Two models were evaluated, one with the bottom temperature and salinity metrics together (Equation 1) and another with the both metric (Equation 2). All models excluded Lower Tampa Bay because of minimal seagrass change over time. Additionally, total seagrass frequency occurrence was used as a response variable as an aggregate measure of community change to potential stressors given the likelihood that species-specific changes may be more difficult to model and that the majority of seagrass loss in recent years was dominated by *H. wrightii* in the upper bay segments (Figure 2b). The GAM equations in *mgcv* R package notation (Wood 2017) are below, where s is a smoother function for a single predictor and ti is the tensor product interaction between predictors. All GAMs were fit using restricted maximum likelihood evaluation (Wood 2011).

$$Seagrass \sim s(year, by = bay\ segment) + s(light\ att, by = bay\ segment) + s(temperature, by = bay\ segment) + s(salinity, by = bay\ segment) + ti(light\ att,\ year, by = bay\ segment) + ti(temperature, year, by = bay\ segment) + ti(salinity, year, by = bay\ segment) \quad (1)$$

$$Seagrass \sim s(year, by = bay\ segment) + s(light\ att, by = bay\ segment) + s(both, by = bay\ segment) + ti(light\ att,\ year, by, bay\ segment) + ti(both, year, by = bay\ segment) \quad (2)$$

Two other 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 measurements of bottom salinity and temperature as independent variables because the stressor metrics could not be calculated using the sampling designs from these monitoring programs (i.e., each sample was a distinct location). GAMs were used for the FIM data to evaluate annual average seagrass percent cover using year, temperature, and salinity as independent variables. Separate smoothed terms for each predictor and interactions with year for each predictor for each bay segment were included as above. GAMs for the PDEM data were constructed similarly, except only Old Tampa Bay was evaluated due to spatial limitations of the data. Neither the FIM nor PDEM models used light attenuation as a predictor variable given that a large percentage of Secchi observations were measured on the bottom (92% and 45%, respectively), providing further support that light environments have not been limiting for seagrasses. Model input data were further subset to include only months from July to October to describe seagrasses during the growing season and to reduce potential seasonal effects. Lastly, all data were averaged annually for the monthly subsets for comparability of sample size (i.e., power) with the EPC models. For the PDEM models, presence/absence was converted to frequency occurrence as the number of sites with seagrass in a year divided by the total number of sites and all data were subset to less than 2 meters to better characterize locations where seagrass occurs. The GAM equations for the FIM (Equation 3) and PDEM (Equation 4) datasets, respectively, were as follows:

$$Seagrass \sim s(year, by = bay\ segment) + s(temperature, by = bay\ segment) + s(salinity, by = bay\ segment) + ti(temperature, year, by = bay\ segment) + ti(salinity, year, by = bay\ segment) \quad (3)$$

$$Seagrass \sim s(year) + s(temperature) + s(salinity) + ti(temperature, year) + ti(salinity, year) \quad (4)$$

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 (n = 48, p < 0.001, 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 2.21 mm per year, although the trend was weak (n = 47, p = 0.104, R^2 = 0.04). Removing September from the rainy season showed a slightly larger trend of 2.4 mm per year and slightly more powerful trend model (n = 48, p = 0.039, R^2 = 0.07), suggesting precipitation increases were driven by the earlier months (June - August). Using this model, mean precipitation for June to August in 1975 was 559.1 (+/-30.72 st. err.) mm, whereas current mean precipitation for June to August in 2022 was 669.3 (+/-29.76 st. err.) mm, showing an overall increase in the period of record of 110.3 mm. Notably, rainfall during the dry season (October through May) has decreased slightly over time at 1.97 mm per year, although the trend model was weak (n = 48, p = 0.261, R^2 = 0.01). The SPI showed notable anomalies in precipitation, with pronounced rainy periods in the early 1980s, late 1990s, 2005, and 2015-2020 (third row, Figure 4).

Increasing water temperature and decreasing salinity generally followed the meteorological trends for all three *in situ* datasets (EPC, FIM, and PDEM, Figure 4, Tables 2, 3, Figures S1, S2, S3). Note that for tables 2 and 3, comparable time periods were evaluated between the datasets when possible given the different sample sizes, and therefore power, to detect trends (starting year 1975: n = 48, 1996: n = 27, 2004: n = 19). The strongest trends were observed for the EPC dataset based on the standard errors for the slope estimates. 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 large 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 2). 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 notable decreasing trends, with 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. Increasing temperature trends and decreasing salinity trends were also observed for the FIM and PDEM dataset, although confidence in the slope estimates (larger standard errors) generally decreased for shorter time periods of assessment.

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 increasing temperature and decreasing salinity from 1975 to present, although some stations in HB had weak salinity trends (Figure 5a). Seasonally, most bay segments had more stronger trends in the early fall/winter periods for both temperature and salinity (Figure 5b), although some variation was observed throughout the bay. Temperature trends were generally stronger in the fall for OTB, whereas the remaining bay segments showed the strongest trends in the winter (February). Seasonal trends in salinity showed the largest decreases in the fall following the rainy season, with trends being especially strong in OTB. Small increases in salinity were observed in the spring for all but LTB.

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 were similar between bay segments, with an average increase of 48 days across the bay segments for the period of record (Table 4, Table S5, see Table S6 for 1998 to 2022). The increase in the number of 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 strong trends in the number of days per year when salinity was below 15 ppt. Both OTB and HB had models showing an increasing number of days when salinity was below 20 or 25 ppt, whereas MTB only showed an increase in the number of days when salinity was below 25 ppt. Some salinity models for LTB had 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 threshold, particularly for 25 ppt, where the change was 86 days per year from 1975 to 2022 (130 to 216 days, Table 4, Table S5, see Table S6 for 1998 to 2022).

Across all bay segments, the average increase in the number of days salinity was below 25 ppt from 1975 to 2022 was 36 days, although a distinct gradient towards the mouth of the bay was observed.

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, 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 (Table S5; 4 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 between segments, whereas the number of days when salinity was below the threshold decreased with proximity to the Gulf of Mexico (Table 4). 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 to more rigorously develop seagrass response models and the chosen thresholds were based primarily on statistical considerations.

3.3 Seagrass response

GAMs to assess the potential effects of year, light attenuation, temperature and salinity on seagrass change provided some evidence that seagrass change was influenced by climate stressors, particularly for the FIM and PDEM datasets. The model for the EPC data (n = 75, Adj. R^2 = 0.93, Deviance explained = 97%) had notable associations with year for the OTB and MTB bay segments and for light attenuation for the HB bay segment (Figure 7, Table S7). Associations of seagrass change with the temperature and salinity metrics were weak and somewhat contrary to expectation (i.e., an increasing partial effect on seagrass as the stressor metrics increased). The EPC model using the "both" stressor metric was similar (n = 75, Adj. R^2 = 0.97, Deviance explained = 99%, Figure S7, Table S8), suggesting no added benefit of evaluating times when both temperature was above and salinity was below the threshold.

The GAMs for the FIM and PDEM datasets had notable associations with temperature and salinity stress, where an increase in temperature and a decrease in salinity was often associated with a decline in seagrass depending on bay segment. The FIM model (n = 81, Adj. R^2 = 0.81, Deviance explained = 89%, Figure 8a, Table S9) showed a slight decrease in seagrass with increasing temperature for HB and MTB, with a similar change as salinity decreased. The PDEM model (n = 19, Adj. R^2 = 0.69, Deviance explained = 81%, Figure 8b, Table S10) that evaluated only OTB showed a distinct decrease with seagrass as temperature increased, but no association with salinity. Some of the interaction terms of the predictors with year suggested the relationships with seagrass may have changed over time, but the results were not consistent between bay segments or models (Tables S7 to S10).

4 Discussion

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. The number of days each year when temperature was above 30 ˚C or salinity was below 25 ppt has also increased consistently, with an average increase baywide of 48 and 37 days, respectively, since 1975. 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 fall, winter period provided a finer-scale comparison, where trends were most notable for OTB and northern stations of HB (Figure 5) – 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 explanation that increasing temperature and decreasing salinity were key (or the sole) drivers. However, evaluating all models together as weight-of-evidence suggests there is value in considering multiple datasets and models to interpret noisy patterns and compounding ecological processes. Model results for the FIM and PDEM datasets both suggested that increasing temperature and decreasing salinity were associated with potential seagrass loss in recent years

(Figure 8). For the EPC model, none of the stressor metrics were associated with seagrass change. An important distinction between the EPC and other 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. Additionally, the EPC models evaluated water quality changes from data collected at relatively deeper locations than where seagrass typically grows (Figure 1b), suggesting a potential disconnect in relating the two. 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 sub-tropical environments suggest that the limits of the ecological niche for seagrasses are higher for temperature and lower for salinity (Phillips 1960; McMillan and Moseley 1967; Zieman 1975; Lirman and Cropper 2003). Because we did not see a dramatic increase in the number of days each year when the thresholds were crossed at more stressful values, conditions in Tampa Bay in recent years are likely suboptimal but within the ecological niche for seagrasses. This is especially true for *H. wrightii* that had the greatest changes over the period of record and is tolerant of a wide range of salinity. However, 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). Without more continuous, diel observations of these metrics over the period of record, we were heavily reliant on these model outputs to determine relevant thresholds for Tampa Bay seagrass. This further highlights a long-standing data gap and need for the estuary. Regardless, our thresholds seem to be indicative of the potential for chronic sublethal effects of stress on seagrasses, reducing their resilience to other stressors. Further, our models suggested that temperature and salinity changes are at least associated with seagrass loss and, if so, long-term trends in both are set to amplify their effect in the future.

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). Our inclusion of light attenuation in the EPC models was meant to account for how the light environment may be influencing seagrass growth, in addition to climate-related stressors. However, light attenuation has improved over the period of record and is currently within the limits estimated to be supportive of seagrass growth in Tampa Bay (Janicki and Wade 1996; Greening et al. 2011), particularly in OTB where the most loss occurred (Figure 2c). Additional water quality parameters could be included in the models to provide further evidence that light-limitation is not the present driver for seagrass change (e.g., nitrogen loading, chlorophyll-a, color), although nutrient management for the benefit of seagrass growth will likely continue to be a dominant management paradigm for Tampa Bay. 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 large-scale 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 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 (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* *testudinum* 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. testudinum* 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 (Hall et al. 2016). However, elevated summertime temperatures were also implicated in the decline. 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 inflows. 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 long-term increase is inversely associated with the decrease in salinity.

4.1 Conclusions

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. GAMs provided partially-supporting evidence that these changes can be linked to recent seagrass losses. Future analyses may show stronger 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. Ongoing work in OTB using continuous data loggers in shallow areas where seagrass has been gained or lost will provide insights into short-term diurnal changes as potential acute temperature stress (see https://tbep-tech.github.io/otb-temp/tempeval). Morphological or physiological measurements at the individual level could also provide early indications of heat and osmotic stress (Congdon et al. 2023).

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 become less so, resulting in diminished ecosystem resilience compounded by climate change. For example, nitrogen load reductions have historically been the most effective strategy to restore seagrass in Tampa Bay (Greening and Janicki 2006; Greening et al. 2014). As Tampa Bay becomes hotter and fresher, current nutrient load targets may no longer be effective, resulting in further shifts in algal and seagrass ecology dynamics. Strategies that mimic or restore pre-development 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 seagrasses responding to climatic changes in Tampa Bay. These considerations are especially critical for upper 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, especially since the challenges of restoring these long-lived foundation species once lost will be exacerbated by ongoing development in the watershed and the current climate trajectory.

Figures

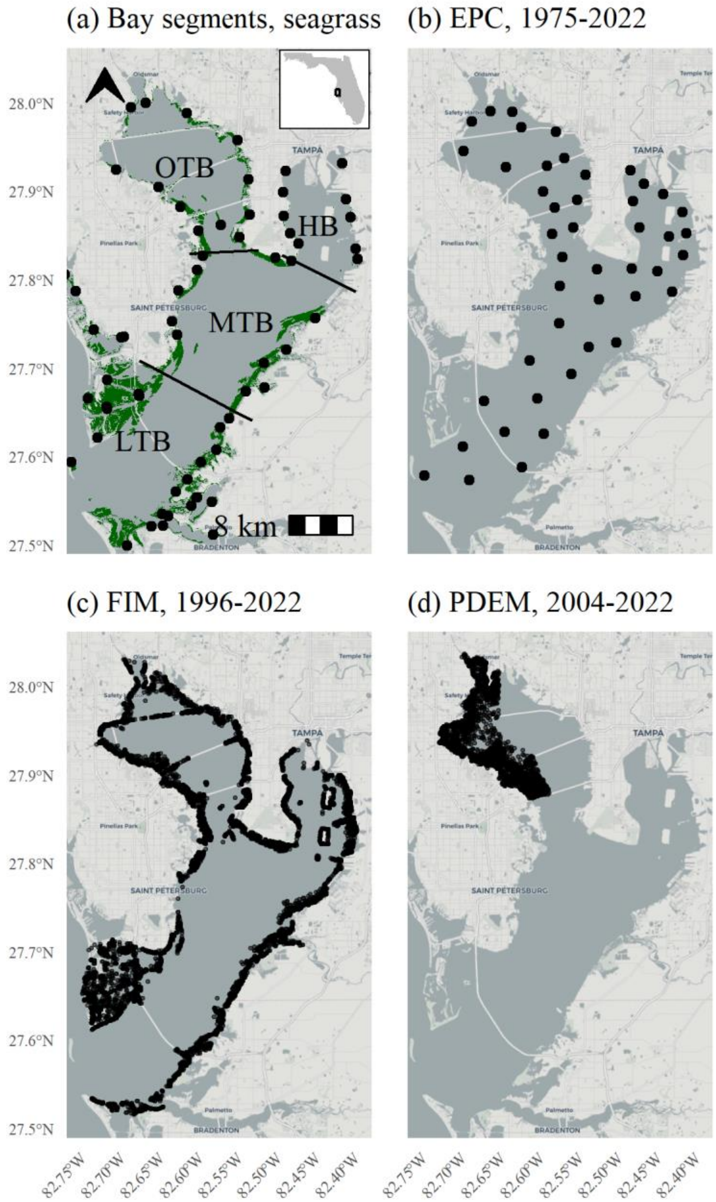

Fig. 1: Map of Tampa Bay and the three datasets used for trend analysis, including (a) bay segments, 2022 seagrass coverage (green), and transect starting points (black), (b) Environmental Protection Commission (EPC) long-term monitoring sites, (c) Fisheries Independent Monitoring (FIM) random sampling 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.

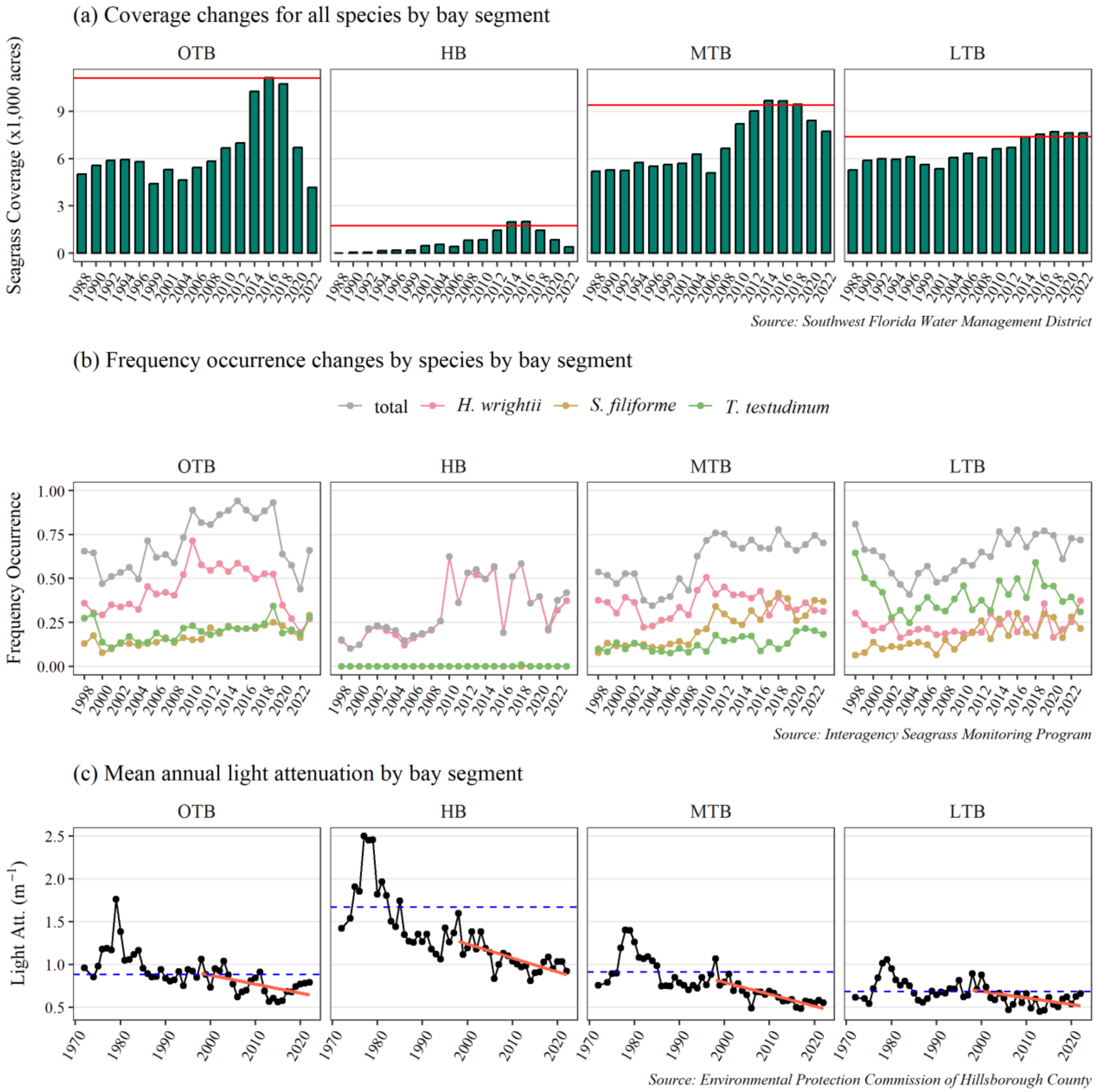

Fig. 2: Seagrass changes over time in Tampa Bay for (a) areal coverage 1988 - 2022 from mapping, (b) frequency occurrence of major species 1998 - 2022 from annual transect monitoring, and (c) mean annual light attenuation from 1970 to 2022. Changes are shown for major bay segments. Red lines in (a) show approximate capacity of seagrass coverage based on the baywide target of 40,000 acres and red lines in (c) show the approximate linear trend for the period of record covered by the transect data in (b). The dashed line in (c) shows the light attenuation threshold to support seagrass growth in each bay segment. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

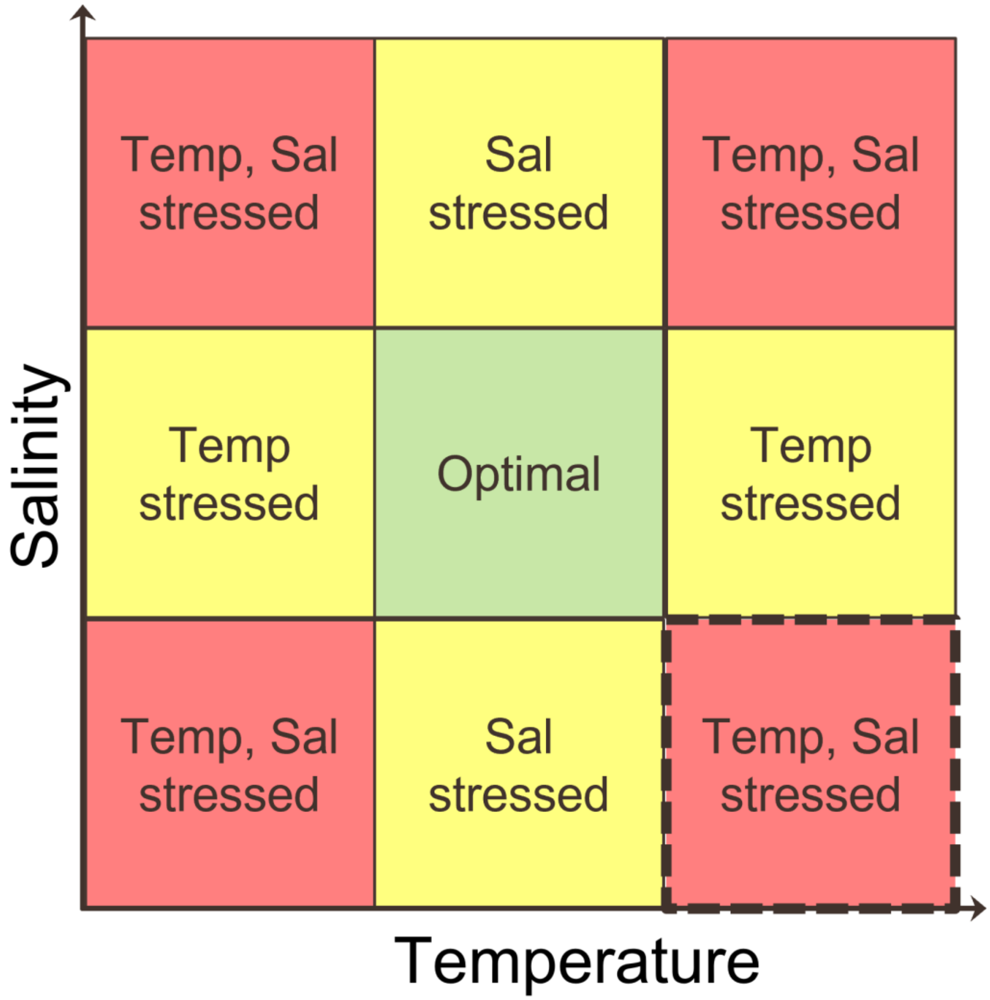

Fig. 3: Conceptual stressor diagram demonstrating a two-dimensional niche space for temperature and salinity. Tampa Bay is trending towards the bottom right.

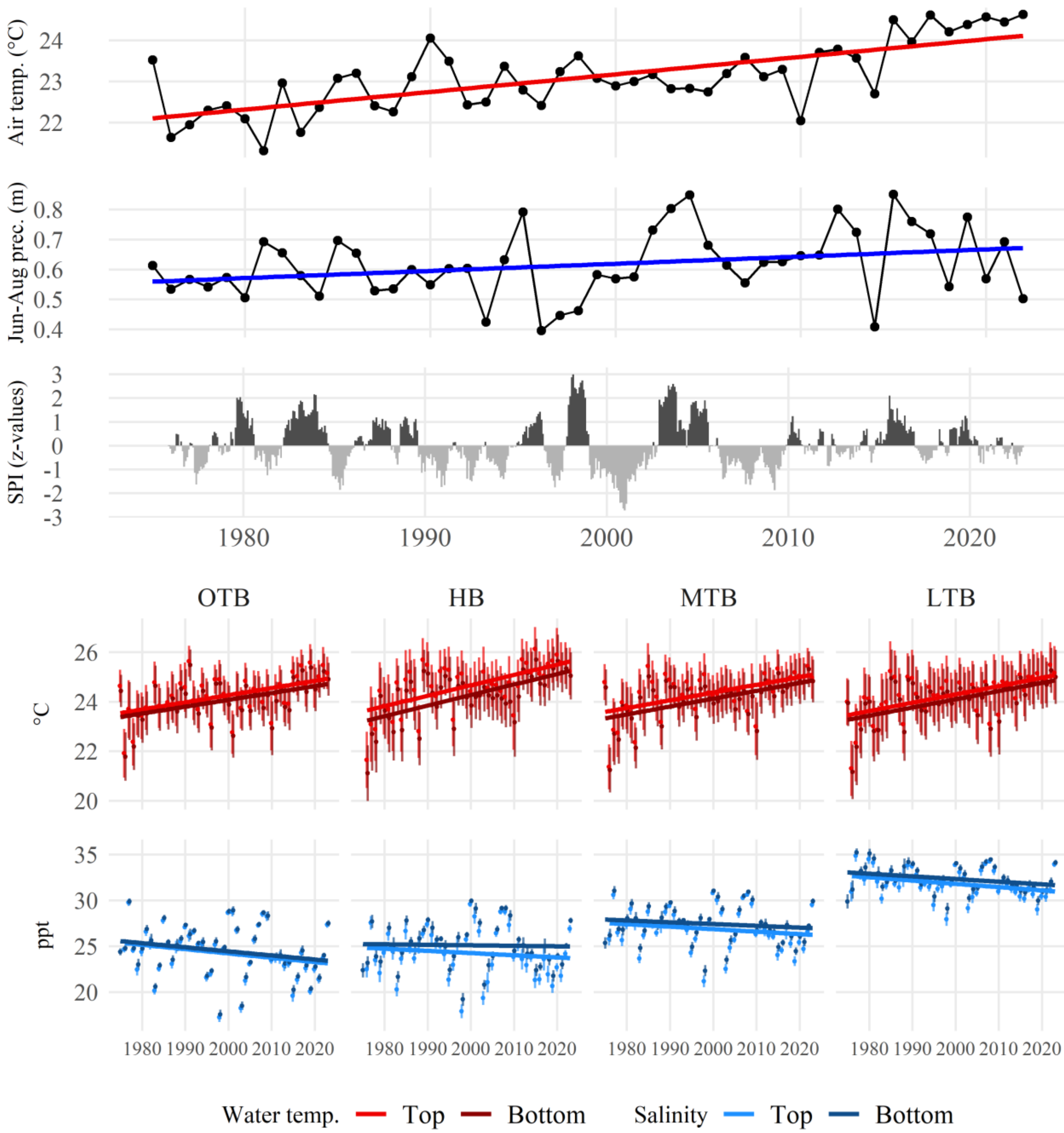

Fig. 4: Long-term air temperature, precipitation (Jun-Aug), Standard Precipitation Index (SPI), water temperature, and salinity trends from 1975 to 2022. The color shades for water temperature and salinity indicate sampling location and values shown are the averages (95% confidence interval) across all 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.

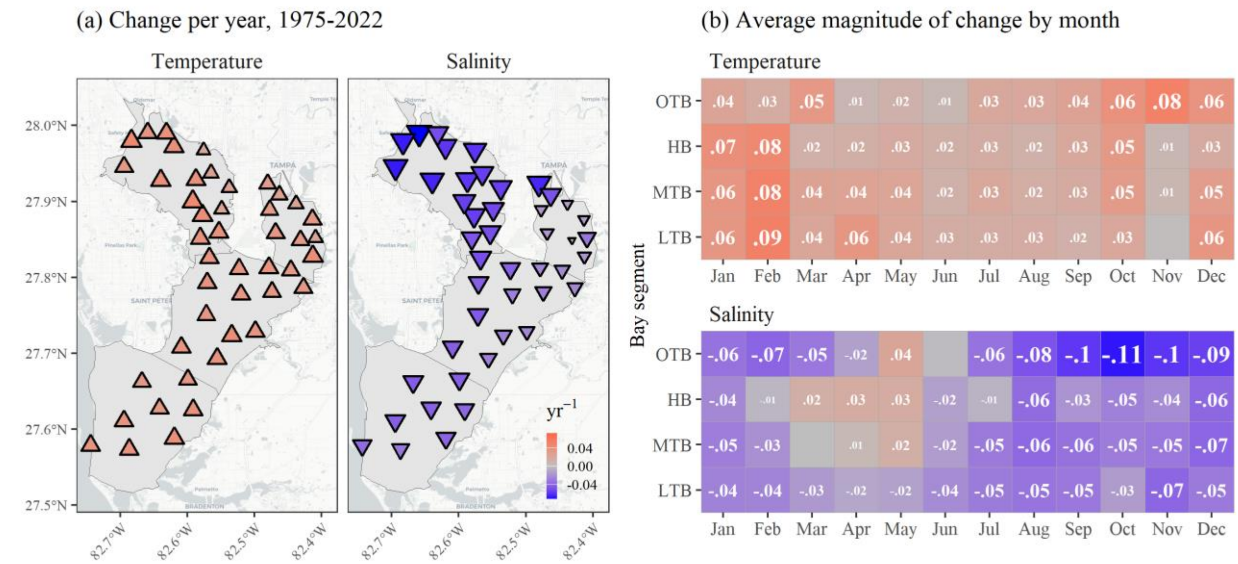

Fig. 5: Trends from 1975 to 2022 for bottom water temperature and salinity measurements at long-term monitoring stations in Tampa Bay. Results for seasonal Kendall tests by station are shown in (a) with color, size, and shape corresponding to the estimated annual slope as change per year (yr^{-1}). Summarized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

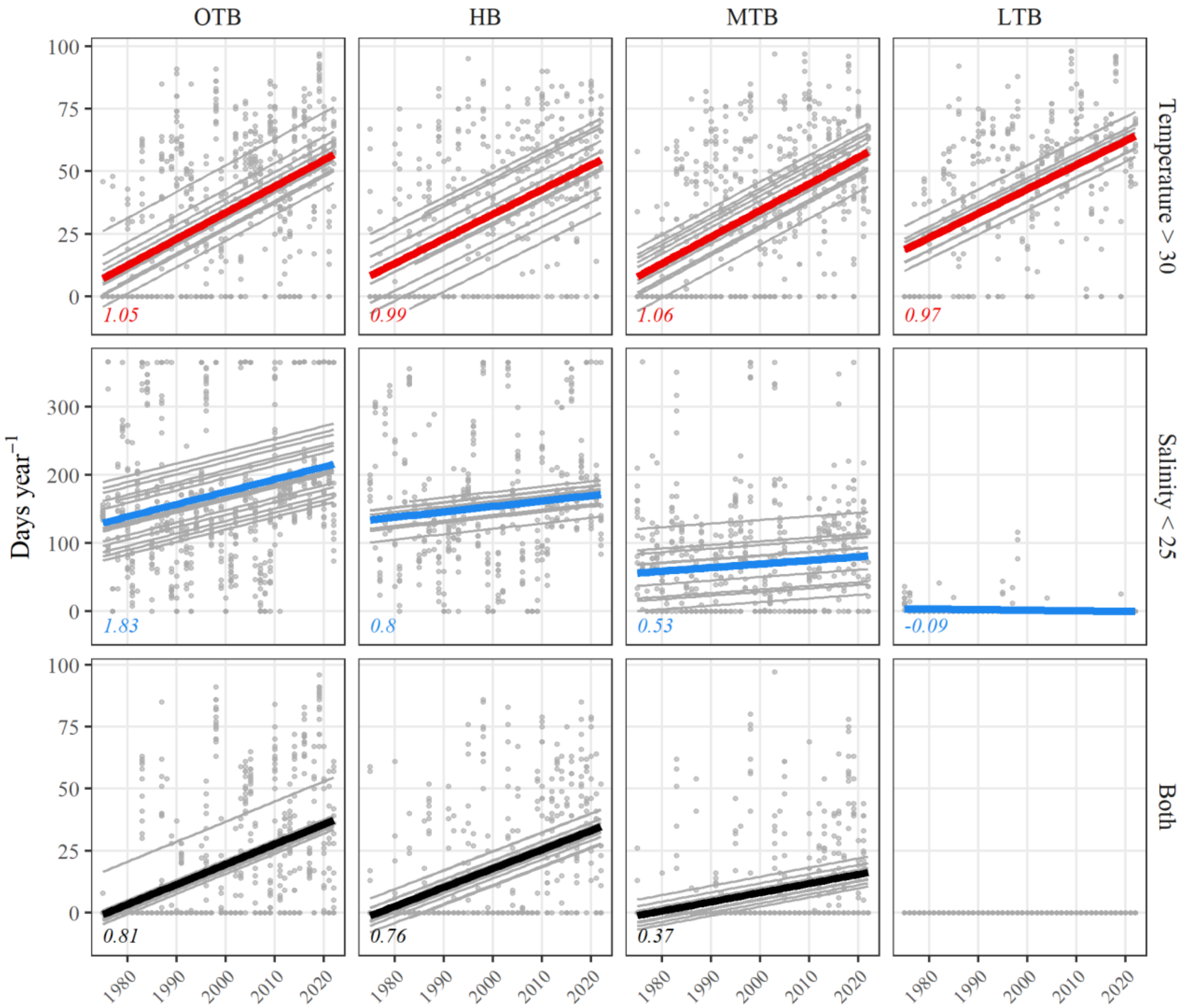

Fig. 6: Example of mixed effects models for the estimated number of days per year that bottom temperature (red) or salinity (blue) were above or below thresholds of 30 ˚C or 25 ppt, respectively, from the EPC data. The bottom row (black) shows the number of days when both temperature was above and salinity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

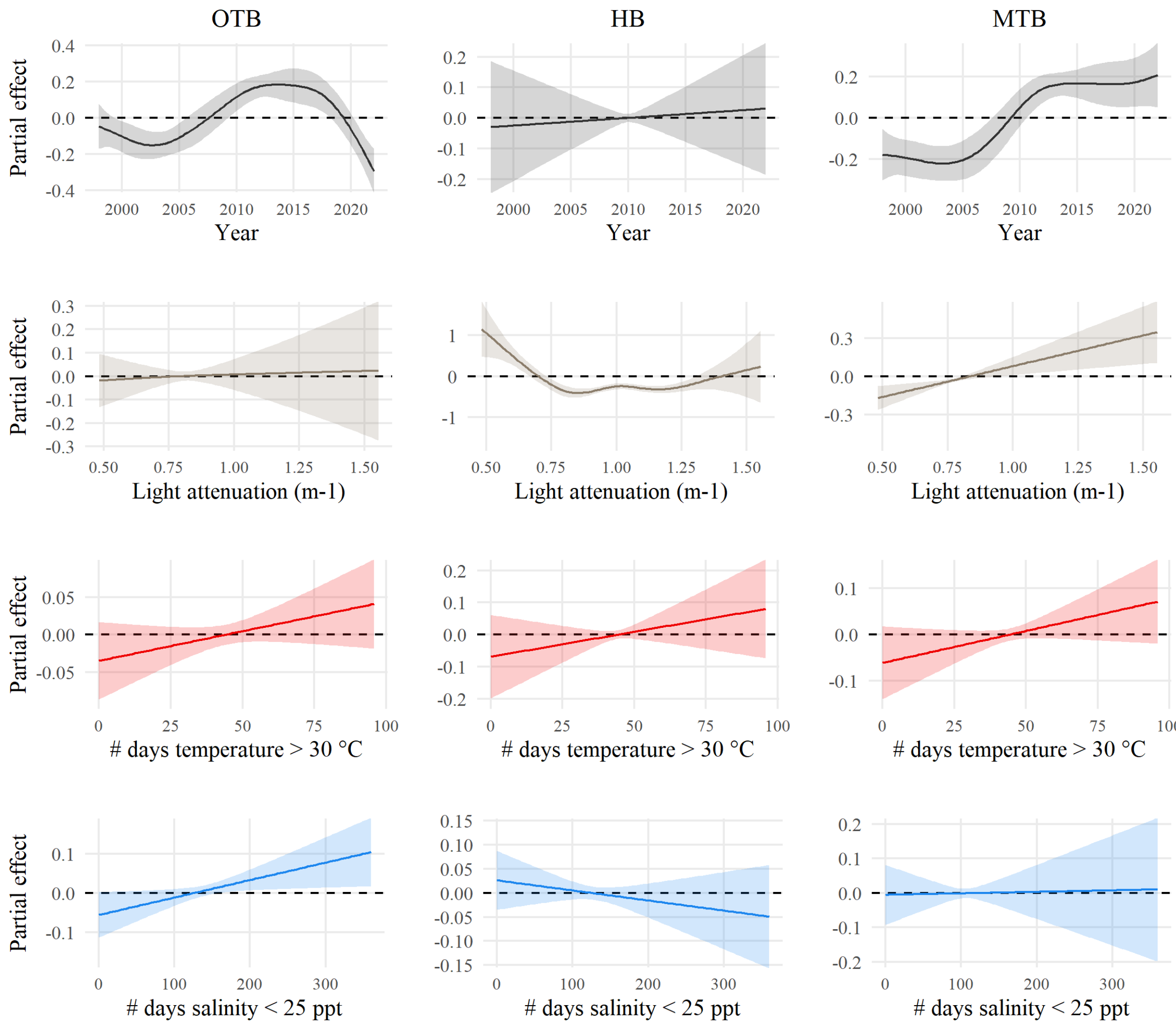

Fig. 7: Partial effects of smoothers (rows) by bay segment (columns) from a Generalized Additive Model used to describe seagrass change relative to year, light attenuation, the number of days each year when bottom temperature was above 30 °C, and the number of days each year when bottom salinity was below 25 ppt. The EPC data were used for the independent variables in the model. Partial effects describe the modeled association between each predictor and seagrass frequency occurrence after accounting for the effects of the other predictors. The model also included smoothed interaction terms with year (not shown). See Table S7 for additional model fit statistics. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay. n = 75, Adj. R^2 *= 0.93, Deviance explained = 97%*

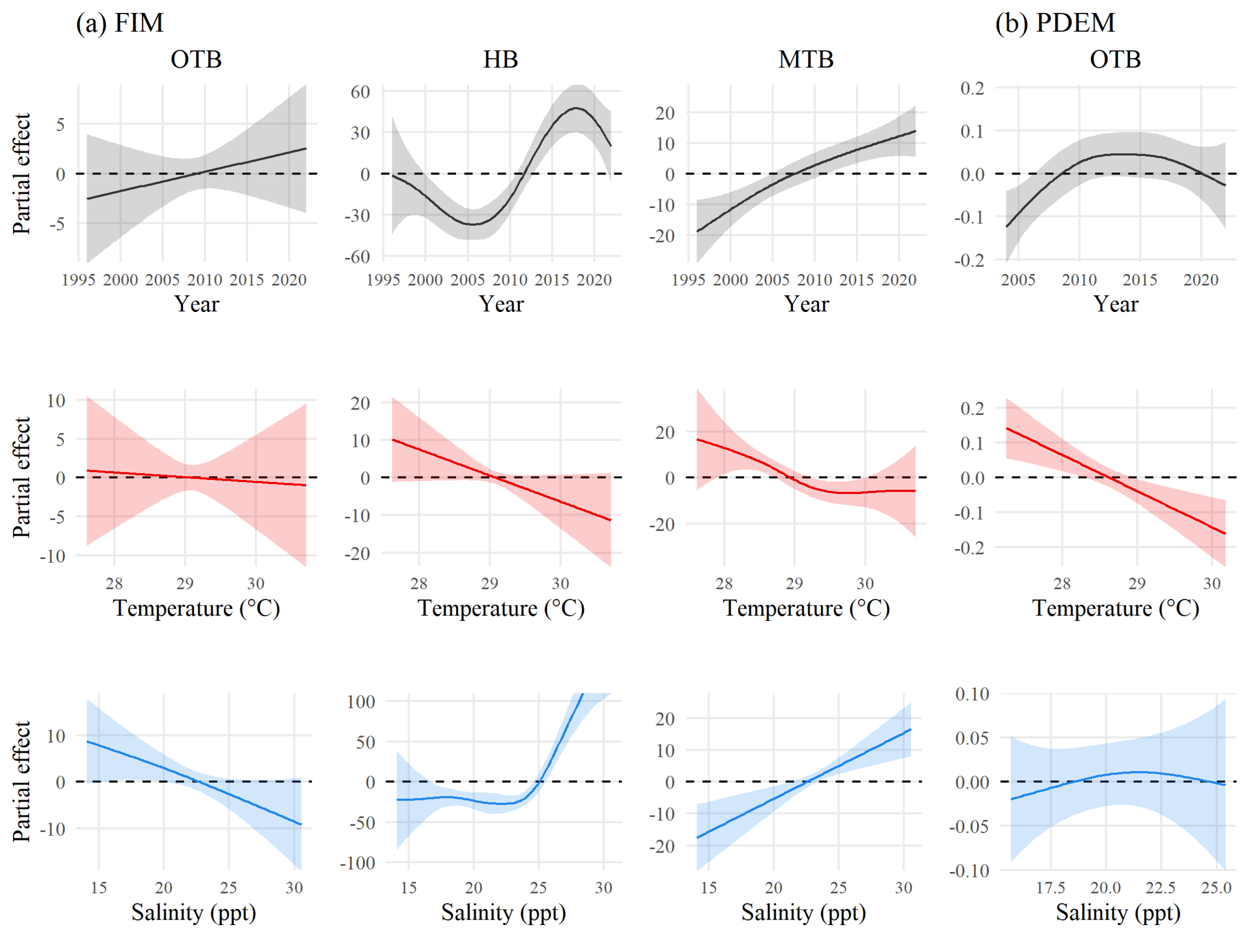

Fig. 8: Partial effects of smoothers (rows) by bay segment (columns) from Generalized Additive Models evaluating seagrass changes versus year, bottom temperature, and bottom salinity for the (a) FIM (first three columns evaluating mean annual percent cover from 0-100, n = 81, Adj. R^2 = 0.81, Deviance explained = 89%) and (b) PDEM (right column evaluating annual frequency occurrence from 0-1 in OTB only, n = 19, Adj. R^2 = 0.69, Deviance explained = 81%) datasets. Partial effects describe the modeled association between each predictor and seagrass change after accounting for the effects of the other predictors. The models also included smoothed interaction terms with year (not shown). See Tables S9 and S10 for additional model fit statistics. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay.

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Tables

Table 1: Summary of datasets used for trend analyses and comparisons with seagrass datasets. Temporal and spatial scales are those used for analysis. SWFWMD: Southwest Florida Water Management District, EPC: Environmental Protection Commission of Hillsborough County, FIM: Florida Fish and Wildlife Comission, Fisheries Independent Monitoring, PDEM: Pinellas County Department of Environmental Management.

Dataset	Description	Temporal	Spatial	Analysis
SWFWMD aerial maps	Seagrass coverage in acres	1988 - 2022, ~biennial	Whole bay	Biennial trends by bay segment, visual only
Transect data	Seagrass frequency occurrence by species	1999 - 2022, annual	Whole bay, 62 transects	Annual trends by bay segment and species, comparison with temperature, salinity, and light attenuation as stressor metrics or observed data at annual scale
EPC	Water quality monitoring samples	1975 - 2022, monthly	Whole bay, fixed sites	Trends in annual change and seasonal Kendall tests, estimate of stressor metrics as number of days above/below threshold
FIM	Nearshore temperature and salinity, seagrass species and cover	1996 - 2022, monthly	Whole bay shallow, stratified random sites	Trends in annual observed temperature, salinity, comparison to annual seagrass % cover
PDEM	Water quality and seagrass presence/absence	2003 - 2022, monthly	Old Tampa Bay, stratified random sites	Trends in annual observed temperature, salinity, comparison to annual seagrass frequency occurrence
Tampa International Airport	Air temperature	1975 - 2022, annual	27.979°N, 82.535°W	Annual trend
SWFWMD precipitation	Area-weighted precipitation for the wet season (June-September) for the Tampa Bay watershed	1975 - 2022, annual	Whole watershed	Annual trend

Table 2: Bottom temperature trends (°C) by bay segments and datasets. Change per year is the model slope with standard error in parentheses. Start year describes the range of the trend test to 2022 (1975: n = 48, 1996: n = 27, 2004: n = 19). 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.

Start year	Bay segment	Dataset	Change / year	Start value	End value	Total change
1975	OTB	EPC	0.03 (0.01)	23.4	24.7	1.3
	HB	EPC	0.04 (0.01)	23.4	25.1	1.7
	MTB	EPC	0.03 (0.01)	23.3	24.8	1.5
	LTB	EPC	0.03 (0.01)	23.3	24.8	1.5
1996	OTB	EPC	0.04 (0.02)	23.6	24.8	1.2
		FIM	0.05 (0.02)	23.5	24.8	1.3
	HB	EPC	0.06 (0.01)	23.8	25.3	1.5
		FIM	0.05 (0.02)	24.0	25.3	1.3
	MTB	EPC	0.04 (0.01)	23.8	24.8	1.0
		FIM	0.03 (0.02)	24.0	24.8	0.8
	LTB	EPC	0.03 (0.01)	23.9	24.8	0.9
		FIM	0.02 (0.02)	24.1	24.6	0.5
2004	OTB	EPC	0.06 (0.02)	23.8	25.0	1.2
		FIM	0.06 (0.03)	23.8	24.9	1.1
		PDEM	0.04 (0.03)	24.4	25.2	0.8
	HB	EPC	0.09 (0.02)	23.9	25.5	1.6
		FIM	0.1 (0.04)	23.9	25.7	1.8
	MTB	EPC	0.07 (0.02)	23.8	25.0	1.2
		FIM	0.07 (0.04)	23.8	25.1	1.3
	LTB	EPC	0.06 (0.02)	23.8	25.0	1.2
		FIM	0.05 (0.03)	23.9	24.8	0.9

Table 3: Bottom salinity trends (ppt) by bay segments and datasets. Change per year is the model slope with standard error in parentheses. Start year describes the range of the trend test to 2022 (1975: n = 48, 1996: n = 27, 2004: n = 19). 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.

Start year	Bay segment	Dataset	Change / year	Start value	End value	Total change
1975	OTB	EPC	-0.06 (0.03)	25.7	23.1	-2.6
	HB	EPC	-0.01 (0.02)	25.4	24.7	-0.7
	MTB	EPC	-0.03 (0.02)	28.0	26.7	-1.3
	LTB	EPC	-0.04 (0.01)	33.1	31.5	-1.6
1996	OTB	EPC	-0.08 (0.07)	24.9	22.8	-2.1
		FIM	-0.05 (0.08)	24.1	22.7	-1.4
	HB	EPC	-0.08 (0.07)	26.1	23.9	-2.2
		FIM	0 (0.08)	24.0	23.9	-0.1
	MTB	EPC	-0.07 (0.05)	28.0	26.3	-1.7
		FIM	-0.03 (0.06)	26.6	25.8	-0.8
	LTB	EPC	-0.04 (0.03)	32.5	31.4	-1.1
		FIM	0.01 (0.04)	31.6	32.0	0.4
2004	OTB	EPC	-0.2 (0.09)	25.5	22.0	-3.5
		FIM	-0.15 (0.09)	24.6	22.0	-2.6
		PDEM	-0.19 (0.1)	24.3	20.9	-3.4
	HB	EPC	-0.19 (0.08)	26.5	23.1	-3.4
		FIM	-0.08 (0.1)	24.7	23.4	-1.3
	MTB	EPC	-0.17 (0.07)	28.6	25.6	-3.0
		FIM	-0.11 (0.08)	27.2	25.3	-1.9
	LTB	EPC	-0.12 (0.04)	33.0	30.9	-2.1
		FIM	-0.05 (0.05)	32.5	31.5	-1.0

Table 4: Summary of mixed-effects models evaluating increases in the number of days each year from 1975 to 2022 when bottom temperature was above 30 °C, bottom 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 (mean, standard error) in 1975 and 2022 when temperature or salinity were above or below the thresholds. 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	-	-	-

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Statements and Declarations

Data Availability

All data and analysis code for this manuscript is available on GitHub at https://github.com/tbep-tech/temp-manu. A preprint of an earlier version of this manuscript is available at https://doi.org/10.21203/rs.3.rs-3946855/v1.

Author Contributions

Conceptualization: Marcus W Beck, Kerry Flaherty-Walia, Sheila Scolaro, Maya C Burke, Edward T Sherwood; Data Curation: Bradley T Furman, David J Karlen, Christopher J Anastasiou, Christopher Pratt; Methodology: Marcus W Beck, Kerry Flaherty-Walia, Sheila Scolaro, Maya C Burke, Edward T Sherwood; Formal analysis and investigation: Marcus W Beck; Writing - original draft preparation: all authors; Writing - review and editing: all authors; Funding acquisition: Edward T Sherwood.

Ethics Declaration

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

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Figure 1

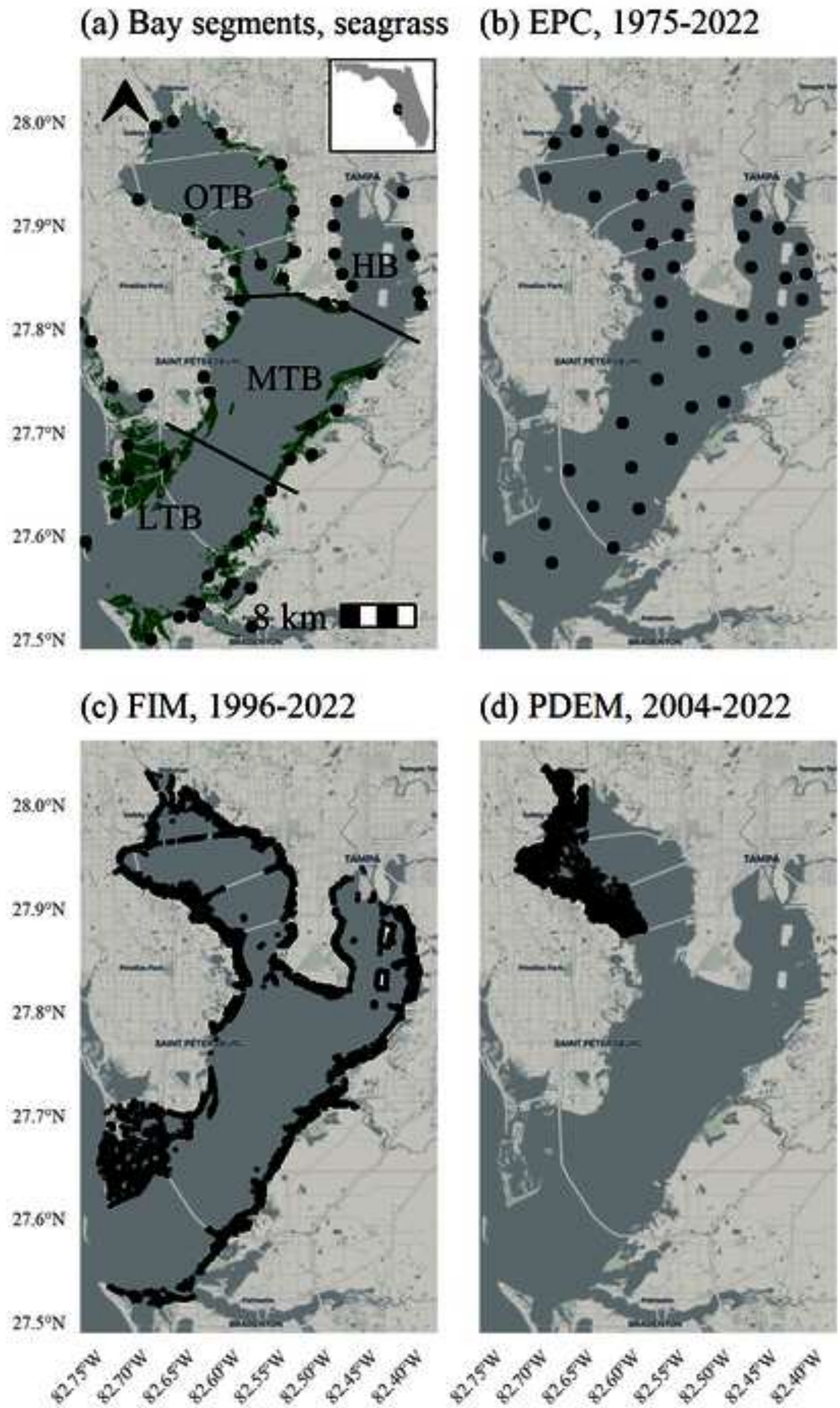

(a) Bay segments, seagrass
(b) EPC, 1975-2022
(c) FIM, 1996-2022
(d) PDEM, 2004-2022
OTB
HB
MTB
LTB
8 km
TAMPA
28.0°N
27.9°N
27.8°N
27.7°N
27.6°N
27.5°N
82.75°W
82.70°W
82.65°W
82.60°W
82.55°W
82.50°W
82.45°W
82.40°W

Figure 2 Click here to access/download;Figure;Fig2.png

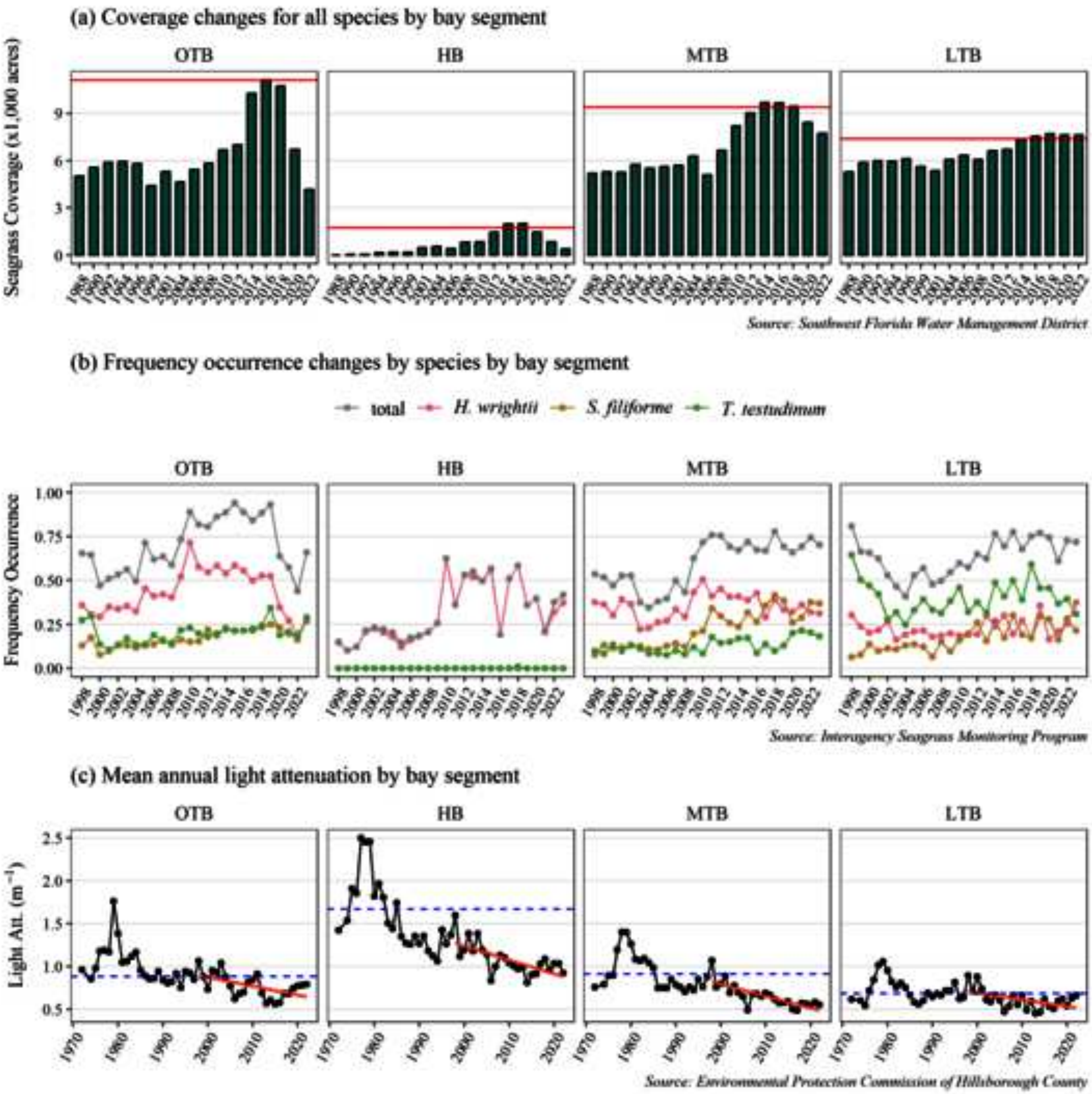


Figure 3

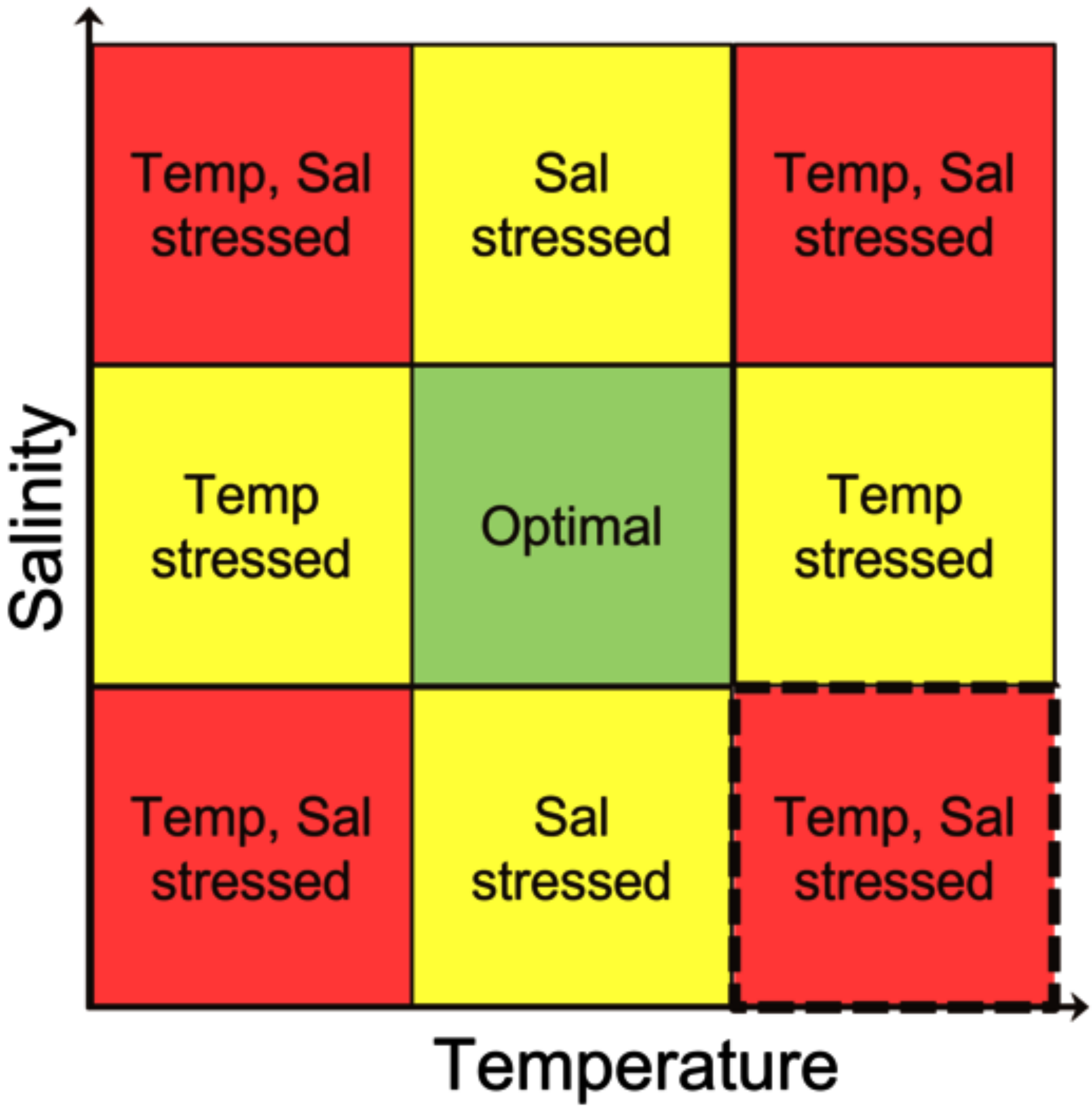

Temp, Sal stressed
Sal stressed
Temp, Sal stressed
Temp stressed
Optimal
Temp stressed
Temp, Sal stressed
Sal stressed
Temp, Sal stressed
Salinity
Temperature

Figure 4 Click here to access/download;Figure;Fig4.png

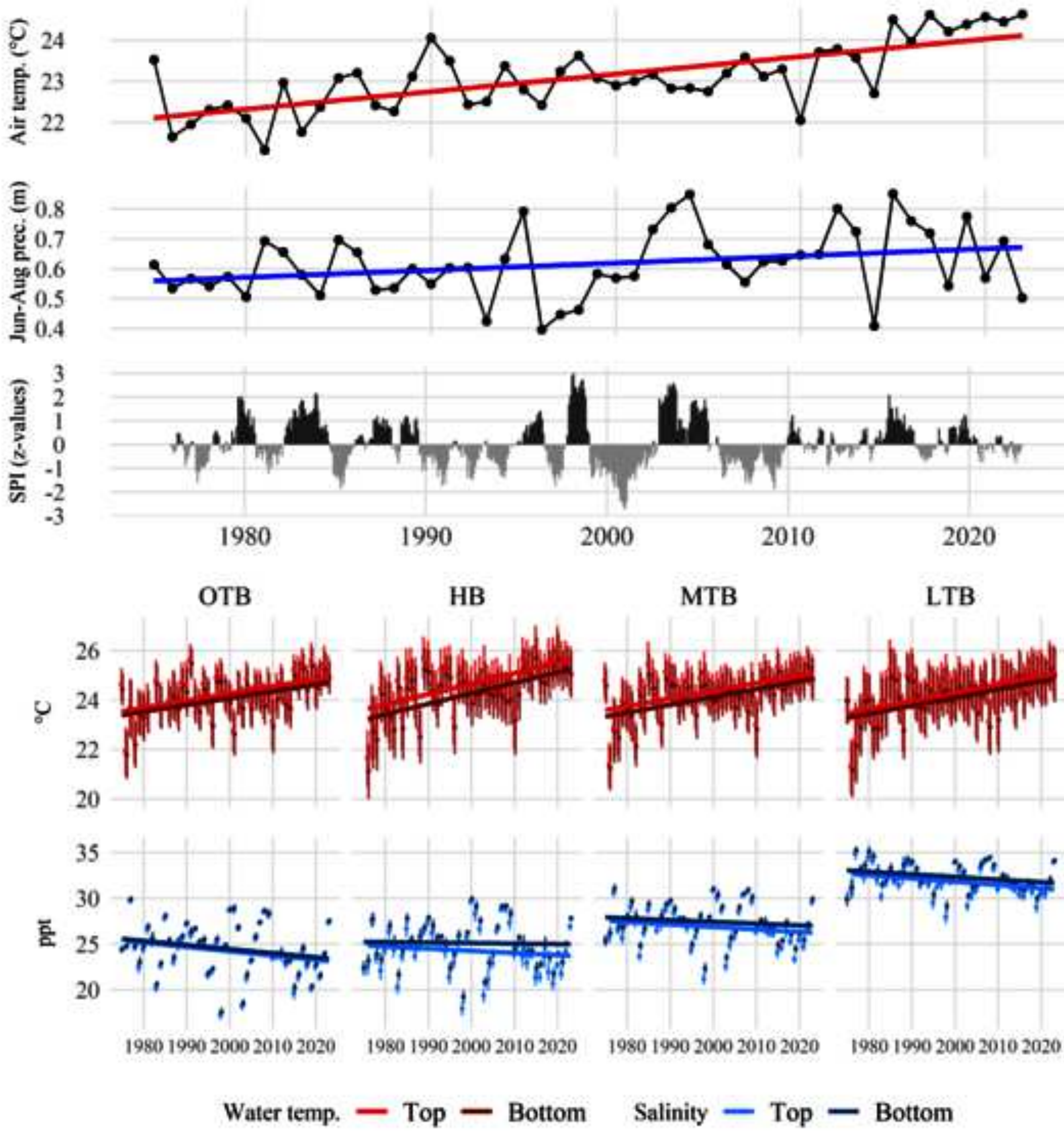


Figure 5

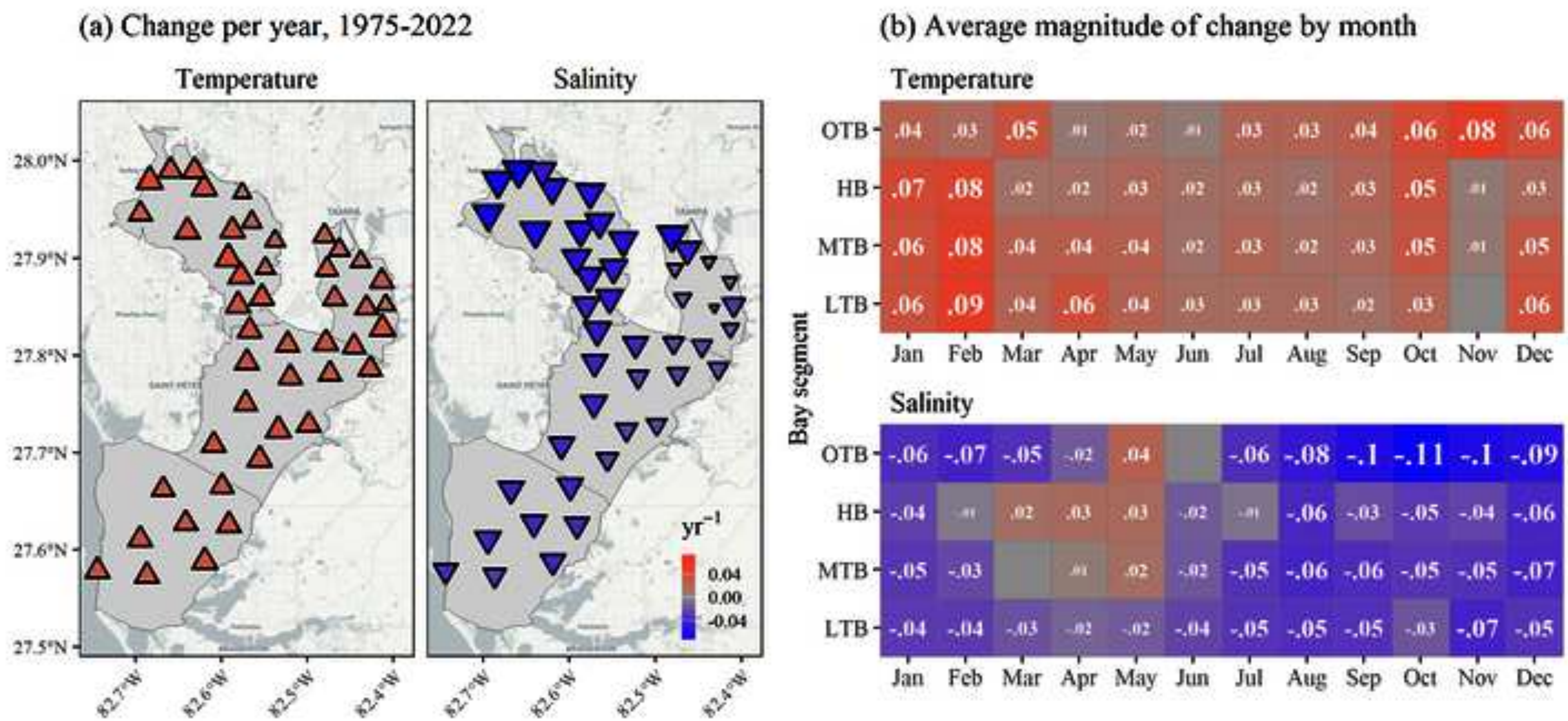

(a) Change per year, 1975-2022
Temperature
Salinity
28.0°N
27.9°N
27.8°N
27.7°N
27.6°N
27.5°N
82.7°W
82.6°W
82.5°W
82.4°W
yr⁻¹
0.04
0.00
-0.04
(b) Average magnitude of change by month
Temperature
Salinity
Bay segment
OTB
HB
MTB
LTB
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Figure 6

Click here to access/download;Figure;Fig6.png

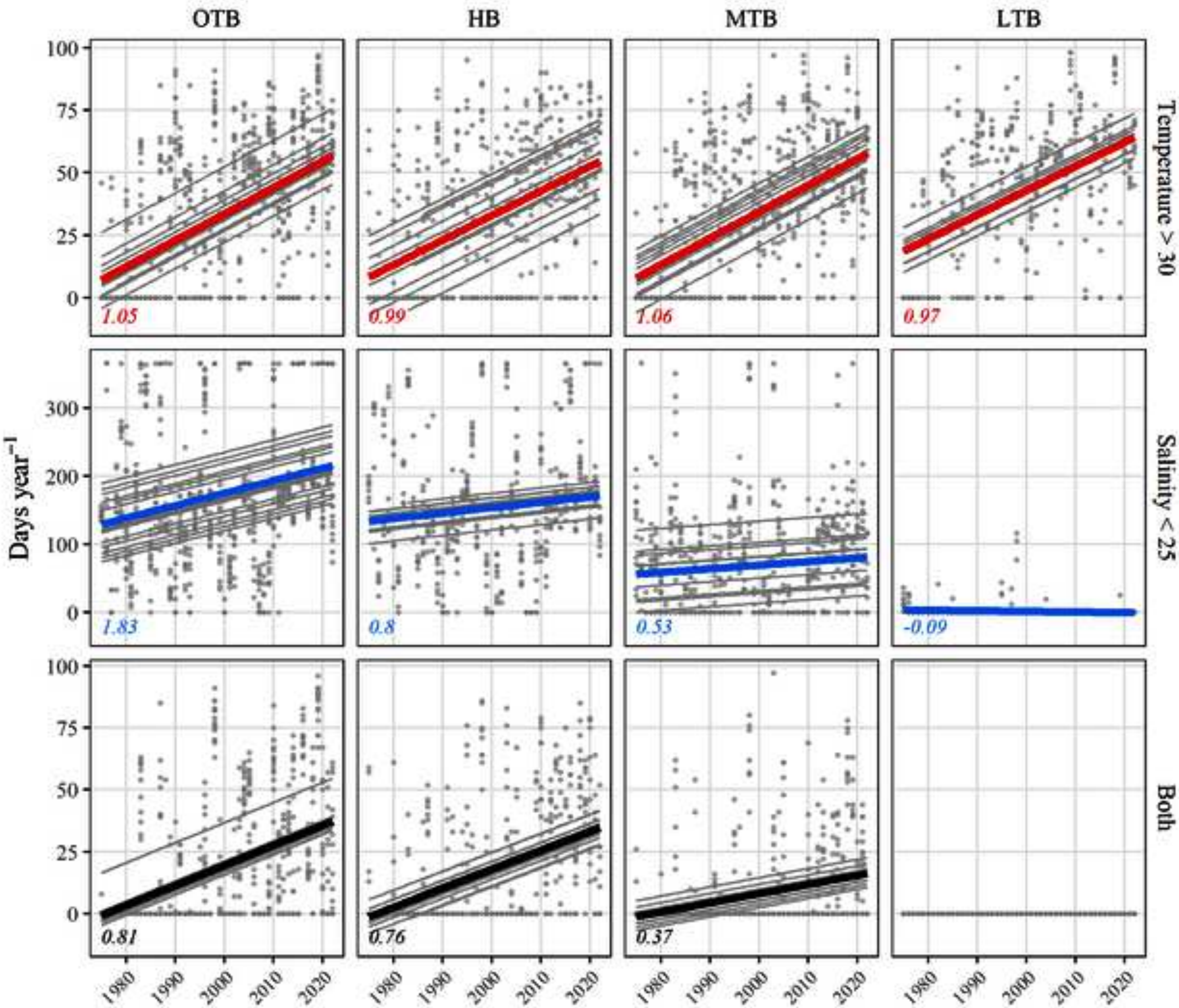


Figure 7

Click here to access/download;Figure;Fig7.png

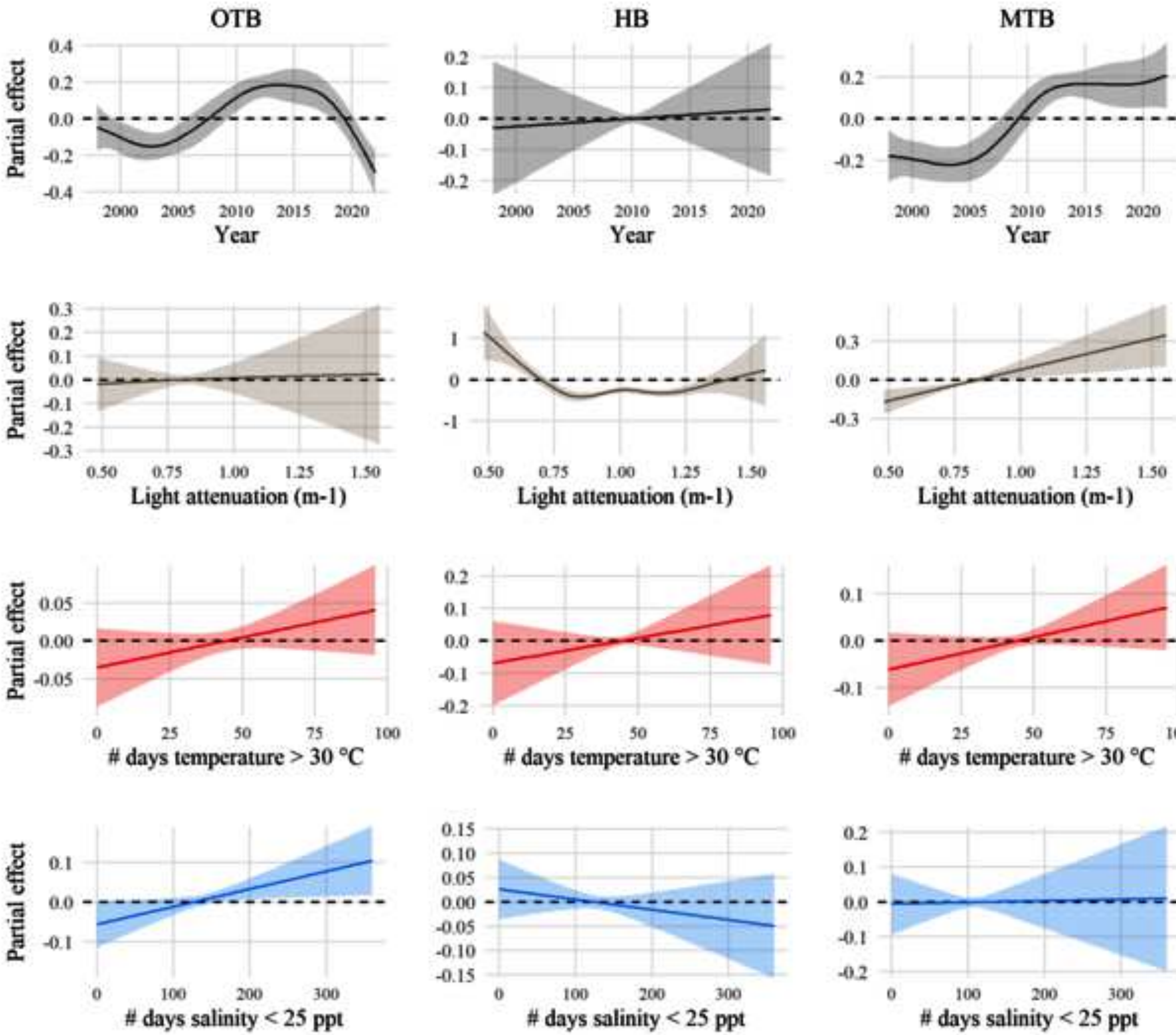
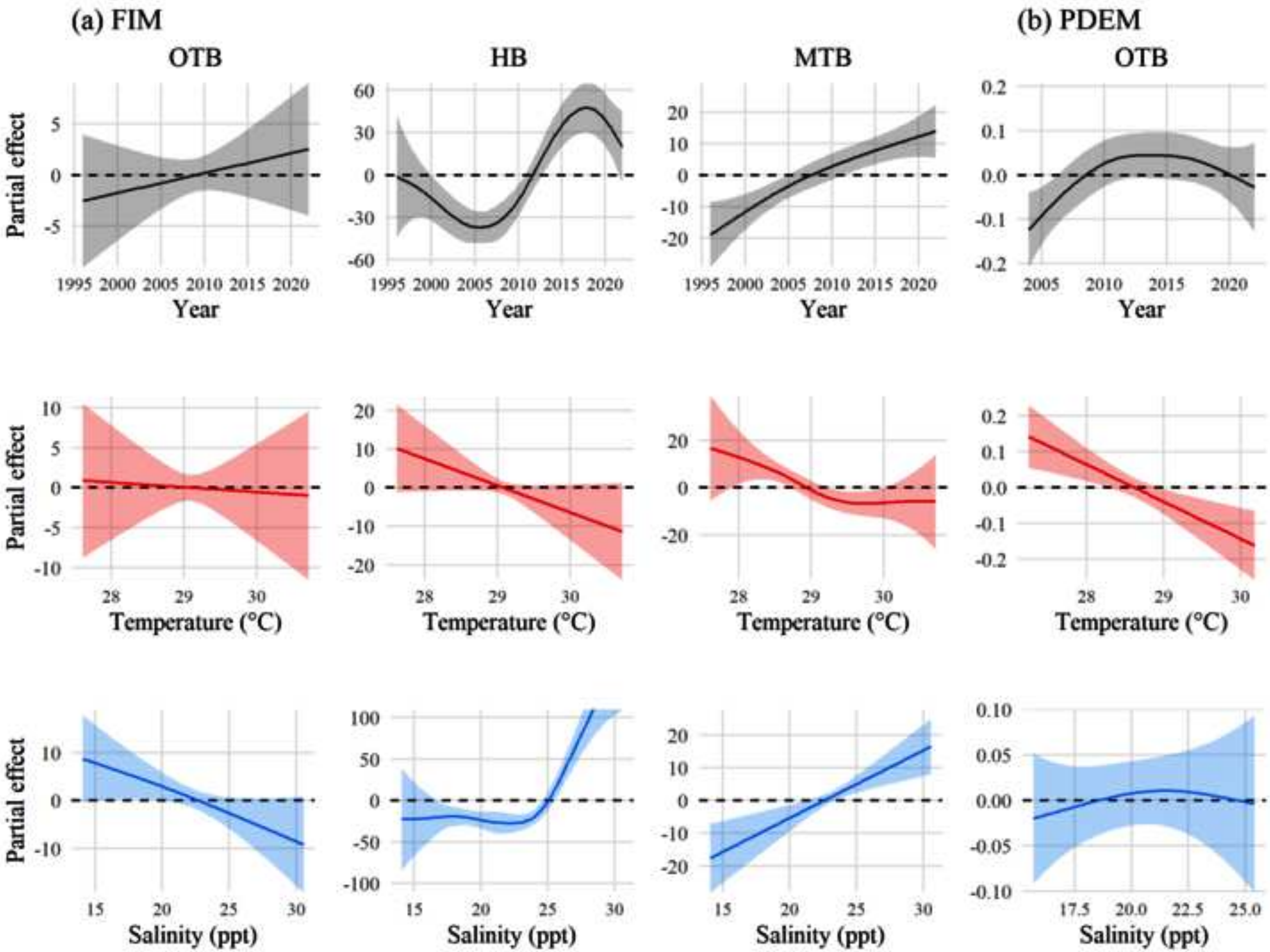


Figure 8

Click here to access/download;Figure;Fig8.png



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

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Figures

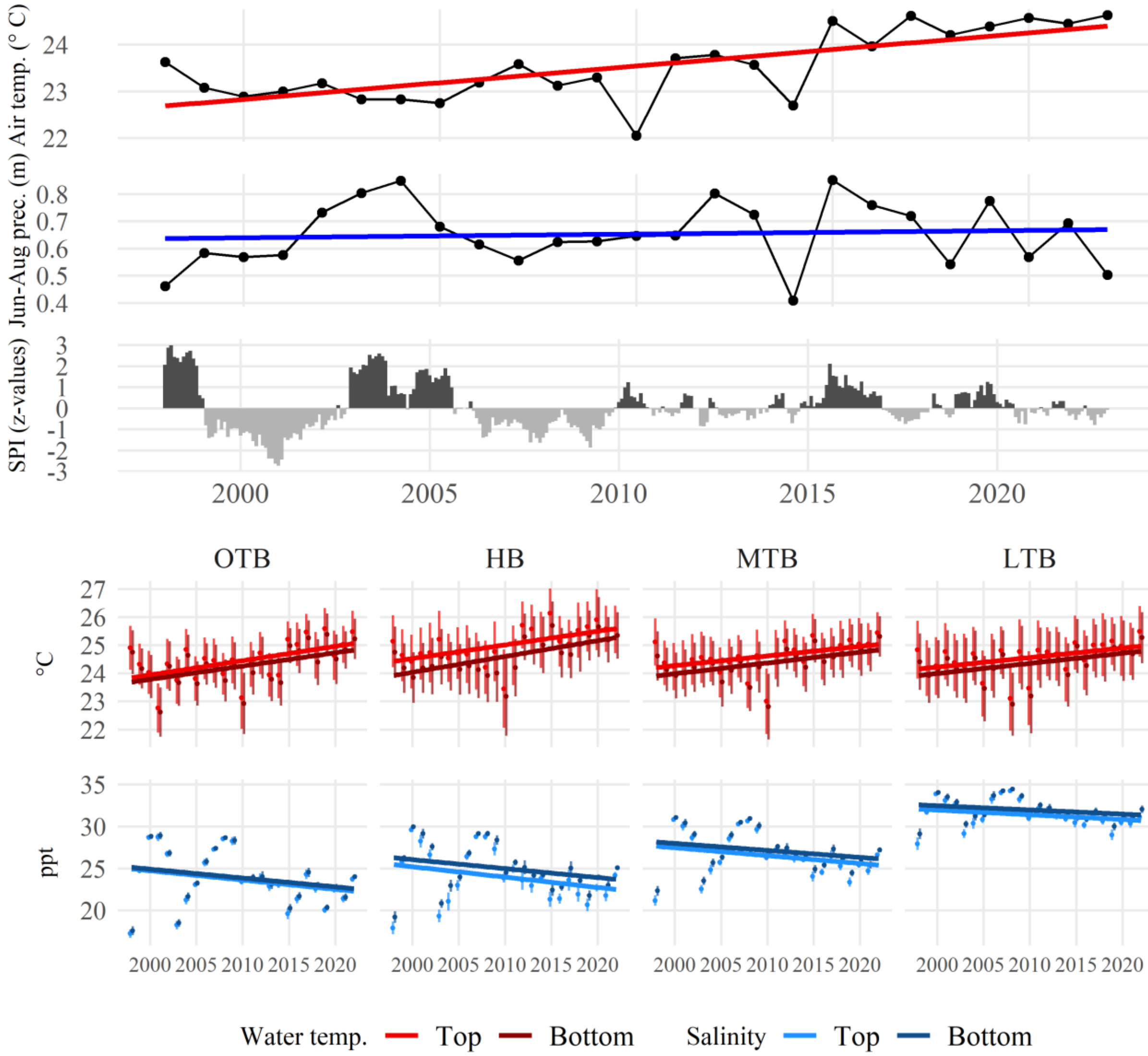

Fig. S1: Air temperature, precipitation (Jun-Aug), Standard Precipitation Index (SPI), water temperature, and salinity trends from 1998 to 2022. The color shades for water temperature and salinity indicate sampling location and values shown are the averages (95% confidence interval) across all Environmental 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.

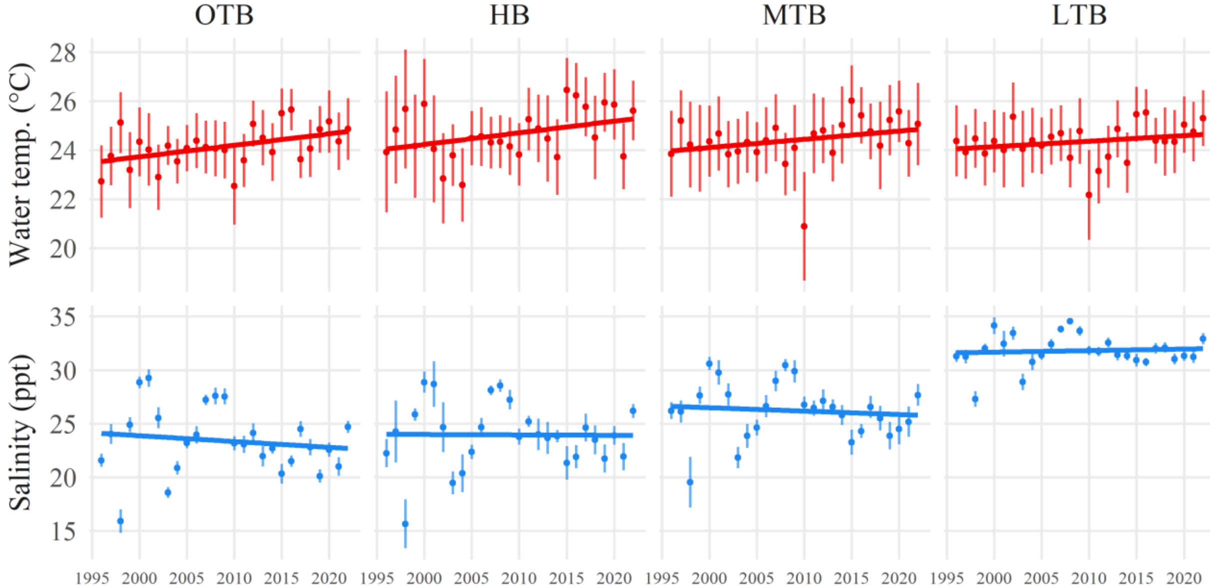

Fig. S2: Bottom water temperature and salinity trends from 1996 to 2021 for stations from the Fisheries Independent Monitoring program. Points show the average (95% confidence interval) across all stations in each bay segment and sampling months for each year. OTB: Old Tampa Bay, HB: Hillsborough Bay,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

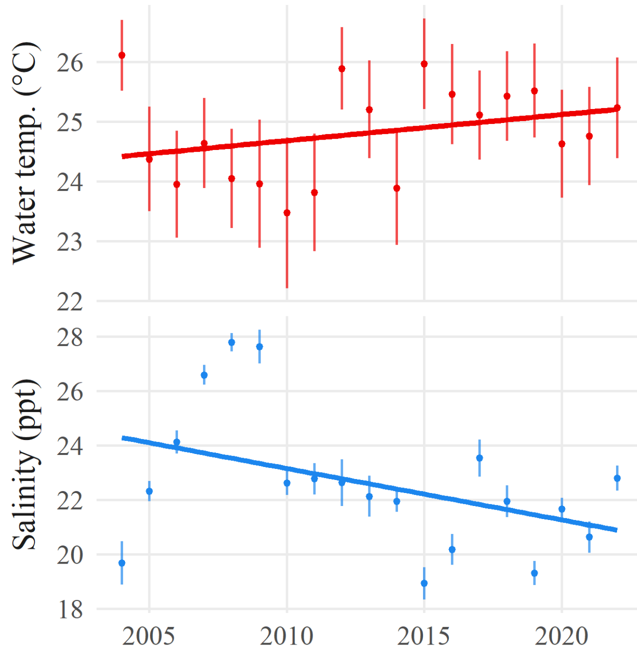

Fig. S3: Bottom water temperature and salinity trends from 2004 to 2022 for stations from the Pinellas County Department of Environmental Management in Old Tampa Bay. Points show the average (95% confidence interval) across all stations in each bay segment and sampling months for each year.

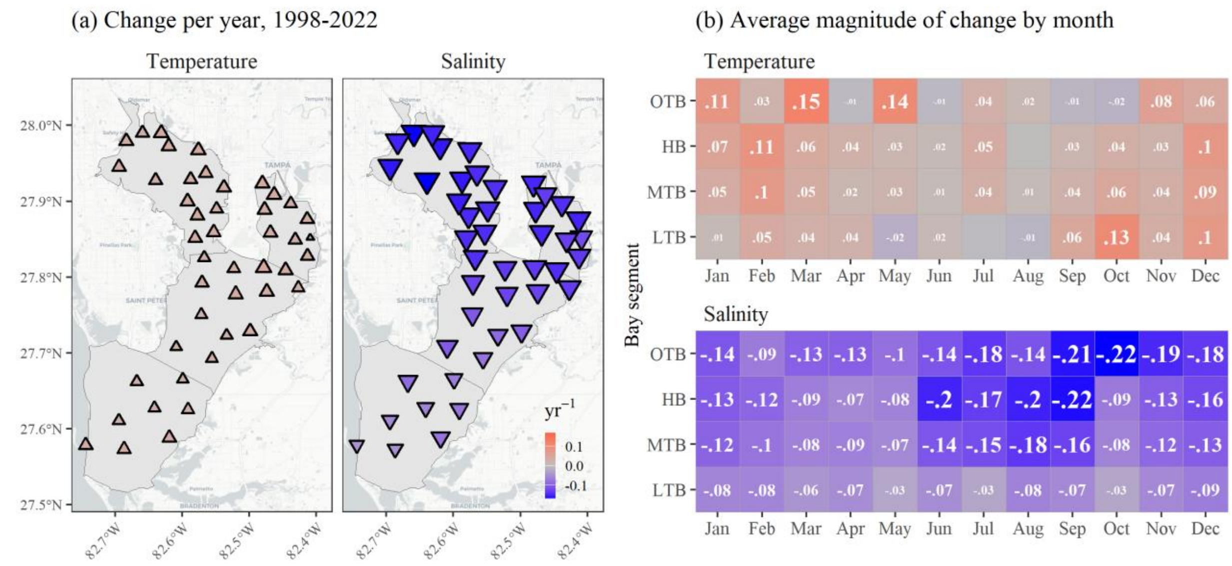

Fig. S4: Trends from 1998 to 2022 for bottom temperature and salinity measurements at long-term monitoring stations in Tampa Bay. Results for seasonal Kendall tests by station and monitoring location (top or bottom of the water column) are shown in (a) with color, size, and shape corresponding to the estimated annual slope as change per year (yr^{-1}). Summarized seasonal trends by month are shown as (b) the average magnitude of change (slope) for all stations in each bay segment for temperature and salinity, indicated by color and text scaled by absolute magnitude. Bay segment outlines are shown in (a); OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

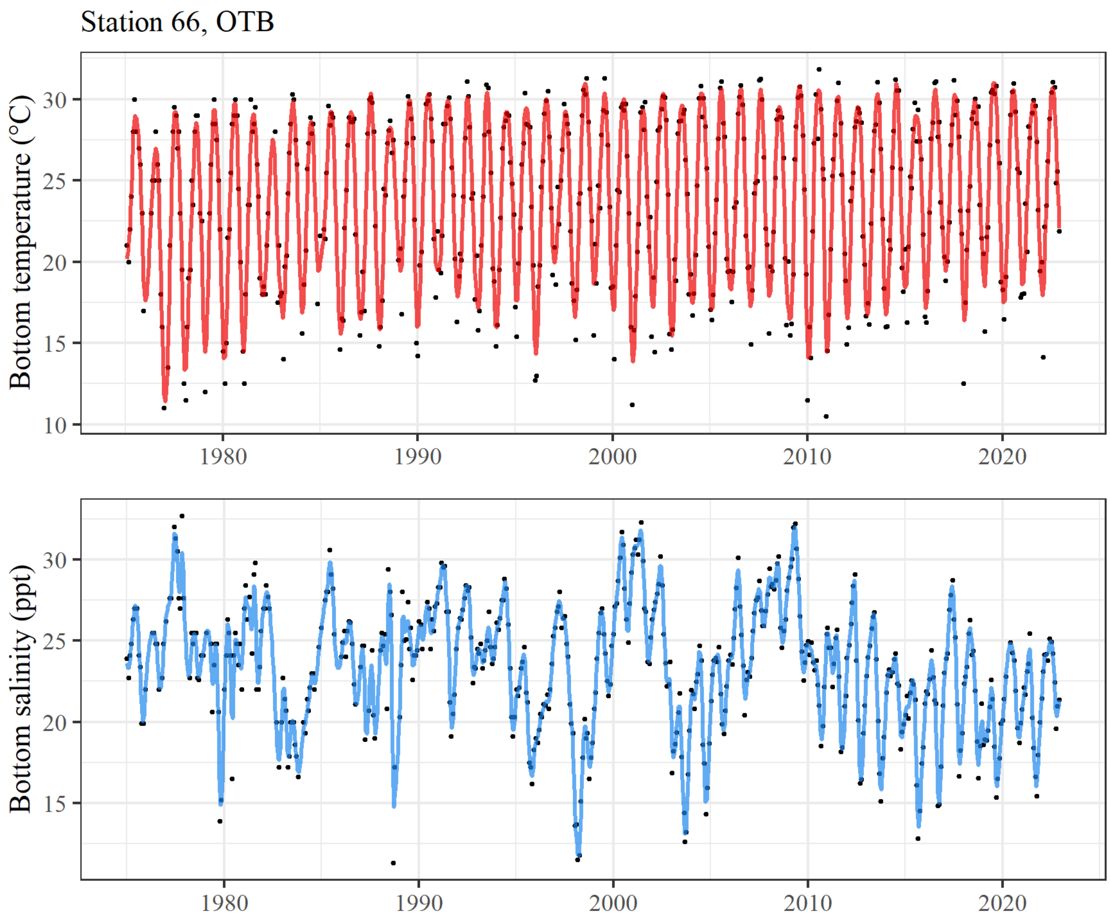

Fig. S5: Examples of Generalized Additive Models of bottom water temperature and salinity for station 66 in Old Tampa Bay. Models were fit using a continuous smoothing spline for decimal year. Monthly observations are shown as points and daily predictions from the models as lines.

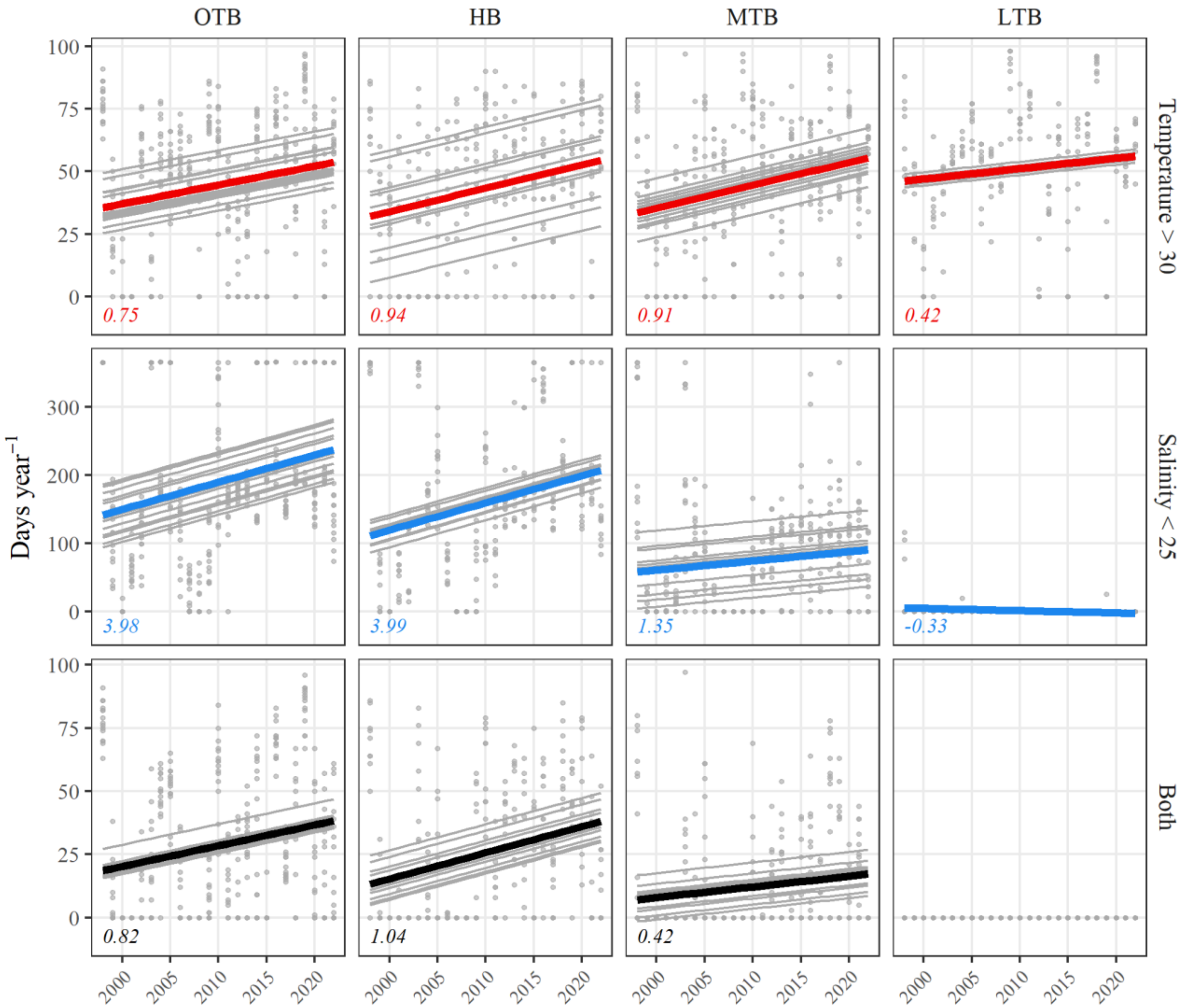

Fig. S6: Example of mixed effects models for the estimated number of days per year that bottom temperature (red) or salinity (blue) were above or below thresholds of 30 ˚C or 25 psu, respectively, from 1998 to 2022. The bottom row (black) shows the number of days when both temperature was above and salinity 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 are shown in the bottom left of each facet. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

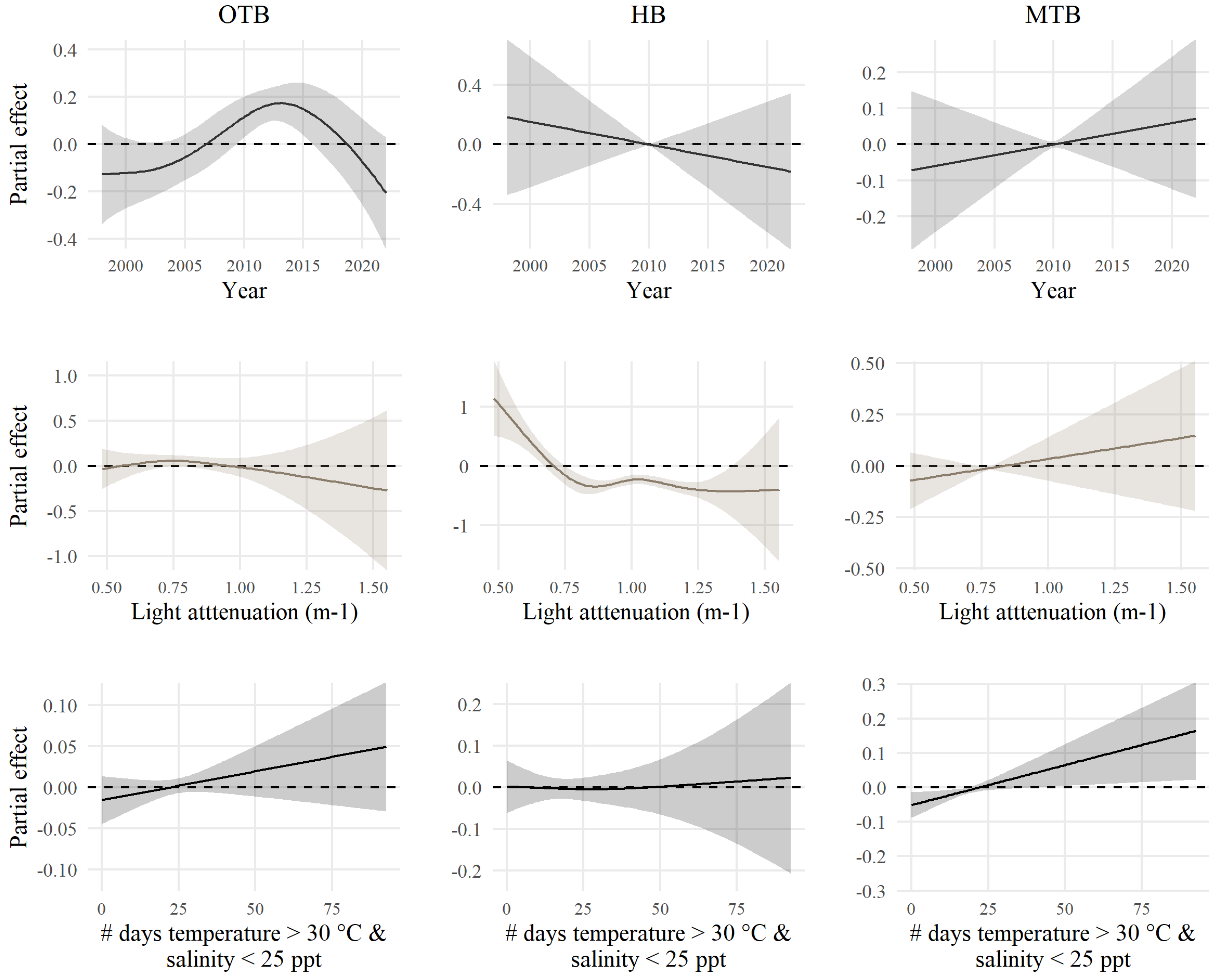

Fig. S7: Partial effects of smoothers (rows) by bay segment (columns) from a Generalized Additive model used to describe seagrass change relative to year, light attenuation, and the number of days each year when both bottom temperature was above 30 °C and bottom salinity was below 25 ppt. The EPC data were used to develop the model. Partial effects describe the modeled association between each predictor and seagrass frequency occurrence after accounting for the effects of the other predictors. The model also included smoothed interaction terms with year (not shown). See Table S8 for additional model fit statistics. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay. n = 75, Adj. R^2 *= 0.97, Deviance explained = 99%*

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.

Bay Segment	Station	Lon	Lat	AIC	GCV	R^2
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 bottom 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. - 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
HB					
	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	-
	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 bottom 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. - 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
HB					
	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	-	-
	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 bottom 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. - no model. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

	Thresholds		Temperature		Salinity		Both	
Bay	Temperature	Salinity	Start	End	Start	End	Start	End
OTB								
	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)
HB								
	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)
MTB								
	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)	-	-
	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 bottom 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. - no model. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay.

	Thresholds		Temperature		Salinity		Both	
Bay	Temperature	Salinity	Start	End	Start	End	Start	End
OTB								
	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)
HB								
	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)	-	-
	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)	-	-

Table S7: Summary of smoother terms in the Generalized Additive Model used to evaluate seagrass response for the EPC data in relation to bottom temperature (temp) and salinity (sal) stress metrics, with additional smoothers for year (yr) and light attenuation (la). Separate smoothers were fit for each bay segment. s = invidual smoother, ti = interaction term. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay. n = 75, Adj. R^2 = 0.93, Deviance explained = 97%.

Smoother	edf	Ref.df	F	p
s(yr):bay_segmentOTB	4.986	5.902	5.042	< 0.001
s(yr):bay_segmentHB	1.000	1.000	0.076	0.785
s(yr):bay_segmentMTB	4.625	5.693	11.264	< 0.001
s(la):bay_segmentOTB	1.055	1.103	0.015	0.979
s(la):bay_segmentHB	5.314	5.851	15.321	< 0.001
s(la):bay_segmentMTB	1.000	1.000	9.237	0.004
s(temp):bay_segmentOTB	1.000	1.000	1.898	0.177
s(temp):bay_segmentHB	1.000	1.000	1.067	0.309
s(temp):bay_segmentMTB	1.000	1.000	2.403	0.130
s(sal):bay_segmentOTB	1.000	1.000	4.843	0.034
s(sal):bay_segmentHB	1.000	1.000	0.788	0.381
s(sal):bay_segmentMTB	1.000	1.000	0.011	0.918
ti(la,yr):bay_segmentOTB	1.000	1.000	0.033	0.857
ti(la,yr):bay_segmentHB	1.000	1.000	3.379	0.075
ti(la,yr):bay_segmentMTB	1.000	1.000	0.010	0.923
ti(temp,yr):bay_segmentOTB	1.791	2.190	0.828	0.484
ti(temp,yr):bay_segmentHB	1.000	1.000	0.051	0.823
ti(temp,yr):bay_segmentMTB	1.000	1.000	0.272	0.605
ti(sal,yr):bay_segmentOTB	1.000	1.000	0.601	0.443
ti(sal,yr):bay_segmentHB	6.672	8.147	5.716	< 0.001
ti(sal,yr):bay_segmentMTB	1.000	1.000	0.860	0.360

Table S8: Summary of smoother terms in the Generalized Additive Model used to evaluate seagrass response for the EPC data in relation to "both" stressors (both bottom temperature above and salinity below thresholds), with additional smoothers for year (yr) and light attenuation (la). Separate smoothers were fit for each bay segment. s = invidual smoother, ti = interaction term. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay. n = 75, Adj. R^2 = 0.97, Deviance explained = 99%.

Smoother	edf	Ref.df	F	p
s(yr):bay_segmentOTB	3.854	4.382	0.905	0.432
s(yr):bay_segmentHB	1.000	1.000	0.464	0.502
s(yr):bay_segmentMTB	1.000	1.000	0.411	0.527
s(la):bay_segmentOTB	2.040	2.326	0.463	0.770
s(la):bay_segmentHB	5.020	5.515	9.597	< 0.001
s(la):bay_segmentMTB	1.000	1.000	0.726	0.402
s(both):bay_segmentOTB	1.000	1.000	1.451	0.239
s(both):bay_segmentHB	1.126	1.178	0.021	0.967
s(both):bay_segmentMTB	1.000	1.000	5.645	0.025
ti(la,yr):bay_segmentOTB	5.235	6.153	1.582	0.213
ti(la,yr):bay_segmentHB	1.942	2.085	1.584	0.286
ti(la,yr):bay_segmentMTB	7.630	8.854	3.315	0.007
ti(both,yr):bay_segmentOTB	3.605	4.585	1.797	0.121
ti(both,yr):bay_segmentHB	10.462	11.667	8.077	< 0.001
ti(both,yr):bay_segmentMTB	1.000	1.000	0.108	0.745

Table S9: Summary of smoother terms in the Generalized Additive Model used to evaluate seagrass response for the FIM data in relation to bottom temperature (temp), bottom salinity (sal), and year (yr). Separate smoothers were fit for each bay segment. s = invidual smoother, ti = interaction term. OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay. n = 81, Adj. R^2 *= 0.81, Deviance explained = 89%.*

Smoother	edf	Ref.df	F	p
s(yr):bay_segmentOTB	1.000	1.000	0.625	0.433
s(yr):bay_segmentHB	4.809	5.329	14.012	< 0.001
s(yr):bay_segmentMTB	1.601	1.977	0.819	0.440
s(temp):bay_segmentOTB	1.000	1.000	0.034	0.855
s(temp):bay_segmentHB	1.000	1.000	3.184	0.081
s(temp):bay_segmentMTB	2.227	2.794	1.546	0.352
s(sal):bay_segmentOTB	1.084	1.161	3.947	0.050
s(sal):bay_segmentHB	4.384	4.880	11.480	< 0.001
s(sal):bay_segmentMTB	1.000	1.000	12.716	< 0.001
ti(sal,yr):bay_segmentOTB	1.342	1.614	0.126	0.787
ti(sal,yr):bay_segmentHB	8.259	9.086	11.740	< 0.001
ti(sal,yr):bay_segmentMTB	2.846	3.332	4.644	0.005
ti(temp,yr):bay_segmentOTB	1.003	1.006	1.355	0.248
ti(temp,yr):bay_segmentHB	2.647	3.080	0.752	0.530
ti(temp,yr):bay_segmentMTB	1.000	1.000	4.166	0.047

Table S10: Summary of smoother terms in the Generalized Additive Model used to evaluate seagrass response for the PDEM data in relation to bottom temperature (temp), bottom salinity (sal), and year (yr). s = invidual smoother, ti = interaction term. n = 19, Adj. R^2 = 0.69, Deviance explained = 81%.

Smoother	edf	Ref.df	F	p
s(yr)	2.428	2.970	0.277	0.831
s(temp)	1.000	1.000	11.556	0.006
s(sal)	1.375	1.612	0.042	0.949
ti(yr,temp)	1.000	1.000	1.996	0.185
ti(yr,sal)	1.372	1.598	3.824	0.075