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The Influence of Bottom Type and Water Column Stratification on Reef Fish Community Structure at Gray's Reef National Marine Sanctuary

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Variable Terms and Definitions

Aggregation index (m^{-1}): The reciprocal of the values that would represent the area inhabited if every data cell contained the mean density of fish (Urmy et al., 2012).

Buoyancy Frequency (1/s): Local density stratification; Characterizes the strength of stratification in a water column (N) (Thorpe, 2007).

Height mean (m): The height, or depth, of the analyzed domain.

Normalized Bottom Hardness: Hardness of the substrate, as determined by the presence or absence of the epi-benthos (Siwabessy et al., 1999).

Normalized Bottom Roughness: Roughness of the substrate, as determined by the smoothness or roughness of the seabed surface and the presence or absence of sand waves (Siwabessy et al., 1999).

Proportion Occupied: Proportion of the water column being occupied by fish (Urmy et al., 2012).

Sample Size: The number of replicates, indicated by 'n', used in the statistical tests.

S_v Mean: Value proportional to the detected fish biomass in the water column (Boswell et al., 2010).

Abstract

Understanding the physical and oceanographic differences across reef habitats can help researchers assess how those differences influence fish distribution and community structure, which leads to a better understanding of what a healthy reef system looks like. The traditional methods used to assess fish communities on temperate reefs are limited and often focus solely on either the reef structure or water column conditions alone. An assessment of both data sets would yield a more complete understanding of the ecosystem as a whole. In this study, Gray's Reef National Marine Sanctuary (GRNMS) was surveyed both inside and outside a Marine Protected Area (MPA) using echosounder technology and a CTD profiler to assess physical and environmental controls of the reef and water column that may be driving reef fish communities. Physical characteristics of the reef such as bottom type and depth were able to be identified and classified, however they did not show a significant relationship with the fish parameters of S_v mean, proportion occupied, and aggregation index ($p > 0.05$). Stratification of the water column, identified from the CTD data, did have a significant relationship with the percentage of the water column being occupied by fish ($p = 0.004$). This suggests that the environmental conditions are the main drivers of temperate reef fish distribution however, differences in stratification between the analyzed tracks may have kept other controlling factors from being observed.

Introduction

Reef systems in both temperate and tropical environments are vital habitats when it comes to supporting marine life. They are made up of a mix of habitats that provide food and shelter to countless organisms in all stages of life (Fernandes et al., 2005). Supporting so much

biodiversity requires a complex system, one where physical and biological characteristics of the reef will act as controls on the marine life that is present (Ebeling & Hixon, 1991). By understanding these controls, the biological factors of the reef can be better assessed.

One physical control on reefs is temperature. All marine organisms have certain physiological thresholds related to the temperature of their surroundings. When the conditions of the environment do not line up with physiological limitations of the organism, their behaviors may change, causing them to move to a new location that is better suited to their needs (Koslow et al., 2013). Alternatively, when temperatures are suitable for the organisms, they may be encouraged to gather in particular areas or exhibit certain behaviors (Koslow et al., 2013). A study of a temperate reef off the coast of North Carolina in the USA, found that how the temperatures changed with depth was an important factor in determining the structure and composition of the fish community, which included invasive lionfish (*Pterois volitans*), black sea bass (*Centropristis striata*), and spottail pinfish (*Diplodus holbrookii*) (Whitfield et al., 2014). By knowing how the temperature changed throughout the water column, researchers were able to predict how rising temperatures from climate change may change the local fish community structure, as tropical fish are able to move farther into warming temperate waters (Whitfield et al., 2014). Sea surface temperatures have also been found to influence the density of Pacific saury (*Cololabis saira*) in the western North Pacific, where the density of the species was highest in waters where the sea surface temperatures were between 7 – 15^{o}C (Hashimoto et al., 2020). This knowledge was used to estimate the distribution and biomass of Pacific saury for population dynamics models (Hashimoto et al., 2020). Responses to water temperatures in fish structure occur in all types of marine environments and by knowing this physical control, it has been possible in past studies to predict the local biological factors of the habitat.

Reefs are also subjected to the physical control of salinity levels. Although the salinities of a reef may not vary on a daily or seasonal basis, like those that have been observed in estuaries, factors such as precipitation, currents, or surface mixing over the habitat can all influence the salinity levels in the water column and the depth of the pycnocline (Greenwood, 2007). The pycnocline, in particular, may act as a barrier to certain fish species or larvae with narrow salinity tolerances, thus making it more difficult for them to navigate a stratified water column (Wellenreuther et al., 2008). One study did observe a correlation between increased salinity levels and increased abundances of several fish species, including *Dentex dentex*, annular sea bream (*Diplodus annularis*), and damselfish (*Chromis chromis*), in a coastal environment of the Mediterranean Sea (Aguzzi et al., 2015). The abundances of other species in the area showed a positive response to increased salinities, however the changes were also found to be correlated with increased temperatures, indicating that fish activity is not always tied to just one environmental parameter (Aguzzi et al., 2015). Unfortunately, many studies on the salinity tolerances of fish focus on estuarine species since they experience the most variability, but as these studies have shown, changes in the marine environments will also have an effect on the local fish fauna. By knowing the salinity tolerances of local species at all life stages and comparing those to changes in salinity levels throughout the water column and across seasons, researchers may be able to predict fish behavior based on this physical control.

The structure of the reef itself is another physical control on the reef system. The location, climate, and depth of the reef can lead to a wide variety of reef structures, which in turn influence the fish fauna in the area (Ebeling & Hixon, 1991). In an acoustic survey of the northern Gulf of Mexico, one study found that acoustic estimates of fish biomass were highest right over the reef complex but then decreased as the distance from the structure increased

(Boswell et al., 2010). This was evident as fish biomass was highest in the region closest to the reef, where depths were greater than 10 meters, then decreased in the mid-water region with depths from 10 meters to 6.1 meters (Boswell et al., 2010). These results indicate that the presence of the reef structure will increase the biomass in the water column, but fish diversity and abundance may also change based on the bottom relief of the reef. A comparison between tropical and temperate reefs noted that tropical reefs exhibit a higher bottom relief due to the complexity in their structures that the coral skeletons provide, leading to an increase in habitat variety and spatial heterogeneity (Ebeling & Hixon, 1991). The differences between temperate and tropical reefs is also what leads to higher fish abundance and diversity in tropical reefs, as the high bottom relief bolsters the diversity of life around it (Ebeling & Hixon, 1991). Not only does the presence of a reef lead to an increase in fish biomass in the water column, but an understanding of the structure's bottom relief can also provide a way to predict the expected fish abundance and diversity over the reef.

Biological characteristics of a reef, or the lack of them, will also play a role in controlling fish fauna around the reef structure. A study at Gray's Reef National Marine Sanctuary (GRNMS) off the coast of Georgia, assessed two different habitats, one of rippled sand and low relief and another with several mixed benthic habitat types, including sparsely and densely colonized live bottom (Kracker, 2007). The acoustic surveys of these habitats revealed that the mixed habitat of high relief had a fish biomass density approximately two orders of magnitude greater than the rippled sand habitat of low relief, in addition to showing a greater range in the sizes of targets detected in the water column above the mixed habitat (Kracker, 2007). The very presence of a colonized live bottom habitat leads to higher fish biomass and diversity.

One way to differentiate between bottom habitats is to assess their structures through an analysis of video data and sonar imagery and then categorize them based on differences in those structures, as they are often the most defining features of the reef (Kendall et al., 2005). When making these assessments however, the composition of the local community must also be considered, as the behaviors of these organisms living on temperate reefs help to shape the environment, often influencing the resulting habitat structure and functionality (Jones & Andrew, 1993). For example, habitat formers, such as sedentary, encrusting organisms like macroalgae, are often ecologically dominant and will characterized the habitat while grazing or predatory organisms, known as habitat determiners, will control the presence or absence of the habitat formers (Jones & Andrew, 1993). Feeding habits, large aggregations of sedentary organisms, and interactions between organisms are all potential ways that the habitat may be changed by the biotic elements of the reef (Jones & Andrew, 1993). As the bottom habitat changes, do so the organisms that frequent it, so the more stable the environment is, the more stable the population will be (Ebeling & Hixon, 1991).

With the scope and complexity of reefs, it is difficult to assess the characteristics relating to their biomass, bottom types, and water quality, as oftentimes the locations are difficult to access and conditions are too extreme for prolonged direct observation (Kracker et al., 2008). Likewise, the use of cameras is limited to a fixed location and identification must be performed manually, so data collection is often done through the use of acoustics (Kendall et al., 2005). Underwater acoustics has been an increasingly reliable way to document aspects of the oceanic environment such as fish abundance, bottom habitats, and other physical features beneath the surface and compared to past methods, it is relatively non-invasive (Misund, 1997). Echosounders mounted to ships or moorings transmit sound beams of frequencies between 12

kHz and 200 kHz and when the echo signals are reflected back to the source, the transducer is able to convert the backscatter signal into data that can be interpreted to learn more about the environment (Misund, 1997). This method lowers the level of disturbance that the environments experience, compared to when bottom or mid-water trawls are used, and records many different types of data from information on fish distribution to level of bottom hardness (Kracker, 2007). Use of acoustics can allow researchers to cover larger areas of a reef, including several different bottom types in their surveys, so that the sources of the data are varied and more representative of the study site (Kendall et al., 2005; Kracker, 2007).

The environment of the fish is not just limited to the bottom habitats they rely on for food and shelter but also includes the conditions within the water column, such as temperature, conductivity, and pressure. The physical properties of these variables have already been established as components that exert some control on the biological and structural variables in a reef (Aguzzi et al., 2015). These properties however, change not only across time but also through space. The temperature at the surface of a body of water, for instance, is not always the same as the temperature of the bottom water, particularly in areas of greater depth. This applies to the other physical properties as well. To measure these properties, one commonly used method is to gather samples with a conductivity-temperature-depth (CTD) profiler. The CTD profiler collects readings on regular intervals from throughout the water column, so that the area might be characterized based on how it changes as the depth increases (Fakhrudin et al., 2019). By understanding the environmental gradients of the water column, through the use of a CTD profiler, researchers in a previous study were able characterize the differences in the conditions between a healthy reef system and one that has been damaged by anthropogenic and natural disturbances (Rowley, 2018). The data collected with this equipment characterizes the study area

in terms of environmental factors, such as the values of salinity, which can then be used to further understand what forces must be considered when assessing fish distribution patterns and behaviors.

For this study, acoustic SONAR and CTD data were collected at GRNMS, a live-bottom reef that is located 20 nautical miles off the coast of Georgia, in the United States (Campanella et al., 2019). It was established as a zoned National Marine Sanctuary with a natural heritage conservation focus, several No-Take areas, and a general restriction on commercial and recreational fishing (NOAA, 2020). This means that the ecosystems included within GRNMS experience lower levels of disturbances and less interference from human impacts. Some of the most common fish fauna found throughout the reef include species of jacks and groupers such as almaco jacks (*Seriola rivoliana*) and scamp grouper (*Mycteroperca phenax*), as well as black sea bass (*Centropristis striata*) (Auster & Giacalone, n.d.). The reef bottom itself is mostly made up of unconsolidated sediments (75% of the bottom), the majority of which is rippled sand, while the remaining portion (25% of the bottom) is represented by sparsely colonized regions of flat bottom and densely colonized vertical ledges (Kendall et al., 2005). This system is a good representation of a live-bottom temperate reef, with a variety of habitats present to support a diverse population of marine fauna.

The goal of this study was to assess the structures of a hard bottom reef, as well as the characteristics of the water column, and how they influence the presence of biological life on the reef. Structures of the reef that might have had some control over the fish community structure in the area include the bottom type, which was determined from acoustic values of bottom roughness and bottom hardness. Potentially influencing factors of the water column included the temperature and stratification in the water. Assessments of these factors help to determine if the

differences in biological life are related to the characteristics of the water column or to the reef structure.

Methods and Materials

Data Collection

Data were collected from GRNMS between July 31st, 2018 and August 7th, 2018. For each survey track, data collection began between 0200 and 0300 GMT (1000 and 1100 EDT) and continued for about a 10-hour period into the morning. At that point, the surveys paused for a scheduled stop, before beginning again about 4 hours later to run for 1 hour, then stopping again for 5 hours, before continuing for a final hour of data collection. This was planned in such a way to allow for data collection to take place overnight, at noon, and at dusk. Stops in the surveys also allowed for diving activities to take place and underwater cameras to be deployed for other scientific studies. The tracks consisted of sets of five pre-determined latitudinal survey lines, each of which were repeatedly traversed around 8 to 10 times by the survey vessel during the overnight survey. The surveys at dawn, noon, and dusk covered one set of lines for the track. Survey lines for each track covered 0.5 nautical miles in length of reef area in a longitudinal direction, either inside or outside the boundaries of the local MPA (Figure 1). For the tracks used in this study, Track 05 In was collected first on 7/31/2018, then Track 41 Out was collected on 8/2/2018, Track 40 In was collected on 8/4/2018, and lastly Track 01 Out was completed on 8/7/2018.

Along each track, an EK-60 Split Beam SONAR System collected data continuously with a 120 kHz transducer. Simultaneously, a Teyleyne Marine UCTD Underway Profiling System

was deployed while the ship was in transit along the tracks to collect water column data. A SBE 911plus CTD collected more complete information on the water column, including dissolved oxygen (DO), at two locations, however data from one of the sites was omitted due to an error in the location of deployment relative to the rest of the track.

Data Processing

Raw SONAR data from the EK-60 echosounder were processed in Echoview. For each track, background noise was removed, a surface line and a bottom line were defined, regions of interference due to surface wave activity were labeled as unusable data, and integration and bottom classification data were extracted and exported. For more information on the settings and functions used, see Appendix 1.

After the Echoview Automatic Bottom Classification function was run on each of the four tracks to determine the bottom types that appeared on the reef across each track, the resulting classifications indicated that there were anywhere between 2 to 5 automatically detected bottom classes for each of the tracks. However, a manual assessment of how those values appeared in clusters on a scatter plot and the range of data values they covered determined that there was not that much variability in the detected bottom types. By assessing the range of data values for each cluster, the number of bottom classes for each track were re-evaluated.

Raw data files from the UCTD and CTD profilers were exported using Sea-Bird Scientific (SBE) Data Processing Software. This realigned the data to account for a delay between the temperature and conductivity sensors and saved the data in a format that allowed for importation into MATLAB for analysis and data processing. For more details on the programs and parameters used, see Appendix 2.

Data Analysis

CTD Data, once loaded into MATLAB, were grouped based on the day the casts occurred with the variables exported from SBE (see Appendix 2). The function 'gsw_SP_from_C' used the existing temperature and conductivity variables to calculate salinity data, which were added to the corresponding casts (McDougall & Barker, 2011). Latitude, longitude, and date-time values were added separately using information from the header files. Linear interpolation plots were generated for temperature and salinity data across each track to help describe the physical conditions of the water column. Potential density values were produced in MATLAB by running temperature and conductivity values through the function 'gsw_rho', with a reference pressure of 0 decibars (McDougall & Barker, 2011). A MATLAB script was then written to smooth the potential density with a simple moving average and a window size of 2 meters. The new, smoothed values were used to calculate the buoyancy frequency (N) throughout the water column with units of 1/s, using the following standard formula, where the reference density (ρ_0) is 1028 kg/m^3, Δz is -0.25 m, and the density is the potential density as defined above:

$$N = \sqrt{-\frac{1}{\rho_0}\frac{\partial \rho}{\partial_z}}$$

Integration files of SONAR data from Echoview were opened in MS Excel 2016 where they were assessed for variability. Any variables that did not vary across the tracks were removed from further analysis and those left were saved to new integration data files. The new files from Excel and the bottom classification files for each track were loaded into MATLAB.

Script functions of the program were used to align the data points based on their recorded time of collection and combine variables from both files into one table for each track.

To assess the potential relationships between variables from the SONAR data and CTD data, a MATLAB script was used to identify the SONAR data points that were collected closest to each CTD cast. These values were combined into tables grouped by track and the script function 'pca' was used to generate a Principle Component Analysis for each data table. By assessing how the variables loaded into the biplots, the relationships between them could be explained. The lengths of the lines representing the variables indicated the strength of the contribution of the individual variables to each axis. Variables that loaded very similar to each other indicated that they had a proportional relationship with each other while those that loaded opposite, or nearly opposite, of each other were determined to be inversely proportional to each other. These were the potentially relevant relationships that were identified for each track. Variables loading 90^{o} from each other indicated that there was no relationship between them.

A two-tailed Pearson correlation test in MS Excel 2016 was used to assess the relationships between the variables using all SONAR data from all of the tracks. The relationships explored can be seen in Table 1. For each statistical test, a $n = 4$ was used to assess a significance level. This n-value represented the number of tracks assessed in this research and was the most conservative method of assessing significant to avoid potential pseudo-replication errors. Significance level was assessed at $p < 0.05$ for all paired correlations.

Table 1: Variable relationships identified in the PCA biplots and explored in MS Excel 2016.

X Variable	Y Variable
Bottom Roughness	Aggregation Index
Bottom Hardness	Aggregation Index
Bottom Roughness	Proportion Occupied
Bottom Hardness	Proportion Occupied

Bottom Roughness	S_v Mean
Bottom Hardness	S_v Mean
Time	Proportion Occupied
Time	S_v Mean
S_v Mean	Proportion Occupied

Using MATLAB, line plots of S_v mean vs time and proportion occupied vs time from the acoustic data were generated to provide a visualization of how the values changed over time. The variable S_v mean was obtained from an equation, which took the log of the linear mean S_v, also known as the volume backscattering coefficient (s_v), and then multiplied it by ten ("Echoview Help: Sv_mean," 2020). The resulting values were proportional to the detected fish biomass in the water column (Boswell et al., 2010). On the plots for each track, more negative values mean there was less fish biomass while less negative numbers indicate there was more fish biomass. Proportion occupied reported the proportion of the water column being occupied by fish and was obtained through calculations using the height of the water column (H), the depth of a sample in the analysis domain (z), and the volume backscattering coefficient at z ($s_v(z)$) ("Echoview Help: Proportion_occupied," 2020; Urmy et al., 2012). The plots of these two variables helped to explain how the biological factors might be changing with time, as well as display those changes in a way that could be easily compared to changes in other variables.

CTD data on the buoyancy frequencies were exported from MATLAB and opened in MS Excel 2016 with SONAR data on the fish variables of S_v mean, proportion occupied, and aggregation index. The maximum N value for each site was calculated to represent the point in the water column with the highest stratification. The statistical relationships between this 'max N' variable and the fish variables from Echoview were assessed using the same two-tailed Pearson correlation test as before. However, the n-value for these calculations was n = 37. This

was chosen so each CTD cast would be treated as a replicate. The SONAR data was assessed in groupings of every 100 pings and only the data from the group that was recorded closest to each of the CTD cast sites was used in the statistical analysis test. Significance level was assessed at $p < 0.05$ for all paired correlations.

Results

Bottom Classifications

Across all tracks, there was variability in the number of bottom types classified. Tracks 01 Out and 41 Out both had two distinct bottom types, while Tracks 05 In and 40 In had one bottom type each. Track 01 Out displayed two distinct groupings of data values, with one cluster of mostly green data points at high bottom hardness values (> 6) and low bottom roughness values (< 8.4) and the other cluster of mostly red points at medium bottom hardness values (5.6 – 6.6) and high bottom roughness values (> 8.4) (Figure 2a). These clusters indicate that the major bottom types for Track 01 Out were flat sand with live cover and rippled sand (Figure 2a). Bottom classification results for Track 41 Out also resulted in two general clusters of data points. The first cluster of mostly blue data points had medium bottom hardness values (5.6 – 6.6) and low bottom roughness values (< 8.4) while the second cluster of light blue, red, and some green data points also had medium bottom hardness values but high bottom roughness values (> 8.4) (Figure 2b). These groupings of data points indicated that Tack 41 Out had bottom types of flat sand and rippled sand. All data points for Track 40 In were in the same cluster of red and green data points at medium bottom hardness values (5.6 – 6.6) and high bottom roughness values (> 8.4), indicating that the entire track had a bottom type of rippled sand (Figure 2c). Finally, Track 05 In also had one main clustering of red, green, light blue, and blue data points ranging from

medium to low bottom hardness values (5.5 – 6.3) and variable bottom roughness values (8 – 8.7) (Figure 2d). The data of this track aligns closest to a bottom type with some live cover. Overall, Tracks 41 Out and 40 In were made up of sandy bottom types while both Track 01 Out and 05 Out showed bottom types with sparse live cover.

Fish Parameters

For all of the tracks, the S_v mean plots show that fish biomass was higher at night, with data values between -65 to -55, than during the day (-65 to -75), with the values decreasing at dawn and remaining lower throughout daylight hours (Figure 3). The data points for Track 01 Out showed the greatest difference between the night and day values, as the fish biomass overnight was between data values of -60 to -50 while daytime values mostly fell between -75 to -65 (Figure 3a). In the plots for Track 40 In and 05 In, the fish biomass appeared to be increasing again near the end of the track (Figure 3c,d). These trends are not seen in Track 41 Out, likely due to the lack of daytime data (Figure 3b).

The variable plots for proportion occupied displayed how much of the water column the fish were occupying over the course of the tracks. For this variable, Track 01 Out had the most variation in its data points. The track initially had high variability overnight, ranging from about 0.87 to 1, then increased to show an overall high proportion occupied (~1) during the transition from night into day, before decreasing and remaining low throughout the day with data points falling between 0.75 to 0.85 (Figure 4a). All of the other plots for the remaining tracks showed consistently high proportion occupied values (~ 0.98 – 1) during the night (Figure 4b,c,d). Proportion occupied fell in the morning for Track 05 In from ~1 to ~0.96 and for Track 40 In from ~0.98 to between 0.95 – 0.8, and remained at those low values for the rest of the day

(Figure 4c,d). Again, the trends during the day are not seen in Track 41 Out (Figure 4b). In all of the other proportion occupied plots, the data points were higher at night than during the day.

PCA Variable Correlations

The relationships between the variables in each track were able to be assessed from the PCAs. In Track 01 Out, a strong, proportional relationship was seen between proportion occupied, equivalent area, and S_v mean and another grouping of aggregation index, bottom line depth mean, time, and height mean (Figure 5a). These two groupings of variables also loaded opposite of each other, meaning that the variables of one group had a strong, inversely proportional influence on the variables of the other group. Normalized bottom roughness and hardness variables for Track 01 Out both loaded strongly but at nearly a 90^o angle from the two groups, indicating they have little to no influence on them as a result (Figure 5a). Track 41 Out had some similarities to Track 01 Out, which could be seen where aggregation index loaded proportionally with time and opposite of proportion occupied, as well as where both normalized bottom roughness and hardness loaded with minimal influence on those variables (Figure 5b). Differences could be seen where bottom line depth mean and height mean loaded opposite of aggregation index and time and where S_v mean loaded closest to bottom hardness normalized, indicating it did not have much influence on any of the other variables (Figure 5b). For Track 40 In, variables that loaded together were normalized bottom hardness, average temperature, and proportion occupied, a group which appeared opposite of the time variable (Figure 5c). The variables bottom line depth mean and normalized bottom roughness both loaded strongly, but at roughly a right angle from the group, indicating minimal influence (Figure 5c). The variables in Track 05 In showed proportional influence between S_v mean and proportion occupied, as well as

aggregation index and bottom roughness normalized, though those two groupings of variables had little to no influence on each other (Figure 5d).

CTD Data

There were eight CTD profiler casts for Track 01 Out, twelve casts for Track 41 Out, nine casts for Track 40 In, and eight casts for Track 05 In. Temperatures in all tracks ranged from about 29.4ºC to 27.4ºC and tended to vary across the tracks in both depth and time. In Track 01 Out, the temperature contour plot revealed a clearly defined thermocline between the depths of about 3 to 5 meters throughout most of the track, with temperatures changing rapidly from ~27.6ºC to ~28.8ºC over that depth. (Figure 6a). Between the times of about 06:00 to 12:00, the thermocline for this track fluctuated more between depths of ~3 to ~7 meters and was not as strong as it had been earlier in the track, but it was always present to some degree (Figure 6a). The temperature contour plot for Track 41 Out showed the track to be well-mixed, with no clearly defined thermocline at any time or depth (Figure 6b). Instead, the track began with higher temperatures at night (~28.4ºC), throughout all depths, then rapidly decreased from 28.45ºC to 28.1ºC between about 12:00 to 15:00 and remained low for the rest of the track (Figure 6b). Track 40 In was also well-mixed at all depths until after 15:00, at which point there was some surface heating generating stratification at the end of the track where temperatures changed from 28.4ºC to 27.8ºC over depths from about 3 to 7 meters (Figure 6c). However, it was not as stratified as Track 01 Out. Finally, the temperature contour plot for Track 05 In was most similar to the plot of Track 41 Out, in that temperatures were found to represent a well-mixed water column, where the values changed more with time rather than the depth of the water column. The plot for this track showed initially high temperatures (~29ºC) at about 03:00, then a rapid decrease from 29ºC to 28.85ºC over the next 1.5 hours (Figure 6d). This was followed by a rapid

increase from 28.85ºC back to 29ºC for 2 hours, before finally slowly decreasing again over the final 12 hours of the track to a final temperature of 28.75ºC (Figure 6d). Only Track 01 Out had a consistently and clearly defined thermocline across the entire track while the Tracks 41 Out, 40 In, and 05 In showed more well-mixed water columns.

Salinity values for all tracks ranged between about 35.8 psu to 36.5 psu. Across Track 01 Out, the salinity contour plot revealed a well-defined pycnocline between depths of about 5 to 8 meters, where the salinity values rapidly increase from ~35.8 psu near the surface to 36 psu at depths greater than 8 meters (Figure 7a). The salinity values for Track 41 Out also showed higher values of about 36.12 psu at greater depths, however, there was no single depth interval across the track where the values changed rapidly as they did for Track 01 Out and the overall range of salinities present was smaller (36.04 psu to 36.14 psu) (Figure 7b). Data for Track 40 In produced a contour plot that displayed very consistent levels of salinities across depths and time. For this track, there were only a few changes in salinities in the water column such as between 03:00 to 06:00 and around 12:00 where values ranged from 36 psu to 36.05 psu (Figure 7c). The rest of the changes were isolated in the first 3 meters below the surface and did not show much range in the overall conditions (36 psu to 36.05 psu) (Figure 7c). Track 05 In had a salinity contour plot that revealed several fluctuations between ~36.10 psu to 36.16 psu within 0 to 3 meters in depth before the changes spread out more at greater depths (Figure 7d). These changes however, did not occur at a consistent depth across time and so did not display a well-defined pycnocline, just as Track 41 Out and 40 In did not have one. Track 01 Out was the only one to have a well-defined pycnocline.

Using the maximum N values from the buoyancy frequencies, it could be seen that Tracks 05 In, 41 Out, and 40 In all had relatively similar conditions of low stratification at nearly

all of their cast locations, which ranged from about 0.002 s^{-1} to 0.0048 s^{-1} (Figure 8). The only cast of those tracks to display more stratified conditions was the 8th cast of Track 40 In at a value of 0.0062 s^{-1}, a rapid increase from the conditions recorded during the 6th and 7th casts for that track, ~0.002 s^{-1} and 0.0034 s^{-1}, respectively (Figure 8). The track showing the most stratification was Track 01 Out, which had max N values between ~0.0054 s^{-1} and 0.0088 s^{-1} for all casts, where were greater than all of the max N values for all of the other tracks besides cast 8 in Track 40 In (Figure 8). With these assessments of the buoyancy frequencies of each cast, Tracks 05 In, 41 Out, and 40 In can be best described as representing water columns that are less stratified than that of Track 01 Out, which is consistent with what was observed in the contour plots.

Statistical Analysis of Relationships

As noted in the methods, each track was treated as a replicate when the variable relationships were evaluated, so n = 4. The resulting Pearson coefficient, T-Statistic, and P-Value for each of the variable relationships can be seen in Table 2. Aggregation index, an indication of the mean density of fish, was not found to be significantly correlated with either bottom roughness or bottom hardness, as the analysis of both relationships resulted in a P-Value greater than 0.05. The proportion of the water occupied by fish also did not show a significant relationship with bottom roughness and hardness variables ($p > 0.05$). Similarly, fish biomass in the water column, as represented by S_v mean, had no statistically significant correlation with the bottom type variables ($p > 0.05$). Both S_v mean and proportion occupied were each assessed for a significant relationship with time and neither relationship was found to be significant ($p > 0.05$), however, the P-Value for proportion occupied & time was less than that of S_v mean & time. Finally, S_v mean and proportion occupied were analyzed but were not found to have a significant relationship ($p > 0.05$) While of the P-Values calculated were found to be significant ($p < 0.05$),

it was noted that the relationships between the variables S_v mean & proportion occupied and proportion occupied & time produced the lowest P-Values (Table 2).

Table 2: Statistical analysis of variable relationships using data from the entire track to produce a Pearson coefficient, T-Statistic, and P-Value.

Variables	Pearson coefficient, r	n	T-Statistic	Degrees of Freedom	P-Value
Aggregation Index & Bottom Roughness	-0.024	4	-0.042	3	0.969
Aggregation Index & Bottom Hardness	-0.006	4	-0.011	3	0.992
Proportion Occupied & Bottom Roughness	-0.067	4	-0.116	3	0.915
Proportion Occupied & Bottom Hardness	-0.142	4	-0.248	3	0.820
S_v Mean & Bottom Roughness	-0.098	4	-0.171	3	0.875
S_v Mean & Bottom Hardness	0.095	4	0.166	3	0.879
Proportion Occupied & Time	-0.463	4	-0.906	3	0.432
S_v Mean & Time	0.060	4	0.105	3	0.923
S_v Mean & Proportion Occupied	0.624	4	1.383	3	0.261

As explained earlier the assessment the relationship between the maximum N of the buoyancy frequencies and the fish variables at each CTD cast site used n = 37 because each cast site was treated as a replicate. From this analysis, no significant relationship was found between max N, the most strongly stratified point in the water column, and the fish biomass ($p > 0.05$). There was also no significant correlation between max N and the aggregation index ($p > 0.05$), however the P-Value for the variables max N and proportion occupied was 0.004, indicating the correlation between them is statistically significant (Table 3).

Table 3: Statistical analysis results of the relationships between buoyancy frequencies and fish parameters.

Variables	Pearson coefficient, r	n	T-Statistic	Degrees of Freedom	P-Value

Max N & S_v Mean	-0.009	37	-0.052	36	0.958
Max N & Proportion Occupied	-0.451	37	-3.032	36	0.004
Max N & Aggregation Index	-0.044	37	-0.263	36	0.794

Discussion

Bottom Types

The data from this study showed that Tracks 01 Out, 41 Out, and 40 In were predominately made up of sand-based bottom types. The flat sand bottom of Track 01 Out was determined to have some live cover, but Track 05 In was the only one to have an entire bottom type of sparse live coral cover. For Tracks 01 Out and 41 Out, where two bottom types were present, the distribution of data points indicated that each type made up about equal portions of the tracks.

The results seen here match those of previous studies at GRNMS, where the majority of the bottom habitats were represented by flat or rippled sand and the portion of habitats with some form of live cover make up about a quarter of the surveyed area (Campanella et al., 2019; Kendall et al., 2005). This indicates that even though the data used in this study did not cover the entire reef area, the study sites included are still representative of the features that make up the area as a whole. One bottom type that was not evident in the data was the vertical ledge features characterized by dense live cover. However, a previous study did note that these ledge habitats make up less than 1% of the reef area, so their presence in the data would be minimal in comparison to the amount of space taken up by the other identified reef types (Kendall et al., 2005). With knowledge of the bottom types that were present and the locations of the ledge

habitats, it may be possible to predict the nature of the fish assemblages found across the reef. One such way to accomplish this would be to make spatial plots to compare to previous habitat classification maps to see if the nearby presence of a ledge correlates with higher fish activity or biomass.

Past surveys across reefs found that the distribution of fish fauna over the reef was related to the bottom habitats present, with the highest fish biomass generally associated with the habitats of the highest relief, such as ledges (relief > 0.2 m) or fragmented hard bottom (relief > 0.1 m) (Kracker, 2007; Switzer et al., 2020). When determining the distribution of fish biomass in the mid-water region, areas of reef bottoms with a mix of habitats, such as flat sand, rippled sand, and high to medium ledges, have been found to increase the amount of biomass in the mid-water zone (Kracker et al., 2008). Predictions of fish biomass in the near-bottom zone were found to be significantly related to the distance from the nearest ledge (Kracker et al., 2008). Based on these conclusions, fish biomass would be expected to be higher in Tracks 01 Out and 41 Out, both of which had two different bottom types. In the areas on these tracks, where one of the bottom types transition into the other, there would be a mixture of habitats, like those described in (Kracker et al., 2008). For Track 01 Out, the data did show that it had the highest fish biomass compared to the other tracks, but the same could not be determined for Track 41 Out due to the lack of data. The other track that would be expected to have a higher fish biomass would be Track 05 In due its bottom type of sparse live coral cover. This bottom type would increase the bottom relief along the track. The fish biomass data points for that track do show higher values during the day than the other tracks, supporting that assessment.

By understanding the bottom habitats present over the reef, not only can the fish biomass be predicted but so can other reef features such as the diversity of the fish fauna. Target strength,

the backscatter energy of a returned signal that is equivalent to the size of individual fish, was assessed over two different habitats at GRNMS (Kracker, 2007). Researchers there found that there was a greater range in the size of fish over a mixed habitat of high relief than there was for a habitat of flat sand and low relief (Kracker, 2007). With a greater range in sizes of individuals, the fish over the mixed habitat were determined to represent a greater diversity of species that were likely present due to the higher complexity of the reef, which contributes to an increase in the habitat variety and spatial heterogeneity. Although target strength was not included in the data for this study, the patterns that have been observed in the past over similar habitats should be considered to ensure a full understanding of the data and what it means for the reef system. For instance, by evaluating the complexity of each bottom type detected in each track of this data, the track expected to have the most species diversity might be determined.

Fish Parameters

Fish biomass, across all tracks, was consistently higher in the hours overnight than during the day. For Track 01 Out, the difference of the fish biomass values between night and day was greater than the difference seen in the other tracks. The proportion occupied data also followed a general trend across all of the tracks. In each one, a greater proportion of the water column was being occupied by fish at night than during the day. Track 05 In had the least amount of change in the proportion occupied from night into day, as the values remained very high throughout the entire track. The water column across Track 40 In was consistently occupied at high proportions overnight before showing a clear decrease into the day. The proportion occupied across Track 01 Out showed the most variation as the track began with values that were high, but also more spread out. During the early morning hours however, the water column was almost entirely occupied before the proportion decreased to lower values during the day.

Typical distributions of fish across a reef over the course of 24 hours are usually expected to show higher activity during the day than at night, potentially with short periods showing the transition between diurnal and nocturnal organisms as they both change their behaviors in response to the time of day (Myers et al., 2016). The data show the opposite of this expected pattern as the fish biomass and proportion occupied values are highest at night and lower during the day. Factors that may explain the temporal patterns of fish activity include food availability, predation risk, and use of the reef structure by the local organisms (Khan et al., 2017; Myers et al., 2016). Feeding behaviors of different trophic levels can contribute to patterns of fish activity as a previous study found that benthic invertivores were typically more active during the day while planktivores were more abundant at night (Myers et al., 2016). Even the perceived risk of predation has been observed to alter the feeding habits of prey species (Catano et al., 2017). Herbivory by surgeonfishes (Acanthuridae) and parrotfishes (Scaridae) decreased when the distance between them and fiberglass models of grouper (*Mycteroperca bonaci*) and barracuda (*Sphyraena barracuda*) decreased, simulating increased predation risk on the foraging species (Catano et al., 2017). Knowledge from these studies and of the diets of local species at GRNMS would help to explain the temporal patterns of fish activity based on the interactions between the trophic levels.

The other behavioral factor that influences reef fish activity is the use of shelter during certain times of the day. Large reef fish species, including the Spanish flag snapper (*Lutjanus carponotatus*) and the black sweetlips (*Plectorhinchus gibbosus*) have been found to consistently use reef structures for shelter during the day while traveling over 1 km at night, possibly to hunt for prey (Khan et al., 2017). While previously this strategy was attributed to UV avoidance, the long-term occupation of these shelters at all times in the diurnal period and in all types of

weather indicate that there is likely another reason for the use of the shelters (Khan et al., 2017). Regardless, this behavior of reef fish species might help to explain why the data seen in this study differs from those of previous research. How the local species at GRNMS use the reef structure, as well as patterns of that use, would need to be assessed in order to see if this type of behavior is a contributing factor to the temporal patterns of fish activity.

Statistical Analysis of Relationships

The statistical analysis performed on relationships between the fish variables, the bottom variables, and time, using all of the data collected across the tracks, did not find any significant relationships, indicating that none of the variables influenced any of the others. An analysis of the relationship between the stratification and variables relating to fish presence and location revealed that only the correlation between the stratification and proportion occupied showed any statistical significance ($p < 0.01$). This indicated that a stratified water column can reduce the proportion of the water column occupied by fish, but is less likely to affect the fish biomass or aggregation levels.

Although there was only one relationship showing statistical significance in the data, not all of the stations were represented in the data used. With only four sites, each varying in time, space, and conditions of the water column, it is not entirely surprising that more significant relationships were not found. However, without these limitations, relationships between variables such as bottom types and fish biomass might have been observed. A previous study on coral reefs in Hawaii did find that the complexity of the reef structure, as well as the presence of bottom types such as sand and turf, had a significant influence on the fish biomass of the water column (Wedding et al., 2019). Another study went even further and used the species-habitat relationships identified at a temperate reef to develop models to show how different

environmental variables will effect species densities and assemblages (Young & Carr, 2015). The data in this study may have only revealed one significant relationship, but there have obviously been other instances where these correlations have been identified. The reason for this difference could be attributed to the low n value used in the analysis of the fish, bottom, and time variables. The study that developed the model for species-habitat relationships combined tracks of data collected over the course of two years for an n value of 265 while this study only had four tracks of data (Young & Carr, 2015). Another factor that may have contributed to the resulting low number of significant relationships might have been the conditions within the water column. The stratification of the environment varied between the tracks and it was only when the relationships of the maximum buoyancy frequencies and fish variables were assessed that any significance was seen between proportion occupied and max N.

Stratification

Based on the interpretations of the temperature and salinity plots, the tracks can be divided into categories of stratified and well-mixed tracks. First is Track 01 Out, the only track to show strong and consistent stratification. The contour plots for this track both showed a clearly defined thermocline and pycnocline throughout the entire track and at depths that were relatively consistent across time. In the other category of well-mixed tracks, Tracks 41 Out, 40 In, and 05 In showed inconsistent changes between temperature values and salinity values across both depths and time. These assessments were backed up by the calculated maximum N values from the buoyancy frequency data, which showed that Track 01 Out was most strongly stratified throughout all of the CTD cast sites. From the UCTD casts, DO values were found to be 6 mg/L on 8/1/2018 and 8/2/2018. However, since these data were only collected on those two days, they did not reflect the conditions of the entire study area or timeframe.

Due to the location of the study site, it is not expected that the local fish fauna would be accustomed to large fluctuations in temperatures or salinities in short timescales like those that may be seen in habitats such as estuaries (Greenwood, 2007). Changes to oceanic stratification have been documented in the past with attention given to how the environment reacts to a storm that passed through the area. In one particular case, it was found that the mixing of the surface region increased due to waves generated by the storm and that resulted in a deepening of the thermocline as the base of the ocean surface boundary layer weakened (Lucas et al., 2019). It is likely that the well-mixed contour plots that were seen from data in this study were also the result of interference from a storm. Data collected at the study site showed that wind speeds were higher between 7/29/2018 to 8/4/2018, which was when Tracks 05 In, 41 Out, and 40 In were surveyed, than between 8/5/2018 to 8/8/2018, which was when Track 01 Out was surveyed (Figure 9a). In addition, data in Figure 9b shows that there was a low pressure system over the area at the same time as the high winds, indicating the presence of a storm, which would have led to water column mixing. These conditions contributed to the distinction between the well-mixed water columns and the stratified water column.

When considering stratification in this data, the local fish species may have found it more difficult to occupy a greater portion of the water column during stratified conditions such as those in Track 01 Out. Although individual species were not identified and assessed in this data for specific behaviors, previous studies have noted changes in marine fishes when the conditions of the environment are no longer best suited to them. One such study found that fish assemblages in the southern California Current were primarily driven by the local environmental conditions, including the DO levels of the mid-water region and the sea surface temperatures (Koslow et al., 2013). This is likely why the only significant correlation seen in this data was between the level

of stratification in the water column and the portion of water column that was being occupied by the fish. However, more data representing conditions in a stratified water column would be needed to truly understand the effects of these environmental factors at GRNMS.

Fish Fauna Thermal Tolerances

Looking back at the data presented here, the potential connection between the stratification of the water column and the proportion occupied by fish is most clearly seen in Track 01 Out. The temperature contour plot for Track 01 Out shows a well-defined thermocline for nearly the entire time, while the plot for the proportion occupied across Track 01 Out has the lowest proportion occupied values overall out of all of the tracks. In the other tracks, there was low stratification and their proportion of the water column being occupied was higher than that of Track 01 Out. The local fish fauna were likely responding to those changes and taking advantage of the opportunity to move more freely in the water column, which could be explained by the conditions of the environment becoming more suitable for them.

As mentioned before, data on specific species were not gathered, however several of the most common local fish species include blue runner (*Caranx crysos*), red snapper (*Lutjanus campechanus*), and black sea bass (*Centropristis striata*) (Auster & Giacalone, n.d.; Campanella et al., 2019; Kracker, 2007). By knowing a few of these species, some predictions relating to their thermal tolerances and how those may drive the fish distribution can be made. Juvenile black sea bass for instance, have been found to have a 50% mortality rate when temperatures reach 33.3ºC, with mortalities beginning at temperatures as low as 29.7ºC (Atwood et al., 2001). While the data indicate that temperatures did not reach this threshold, the local populations may be unwilling or unable to move across temperature gradients of 1 to 2 decrees, like the one seen in Track 01 Out (Figure 6a). Instead, the species may prefer to move when the temperature

changes slowly with the diurnal cycle. A few temperature ranges that have been observed to encourage more activity for other local species include between ~25.5 – 30ºC for the blue runner and ~22 – 28ºC for the red snapper (Bolser et al., 2020). A closer examination of the fish species present, their individual thermal tolerances, and how those tolerances may change throughout their development would help further explain fish activity in the area in relation to the environmental conditions such as temperatures.

Conclusions

This study intended to characterize the bottom habitats that exist in a temperate reef, as well as record the conditions of the water column, in order to assess how those components of the reef may influence the biological factors such as fish distribution. A complete assessment of the structures of this reef led to the classifications of the bottom types based on bottom hardness and roughness, while the characteristics of the water column revealed that conditions of both a stratified and well-mixed environments were present. Despite these characterizations of the temperate reef, only the stratification of the water column was found to have a statistically significant relationship with one of the fish variables. As stratification increased so did the proportion of the water column that was occupied by fish, suggesting that the differences in stratification between the tracks may have been the primary driver of fish distribution. However, further analysis of data from GRNMS would be needed to identify if this correlation exists between other stratified tracks. In addition, an assessment of surveys with the same level of stratification may provide a way to observe other driving factors across the reef.

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Literature Cited

Aguzzi, J., Doya, C., Tecchio, S., de Leo, F. C., Azzurro, E., Costa, C., et al. (2015). Coastal observatories for monitoring of fish behaviour and their responses to environmental changes. *Reviews in Fish Biology and Fisheries*, *25*(3), 463–483. https://doi.org/10.1007/s11160-015-9387-9

Atwood, H. L., Young, S. P., Tomasso, J. R., & Smith, T. I. J. (2001). Salinity and Temperature Tolerances of Black Sea Bass Juveniles. *North American Journal of Aquaculture*, *63*(4), 285–288. https://doi.org/10.1577/1548-8454(2001)063<0285:sattob>2.0.co;2

Auster, P. J., & Giacalone, L. (n.d.). Virtual Reality Camera Technology Facilitates Sampling of Interactions Between Reef Piscivores and Prey. *Marine Technology Society Journal*, 2021.

Bolser, D. G., Egerton, J. P., Grüss, A., Loughran, T., Beyea, T., McCain, K., & Erisman, B. E. (2020). Environmental and Structural Drivers of Fish Distributions among Petroleum Platforms across the U.S. Gulf of Mexico. *Marine and Coastal Fisheries*, *12*(2), 142–163. https://doi.org/10.1002/mcf2.10116

Boswell, K. M., David Wells, R. J., Cowan, J. H., & Wilson, C. A. (2010). Biomass, density, and size distributions of fishes associated with a large-scale Artificial Reef complex in the Gulf of Mexico. *Bulletin of Marine Science*, *86*(4), 879–889. https://doi.org/10.5343/bms.2010.1026

Campanella, F., Auster, P. J., Christopher Taylor, J., & Muñoz, R. C. (2019). Dynamics of predator-prey habitat use and behavioral interactions over diel periods at sub-tropical reefs. *PLoS ONE*, *14*(2). https://doi.org/10.1371/journal.pone.0211886

Catano, L. B., Barton, M. B., Boswell, K. M., & Burkepile, D. E. (2017). Predator identity and time of day interact to shape the risk–reward trade-off for herbivorous coral reef fishes. *Oecologia*, *183*(3), 763–773. https://doi.org/10.1007/s00442-016-3794-z

Ebeling, A. W., & Hixon, M. A. (1991). Tropical and Temperate Reef Fishes: Comparison of Community Structures. In Peter F. Sale (Ed.), *The Ecology of Fishes on Coral Reefs* (pp. 509–563). San Diego: Academic Press.

Echoview Help: Proportion_occupied. (2020, October 16). Retrieved May 1, 2021, from https://support.echoview.com/WebHelp/Echoview.htm

Echoview Help: Sv_mean. (2020, October 16). Retrieved May 1, 2021, from https://support.echoview.com/WebHelp/Echoview.htm

Fakhrudin, M., Subehi, L., Jasalesmana, T., & Dianto, A. (2019). Dissolved oxygen and temperature stratification analysis for early warning system development in preventing mass mortality of fish in lake Maninjau, West Sumatera-Indonesia. *IOP Conference Series: Earth and Environmental Science*, *380*(1). https://doi.org/10.1088/1755-1315/380/1/012002

Fernandes, L., Day, J., Lewis, A., Slegers, S., Kerrigan, B., Breen, D., et al. (2005). *Establishing Representative No-Take Areas in the Great Barrier Reef: Large-Scale Implementation of Theory on Marine Protected Areas* (Vol. 19).

Greenwood, M. F. D. (2007). Nekton Community Change Along Estuarine Salinity Gradients: Can Salinity Zones be Defined?, *30*(3), 537–542. Retrieved from https://about.jstor.org/terms

Hashimoto, M., Kidokoro, H., Suyama, S., Fuji, T., Miyamoto, H., Naya, M., et al. (2020). Comparison of biomass estimates from multiple stratification approaches in a swept area method for Pacific saury Cololabis saira in the western North Pacific. *Fisheries Science*, *86*(3), 445–456. https://doi.org/10.1007/s12562-020-01407-3

Jones, G. P., & Andrew, N. L. (1993). Temperate reefs and the scope of seascape ecology. In *The Ecology of Temperate Reefs* (pp. 63–76). NWIA Publications. Retrieved from https://www.researchgate.net/publication/261486137

Kendall, M. S., Jensen, O. P., Alexander, C., Field, D., McFall, G., Bohne, R., & Monaco, M. E. (2005). Benthic mapping using sonar, video transects, and an innovative approach to accuracy assessment: A characterization of bottom features in the Georgia Bight. *Journal of Coastal Research*, *21*(6), 1154–1165. https://doi.org/10.2112/03-0101R.1

Khan, J. A., Goatley, C. H. R., Brandl, S. J., Tebbett, S. B., & Bellwood, D. R. (2017). Shelter use by large reef fishes: long-term occupancy and the impacts of disturbance. *Coral Reefs*, *36*(4), 1123–1132. https://doi.org/10.1007/s00338-017-1604-7

Koslow, J. A., Goericke, R., & Watson, W. (2013). Fish assemblages in the Southern California Current: Relationships with climate, 1951-2008. *Fisheries Oceanography*, *22*(3), 207–219. https://doi.org/10.1111/fog.12018

Kracker, L. (2007). *Hydroacoustic surveys: A non-destructive approach to monitoring fish distributions at National Marine Sanctuaries. NOAA Technical Memorandum NOS NCCOS 66*. Charleston.

Kracker, L., Kendall, M., & McFall, G. (2008). Benthic features as a determinant for fish biomass in Gray's Reef National Marine Sanctuary. *Marine Geodesy*, *31*(4), 267–280. https://doi.org/10.1080/01490410802466611

Lucas, N. S., Grant, A. L. M., Rippeth, T. P., Polton, J. A., Palmer, M. R., Brannigan, L., & Belcher, S. E. (2019). Evolution of oceanic near-surface stratification in response to an autumn storm. *Journal of Physical Oceanography*, *49*(11), 2961–2978. https://doi.org/10.1175/JPO-D-19-0007.1

McDougall, T. J., & Barker, P. M. (2011). Getting started with TEOS-10 and the Gibbs Seawater (GSW) Oceanographic Toolbox.

Misund, O. A. (1997). Underwater acoustics in marine fisheries and fisheries research. *Reviews in Fish Biology and Fisheries*, *7*, 1–34.

Myers, E. M. V., Harvey, E. S., Saunders, B. J., & Travers, M. J. (2016). Fine-scale patterns in the day, night and crepuscular composition of a temperate reef fish assemblage. *Marine Ecology*, *37*(3), 668–678. https://doi.org/10.1111/maec.12336

National Data Buoy Center. (2021, April 1). Station 41008 - Gray's Reef. Retrieved May 4, 2021, from https://www.ndbc.noaa.gov/station_page.php?station=41008

NOAA. (2020). The MPA Inventory: National Marine Protected Areas Center. Retrieved May 6, 2021, from https://marineprotectedareas.noaa.gov/dataanalysis/mpainventory/

Rowley, S. J. (2018). Environmental gradients structure gorgonian assemblages on coral reefs in SE Sulawesi, Indonesia. *Coral Reefs*, *37*(2), 609–630. https://doi.org/10.1007/s00338-018-1685-y

Siwabessy, P. J. W., Penrose, J., Kloser, R., & Fox, D. (1999). *Seabed habitat classification*. Sydney. Retrieved from https://www.researchgate.net/publication/237064031

Switzer, T. S., Tyler-Jedlund, A. J., Keenan, S. F., & Weather, E. J. (2020). Benthic Habitats, as Derived from Classification of Side-Scan-Sonar Mapping Data, Are Important Determinants of Reef-Fish Assemblage Structure in the Eastern Gulf of Mexico. *Marine and Coastal Fisheries*, *12*(1), 21–32. https://doi.org/10.1002/mcf2.10106

Thorpe, S. A. (2007). Ocean stratification and buoyancy. In *An Introduction to Ocean Turbulence* (pp. 22–23). Cambridge University Press.

Urmy, S. S., Horne, J. K., & Barbee, D. H. (2012). Measuring the vertical distributional variability of pelagic fauna in Monterey Bay. *ICES Journal of Marine Science*, *69*(2), 184–196. https://doi.org/10.1093/icesjms/fsr205

Wedding, L. M., Jorgensen, S., Lepczyk, C. A., & Friedlander, A. M. (2019). Remote sensing of three-dimensional coral reef structure enhances predictive modeling of fish assemblages. *Remote Sensing in Ecology and Conservation*, *5*(2), 150–159. https://doi.org/10.1002/rse2.115

Wellenreuther, M., Syms, C., & Clements, K. D. (2008). Consistent spatial patterns across biogeographic gradients in temperate reef fishes. *Ecography*, *31*(1), 84–94. https://doi.org/10.1111/j.2007.0906-7590.05270.x

Whitfield, P. E., Muñoz, R. C., Buckel, C. A., Degan, B. P., Freshwater, D. W., & Hare, J. A. (2014). Native fish community structure and Indo-Pacific lionfish Pterois volitans densities along a depth-temperature gradient in Onslow Bay, North Carolina, USA. *Marine Ecology Progress Series*, *509*, 241–254.

Young, M., & Carr, M. H. (2015). Application of species distribution models to explain and predict the distribution, abundance and assemblage structure of nearshore temperate reef fishes. *Diversity and Distributions*, *21*(12), 1428–1440. https://doi.org/10.1111/ddi.12378

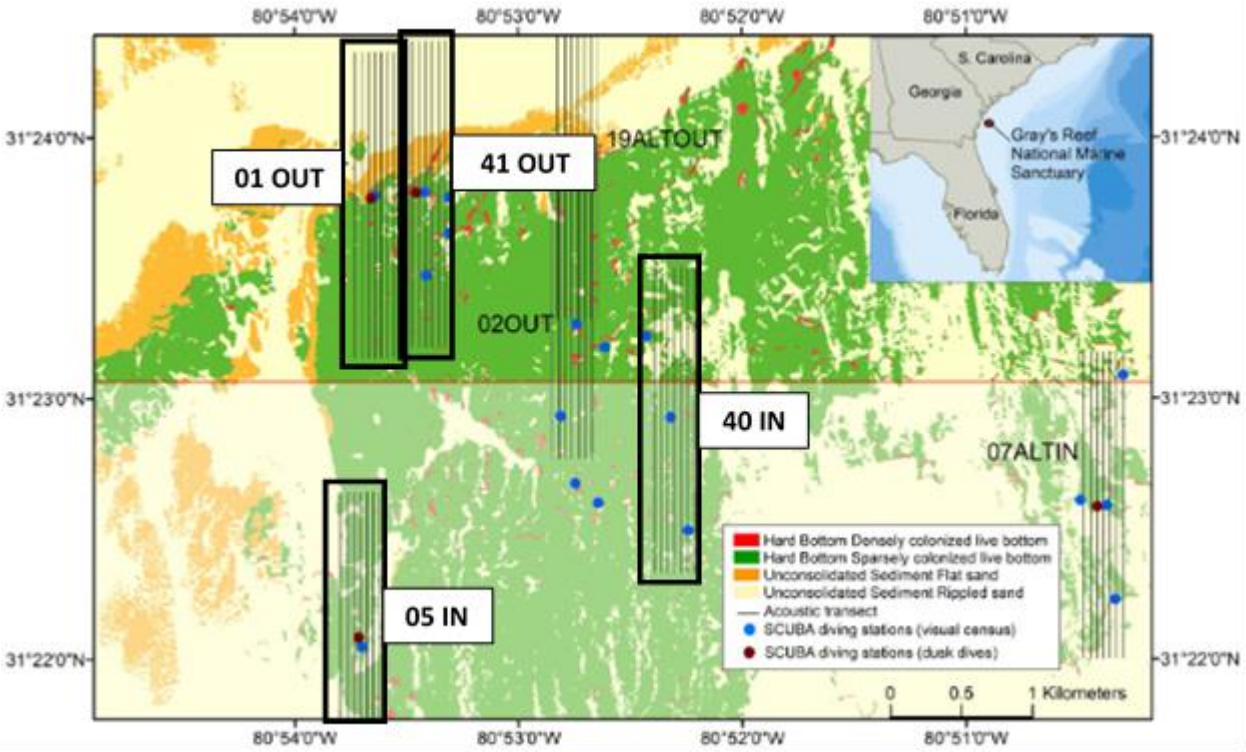
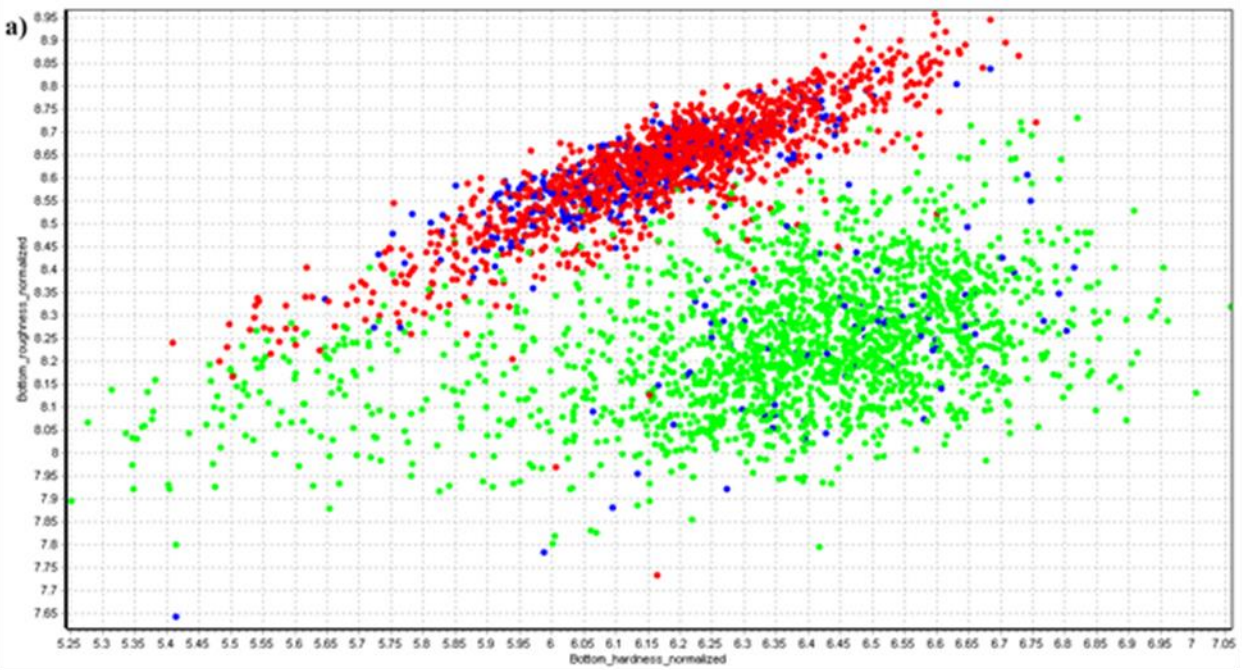
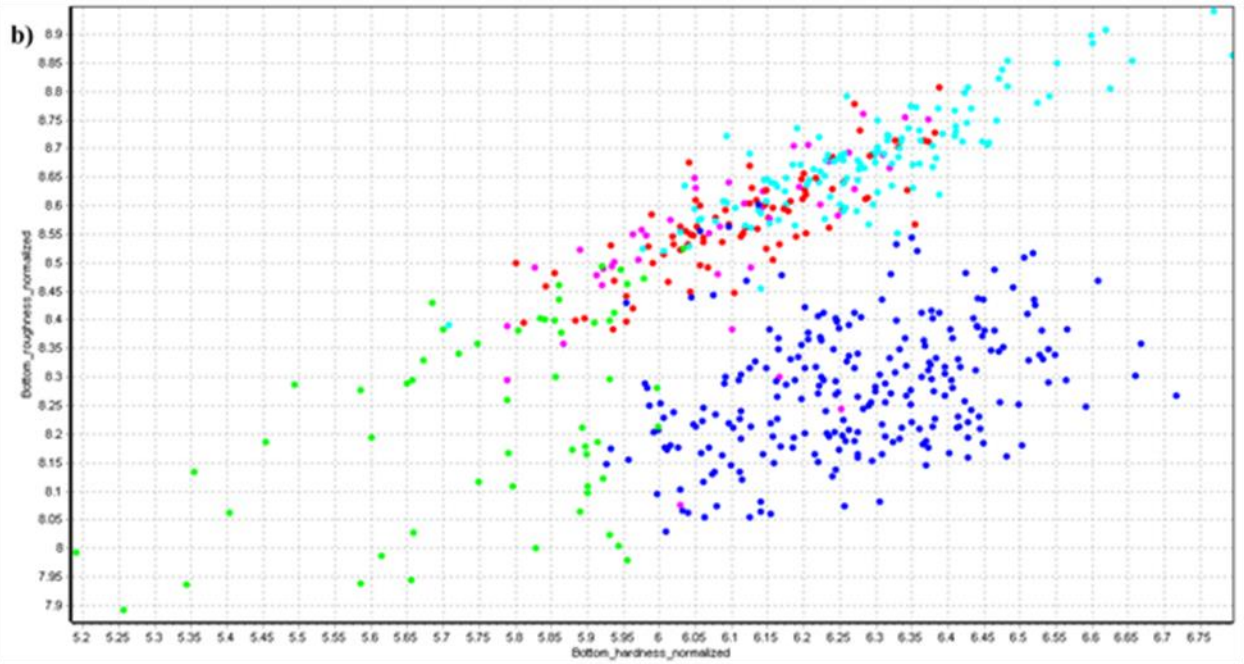


Figure 1: Map of Gray's Reef National Marine Sanctuary showing the acoustic transect lines, dive sites, boundary of the MPA, and perviously mapped bottom types (Campanella et al., 2019). Edited to highlight the tracks used in this study.


a)
Bottom_roughness_normalized
Bottom_hardness_normalized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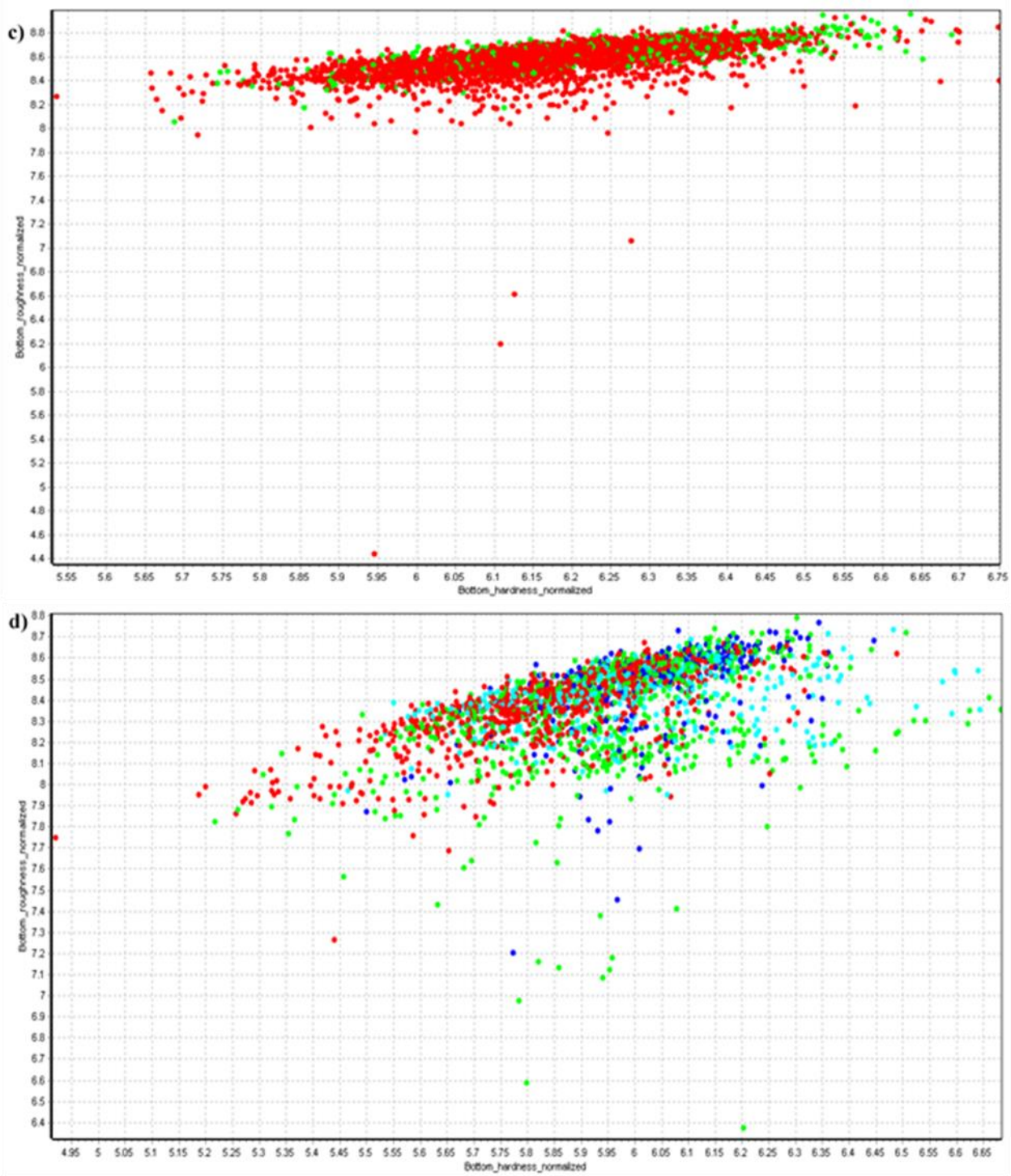

Figure 2: Bottom Classification graphs for (a) Track 01 Out, (b) Track 41 Out, (c) Track 40 In, and (d) Track 05 In, showing Normalized Bottom Roughness vs Normalized Bottom Hardness.

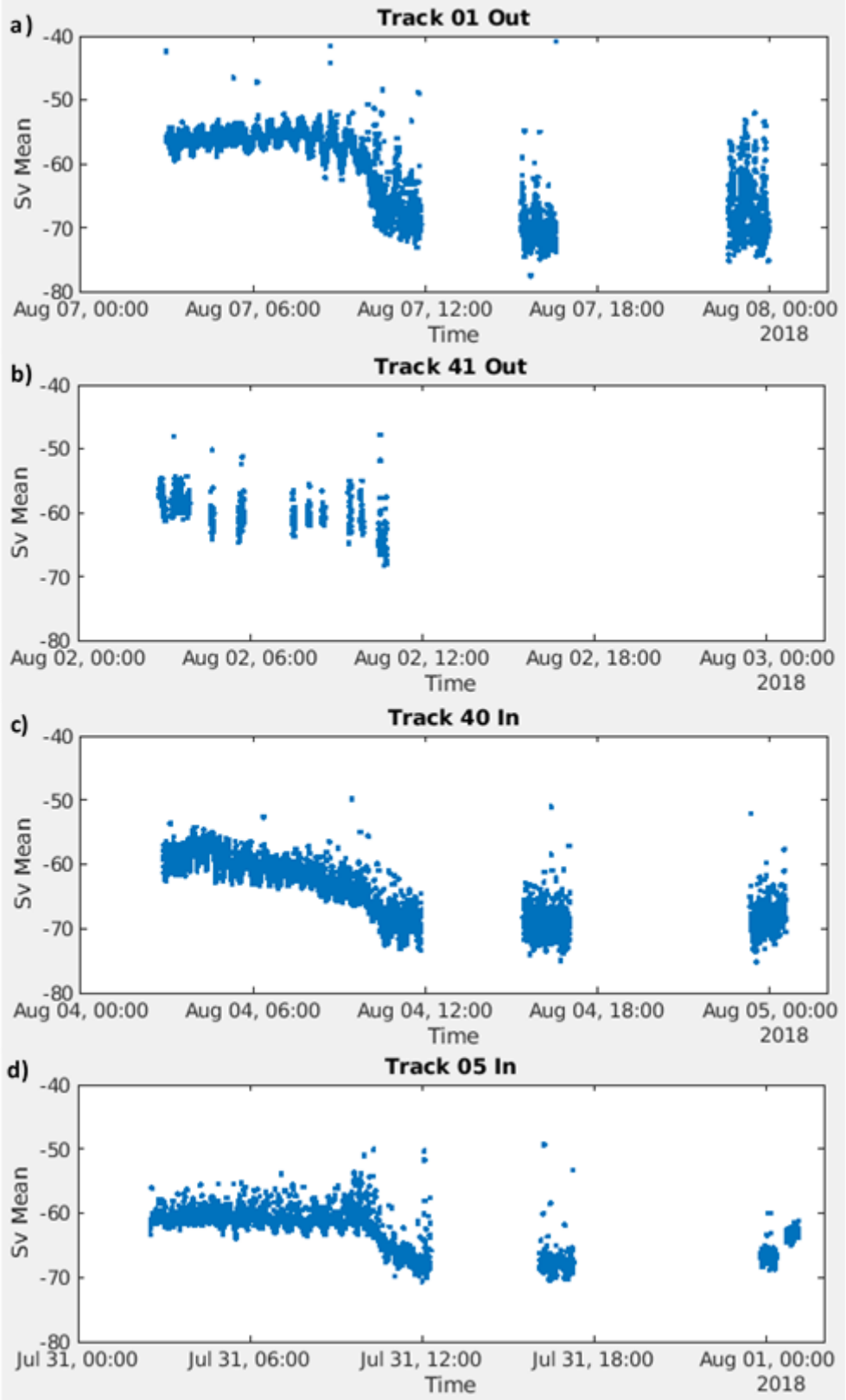

Figure 3: S_v Mean plots for (a) Track 01 Out, (b) Track 41 Out, (c) Track 40 In, and (d) Track 05 In with time as GMT.

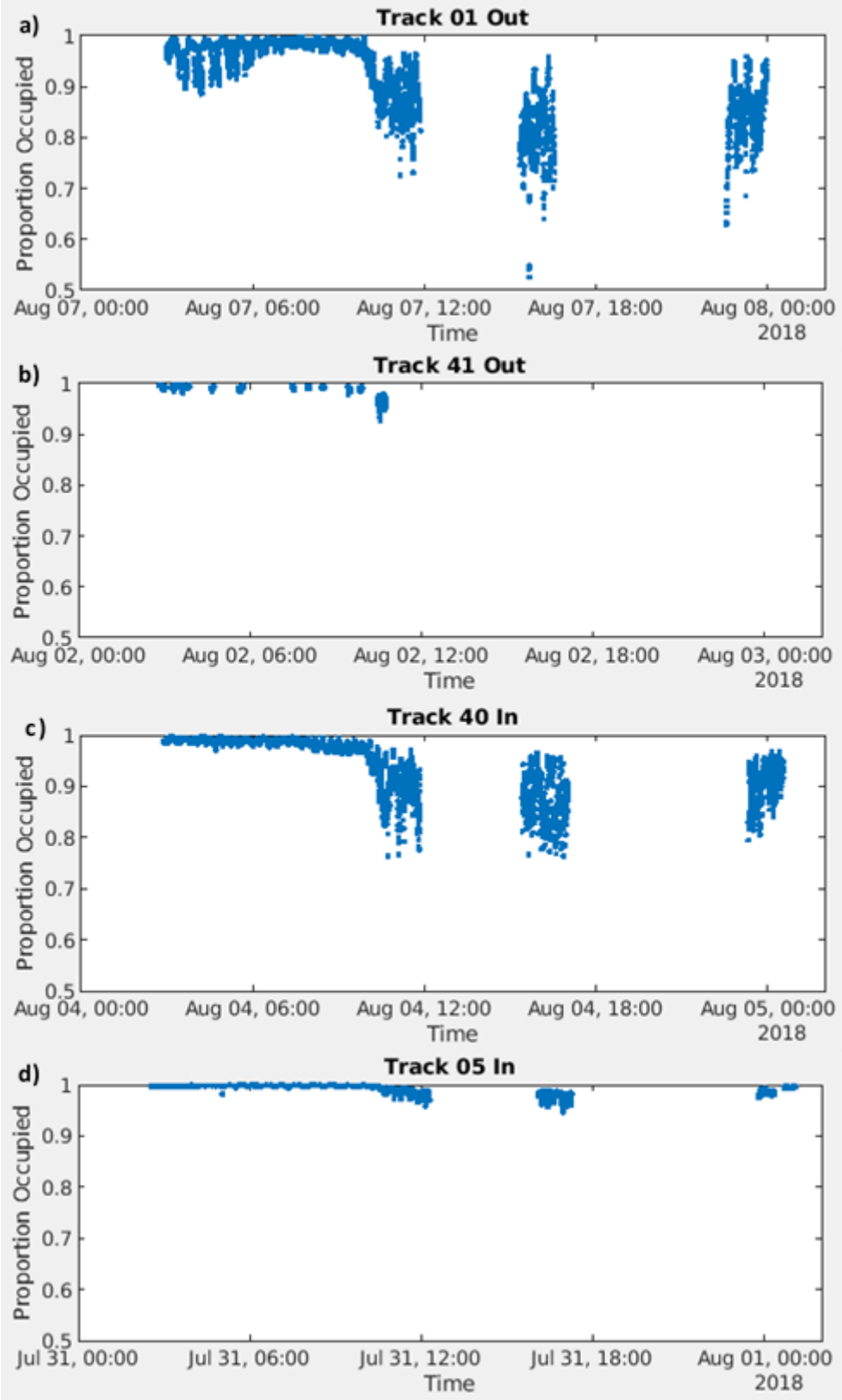

Figure 4: Proportion Occupied plots for (a) Track 01 Out, (b) Track 41 Out, (c) Track 40 In, and (d) Track 05 In with time as GMT.

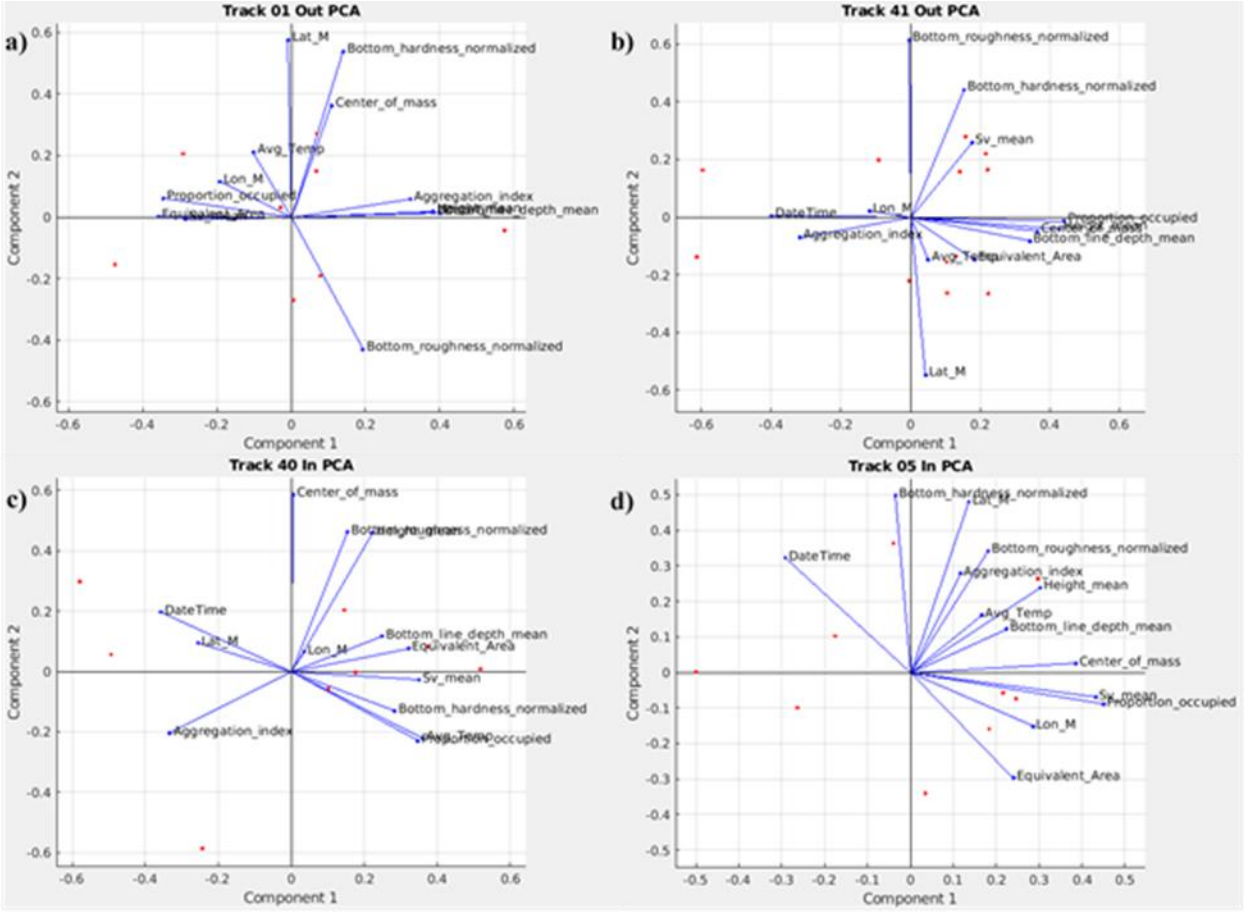

Figure 5: PCA biplots for a) Track 01 Out, (b) Track 41 Out, (c) Track 40 In, and (d) Track 05 In

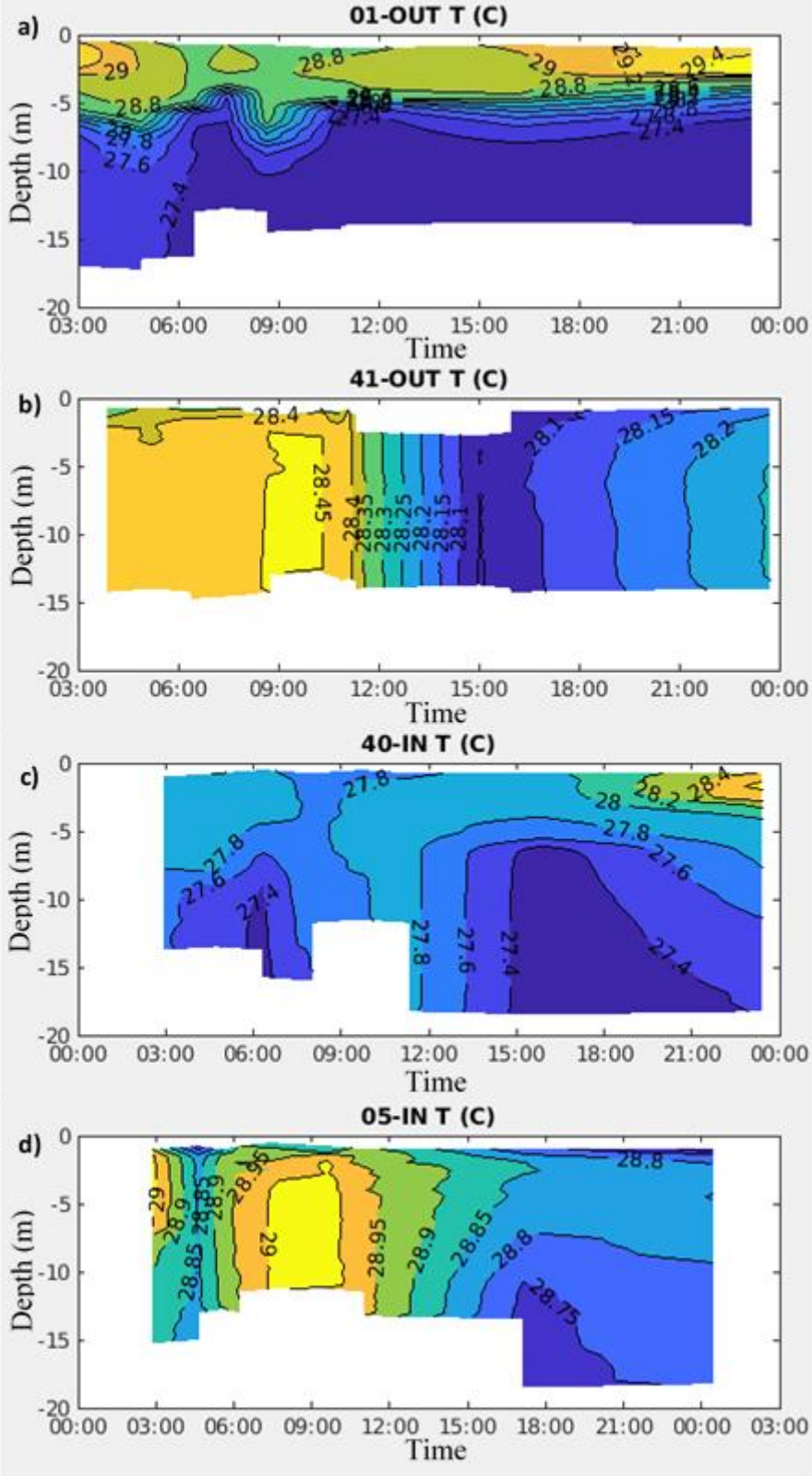

Figure 6: Temperature contour plots (°C) for (a) Track 01 Out, (b) Track 41 Out, (c) Track 40 In, and (d) Track 05 In. Warm colors represent higher temperatures and cool colors represent lower temperatures, however they are not set to standardize across the plots. Time in GMT.

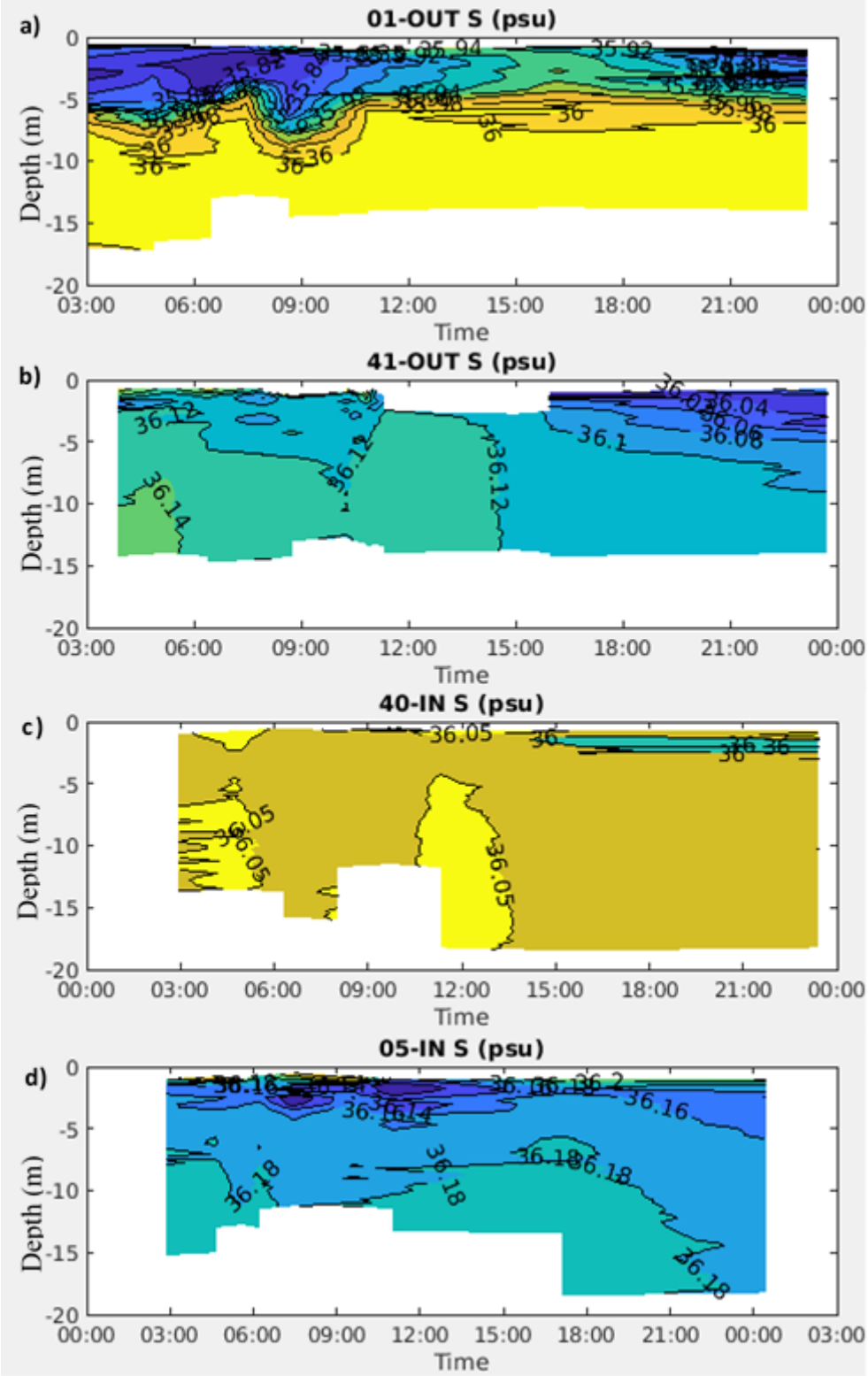

Figure 7: Salinity contour plots (psu) for (a) Track 01 Out, (b) Track 41 Out, (c) Track 40 In, and (d) Track 05 In. Warm Warm colors represent higher temperatures and cool colors represent lower temperatures, however they are not set to standardize across the plots. Time in GMT.

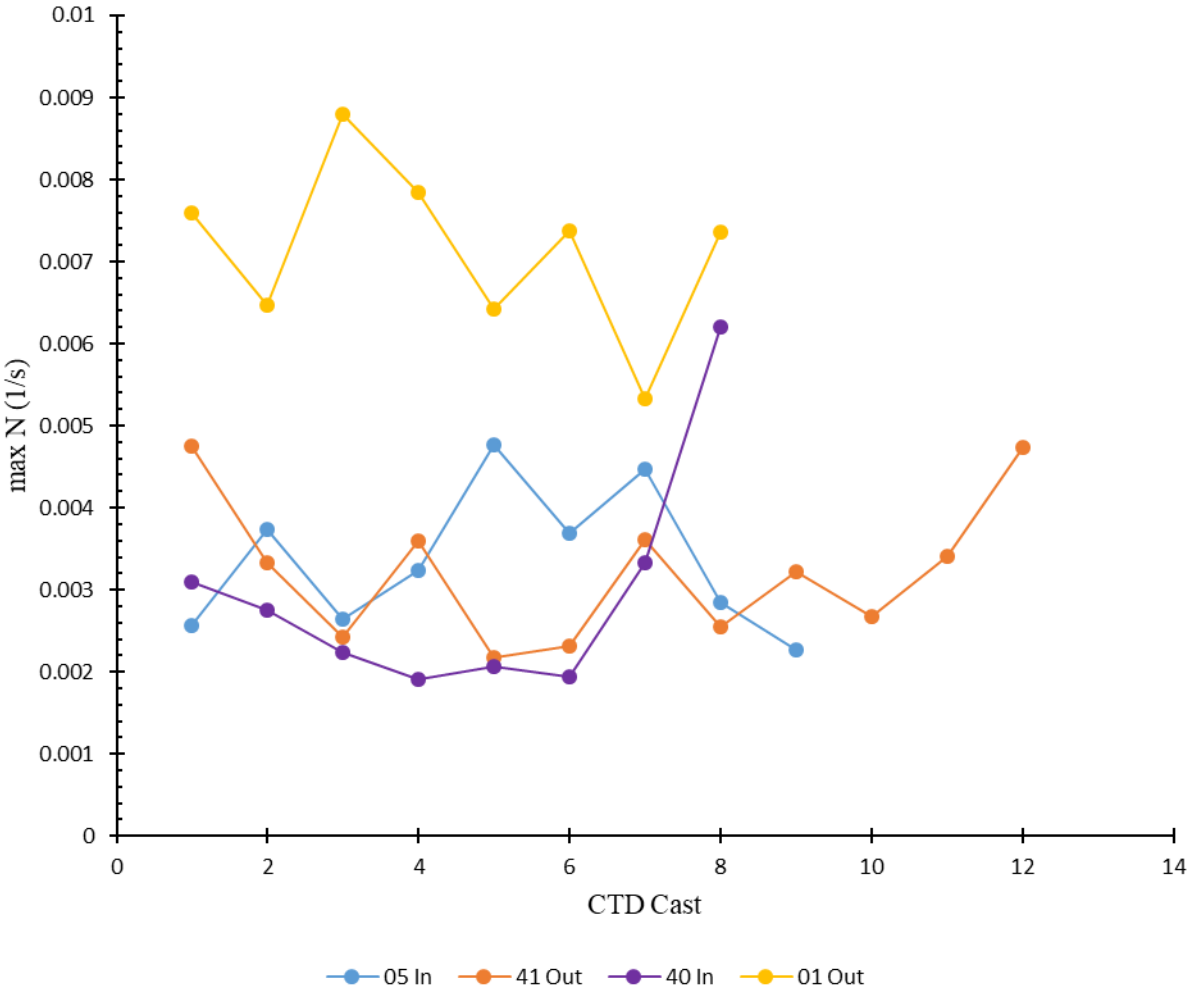

Figure 8: Maximum buoyancy frequencies for each of the CTD casts of each track.

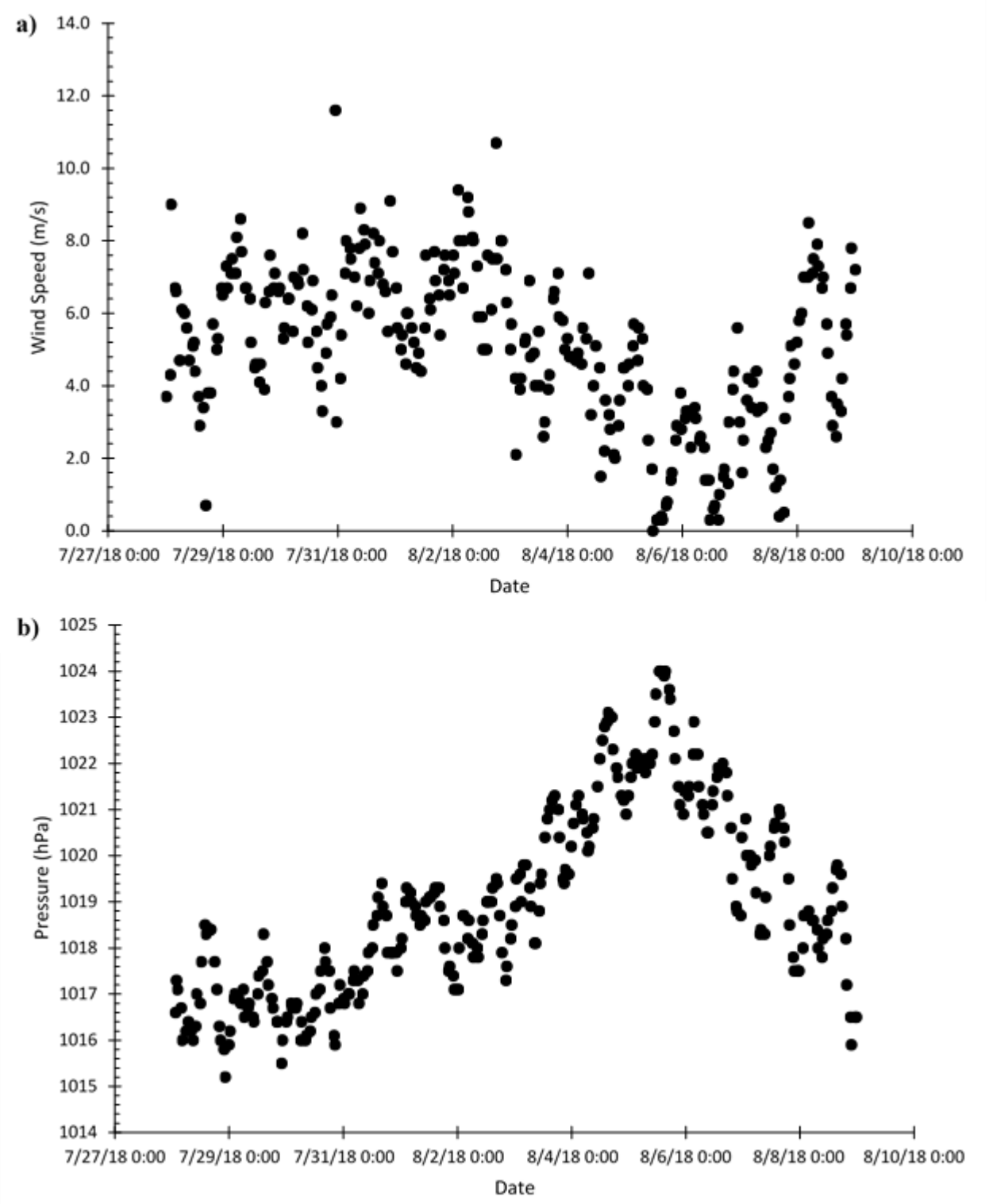

Figure 9: Local wind speeds (a) and atmospheric pressure (b) from NDBC's Station 41008 at Gray's Reef (National Data Buoy Center, 2021).

Appendices

Appendix 1. Steps taken with the SONAR data in Echoview

1. SONAR .raw files from each track were loaded into Echoview
2. Background noise removal was applied to the 120 kHz echogram
    a. In Dataflow window, on the Shortcut menu select New → Variable
    b. In the New Variable dialog box:
        i. Operand 1 List: Sv raw pings T4
        ii. Click OK
    c. On Dataflow window, select Background noise removal 1
        i. One the Shortcut menu, select Echoview
3. Line-pick algorithm to identify the start of the bottom
    a. In the View menu, select EV File Properties
    b. In the EV File Properties box, open the Single Beam tab on the Line and Surfaces page
        i. Start depth (m): 0.50
        ii. Stop depth (m): 30.00
        iii. Minimum SV for good pick (dB): -70.00
        iv. Select: Use Backstep
        v. Discrimination level (db): -60.00
        vi. Peak threshold (db): -55.00
        vii. Click OK
4. On Background Noise Removal echogram window, select the New Editable Line from the Line draw tool arrow
    a. In the Create a new line box: Bottom Line
    b. Select: Pick from current variable
    c. Select: Span gaps
    d. Click OK
5. Editing the Bottom Line

a. Open working echogram and select the Line and Surface tool → Choose defined bottom line
b. Click from left to right on echogram window to make the correction
c. On the Shortcut menu, select Update Active Line

6. Automatic Bottom Classification function settings
    a. On the Dataflow window, select the Bottom Line variable
        i. In the Shortcut menu, select Graph
            1. Use displayed line graph of Bottom Line to estimate the average depth
    b. On the Bottom Noise Removal echogram, open Variable Properties through the Shortcut menu
    c. On the Analysis page of Variable Properties:
        i. Bottom Line: Select name of correct bottom line
        ii. Bottom echo threshold at 1 m (dB): -500
        iii. Depth normalization reference depth (m): Enter estimated average depth of the bottom line from the graph
        iv. Click OK
    d. On the Bottom Classification of the EV File Properties:
        i. Under Features to Extract: Select all features
        ii. Cluster Dimension Selection: Automatic (Principle component analysis)
        iii. Bottom Class Allocation: Automatic detection
        iv. Method: Calinski-Harabasz
        v. Clustering iterations: 100
        vi. Click OK
    e. Display the Background Noise Removal echogram
    f. On the Echogram menu, select Classify Bottom
        i. Variable name: enter name for bottom classification variable
        ii. Select Show bottom classes on integram
        iii. Click Classify
    g. To see the bottom classification results graphed based on bottom roughness normalized and bottom hardness normalized:

i. On the Dataflow window, select the Bottom Classification variable
ii. In the Shortcut menu, select Graph to display Bottom Classification results as a scatterplot

h. To export the bottom classification data:
i. On the Dataflow window, select the Bottom Classification variable
ii. In the Shortcut menu, select Export → Data Values
iii. Under Select range to export: All measurements
iv. Click Export
v. Name the file and click Save

7. Creating the surface line as a fixed-depth line
a. Open working echogram and select Line and Surface tool → New Editable Line
i. Under Destination, select Create new line
ii. In the Create new line box, enter a name for the line
iii. Fixed depth: 0.5
iv. Click OK
8. Defining Exclude above and below lines in preparation for integration
a. On Echogram menu, select Variable Properties to open the dialogue box
b. Open the Analysis page
i. Select the correct line for the relevant box
ii. Click Apply
9. Excluding regions of bad data or surface activity interference
a. From the echogram tool bar, select the option best suited for the region:
i. Rectangle tool, Horizontal band tool, Vertical band tool, Parallelogram tool, Polygon tool
b. Make a selection around the area of interest on the echogram
c. Open the Shortcut menu and select Define Region
d. In the Region Browser dialog box:
i. Name: Type in appropriate name for region
ii. Type: Select option from list that best suits the region
10. Exporting Integration Results
a. On Echoview menu, open the Variable Properties dialogue box to the Grid page

i. Show time/distance grid: Ping Number
ii. Distance between grid lines (pings): 100
iii. Show depth/range grid box: Water surface (depth of zero
iv. Separation (m): 150
v. Click OK

b. Exporting integration by cells
    i. On Echoview menu, select Export → Analysis by Cells → Integration
    ii. In Export Integration by Cells (all) window:
        1. Save in: Select folder for exported file
        2. File name: Name for exported file
        3. Click Save

Appendix 2. Steps taken with the UCTD and CTD data files in Sea-Bird (SBE) Data Processing Software

1. UCTD .asc files were converted to .cnv files in the SBE's ASCII In program (14)
    a. Under the File Setup page:
        i. Select UCTD .asc files through the Input directory
        ii. Select the destination for the UCTD .cnv files in the Output directory
        iii. Optional: Fill in Name append and Output file boxes in the Output directory
    b. Under the Data Setup page:
        i. Scan interval variable: Time, seconds
        ii. Scan interval value: 0.0625
        iii. Select Column Names → Variable Name [unit]: Scan Count, Conductivity [S/m], Temperature [ITS-90, deg C], Pressure [db]
    c. Click Start Process
2. Converted UCTD .cnv files were processed by the SBE's Align CTD program (3)
    a. Under File Setup page:
        i. Follow same instructions as Step 1, using UCTD .cnv files
    b. Under Data Setup page → Enter Advanced Values:
        i. Conductivity [S/m]: 0
        ii. Temperature [ITS-90, deg C]: 0.10 s
        iii. Click OK
    c. Click Start Process
3. Aligned UCTD .cnv files were run through SBE's Section program (16)
    a. Under File Setup page:
        i. Follow same instructions as Step 1, using UCTD .cnv files
    b. Under Data Setup page:
        i. Section based on: Pressure
        ii. Pressure section cast: Downcast
        iii. Minimum value: 0.5
        iv. Maximum value: 30

    c. Click Start Process
4. UCTD .cnv files were converted again in SBE's ASCII Out program (15)
    a. Under File Setup page:
        i. Select UCTD .cnv files through the Input directory
        ii. Select the destination for the UCTD .asc and .hdr files in the Output directory
        iii. Optional: Fill in Name append and Output file boxes in the Output directory
    b. Under Data Setup page:
        i. Select: Output header file and Output data file
        ii. Lines per page: 60
        iii. Label columns: No column labels
        iv. Column separator: Space
        v. Julian days conversion format: Julian days
        vi. Select Output Variables: Scan Count, Conductivity [S/m], Temperature [ITS-90, deg C], Pressure [db], Flag
    c. Click Start Process
5. Converting the CTD files began with using SBE's SBE 911plus/917plus CTD program (13) (found under the Configure menu)
    a. Select Open
        i. Choose .xmlcon files to load into the program
    b. Sensor list should include: conductivity, temperature, pressure, altimeter, fluorometer, turbidity, oxygen, and more
    c. Click Save
6. CTD .hex files were converted to .cnv files using SBE's Data Conversion program (1)
    a. Under File Setup:
        i. Instrument Configuration file should match the file being processed
        ii. Select CTD .hex files through the Input directory
        iii. Select the destination for the CTD .cnv files in the Output directory
        iv. Optional: Fill in Name append and Output file boxes in the Output directory

b. Under Data Setup page:
    i. Output format: ASCII output
    ii. Convert data from: Downcast
c. Under Data Setup, Select Output Variables → Variable Name [unit]:
    i. Depth [salt water, m]
    ii. Pressure, Digiquartz [db]
    iii. Temperature [ITS-90, deg C]
    iv. Time, Elapsed [seconds]
    v. Conductivity [uS/cm]
    vi. Density [density, kg/m^3]
    vii. Latitude [deg]
    viii. Longitude [deg]
    ix. Fluorescence, Seapoint
    x. Oxygen, SBE 43 [mg/l]
    xi. Oxygen, SBE 43 [% saturation]
    xii. Potential Temperature [ITS-90, deg C]
    xiii. Salinity, Practical [PSU]
    xiv. Seafloor depth [salt water, m]
    xv. Turbidity, Seapoint [FTU]
d. Click Start Process

7. CTD .cnv files were converted to .asc and .hdr files using SBE's ASCII Out program (15)
    a. Under File Setup page:
        i. Select CTD .cnv files through the Input directory
        ii. Select the destination for the CTD .asc and .hdr files in the Output directory
        iii. Optional: Fill in Name append and Output file boxes in the Output directory
    b. Under Data Setup page
        i. Label columns: Top of the page
        ii. Column Separator: Comma

iii. Select Output Variables: All the same ones as Data Conversion (Step 6c) plus Flag

c. Click Start Process