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# Characterization of Fish Assemblages on an Upper Mesophotic Reef in South Florida Using a Commercial Grade Mini Rov

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# Characterization of Fish Assemblages on an Upper Mesophotic Reef in South Florida Using a Commercial Grade Mini ROV

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**Abstract**

Mesophotic coral ecosystems, or MCEs, are coral ecosystems at approximately 30-150m depths and represent the transitional zone where benthic organisms rely less on photosynthesis and more on filter-feeding or other feeding habits in order to sustain themselves. Fish assemblages in MCE's have been poorly studied and may possibly provide a connection to shallow water reefs (SWR's). Mesophotic reef fish communities from 32-37 m depths were assessed using video footage recorded by a remotely operated vehicle (ROV). Video transects were performed at three locations: unburied areas north and south of Port Everglades, as well as an area which experienced burial from dredging material produced during the creation of Port Everglades at the turn of the 20$^{th}$ century. The results of this research are significant as they have added to a large data gap which existed concerning local MCE fish communities. Results showed evidence of shallow water reef overlap to the upper mesohphotic zone as well as novelties regarding ROV sampling. Additionally, baited versus un-baited ROV transects showed no significant difference in fish assemblages. Particularly, this information provides insight into the connectivity of MCEs to SWRs, as well as long term differences of fish assemblages in areas buried by dredge materials. Local MCE administrators may now utilize this data to more efficiently manage these deeper environments which have previously relied only on SWR data.

Keywords: Mesophotic, ROV, fish assemblages, refugia

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# Introduction

## ***Mesophotic Coral Ecosystem***

Mesophotic coral ecosystems, have been defined as light-dependent, tropical and sub-tropical reef systems beginning at depths of 30-40m and extending down to the end of the photic zone typically near 150m (Hinderstein et al. 2010, Kahng et al. 2010, Kahng et al. 2014, Turner et al. 2017, Baldwin et al 2018, Pyle & Copus 2019). The mesophotic zone can be broken into two portions, the upper mesophotic ranging from 30-60m and the lower mesophotic ranging from 60-150m (Slattery et al. 2011, Loya et al. 2016). Mesophotic reef ecosystems have more recently proven to be potential refugia for many shallow water species, hypotheses suggest MCEs provide potential alternative habitat to degraded shallow reefs (Lindfield et al. 2016, Baldwin et al. 2018, Laverick et al. 2018, Rocha et al. 2019). This has led to the development of the deep-reef refugia hypothesis (DRRH), which suggests that mesophotic ecosystems are more stable than their shallow water counterparts in both environmental resilience and human impact. The stability of MCEs stem from their resilience to climatic variations, anthropogenic impact, disease and sedimentation (Loya et al. 2016). Reigl and Piller (2003) and Linderfield et al. (2016) state that the spatial framework for these ecosystems to be refugia is there.

A noticeable turnover or shift in both fish and coral communities from shallow water reefs (SWRs) to MCEs has been noted in the literature (Kahng et al. 2010, Kahng et al. 2014, Pearsons & Stevens 2015, Abesamis et al. 2018, Baldwin et al. 2018). There are two main reasons for this: 1) species composition overlap and 2) MCEs maybe less susceptible to anthropogenic effects compared to SWRs (Hinderstein et al. 2010, Rocha et al. 2019) and 3) a trophic structure change that occurs with depth gradients. Considering MCEs are typically neighboring SWRs it allows for fish to move between either reef as long as the fish's physiology allows for the increase in depth. As for why MCEs maybe less susceptible to anthropogenic effects, the increase in depth acts as an additional boundary from surface level impacts such as heavy boat traffic, some recreational divers, and beach activity. The third reason for a turnover in fish community maybe tied to trophic structuring. The farther you move offshore typically the trophic style changes from herbivore/omnivore to more Piscivore and carnivore due to the food availability in low relief environments (Fukunaga et al. 2016).

Factors that contribute to the structure of mesophotic communities are as varied as those for shallow water reefs. Depth gradient, substrate, geolocation, shallow water connectivity, thermal stresses and water movement can all effect community structure (Bongaerts et. al 2010, Kahng et al. 2010, Turner et al. 2017). Strong ecosystem connectivity allows for high biodiversity, in this case for both reef building corals and the fishes that rely on them. The drivers which structure mesophotic communities are still being researched because only a few regions have studied MCEs, only generalized understandings can be formulated. Fukunaga et al. (2016) investigated the structuring of mesophotic assemblages in the Northern Hawaiian islands at depths 1m to 67m and found that fish assemblages from mesophotic depths had higher total densities than those in shallower reefs and also exhibited high endemsism. Another study from the Caribbean showed how light gradient and nutrients acted as structuring forces allowing the upper (30-60m) mesophotic to still share connectivity to shallower waters while past 60m (the lower mesophotic) had to rely on different feeding methods due to the continuing decline of light and nutrients (Slattery & Lesser 2012).

While the debate goes on about whether SWR species can utilize deeper waters, it was noted that fish species may use SWRs for a form of nursery ground before making their way to upper mesophotic depths (Kahng et al. 2014,Loya et al. 2016). If so, than mesophotic habitats could be imperative to developing life stages in fish species. The exploitation of fish species is often the reason for reef-health decline, and deep reefs can only act as refuge if community overlap is sufficient and fish larval overlap between shallow water and deep-water reefs can occur (Bongaerts et. al 2010).

Management and preservation of mesophotic reef habitats need to be considered separately from that of shallow water reefs. Just because assumptions of connectivity and overlap have been made for MCEs and SWRs, does not mean that they experience impacts equally. MCEs may be more resistant to abiotic and biotic impacts, but they are not immune (Loya et al. 2016). Not all MCEs are the same and thus cannot be monitored or managed as such. And although most of the “primary threats” of SWRs overlap with MCEs, the magnitude of the impact may vary, as well as, the uncertainty of long term exposure.

A review of the current literature focused on MCEs reveals there is little known of these areas and additional research is necessary to better understand its ecological significance. The majority of studies focus on overall composition MCEs and possible refugia use, while human impacts were poorly documented. Development and implementation of new MCE research will provide critical data to build upon our baseline understanding of how these reef-systems function.

### *Fish Assemblages*

Mesophotic studies typically look at patterns in fish abundance, species composition and trophic ecology. Significant differences between SWR and MCE assemblages has been documented, but overlapping species between these two areas may indicate greater connectivity, which may vary from region to region (Slattery et al. 2011, Wagner et al. 2014, Williams et al. 2016, Abesamis et al. 2018, Chaloux et al. 2020).

Fish assemblage structure can be influenced by many factors including location, prey availability and survivorship. Pearson & Stevens (2015) demonstrated cross-shelf gradients and depth intensity (inshore versus offshore reefs) were the major and independent drivers for fish assemblages in their research. Bottom composition, coral coverage and habitat rugosity, also strongly correlate to fish species richness and overall community diversity (Khang et al. 2010, Fukunaga et al. 2016, Hollarsmith et al. 2020).

Fish endemism on mesophotic reefs is not controlled by the same factors which drive endemism on shallow reef counterparts. Factors that play a role in endemism at mesophotic depths are understudied, most endemism studies are limited to reefs shallower than 30m. Geographic isolation is a driver in fish endemism at mesophotic depths and a study conducted by Kane et al. (2014) explored endemism in Northern Hawaii using closed-circuit technical diving. They found that there were statistically more endemic reef fishes in mesophotic zones than in traditional reef zones with 46% of fish sampled being endemic. Several reasons for this finding were suggested and included limited movement of these species and their likelihood to congregate. It may also be due to larval transport and survivorship differences compared to more shallow water dominant species. One proposed conclusion was the probability of unique evolutionary processes possessed by mesophotic assemblages.

Trophic structure in fishes also appears to shift with depth. Groups like herbivores and coralivores decrease with depth while groups such as piscivores and planktivores may increase (Khang et al. 2010, Abesamis et al. 2018). Due to this depth gradient between shallow reefs and mesophotic reefs differences in trophic guilds have been observed (Bejarano et al. 2014). A study conducted in the Philippines showed species richness of planktivores, herbivores, omnivores, and generalists declined with depth while piscivores were unaffected (Abesamis et al. 2018). In a study conducted of mesophotic habitats off South Florida, out of the 85 fishes species sampled, most were either planktivorous or carnivorous (Bryan et al. 2013).

Some common families found in many mesophotic studies from the Atlantic include: Haemulidae, Pomacanthidae, Pomacentridae, Lutjanidae, Serranidae, Labridae, and Gobiidae (Bejarano et al. 2014, Soares et al. 2018). In Puerto Rico MCEs the bicolor damselfish (*Stegastes partitus)* was one of the most common upper mesophotic reef species observed (Bejarano et al. 2014).

### ***Anthropogenic Effects***

The continuous degradation of coral reef systems due to human impact is steadily increasing. Between the pressures of major fisheries, to the destruction of reef systems due to channel dredging, coral reefs face more threats today than in any time in history. Nearly 19% of coral reef ecosystems have already been lost globally with an estimated 35% to be lost within the next 40 years (Loya et al. 2016). Additionally, the sixth *Status of Coral Reefs of the World: 2020* report indicated a 14% global loss of coral reefs between 2009 and 2018, primarily due to recurring large-scale coral bleaching events caused by elevated sea surface temperatures.

As suggested by Loya et al. (2016), MCEs are suspected to be less vulnerable and more resistant to disturbances such as bleaching and sedimentation, which may help combat these anthropogenic impacts taking place on neighboring SWRs. MCEs may prove vital if they can act as refuge for fishes from reefs that face the unending human induced impacts. The cooler more stable conditions of these deep reefs may act as thermal protection from temperature related stress events, such as human-induced climate change (Reigl & Piller 2003, Bongaerts et al. 2010).

Erftemeijer et. al ‘s (2012) comprehensive review on the effects of dredging emphasizes the impacts of high sedimentation events have. Dredging operations re-suspend fine particles in the water column only to settle and smother many of the reef species nearby. Dredging and port construction can have lethal side effects on reef ecosystems if they are not given adequate time to recover. Erftemeijer et. al (2012) suggested SWRs that face disturbances more frequently are more resilient to these effects compared to mesophotic reefs who have less stress tolerance. How a reef reacts to dredging events is related to: existing conditions, levels of fishing, bio-eroders, species diversity, connectivity to neighboring reefs, and seasonality.

Miller et al. (2016) examined sedimentation effects from the dredging of Port of Miami. They found that most of the sedimentation occurred north of Miami's reef tract, heavily impacting the inner, shallowest reef. Sedimentation levels were significantly higher within 200m from the channel opening, and the coral colonies within these transects showed almost complete mortality from sedimentation burial. Similar to Erftemeijer et. al (2012), they found that prolonged periods of sedimentation and dredging events are what led to reef developmental failure. Port expansion has been proposed for Port Everglades (very close proximity to the study site) and will follow similar construction plans from Port of Miami. However, unlike Port of Miami, Port Everglades is already under stress with coral disease, meaning that any sedimentation stress will likely be more detrimental than what was seen in Miami (Miller et. al 2016).

A study conducted by Walker et al. (2012) examined the dredging and shipping impacts on coral reefs in southeast Florida. The researched showed 8.1 million corals covering 11.7 ha of live cover were impacted by the burial. The study included looking at the dredging that took place in the 1920s of Port Everglades and discussed how substantial the effects were on the benthic community (Walker et al. 2012). They concluded that large-scale impacts such as dredging, port expansion, and anchoring can greatly impact the surrounding ecosystem.

Moustaka et al. (2018) analyzed how suspended sedimentation effect reef fish assemblages and feeding guild structure. The study took place in north-west Australia finding that species richness of fishes greatly declined with high levels of turbidity/sedimentation. Planktivores and herbivores decreased with high sedimentation allowing Moustaka et al. (2018) to conclude that changes in these abundances and functional groups can have severe consequences for coral reef recovery in high impact events.

### *A Brief Overview of ROVs*

One of the major challenges with surveying mesophotic coral reefs is the depth at which they reside. Because they occur deeper than the maximum depth of open-circuit SCUBA, alternative survey tools are needed. Options for research include autonomous underwater vehicles (AUVs), remotely operated vehicles (ROVs), human-occupied vehicles (HOVs), and closed-circuit re-breather diving. Although each option has advantages, as well as disadvantages such as the costs or specialized training required.

ROVs were first invented in 1953 by French scientist and explorer Dimitri Rebikoff with the creation of the "Poddle", one of the first recorded ROVs (Christ & Wernli 2011, Patiris 2015). By the 1960s the US Navy began funding their first attempt known as CURV, or "cable-controlled underwater recovery vehicles" used for recovery missions of equipment or objects on the seafloor unattainable by divers (Patiris 2015). In the 1970s, many ROVs were constructed and tested by the Navy's funding including the SNOOPY, the first hydraulically powered small scale portable ROV (Christ & Wenil 2011). Today, many ROV classifications exist from "micro-ROVS" to large "trenching ROVs". By the end of the late 1980s and early 1990s the first low cost mini-ROV developed by Chris Nicholson offered observation style ROVs. Globally ROV technology is still fairly new, with Japan as a leader in this developing technology having designed an ROV that reached the deepest point in the Mariana Trench. That being said, the global use of small class ROV technology remains limited. According to Patiris 2015 a mini-class ROV would classify as a ROV under 15kg larger than micro-class (used for exploration in pipelines) but smaller than "light class" ROVs (used in 1000m depths).

ROVs can be a great tool for researchers to collect both observational data, as well as small sample collection. Depending on the needs and environment, ROVs can range in price, size, and complexity. Small grade ROVs can range from $10,000 to $100,000 and can operate at depths less than 300m (Pacunski et al. 2008). Pacunski et al. (2008) stated, "the greater affordability, lower operating costs, and relative simplicity of small ROVs makes them especially suited for use by natural resource agencies and academic institutions".

When choosing to use a ROV as a surveying method many factors should be taken into consideration to understand how the ROV will best function in the needed environment. ROVs can be affected by things like, ocean disturbances, centers of gravity, buoyancy control, timed deployments, sensors, and overall calibration limitations. (Yuh 2000), which requires detailed review to choose the correct technique for each individual survey and data type being obtained.

A survey conducted by Pagan & Appledoorn (2010) demonstrated the successful use of small ROVs to identify large reef fish, count and measure coral colonies, and record video of mesophotic fish communities. The study emphasized the practicality of using small ROVs in mesophotic studies. Hollarsmith et al. (2020) conducted a study using small commercial ROVs to both prove the effectiveness of this method and demonstrated how depth and temperature, as well as factors such as water-column mixing, and food availability shape fish communities on mesophotic reefs. Additionally, Chaloux et al. (2020) used ROVs to highlight their ability to collect fish samples with a low cost and compact method. Chaloux et al. (2020) concluded that not only was the use of ROVs a highly effective sampling method but it also was a useful tool when surveying mesophotic reefs.

In addition to ROVs is the use of BRUV, or baited remote underwater video has been effectively utilized in MCEs. BRUV surveys are a more suitable observational technique at mesophotic depths to attract organisms for video data collection without harming or directly impacting their natural behavior (Abesamis et al. 2018, Williams et al. 2019). It is important to understand how attracting organisms such as fish may add to survey bias. Using ROVs as a surveying technique may lead to minor changes in fish behavior, depending on reaction to the presence of bait or the ROV which can attract or repel fish from the area (Stoner et al. 2019). The operator should be aware of the speed at which the ROV is travelling, should observe if the conditions of the ROV change, or if a baited ROV component is leading to survey bias. Conducting both, baited and unbaited ROV surveys, will help illuminate the effects on ROVs on fish behavior, although surveying biases are often unavoidable as a whole, certain biases ROVs present may still be deemed appropriate for the surveying aims.

The use of underwater ROVs, can help us better understand marine issues, help us understand conservation efforts, and efficiently help researches in surveying deep water habitats (Yuh 2000, Hollarsmith et. al 2020). As the consumer-grade/mini ROV industry grows the use of ROVs in marine and freshwater surveys should expand.

## Objectives

The study focuses on three main goals: 1) to provide baseline information on an understudied upper MCE fish assemblages off South Florida 2) to determine if fish assemblages differ in upper MCEs historically buried by dredging events from creation of Port Everglades to those areas that never experienced burial and 3) to determine differences in baited and non-baited ROV surveys in upper MCEs.

This study will provide important information on upper MCE fish communities. The upper MCE is one of the least studied zones in our region and the results will reveal whether there are unique assemblages or if it is similar to SWRs and thus may provide additional refugia for species typical of those shallower habitats. Additionally, the novel methodology presented using a small-scale ROV for reef surveys will serve as a pilot study showing the benefits and limitations of this method. The DTG3 ROV used in this study has never before been used for this type of scientific surveying and will show how access to this technology maybe helpful to observational baseline surveys. The ROV will be used for both mobile baited and mobile non-baited surveying, a technique not deeply researched in its comparative effectiveness.

### *Hypotheses*

**$H_0^1$:** There is no difference between trophic groups between baited and non-baited surveys.

**$H_A^1$:** There is significant difference between trophic group structure between baited and non-baited surveys.

A study conducted by Stobart et al. (2007) used baited video transects to asses shallow water fish assemblages in the Mediterranean at approximately 10-20m depth. Stobart et al. (2007) was able to compare the effectiveness of baited surveying to a visual census method and found that although baited surveying is reliable to represent species richness the visual census was better suited for estimating fish abundance. Another study from south-western Australia compared three transect types, baited stereo-video, non-baited stero-video, and diver operated video (Watson et al. 2005). The baited surveying method was able to capture the larger more predatory species, but all three techniques were able to capture relative species richness consistently (Watson et al. 2005). Andradi-Brown et al. (2016) looked at the comparison between baited surveys and diver-operated surveys in the mesophotic environment, finding that BRUVs produced higher species richness and family richness while diver surveys had a greater representation of the fish communities by reporting a higher biomass of herbivores in the area.

**$H_0^2$:** There is no difference between trophic groups between depth effects.

**$H_A^2$:** There is a difference between trophic group structure between the two depth effects.

A study investigating the MCEs overall functional structure in the Hawaiian Archipelago found that fish species present in SWRs and MCEs had similar population and trophic structures (Pyle et al. 2016). Pyle et al. (2016) goes on to say how MCE endemism increases with depth. Similarly, Kane et al. (2017) conducted a study in Hawaii looking at the trophic designation and fish community structure from shallow water reefs to mesophotic reefs (3-50m depth), and found changes in herbivore abundance even though 78% of the recorded species from shallow water reefs also appeared in the mesophotic sites.

**$H_0^3$:** There will be no differences between fish communities at upper MCE sites buried over 100 years ago to sites which have never been buried.

**$H_A^3$:** Historically buried MCE sites should have an effect on fish communities.

Miller et al. (2016) explored the sedimentation impacts from local dredging in Port of Miami. The study found that spatial scales and location near the port itself had the biggest impact. They predicted that the planned dredging of Port Everglades will produce similar results leading to sub-lethal consequences on both coral structuring and residing fishes. The increased sedimentation, turbidity, and pollution associated with close proximity to this major port, causes the greatest signs of distress in the adjacent reef (Erftemijer et al. 2012). This study should note differences in assemblages between the unburied and buried sites.

# Materials and Methods

## ***Site Location***

South Florida has three major ports from West Palm beach to Miami, including Port Everglades. Three sites have been chosen adjacent to Port Everglades for surveying: a buried region running along the side of Spur and Grove and Aggregated Patch Reef (between 30-32m), and Ridge Deep area (between 35-37m)(Figure 1)(Reigl et al. 2004). Figures 1 below shows site specifics and coordinates. For comparison purposes, unburied areas on either sides of the buried strip (~0.8 km north and south) will be surveyed. The region spans about 0.8 km in length running from north to south along the South Florida coast.

South Florida's reef system is the largest reef system in the continental US, and this zone is of high importance for many conservation efforts. The continental shelf off southeast Florida is narrow, steep, and provides low-relief habitat (Bryan et al. 2013), which implies it may be subject to less sedimentation and can support high coral coverage (Khang et al. 2010). There are no major natural features associated with the upper MCEs in the area, the environment often has a low relief substrate and less benthic coverage (Bryan et. al 2013).

Mesophotic habitats are often challenging to survey, this is because limited surveying time that is often associated with such depths. Traditional coral reef surveys utilize SCUBA as a critical technique, this is not always possible for MCE surveys due to depth ranges. Using a ROV will allow for sustained bottom time and video footage that is otherwise unattainable. Furthermore, a comparison of baited versus non-baited ROV sampling will be conducted to determine which survey method attracts a more comprehensive fish community. This will aid in maximizing use of bottom time and surveying efficiency.

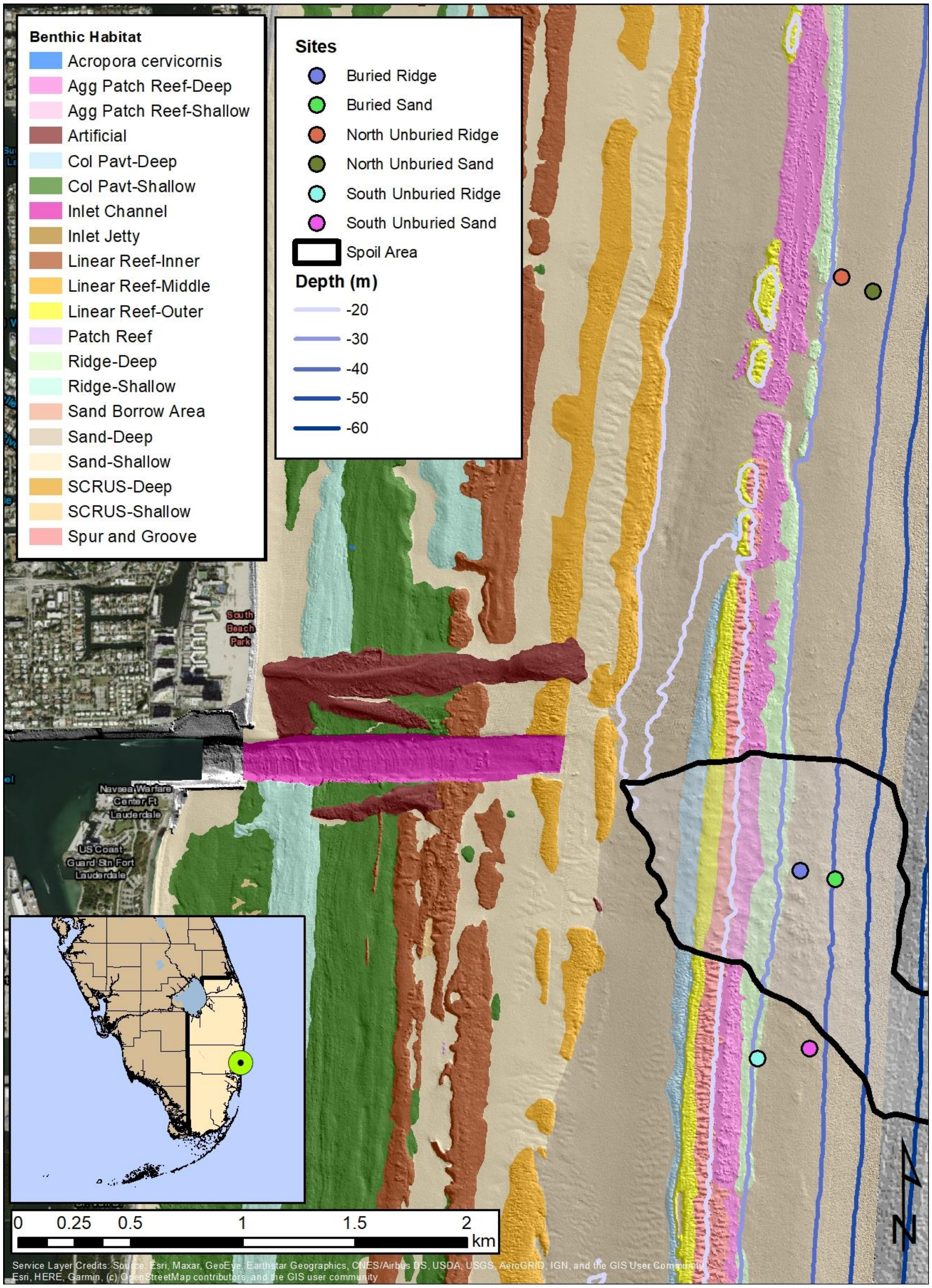


*Figure 1: Map indicating sampling locations and reef zonation. The two northern dots shown in orange and dark green represent the North Unburied sites, the blue and bright green dots in the center represent the Buried sites, and the teal and pink dots at the bottom represent the South Unburied sites. Two dots were used per sampling site to indicate the Deep Ridge sampled depths and the Deep and sampled depths. The black outline shown represents the spoil area. Important to note, the inlet channel in relation to the buried sites.*

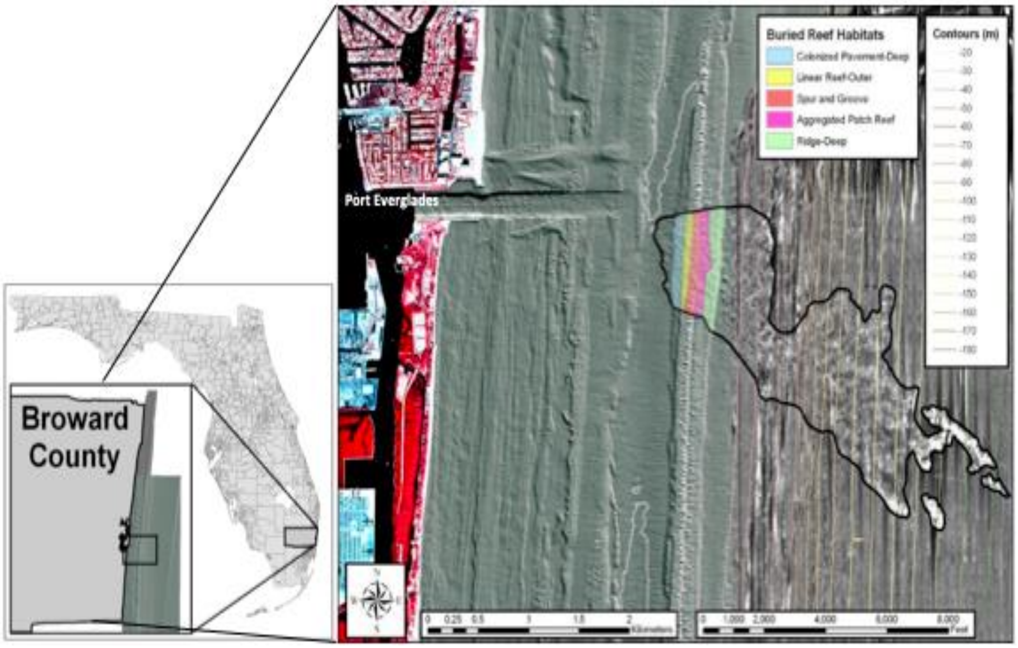


*Figure 2: Sampling locations by colored region. Colors represent reef zonation (before burial). Colonized Pavement-Deep is the back-reef, Linear Reef-Outer is the crest, Spur & Groove is the fore-reef, Aggregated patch reefs are in the fore-reef but not connected, and Ridge-Deep is a deep ridge on outer reef. The black outlined region shows where sedimentation/burial took place. (Walker & Dodge)*

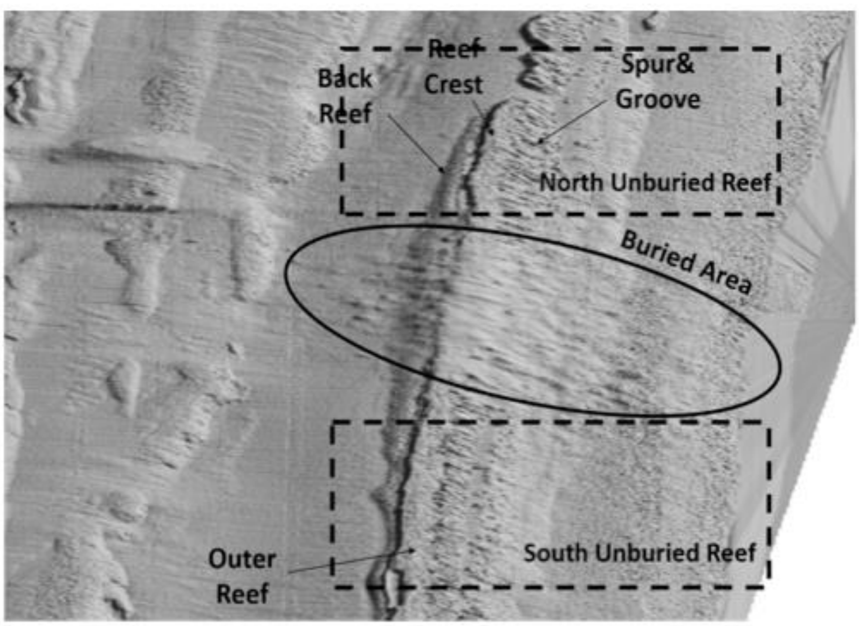


*Figure 3: A 3-D representation looking North. Dashed rectangles correspond to unburied survey locations, solid lined circle is the altered outer reef. (Reigl et al. 2004)*

### *Environmental & Non-environmental Observations*

The North Unburied sampling region, is a low-relief habitat with very little to no plant material. This area is a mix between a sandy and rocky benthos with lot of large industrial cables. The Buried sampling region has lots of coral, rock, and other invertebrate benthic coverage and appears to have more relief compared to the North Unburied sites. The South Unburied sampling location appears patchy in benthic coverage, including a mix of sand flats and clustering corals and sponges. The southern locations offer slight relief, and had signs of anthropogenic interference (i.e. industrial cables present).


*Figure 4: Image depicting the North Unburied site. Image was taken from a deep ridge survey in June of 2020 to show benthic coverage. Note the cable shown diagonally across the image.*

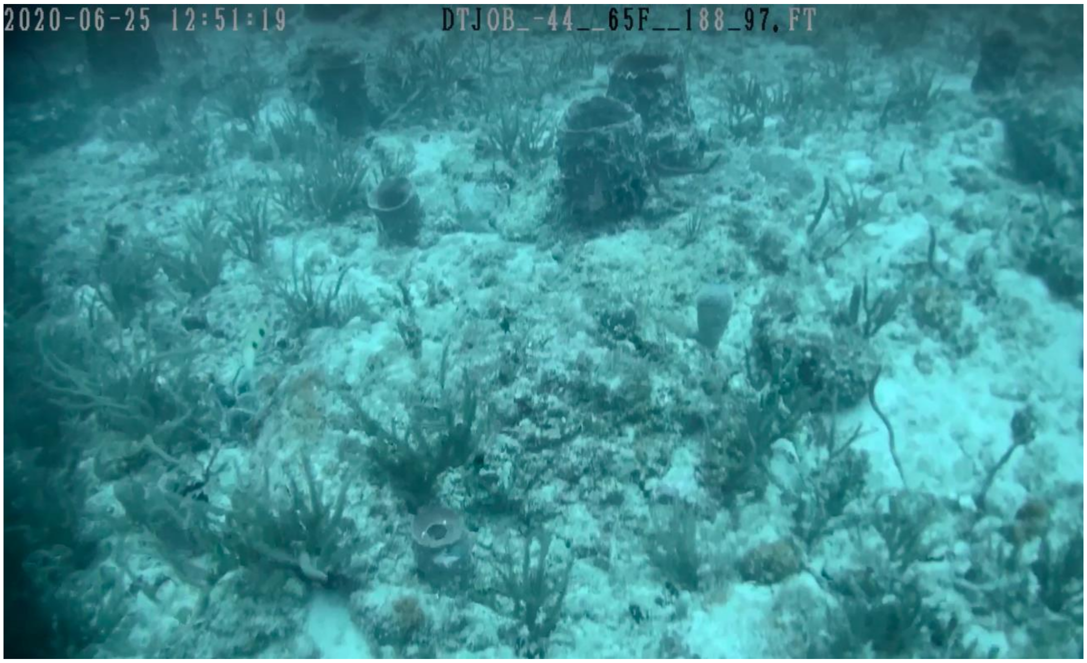


*Figure 5: Image depicting the Buried site. Image was taken from a deep ridge survey in June of 2020 to show benthic coverage.*

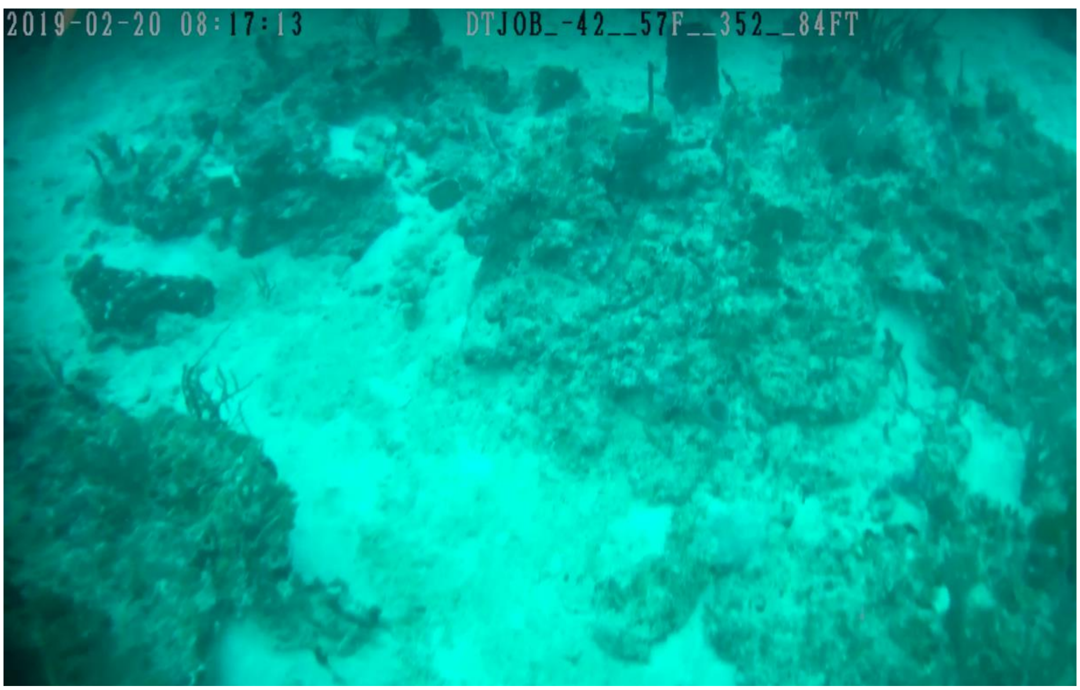


*Figure 6: Image depicting the South Unburied site. Image was taken from a deep ridge survey in November of 2019 to show benthic coverage.*

Occasionally, non-environmental signs of human impact appeared in the footage such as industrial cables, anchor lines, anchors, crates, lobster traps, plastic chairs, and smaller unidentifiable debris. It is worth noting that although sedimentation and burial on the reef is a priority to observe, both environmental and non-environmental observations should be reported to help encompass the scope of the MCE being surveyed. Further studies should look at the intensity these non-environmental observations have on the mesophotic reef health.

### *ROV Data Collection*

Two Deep Trekker DTG3 ROVs (figure 4), equipped with digital video cameras (HD 1080P, 270° total range of view), white lights (up to 1000 lumens LED), and depth sensors were used to gather and record video of the benthic invertebrates and fish assemblages on upper MCEs. The ROVs have 155 minutes of recording time at 4K resolution with a 4-8hour battery life. Considering the speed of the ROV/vessel one transect was surveyed within 4 to 10 minutes, this was due to there being 70 to 100 yards of tether used for each transect out of the available 150m of tether based on current's speed, scope of the tether, and surface level wave height. The ROV once deployed used depth lock to remain at the specified depth and which was approximately 1 meter from the bottom. The transect's footage was not taken into account for surveying until the ROV reached the benthos.

Each of the survey areas were named North Unburied, Buried and South Unburied. At each area two depths were surveyed, "Ridge" sites were at ~32m depth and "Sand" sites were ~37m depth. Sample days typically allowed for two 100m transect surveys at North Unburied Deep Ridge, North Unburied Deep Sand, Buried Deep Ridge, Buried Deep Sand, South Unburied Deep Ridge and South Unburied Deep Sand. Thus, on optimal sample days, a total of 12 transects were conducted. Each of these specific sites included one baited and one unbaited survey with an oblique camera angle positioned at 30-40° from horizontal.

The battery and any additional components, such as lights and lasers, outfitted on the ROV were monitored throughout to avoid differences between sites. The baited surveys had the ROV equipped with two small mesh bags filled with 0.5 kg of bait (mixture of chum and squid) for the baited surveys (Fig. 5).

Sampling started in January of 2020 and concluded in August of 2021, with a overall total of 33 transects. Over the course of sampling, 8 separate sampling events took place, 5 of those containing usable data (many issues with sampling in this region effected data collection). Out of the 33 total transects, 14 were from the North Unburied location, 14 were from the Buried location, and 5 were from the South Unburied location. 17 transects were sampled in the "deep ridge" depth zone of ~32 to 35m and 16 transects were sampled in the "deep sand" depth zone of ~35 to 40 m.


*Figure 7: Deeptrekker Mini ROV, DTG3, with tether and controls.*

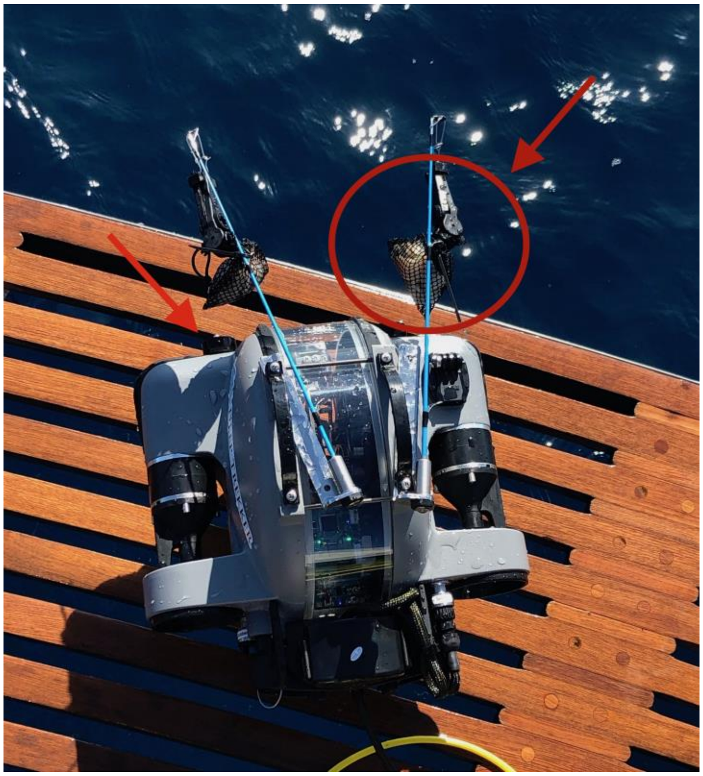

*Figure 8: Deeptrekker Mini ROV outfitted for baited survey. The red arrow on the left indicates where the front facing lights are located and the red arrow and red circle to the right show where the mesh bait bags are located.*

### *Data Analysis*

The project's primary investigator and primary professor viewed the video footage, identifying all observable fishes recorded during baited and oblique view transects. Any additional observations were noted throughout the given transect's video, such as cables, signs of human impact, or mega-fauna. Data was saved as an excel database for future statistical analysis (see 'Statistical Analysis' section for details in analyses used). More specifically, for fish assemblage analysis all fishes recorded were identified to the lowest possible taxon during each transect. Due to the varying clarity in some video, some fishes were identified down to family while others were identified as low as specific species. Trophic guilds were assigned to each species/family identified using Arena et al. (2005) and *FishBase* as a guide. Trophic level was assigned to one of five major trophic types, carnivore, scavenger, herbivore, Piscivore, or omnivore.

## Results

Statistical analysis of collected data was analyzed using a Bray-curtis dissimilarity matrix, ANOSIM (analysis of similarities), nMDS (non-metric multidimensional scaling plot), SIMPROF (similarity profile routine, Clarke et al. 2008), and SIMPER (similarity percentage breakdown, Field et al. 1982). A P-value <0.05 in all tests will be accepted as a significant difference. Data that was not normally distributed and had hetero-scedasticity (i.e., abundance, richness). The transect NUDSO was removed from the data prior to any analysis due to it effects leading to collapse in nMDS analysis. For these tests the Plymouth Routines in Multivariate Ecological Research statistical package (PRIMER v7) was used.

### *Variables & Factors*

Independent variables in the study were nominal (categorical) variables. Site location, depth, and transect type were all categorized as independent. Site location being equal to three major sampling locations (North Unburied, Buried, and South Unburied), depth was represented by two nested sub-locations (Deep Ridge and Deep Sand), and the third independent factor being transect type (Baited versus Un-baited). Dependent variables were discrete (i.e. counts), each identified taxa was a listed dependent species variable; where the absence of a species in a particular site was then represented as zero. Table 1 serves as a reference key for variables/factors.

*Table 1: Key to interpret any site abbreviations. Blue labeled factors are the three major sampling locations, red labeled factors are nested within blue factors, green factors are nested within red factors. i.e. Blue(red(green))).*

| ***Abbreviation Key*** | | |
|---|---|---|
| **Abbreviation** | **Definition** | **Description** |
| NU | North Unburied | North of port entrance |
| B | Buried | Outside of port entrance |
| SU | South Unburied | South of port entrance |
| DR | Deep Ridge | Depth range 30-35 m |
| DS | Deep Sand | Depth range 35-40m |
| -B | Baited | Baited transect (BRUV) |
| -O | Oblique | Un-baited transect, camera at oblique angle |

### ***Descriptive Statistics***

743 individual fish were counted made up of 64 species in the study. Blue chromis (*Chromis cyanea*), Bicolor damsel (*Stegastes partitus*), and Ocean Surgeon *(Acanthurus bahianus*) were the top three most observed species amongst all sites (Table 3). North unburied-deep ridge-oblique (NUDRO), north unburied-deep ridge-baited (NUDRB), and buried-deep ridge-oblique (BDRO) were the top three transect combinations to host the highest level of fish abundance. The south unburied (SU) transects ranked the lowest in fish abundance respectively.

Fishes were classified according to their predominant trophic ecology, either: carnivore, piscivore, herbivore, planktivore, or omnivore. From all transects combined, carnivorous species were most frequently observed, making up 50% of the 64 identified species. Omnivores were 17.19% of the 64 identified species; herbivores were also 17.19% of the 64 identified species; piscivores were 6.25% of the 64 identified species; scavengers were 1.56% of the 64 observed species; planktivores were 7.81% of the observed 64 species. Across all three sampling locations, NU, SU, Buried, carnivores were the dominantly observed species (46.67%, 33.93%, 52.27% respectively). Scavengers had the lowest frequency from all three sites, NU (0%), SU (0%), and Buried (2.27%). In the North Unburied site, carnivores were most dominant out of the 64 observed species with omnivores and piscivores representing the next two most abundant groups, very closely behind were herbivores (Table 2). In the Buried site, carnivores were most frequently represented (52.27%), followed by herbivores (20.45%), then omnivores (15.91%), planktivores (6.82%), piscivores (2.27%), and lastly scavengers (2.27%). The buried site was the only of the three to have scavenger trophic representation. The South Unburied location had carnivores has the highest frequently observed trophic group, followed omnivores, then herbivores, planktivores and piscivores respectively (Table 2).

*Table 2: Trophic breakdown based on 64 identified species. Each of the three sampling uses their own species richness count to determine trophic representation percentages.*

| Trophic Ecology | NU spp. Counts | NU Pct. (out of 45spp) | Buried spp. Counts | Buried Pct. (out of 43spp) | SU spp. Counts | SU Pct. (out of 29spp) | Total Counts (overall) | Total Pct. (out of 64spp) |
|---|---|---|---|---|---|---|---|---|
| **Omnivore** | 8 | 17.78% | 7 | 15.91% | 7 | 24.14% | 11 | 17.19% |
| **Carnivore** | 21 | 46.67% | 23 | 52.27% | 11 | 37.93% | 32 | 50.00% |
| **Herbivore** | 7 | 15.56% | 9 | 20.45% | 5 | 17.24% | 11 | 17.19% |
| **Piscivore** | 4 | 8.89% | 1 | 2.27% | 2 | 6.89% | 4 | 6.25% |
| **Scavenger** | 0 | 0% | 1 | 2.27% | 0 | 0% | 1 | 1.56% |
| **Planktivore** | 5 | 11.11% | 3 | 6.82% | 4 | 13.79 | 5 | 7.81% |

**Trophic Community Composition Based on Overall Fish Abundance**

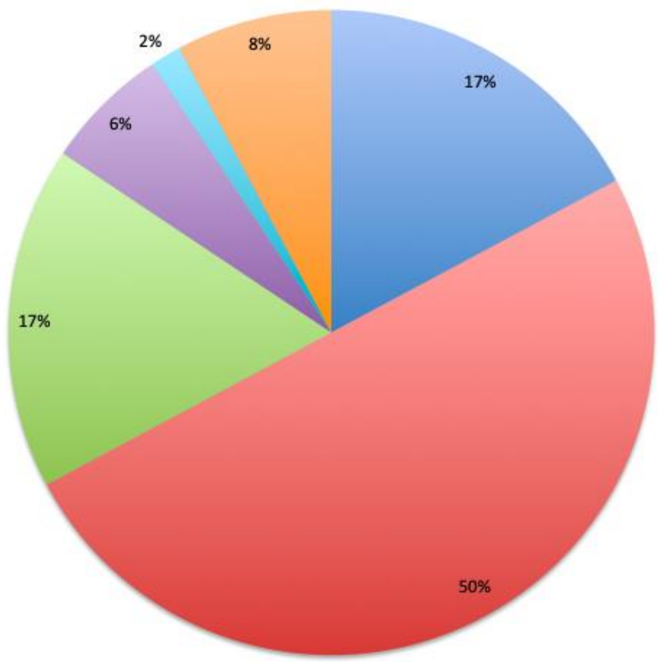


*Figure 9: Trophic community compositions from the three sampling regions and one overall plot. From the top left over and down: Overall composition, South Unburied, Buried, and North Unburied. Red = carnivores, Green = herbivore, Purple = Piscivore, Blue = Omnivore, Orange = Planktivore and Teal = Scavenger.*

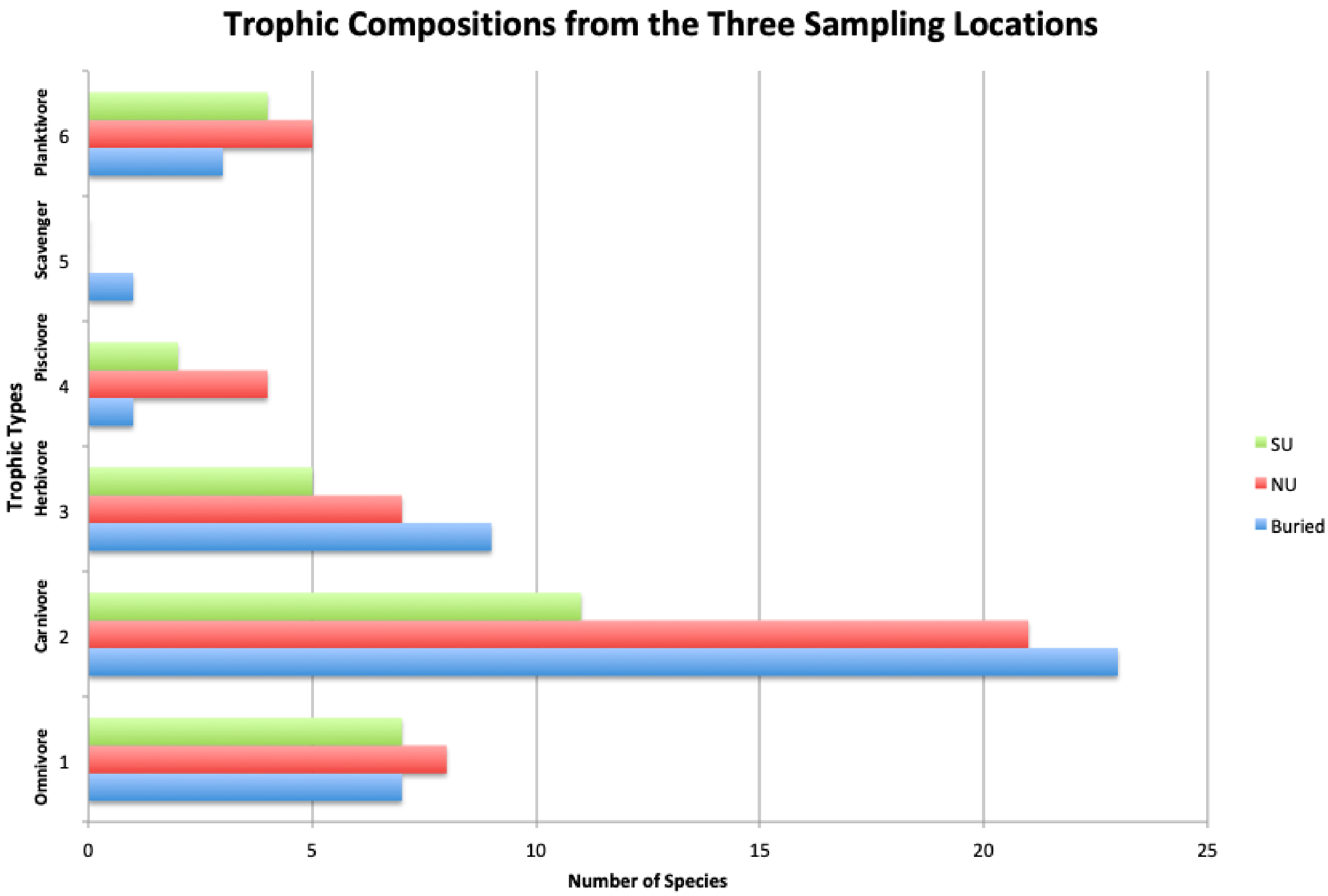


*Figure 10: Trophic compositions from the three sampling locations. The south unburied site is represented in green, the north unburied site is represented in red, and the buried site is represented in blue.*

*Table 3: Comprehensive table of all observed species and their respective trophic groups. Total abundance is based on counts from all sites. If the individual was not able to be identified down to exact genus and species, family name was listed.*
*C = carnivore, O = omnivore, H = herbivore, Pl = planktivore, Pi = Piscivore, S = scavenger*

| Common Name | Scientific Name | Trophic Group | NU Abund. | Buried Abund. | SU Abund. | Total Abundance |
|---|---|---|---|---|---|---|
| Ballonfish | *Diodon holocanthus* | C | 0 | 2 | 0 | 2 |
| Bicolor Damsel | *Stegastes partitus* | O | 43 | 59 | 47 | 149 |
| Bigeye | *Priacanthidae* | C | 5 | 1 | 0 | 6 |
| Black Margate | *Anisotremus surinamensis* | C | 1 | 0 | 0 | 1 |
| Blue Angel | *Holocanthus bermudensis* | O | 0 | 2 | 2 | 4 |
| Blue Chromis | *Chromis cyanea* | C | 36 | 2 | 11 | 49 |
| Blue Tang | *Acanthurus coeruleus* | H | 25 | 3 | 1 | 29 |
| Bluehead Wrasse | *Thalassoma bifasciatum* | Pl | 14 | 4 | 3 | 21 |
| Bucktooth Parrotfish | *Sparisoma radians* | H | 3 | 3 | 0 | 6 |
| Caesar Grunt | *Haemulon carbonarium* | C | 1 | 4 | 0 | 5 |
| Creole Wrasse | *Clepticus parrae* | Pl | 1 | 0 | 0 | 1 |
| Chalk Bass | *Serranus tortugarum* | Pl | 5 | 13 | 3 | 21 |
| Cherub Fish | *Centropyge argi* | O | 0 | 0 | 1 | 1 |
| Cornet Fish | *Fistularia* | C | 0 | 4 | 0 | 4 |
| Damsel (unidentifiable) | *Pomacentridae* | O | 1 | 0 | 0 | 1 |
| Doctorfish | *Garra rufa* | H | 0 | 4 | 0 | 4 |
| Foureye Butterfly fish | *Chaetodon capistratus* | C | 0 | 0 | 1 | 1 |
| French Angel | *Pomacanthus paru* | O | 0 | 0 | 2 | 2 |
| Glass eye Snapper | *Heteropriacanthus cruentatus* | Pi | 0 | 2 | 0 | 2 |
| Greater Soap Fish | *Rypticus saponaceus* | C | 2 | 0 | 0 | 2 |
| Green Turtle | *Chelonia mydas* | H | 0 | 1 | 0 | 1 |
| Grey Snapper | *Lutjanus griseus* | Pi | 1 | 0 | 0 | 1 |
| Grey Angel | *Pomacanthus arcuatus* | O | 6 | 2 | 1 | 9 |
| Grouper | *Epinephelinae* | C | 0 | 1 | 0 | 1 |
| Hamlet | *Hypoplectrus* | Pi | 1 | 0 | 1 | 2 |
| Hogfish | *Lachnolaimus maximus* | C | 8 | 3 | 0 | 11 |
| Jacks | *Carangidae* | Pi | 7 | 12 | 2 | 21 |
| Juvenile Bluehead | *Thalassoma bifasciatum* | C | 0 | 1 | 0 | 11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Wrasse | | | | | | |
| Labrid Wrasse | *Cheilinus undulatus* | C | 0 | 0 | 1 | 1 |
| Lionfish | *Pterois volitans* | C | 2 | 0 | 2 | 4 |
| Mutton Snapper | *Lutjanus analis* | C | 1 | 3 | 0 | 4 |
| Nurse Shark | *Ginglymostoma cirratum* | C | 0 | 0 | 1 | 1 |
| Ocean Surgeon | *Acanthurus bahianus* | H | 42 | 37 | 11 | 90 |
| Orange Spotted Filefish | *Oxymonacanthus longirostris* | C | 0 | 1 | 0 | 1 |
| Parrotfish (unidentifiable) | *Sparisoma* | H | 7 | 11 | 3 | 21 |
| Porgy | *Chrysphrys major* | C | 2 | 2 | 1 | 5 |
| Porkfish | *Anisotremus virginicus* | C | 51 | 11 | 0 | 62 |
| Princess Parrotfish | *Scarus taeniopterus* | H | 2 | 3 | 0 | 5 |
| Purple Reef Fish | *Pentapodus nemurus* | Pl | 10 | 0 | 3 | 13 |
| Queen Angel | *Holocanthus ciliaris* | O | 1 | 6 | 3 | 10 |
| Redfin Parrotfish | *Sparisoma rubripinne* | H | 0 | 2 | 0 | 2 |
| Reef Butterfly | *Chaetodon sedentarius* | C | 20 | 19 | 3 | 42 |
| Remora | *Echeneidae* | S | 0 | 1 | 0 | 1 |
| Rockbeauty | *Holocanthus tricolor* | O | 8 | 12 | 2 | 22 |
| Sandtile Fish | *Malacanthus plumieri* | C | 1 | 0 | 0 | 1 |
| Scrawled Filefish | *Aluterus scriptus* | C | 1 | 0 | 1 | 2 |
| Sharp Nose Puffer | *Canthigaster rostrata* | O | 1 | 4 | 0 | 5 |
| Spadefish | *Chaetodipterus faber* | C | 0 | 2 | 0 | 2 |
| Spanish Hogfish | *Bodianus rufus* | | 3 | 1 | 2 | 6 |
| Spotted Goatfish | *Pseudupenus maculatus* | C | 4 | 12 | 0 | 16 |
| Spotfin Butterflyfish | *Chaetodon ocellatus* | C | 4 | 3 | 0 | 7 |
| Scrawled Cowfish | *Acanthostracion quadricornis* | O | 2 | 0 | 0 | 2 |
| Squirrelfish | *Holocentridae* | C | 1 | 1 | 0 | 2 |
| Stoplight Parrotfish | *Sparisoma viride* | H | 2 | 3 | 0 | 5 |
| Striped Parrotfish | *Scarus iseri* | H | 3 | 1 | 1 | 5 |
| Sunshine | *Chromis insolata* | Pl | 10 | 4 | 1 | 15 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Chromis | | | | | | |
| Tobacco Fish | *Serranus tabacarius* | C | 1 | 0 | 1 | 2 |
| Trunkfish | *Lactophyrs bicaudalis* | O | 2 | 0 | 0 | 2 |
| White Grunt | *Haemulon plumierii* | C | 2 | 2 | 0 | 4 |
| Wrasse (unidentifiable) | *Labridae* | C | 2 | 14 | 2 | 18 |
| Yellow Stingray | *Urobatis jamaicensis* | C | 1 | 2 | 0 | 3 |
| Yellow Tang | *Zebrasoma flavescens* | H | 0 | 0 | 1 | 1 |
| Yellowhead Wrasse | *Halichoeres garnoti* | C | 2 | 2 | 0 | 4 |
| Yellowtail Snapper | *Ocyurus chrysurus* | Pi | 1 | 0 | 0 | 1 |

*Table 4: Top 11 most abundant species that contributed to more than 1% to the abundance. Total counts include counts from all transect types and locations. Total percentage was calculated by total abundance of that species divided by total individuals abundance (i.e. x/743).*

| Top 11 Species Most Abundantly Observed | | | | |
|---|---|---|---|---|
| Common Name | Family | Trophic Group | Total Abundance | Percentage to Total Individuals |
| Bicolor Damsel | Pomacentridae | Omnivore | 149 | 20.05% |
| Ocean Surgeon | Acanthuridae | Herbivore | 90 | 12.11% |
| Porkfish | Haemulidae | Carnivore | 62 | 8.34% |
| Blue Chromis | Pomacentridae | Carnivore | 49 | 6.59% |
| Reef Butterflyfish | Chaetonidae | Carnivore | 42 | 5.65% |
| Blue Tang | Acanthuridae | Herbivore | 29 | 3.90% |
| Rock Beauty | Pomacanthidae | Omnivore | 22 | 2.96% |
| Blueheaded Wrasse | Labridae | Planktivore | 21 | 2.83% |
| Chalk Bass | Serranidae | Planktivore | 21 | 2.83% |
| Jack | Carangidae | Piscivores | 21 | 2.83% |
| Parrotfish | Scaridae | Herbivore | 21 | 2.83% |

***SIMPROF***

A SIMPROF test with a cluster plot will look if there are any groupings that can be made from the data, in our case, it uses the similarity between species counts from each transect type in order to form groups regardless of any other factors. Using the SIMPROF as parameters for the cluster analysis gave the SIMPROF a significance level of 5% ($p = 0.05$). The SIMPROF similarity profile used the data to produce a significance of 0.1% ($p<0.05$), indicating there is significant similarity amongst species-transect groupings. As depicted in figure 8, red lines connecting samples are not significantly differentiated by SIMPROF, those connected by black lines are the focus of the dendrogram. The high level of similarity amongst transects is why nearly all groups are connected by red lines, note that the NUDSO transect type is in black meaning it is significantly different compared to the other transect types and their respective species groupings.

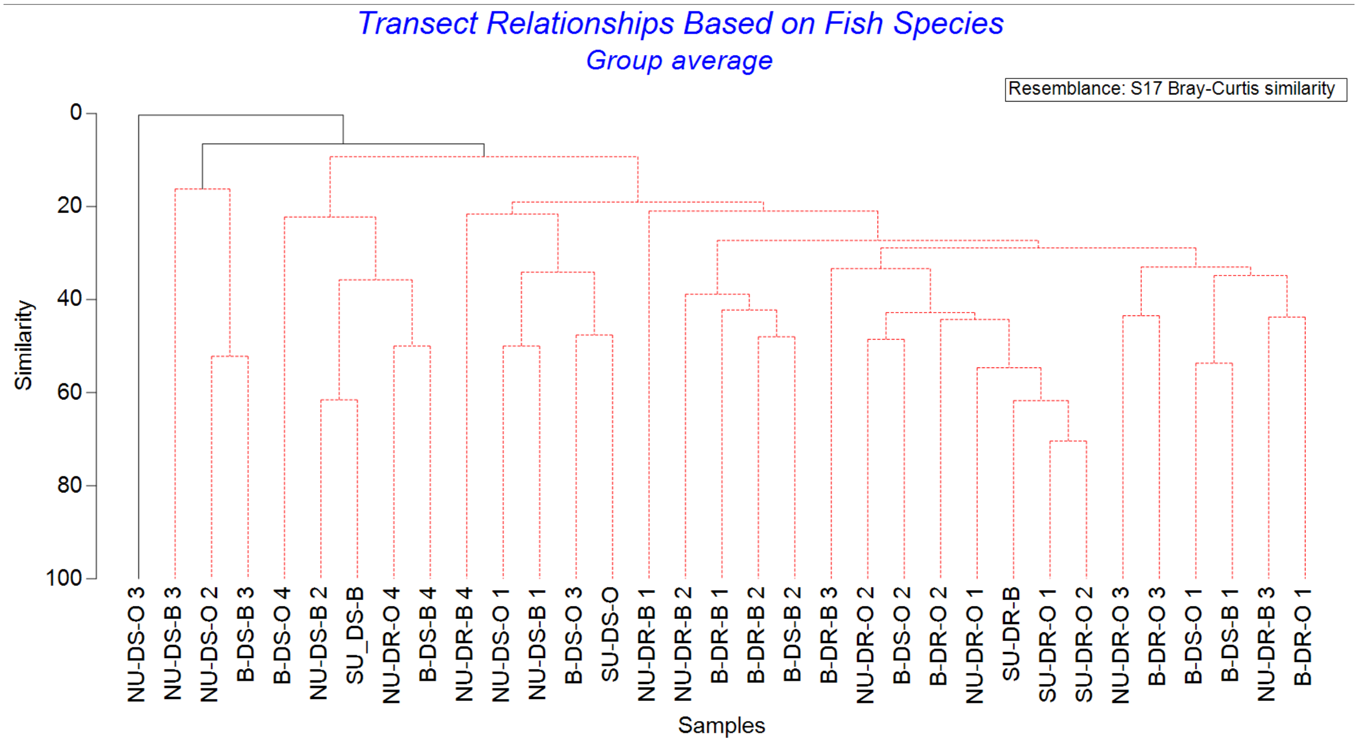


*Figure 11: Cluster Plot representing similarity amongst all transects.*

### *Kruskal-Wallis*

The top five most abundant species were used in this test to represent the population as a whole, and data was treated as non-parametric. A Kruskal-Wallis test indicated that "site" as a factor was a significant influencer of species counts ($p=0.02826 <0.05$) and a separate Kruskal-Wallis test indicated that whether or not the transect was baited did not have a significant affect on species count ($p=0.5999 >0.05$). Given that only the sampling site location served as a significant influence only that factor was needed to run a post-hoc test, in which case, none of the three sites test significantly different from one another. Figure 9 shows how the top five species shape the three site locations. As shown in the plot, the south unburied site has a lower average than the other two sites.

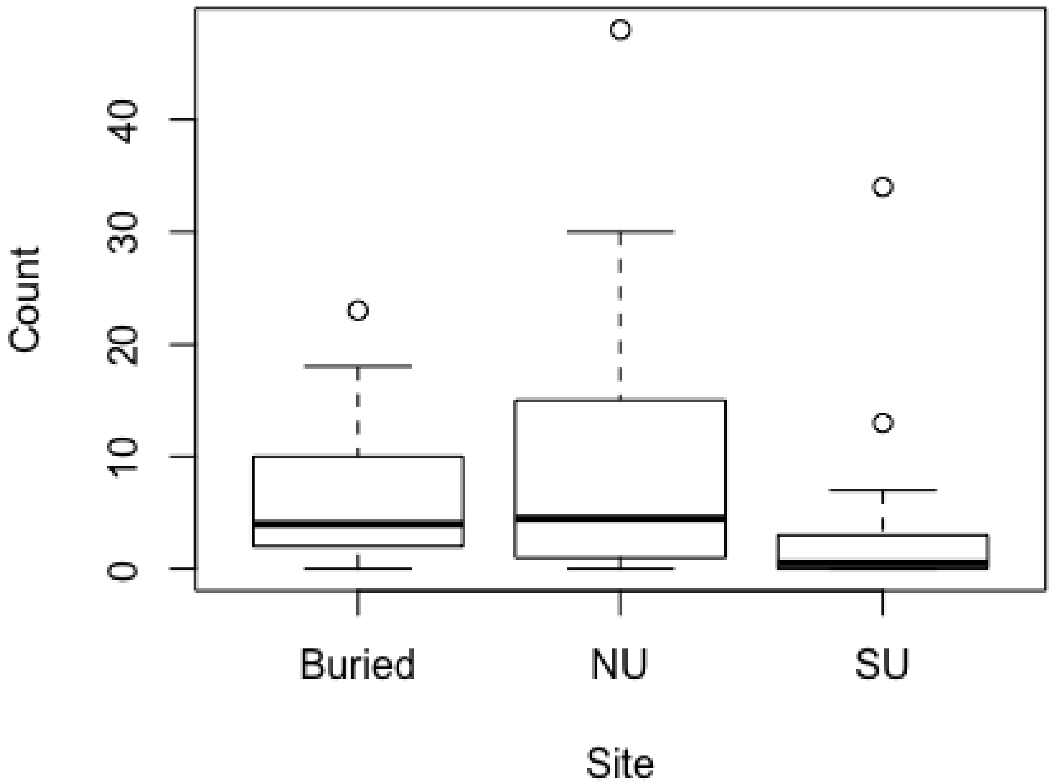


*Figure 12: A boxplot depicting the three sampling locations (NU, SU, and Buried) based on their counts from the top five most abundant species.*

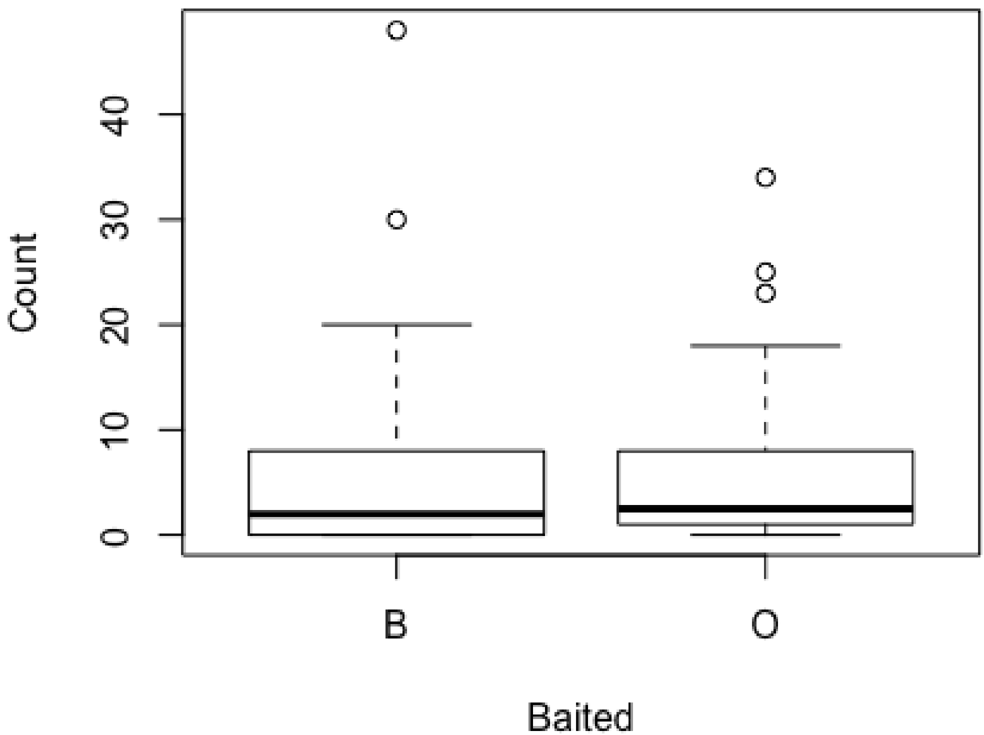


*Figure 13: A boxplot depicting baited and non-baited surveying types using the top five most abundant species.*

***nMDS***

A Multi-dimensional Scaling plot, or MDS, is a means of visualizing levels of similarity for individuals in a data set represented by distances amongst plotted points. Points closest together will therefore have high levels of similarity while points farther apart will show less similarity amongst their species richness. MDS stress values indicate if the ordination is suspect or not, data from the 3-d ordination tested as a good fit, 0.05; data from the 2-d ordination tested as fair, 0.14. These stress levels indicate points fitting closely on the plots indicating similarity amongst transects.

Figure 12 shows the nMDS with a focus on baited versus un-baited transects. Baited transects are shown in red and un-baited transects are shown in blue. Generally, there does appear to be major clustering seen in Figure 12 (stress level 0.14) meaning baited and non-baited transects did appear to have any major overlap on species richness. Figure 13 shows the nMDS with a focus on Deep Ridge versus Deep Sand transect types. Figure 13 has a stress value of 0.14 indicating similarity amongst points. This stress level means no major differences were found amongst the two depth sites.

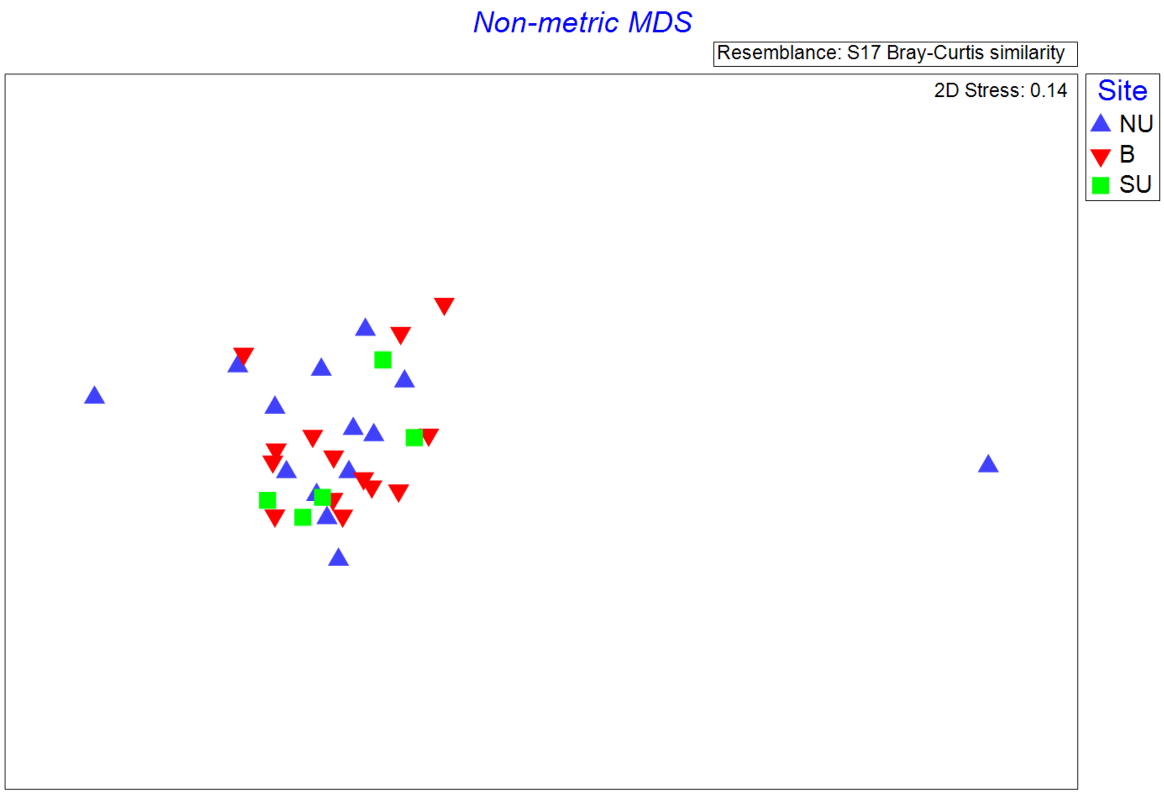


*Figure 14: Non-metric Multidimensional Plot shown in 2-D scale. Transect type is represented by a unique color and shape. Points closest together represent high similarity amongst fish species richness. Data was based on Bray-Curtis similarity matrix.*

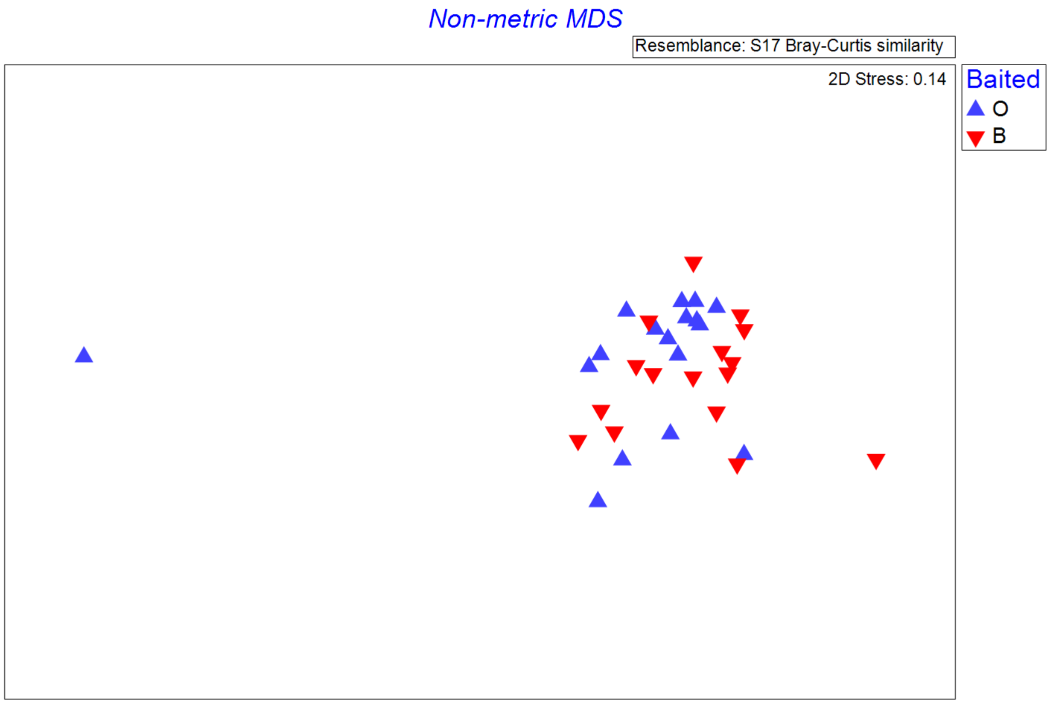


*Figure 15: A non-metric Multidimensional Plot on a 2-D scale. Red symbols indicate baited transect types, and blue symbols indicate un-baited transect types. Data resemblance was based on Bray-Curtis similarity matrix. Stress value = 0.14.*

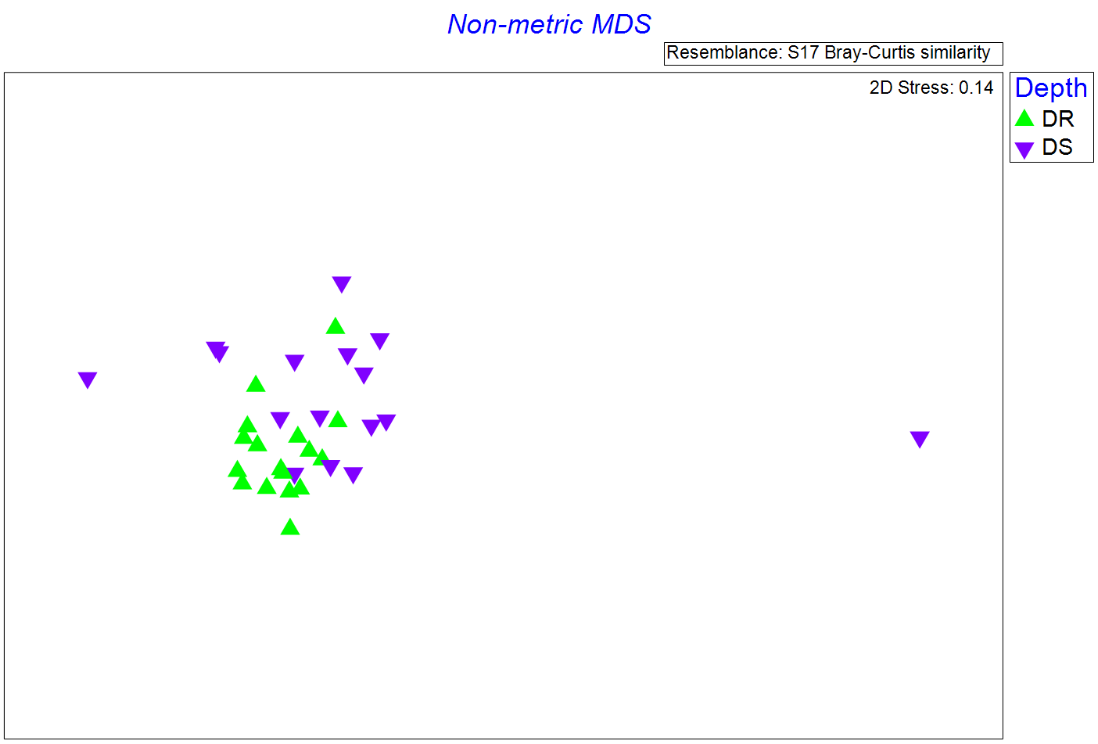


*Figure 16: A non-metric Multidimensional Plot on a 2-D scale. Green symbols indicate Deep Ridge transect types, and purple symbols indicate Deep Sand transect types. Data was resemblance was based on Bray-Curtis similarity matrix. Stress value = 0.14.*

### ***ANOSIM***

The Analysis of Similarities, or ANOSIM, tests to see significant differences between two or more groups; in our case the ANOSIM will test between the different transect types, depth factors, and site locations. A 3-way fully crossed ANOSIM was run, since the baited factor (O or B) occurred at both depth levels (DS or DR) and both depth levels occurred at all three site locations (NU, SU, B). The 3-way ANOSIM showed that the significance level of the sample statistic was 87.1% (R = -0.116), indicating the variables in combination do not have a significant affect on the samples. The R value assesses how well the relationship of variables can be described, this value is showing that the three variables do have a strong similarity or high overlap to one another in the current set up ($0.1 < R < 0.25$).

From this three 2-way crossed ANOSIMs were run pairing each variable together instead of in all in conjunction. The Site versus Depth 2-way ANOSIM showed the significance level of the sample statistic as 64.7% (Site groups across Depth groups, $R = -0.038$) and 2.7% (Depth groups across Site groups, $R = 0.14$). The Site versus Baited 2-way ANOSIM had a significance level of 63.1% (Site groups across Baited groups, $R = -0.031$) and 62.9% (Baited groups across Site groups, $R = -0.022$). The Baited versus Depth 2-way ANOSIM had a significance level of 38.6% (Depth groups across Baited groups, $R = 0.012$) and 0.3% (Baited groups across Depth groups, $R = 0.201$).

### ***SIMPER***

A SIMPER test allows for the similarity amongst groups to be test and displayed as percentages. A two-way analysis was run using Baited (O and B) as well as Depth (DR and DS), alone the DR depth across all baited groups had a similarity of 28.54%. The DS depth across all baited groups showed a similarity of 12.80%. When the two depths were compared to one another, DR and DS had a dissimilarity of 84.73%. From these results we see when the factor DS is included the dissimilarity percentage increases insinuating the DS depth is the more dissimilar to the other depth, DR.

Baited groups (B) has a similarity across all sampling sites of 18.92%. Non-baited groups(O) had a similarity across all sampling sites of 23.18%. O and B groups together had a dissimilarity across all sampling sites of 78.84%.

A one-way SIMPER showing similarity percentages using baited or non-baited factors showed that baited and non-baited surveying had a dissimilarity of 81.75% (Table 5). Individually, the baited survey had a 16.30% similarity across transects and the un-baited survey had a similarity of 19.59%.

*Table 5: SIMPER analysis table depicting the dissimilarity between Baited and Non-Baited groups and what species contribute ~70% to the dissimilarity.*

| Baited Groups: O & B<br>Average Dissimilarity = 81.76% | | | | | |
|---|---|---|---|---|---|
| Species | Group O Avg. Abund. | Group B Avg. Abund. | Avg. Dissimilarity | Contrib. % | Cum. % |
| Bicolor Damsel | 5.47 | 3.63 | 13.28 | 16.24 | 16.24 |
| Ocean Surgeon | 3.24 | 2.19 | 7.36 | 9.37 | 25.61 |
| Jack | 0.35 | 0.94 | 4.39 | 5.37 | 30.98 |
| Reef Butterflyfish | 1.29 | 1.25 | 3.98 | 4.87 | 35.85 |
| Blue Tang | 0.65 | 1.13 | 3.89 | 4.76 | 40.62 |
| Porkfish | 0.65 | 3.19 | 3.75 | 4.58 | 45.20 |
| Blue Chromis | 0.71 | 2.31 | 3.64 | 4.45 | 49.65 |
| Chalk Bass | 0.82 | 0.56 | 3.55 | 4.34 | 53.99 |
| Wrasse | 0.82 | 0.44 | 3.32 | 4.07 | 58.06 |
| Bluehead Wrasse | 0.29 | 0.94 | 2.45 | 2.71 | 61.05 |
| Rockbeauty | 0.88 | 0.44 | 2.22 | 2.71 | 63.76 |
| Hogfish | 0.35 | 0.44 | 2.11 | 2.58 | 66.34 |
| Bigeye | 0.35 | 0.00 | 1.93 | 2.36 | 68.70 |
| Parrotfish | 0.88 | 0.13 | 1.74 | 2.13 | 70.83 |

## Discussion

Based on results, similarities amongst species were seen across all transects with high overlap regardless of factor. A few reasons could have attributed to this, current patterns, seasonality changes, anthropogenic interference, or lack of transects from the each of the areas. The local Navy branch conducting testing in the southern region could effect what fish species are able to reside there and may cause species to congregate more in the buried or north unburied sites. The counter current running north may also affect what food availability is in the three sites skewing species more towards one particular site location over another. Moustaka et al. (2018) found that species richness in sampled areas that experienced high sedimentation impacts had lower richness and changes in biomass, if that is applied to our results we could assume that the southern site is still facing sedimentation impact perhaps due to the nearby port and that is why the diversity in that region was different. Ultimately, the hypothesis stating there would be a difference between species composition in buried versus un-buried sites was not accepted; the buried site shared high similarity to the north unburied and southern unburied site meaning there were other factors influencing the three sampling sites community structure.

Baited versus un-baited surveying did not show any significant difference between sampling methods (*Kruskal-Wallis, $p=0.5999 >0.05$)*. We could infer this from a few things: if not enough bait was used to significantly attract fish, type of bait used was not preferable, or current trophic structure of the habitat did not call for bait type. The dominating trophic group being carnivores across all sampling sites (figure 7) could have been from using baited surveys in the area but similar to other studies carnivorous species are typically more prominent at this depth (Asher et al. 2017, Abesamis et al. 2018, Oliveira Soares et al. 2018). In which case, we will accept the null hypothesis: there is no difference between fish communities between baited and non-baited ROV surveys.

In the 3-way crossed and 2-way crossed ANOSIMS we see the fully crossed 3-way ANOSIM give non-significant results, meaning the three variables in conjunction with one another do not have a strong relationship that affects the samples (fish species). This also means that the three factors share high overlap in the fish richness, and a high significance level percentage indicates that this is mostly not due to chance. The 2-way crossed ANOSIM of Site groups and Depth groups is also do not have a significant relationship, this is not unexpected because the two depth groups, DR and DS, are less than 10m of depth apart. For this result to have been significant perhaps a larger difference in depth would have had to been sampled (i.e. 30m and 60m). In the 2-way ANOSIMS we see that whenever the Baited groups are in the picture the pattern shows significance. Most likely we can say that Depth is marginal in its effects on the data, or at least the depths chosen in this study; Site groups are not significant unless Depth or Baited groups are accounted for, but taken together is potentially suggestive of weak spatial effects.

The results from the ANOSIMs are further backed from the results of the SIMPER. Here we saw how, again, Depth groups have a marginal affect but only when other variables are present. Similar interpretations can be made on the SIMPER's Baited results, Baited groups are more affected when other variables are present. That being said, we do see these pattern form throughout the results but overall the studies sample size is relatively small to extrapolate for an entire area. Our results not yet strong results due to this fact but it still shows potential as to how we might expect these factors to act.

MCEs do show similarities to SWR assemblages as well as community shifts, mainly in regards to overall species richness and diversity (Andradi-Brown et al. 2016, Asher et al. 2017, Abesamis et al. 2018, Oliveira Soares et al. 2018). Species richness and frequency of occurrence results were further compared to data collect by the National Coral Reef Monitoring Program (NCRMP) (Kilfoyle et al. 2018). The NCRMP surveys fish and benthic fauna in the south Florida reef tract. NCRMP data will be representative of species richness and frequency of occurrence from ~320 shallow reef sites in the same sampling location as the proposed study.

NCRMP data was collected over a five-year period (2012 through 2016) and comprised of 305 fish species (70 families). Sites where sample collection took place were all shallower than 33m, approximately just before the mesophotic reefs begin. The studies top ten most frequently reported species included: Sharpnose pufferfish (*Canthigaster rostrata*), Bluehead Wrasse (*Thalassoma bifasciatum*), Bicolor damselfish (*Stegastes partitus*), Ocean Surgeon (*Acanthurus bahianus*), Doctorfish (*Acanthurus chirurgus*), Yellowhead wrasse (*Halichoeres garnoti*), Redband Parrotfish (*Sparisoma aurofrenatum)*, Slippery Dick Wrasse (*Halichoeres bivittatus)*, Reef Butterflyfish (*Chaetodon sedentarius*), and Blue Tang (*Acanthurus coeruleus*).

Kilfoyle et. al. (2018) went on to report that depth was the lead influential factor for fish assemblage structure followed by geographic location. Depth being the lead structuring factor would be reason as to why data from NCRMP had major overlap between our data without being entirely similar. It is important to note that out of the 305 species observed in the NCRMP study, our study had three species not observed by NCRMP, yellow tang (*Zebrasoma flavescens*), glasseyed snapper (*Heteropriacanthus cruentatus*), and balloonfish (*Diodon holocanthus*).

As seen with the comparison from Kilfoyle's data (2018) the connectivity hypothesis is supported, but further data and a larger sample size representing more of the mesophotic depth will further accept (or reject) the idea that there is a high connectivity amongst reefs in the area. Based on current results, we will accept the alternative hypothesis: upper MCE fish communities are distinct from SWRs; we accept this hypothesis because although we have high overlap with SWR communities, not all species were represented in either depth range nor to the same degree of abundance indicating an overall community transition from SWR to MCE. Nine out of the ten most sampled species from the NCRMP surveys matched species we commonly saw in our study, with only one species not observed from their top ten. High connectivity between SWRs in the area and the upper MCEs may allow for these species to move into depths not previously documented. Additionally, we observed three species unique to our study not observed in the NCRMP survey, juvenile atlantic blue tang, balloonfish, and glass eyed snapper. This might be a sign of further recovery on these reefs since burial, the NCRMP study having ended in 2016 puts roughly five years between that survey and ours. Within this five year span additional species could have begun frequenting these depths accounting for our three additional species, also indicating that the MCEs are cable of recovery and expansion.

Abesamis et al. (2018) found both taxonomic and trophic structure differences between MCEs and SWRs near Apo Island (Indo-West Pacific), species richness and abundance of trophic groups declined with increasing depth. Asher et al. (2017) noted that several targeted fish species were more abundant in deeper waters to further highlight the importance of incorporating MCE roles in stock assessments and resource management. Similar to SWRs, MCEs allow for sponges, select corals, and algae growth which help support fish communities, so it is interesting to see studies report depauperate fish communities in MCEs (Lindfield et al. 2016, Asher et al. 2017, Abesamis et al. 2018).

Based on our results as well as results from similar studies the mesophotic zone truly serves as a transitional zone (Hinderstein 2010, Kilfoyle et al. 2013, Andradi-Brown et al. 2016, Abesamis et al. 2018, Abesamis et al. 2020). This habitat may not be entirely seen as an ecological transitional zone because of its high species overlap but a sort of SCUBA transitional zone. Meaning, due to the lack of anthropogenic use from this zone it shows reef recovery, species richness, and refugia purposes differently than habitats within the same depth limits. The definition of mesophotic is often hard to pinpoint because it shares many species with shallower and deeper depths, using community structure can not be the only way to identify the mesophotic zone. South Florida's mesophotic zone might start deeper than traditionally defined (starting at ~30m) because of our high level of connectivity to SWRs and high levels of anthropogenic interference. South Florida's MCE might actually start slightly deeper, ~40-50m depth, because that is where we would see even less overlap of fish species and even less likelihood of SCUBA and fishing. Although our 30m depth MCE is not the best representation of a transitional zone it still functions as one between the coastal SWRs and neighboring shelf drop-off. Slattery and Lesser (2012) made a statement in their study that although there is evidence to support the refugia potential of a MCE, there is not necessarily evidence to support that a MCE is more stable than SWRs. Our results agree with this statement, overlap and refugia evidence seems apparent in the research although we can not say that the area is any more resilient than SWRs.

It is important to note economically important species were surveyed at this depth, including lionfish (*Pterois volitans)*, hogfish (*Lachnolaimus maximus)*, grouper (*Epinephenlinae*), and mutton snapper (*Lutjanus analis).* Each of these species hold economic value in the fisheries in South Florida. To have surveyed these species at our sampling depths further solidifies our knowledge of what habitats they reside in and how we can use this for management strategies.

### *Biases*

Based on the methodology being implemented, it is important to address potential bias in the survey. The ROV itself is equipped with front facing white lights, this could alter fish behavior by either attracting fish or deterring them (Pagan & Appledoorn 2010, Yuh 2000). Additionally, the ROV during baited surveying is equipped with front facing baited mesh bags. This method of a moving baited transect could pose bias if during video review the same fish is counted multiple times as opposed to it following with the ROV and being counted once. To combat that, the camera was moving horizontally along the transect with a front facing camera view to narrow the chances of repeat counts. Additionally with the mobile BRUV style of sampling the potential for the bait plume to trail behind the ROV poses the chance of fish following behind the camera view. That could be reasoning as to why the baited surveying method didn’t show a large significance between surveying methods.

Due to the data composition used in this study, there is potential to have certain species under-represented if said species were cryptic or transient in the area or if the methodology used for the baited surveys was causing interference. This shouldn’t pose any bias currently because the relative proportions between species most likely would be unaffected and results, such as trophic structure, would still remain the same. Although this study took into account certain potential bias, similar set ups from other studies (Bejarano et al. 2015, Lindfield et al. 2016, Kilfoyle et al 2018, Abesamis et al. 2020) encourages the legitimacy of the results.

Finally, a limitation within our survey location means the survey will only be representative of the upper mesophotic (30-40m) and results cannot be extrapolated to represent the entire mesophotic reef system (30-150m). Biases from this study were deemed appropriate considering not all biases are avoidable, and biases from this study help to further show how ROV methodology is used in surveying.

## Conclusion

Mesophotic coral ecosystems are host to a multitude of fish species and may provide additional habitat space in conjunction with shallow water reefs. Many fish species observed in this study are commonly found in shallower depths further supporting the idea that SWRs share a high connectivity to MCEs. The possibility that mesophotic habitats can share as a refuge for shallow water species, or even simply sharing connectivity to these other habitats can highlight their importance in conservation and management.

Connectivity seen amongst sites indicates potential recovery from port dredging and burial in this specific area. No significant difference between buried and unburied sites is a positive sign that MCEs can recover from anthropogenic events and can aide in additional reef habitat to shallow species.

Using mini ROVs to conduct this research aides in expanding methodologies possible for these types of environments. ROVs as a sampling tool shows how different types of data can be collected in a timely and cost-effective manner. This is useful globally as research can be conducted in varying environments with ROVs allowing more research to be completed that otherwise may not have been plausible. The ability to survey using mobile-BRUVs opens a lot of opportunity to fish surveying.

To expand the research in the future, sampling lower mesophotic reefs (100m+) will better paint a comprehensive picture of the mesophotic community in the area. The lower mesophotic can also show connectivity to deeper, larger, or more transient fish species in the south Florida area. The research currently can not be used to describe the MCEs as a whole because sampling only took place in the upper mesophotic. Lower mesophotic sampling would better confirm the refugia hypothesis probability.

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# Appendix A

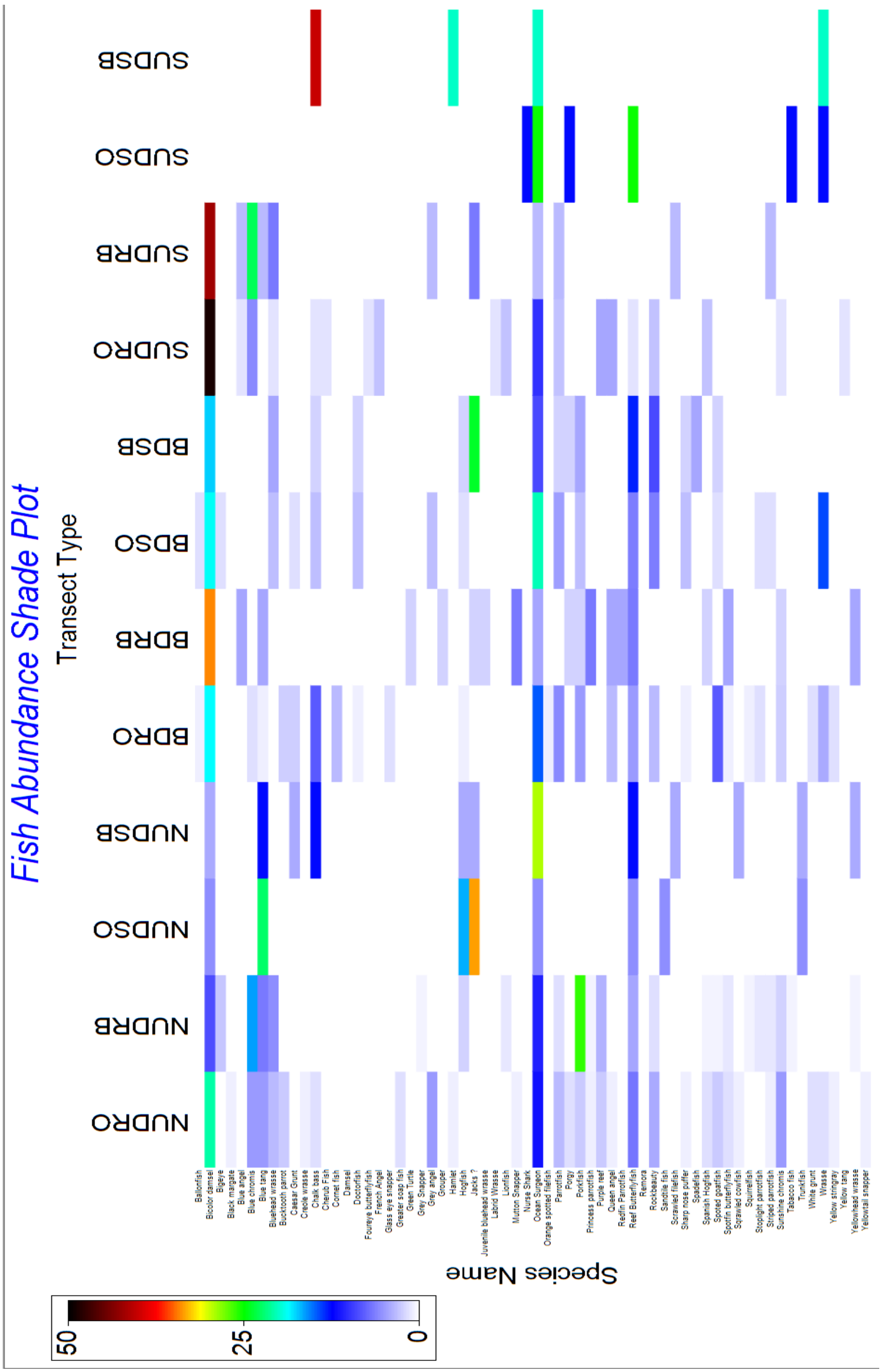
| FISH ABUNDANCE BY TRANSECT LOCATION | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NUDRO | NUDRB | NUDSO | NUDSB | BDRO | BDRB | BDSO | BDSB | SUDRO | SUDRB | SUDSO | SUDSB |
| Ballonfish | | | | | 1 | | 1 | | | | | |
| Bicolor damsel | 25 | 15 | 1 | 1 | 18 | 10 | 11 | 8 | 34 | 13 | | |
| Bigeye | | 5 | | | | | 1 | | | | | |
| Black margate | 1 | | | | | | | | | | | |
| Blue angel | | | | | | | | | 1 | 1 | | |
| Blue chromis | 6 | 30 | | | | | | | 4 | 7 | | |
| Blue tang | 4 | 1 | 4 | 3 | 1 | 2 | | | | 1 | | |
| Bluehead wrasse | 4 | 4 | | | | | 2 | 2 | 1 | 2 | | |
| Bucktooth parrot | 2 | | | | 2 | | | | | | | |
| Caesar Grunt | | | | 1 | 3 | | 1 | | | | | |
| Creole wrasse | 1 | | | | | | | | | | | |
| Chalk bass | | | | 3 | 1 | | | 1 | 1 | | | 2 |
| Cherub Fish | | | | | | | | | 1 | | | |
| Cornet fish | | | | | 4 | | | | | | | |
| Damsel | | | | | | | | | | | | |
| Doctorfish | | | | | | | 2 | 1 | | | | |
| Foureye butterflyfish | | | | | | | | | 1 | | | |
| French Angel | | | | | | | | | 2 | | | |
| Glass eye snapper | | | | | 2 | | | | | | | |
| Greater soap fish | 2 | | | | | | | | | | | |
| Grey Snapper | | 1 | | | | | | | | | | |
| Grey angel | 6 | | | | | | 2 | | | 1 | | |
| Hamlet | 1 | | | | | | | | | | | 1 |
| Hogfish | | 3 | 3 | 1 | 1 | | 1 | 1 | | | | |
| Jacks ? | | | 6 | 1 | | 1 | | 11 | | 2 | | |
| Juvenile bluehead wrasse | | | | | | 1 | | | | | | |
| Labrid Wrasse | | | | | | | | | 1 | | | |
| Lionfish | | | | | | | | | 2 | | | |
| Mutton Snapper | 1 | | | | | 3 | | | | | | |
| Nurse Shark | | | | | | | | | | | 1 | |
| Ocean Surgeon | 14 | 19 | 1 | 7 | 13 | 1 | 13 | 4 | 7 | 1 | 2 | 1 |
| Parrotfish | 4 | 3 | | | 5 | | 3 | 1 | 2 | 1 | | |
| Porgy | 2 | | | | | | | 1 | | | 1 | |
| Porkfish | 3 | 46 | | | 2 | | 2 | 2 | | | | |
| Princess parrotfish | 1 | 1 | | | | 3 | | | | | | |
| Purple reef | 3 | 7 | | | | | | | 3 | | | |
| Queen angel | 1 | | | | 3 | | | | 3 | | | |
| Reef Butterflyfish | 8 | 7 | 1 | 3 | 5 | 3 | 4 | 6 | 1 | | 2 | |
| Remora | | | | | | | | | | | | |
| Rockbeauty | 5 | 3 | | | 1 | | 4 | 4 | 2 | | | |
| Sandtile fish | | | 1 | | | | | | | | | |
| Scrawled filefish | | | | 1 | | | | | | 1 | | |
| Sharp nose puffer | | | | | 1 | | | 1 | | | | |
| Spadefish | | | | | | | | 2 | | | | |
| Spanish Hogfish | 2 | 1 | | | | | | | 2 | | | |
| Spoted goatfish | 3 | 1 | | | 8 | | 1 | 1 | | | | |
| Spotfin butterflyfish | 2 | 1 | | | 1 | | | | | | | |
| Sqrawled cowfish | 1 | | | 1 | | | | | | | | |
| Squirrelfish | | 1 | | | | | | | | | | |
| Stoplight parrotfish | | | | | 2 | | 1 | | | | | |
| Striped parrotfish | 1 | 2 | | | | | 1 | | | 1 | | |
| Sunshine chromis | 6 | 4 | | | 3 | 1 | | | 1 | | | |
| Tabacco fish | | 1 | | | | | | | | | 1 | |
| Trunkfish | | | 1 | 1 | | | | | | | | |
| White grunt | 2 | | | | | | | | | | | |
| Wrasse | 1 | | | | | | 5 | | | | 1 | 1 |
| Yellow stringray | 1 | | | | 1 | | | | | | | |
| Yellow tang | | | | | | | | | 1 | | | |
| Yellowhead wrasse | | 1 | | 1 | | 2 | | | | | | |
| Yellowtail snapper | 1 | | | | | | | | | | | |

# Appendix B

*The table below is a breakdown of all usable sampling days, sampling days that did not have viable data were not included in the table.*

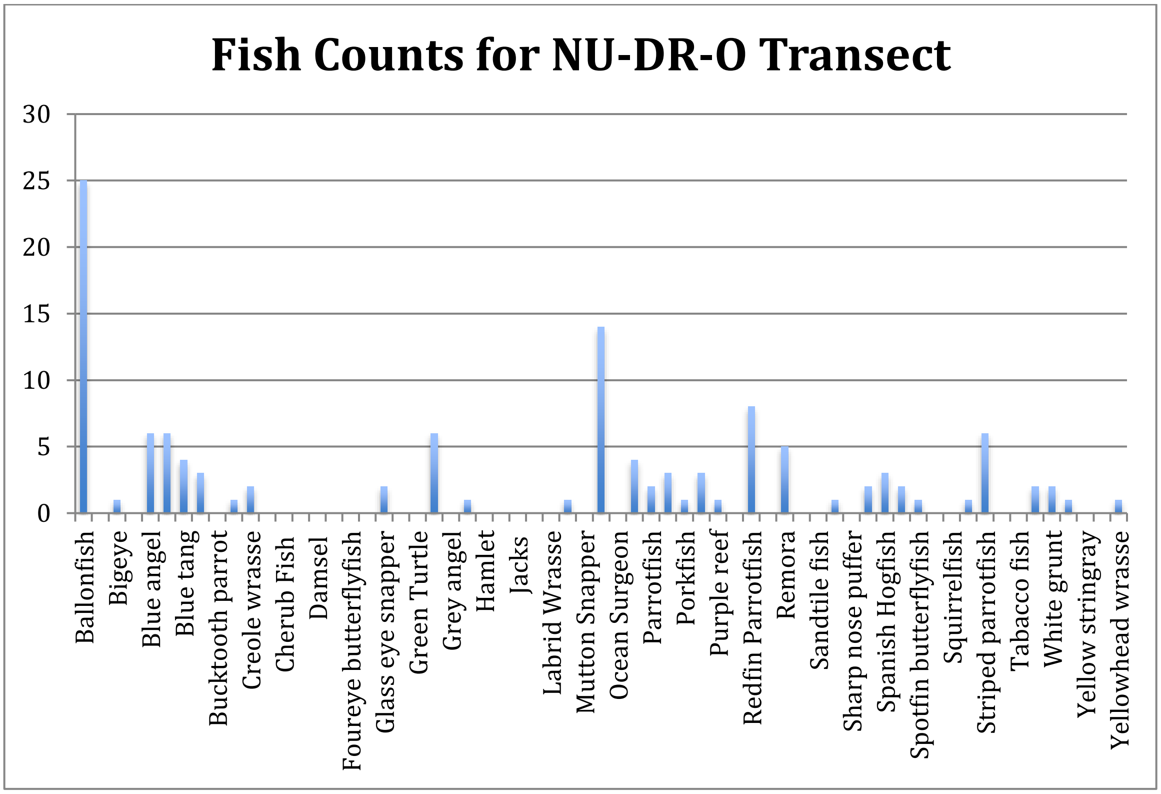
| SAMPLING DATE | TRANSECT TYPE | TRANSECT DURATION | NOTES |
|---|---|---|---|
| November 22, 2019 | SU-DR-B | 3:41 min | Navy Interference |
| | SU-DS-B | 4:17 min | |
| | SU-DR-O | 3:45 min | |
| | SU-DR-O | 3:40 min | |
| | SU-DS-O | 4:16 min | |
| | B-DS-O | 3:23 min | |
| | B-DR-O | 3:55 min | |
| | B-DR-B | 4:54 min | |
| | B-DS-B | 3:28 min | |
| | NU-DR-O | 3:33 min | |
| | NU-DS-O | 3:45 min | |
| | NU-DR-B | 3:34 min | |
| | NU-DS-B | 3:29 min | |
| June 25, 2020 | B-DR-B | 6:36 min | Navy Interference restricted access to SU site<br>Large sargassum patch |
| | B-DS-B | 6:37 min | |
| | B-DR-O | 7:47 min | |
| | B-DS-O | 6:34 min | |
| | NU-DR-B | 8:42 min | |
| | NU-DS-B | 4:00 min | |
| | NU-DR-O | 9:41 min | |
| | NU-DS-O | 9:00 min | |
| June 29, 2021 | NU-DR-O | 2:50 min | Plankton bloom in area<br>Strong currents |
| | NU-DS-O | 2:25 min | |
| | NU-DR-B | 2:10 min | |
| | NU-DS-B | 1:30 min | |
| | B-DS-O | 2:48 min | |
| | B-DS-B | 1:15 min | |
| August 03, 2021 | B-DR-O | 4:55 min | Large storm moved into area<br>Anchor caught on cables, had to cut line and stop sampling |
| | B-DS-O | 6:02 min | |
| | B-DR-B | 3:48 min | |
| | B-DS-B | 4:22 min | |
| | NU-DR-O | 5:12 min | |
| | NU-DR-B | 4:49 min | |

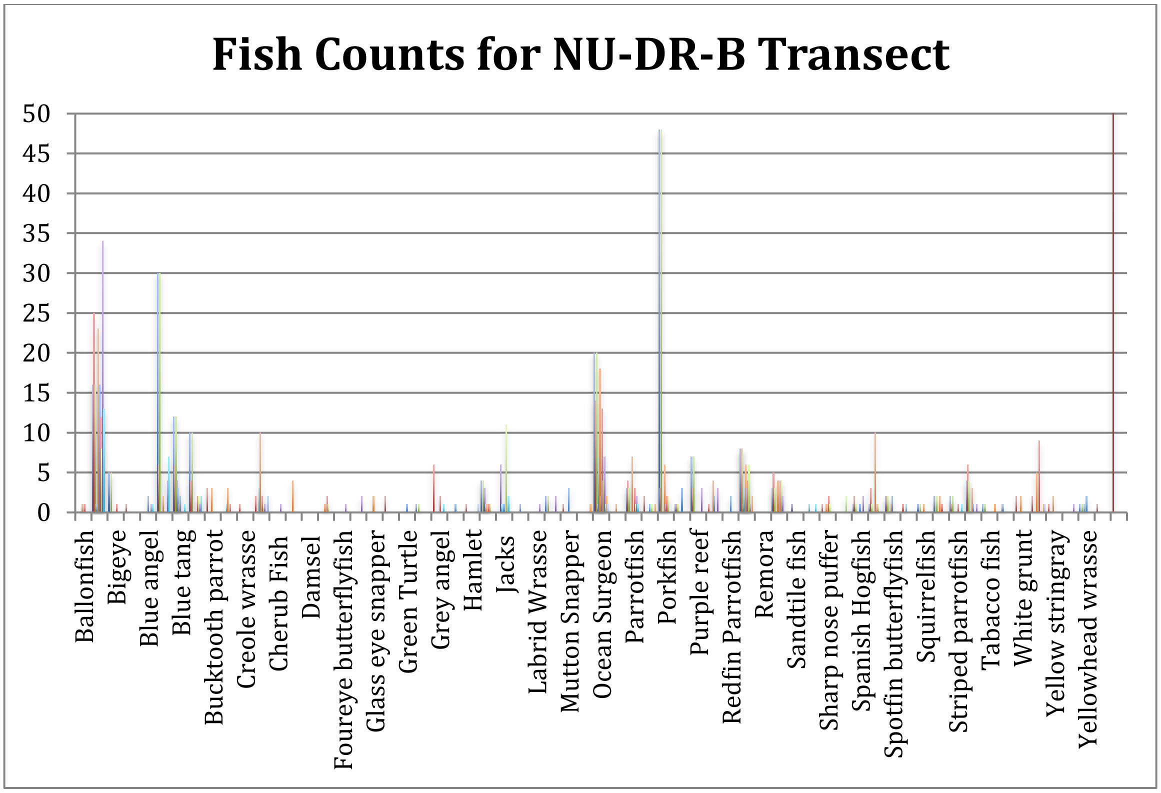
## Appendix C



Fish Abundance Shade Plot
Transect Type
NUDRO
NUDRB
NUDSO
NUDSB
BDRO
BDRB
BDSO
BDSB
SUDRO
SUDRB
SUDSO
SUDSB
Species Name
50
25
0

Appendix D


Fish Counts for NU-DR-O Transect
30
25
20
15
10
5
0
Ballonfish
Bigeye
Blue angel
Blue tang
Bucktooth parrot
Creole wrasse
Cherub Fish
Damsel
Foureye butterflyfish
Glass eye snapper
Green Turtle
Grey angel
Hamlet
Jacks
Labrid Wrasse
Mutton Snapper
Ocean Surgeon
Parrotfish
Porkfish
Purple reef
Redfin Parrotfish
Remora
Sandtile fish
Sharp nose puffer
Spanish Hogfish
Spotfin butterflyfish
Squirrelfish
Striped parrotfish
Tabacco fish
White grunt
Yellow stringray
Yellowhead wrasse


Fish Counts for NU-DR-B Transect
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Blue angel
Blue tang
Bucktooth parrot
Creole wrasse
Cherub Fish
Damsel
Foureye butterflyfish
Glass eye snapper
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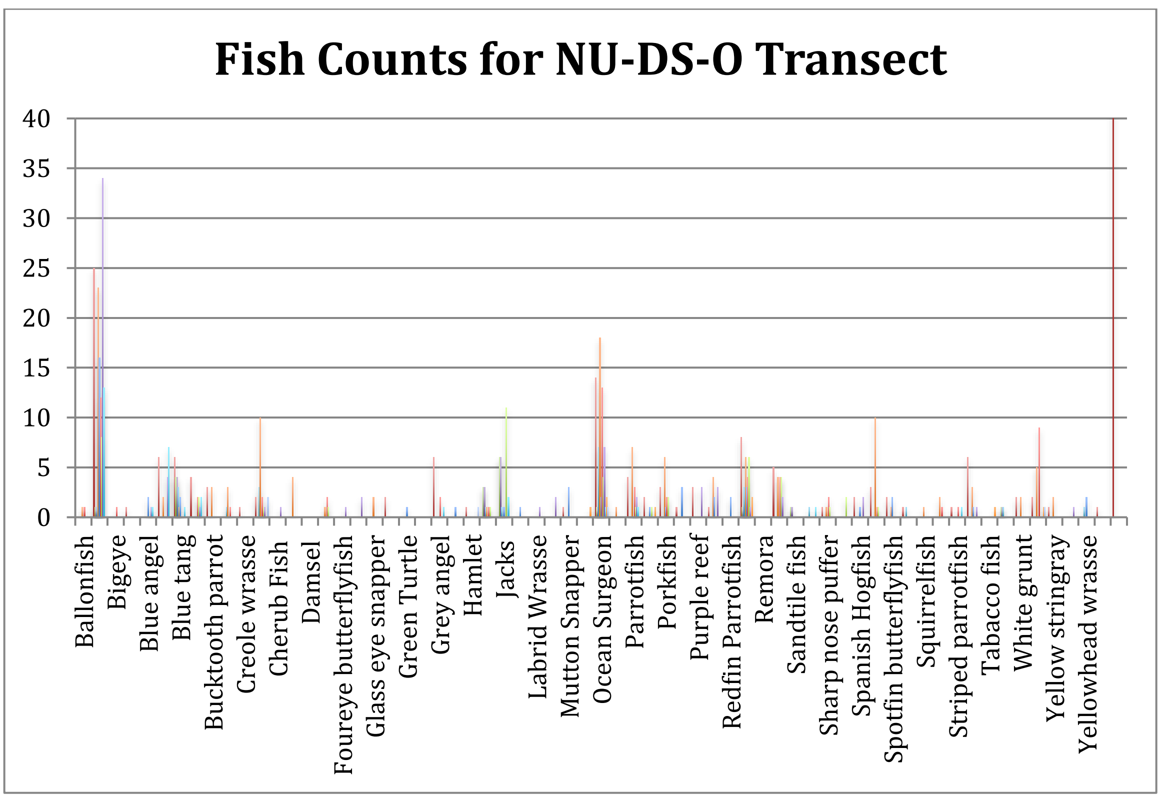
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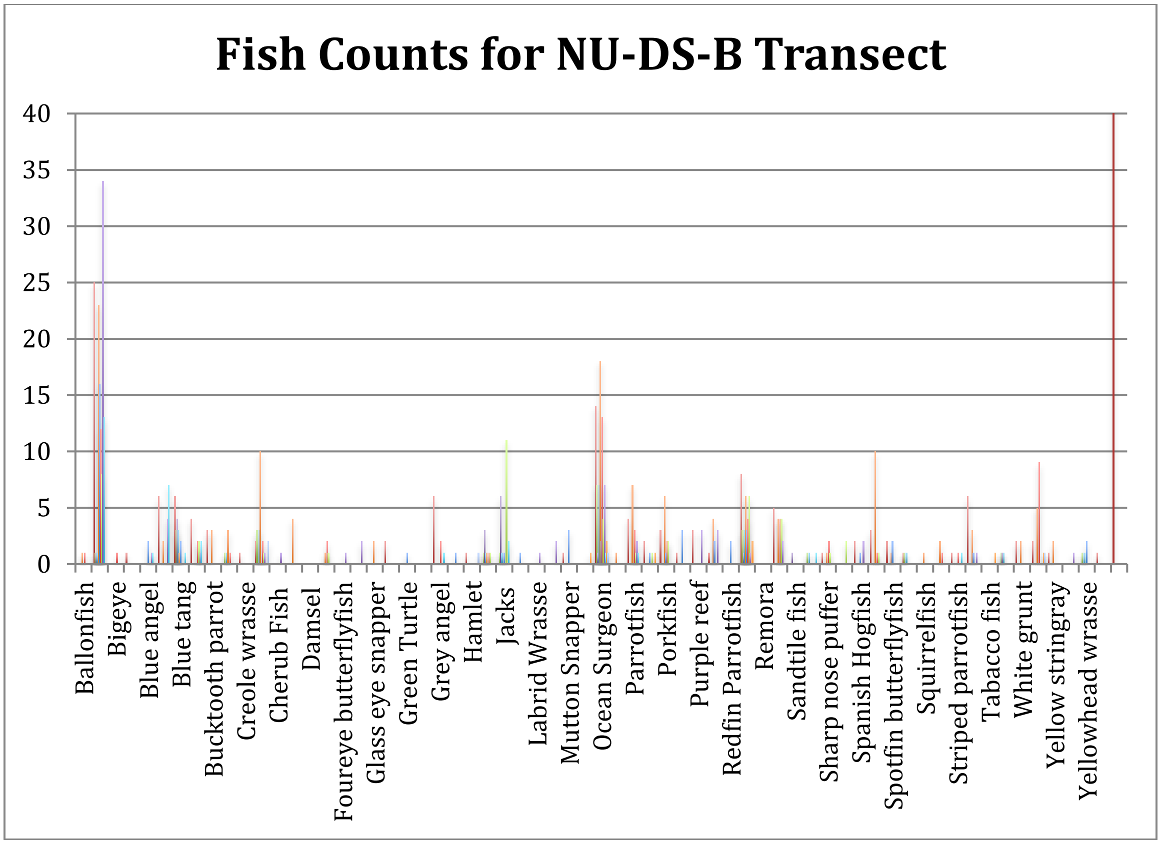
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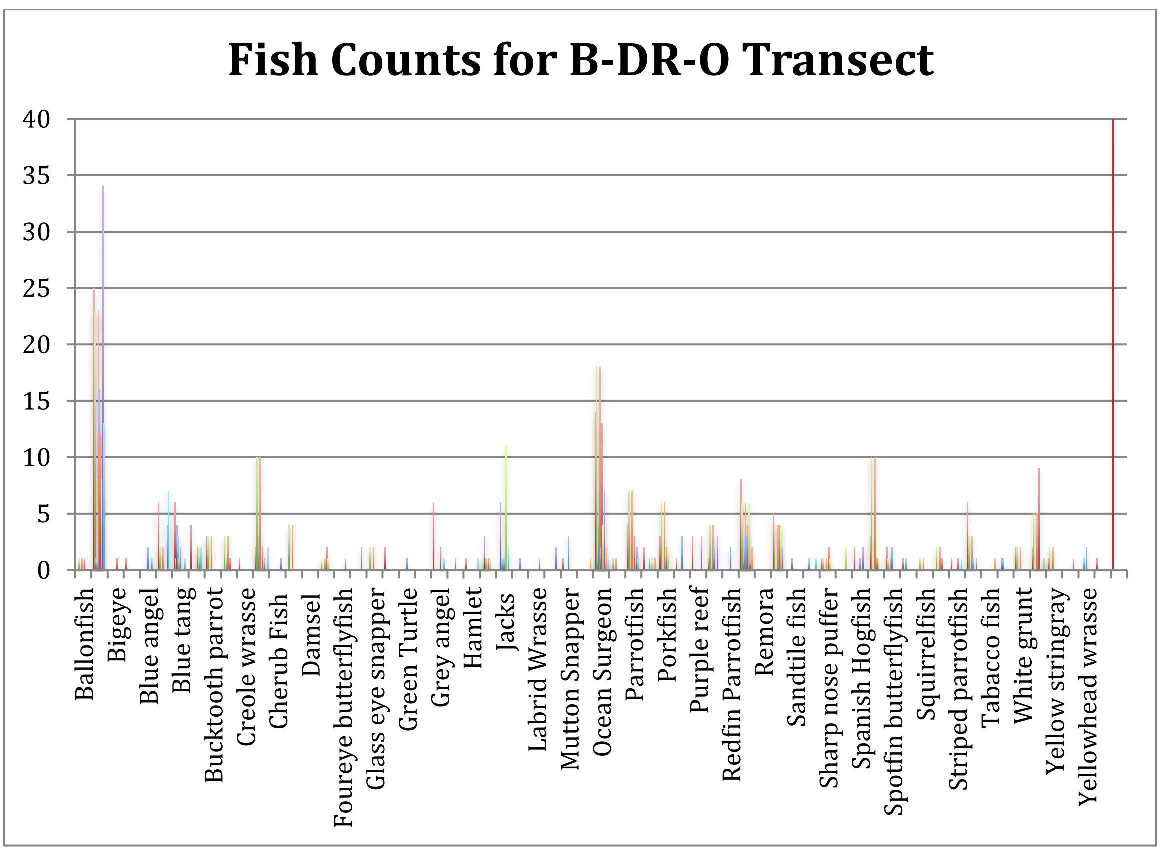
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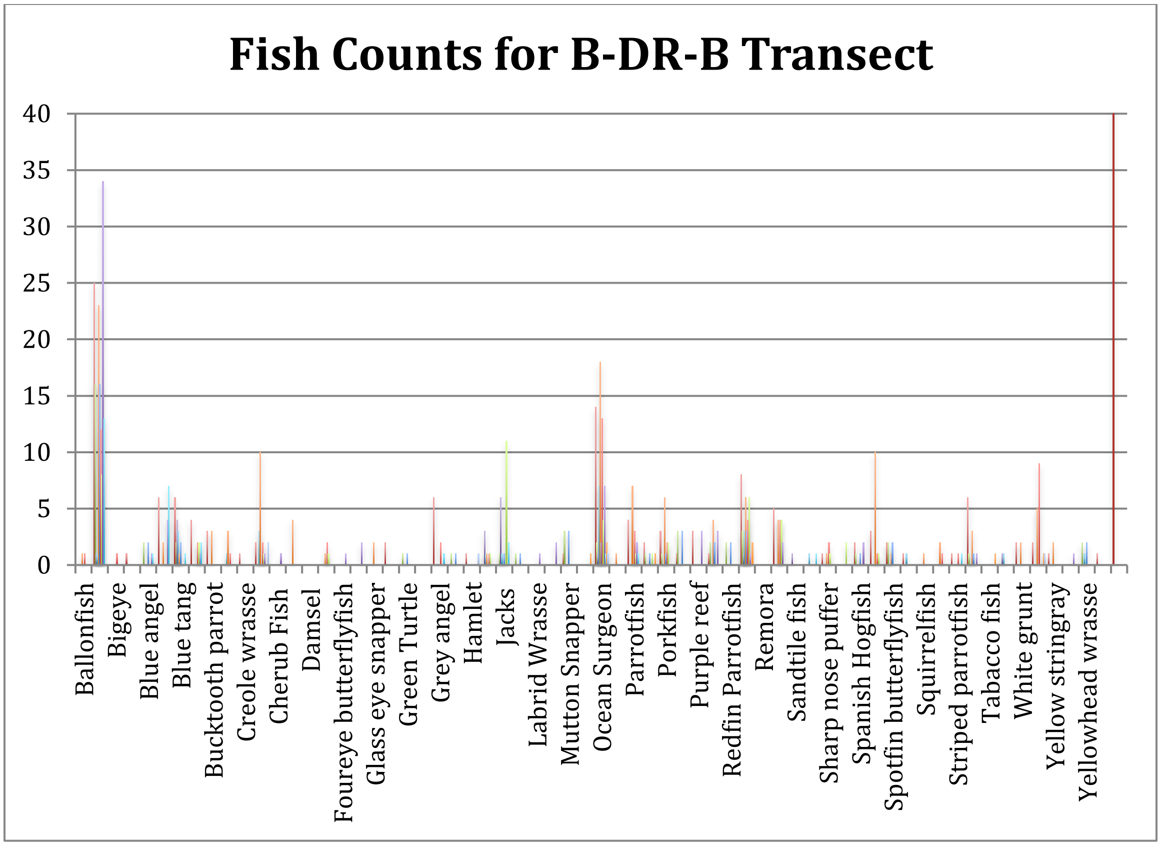
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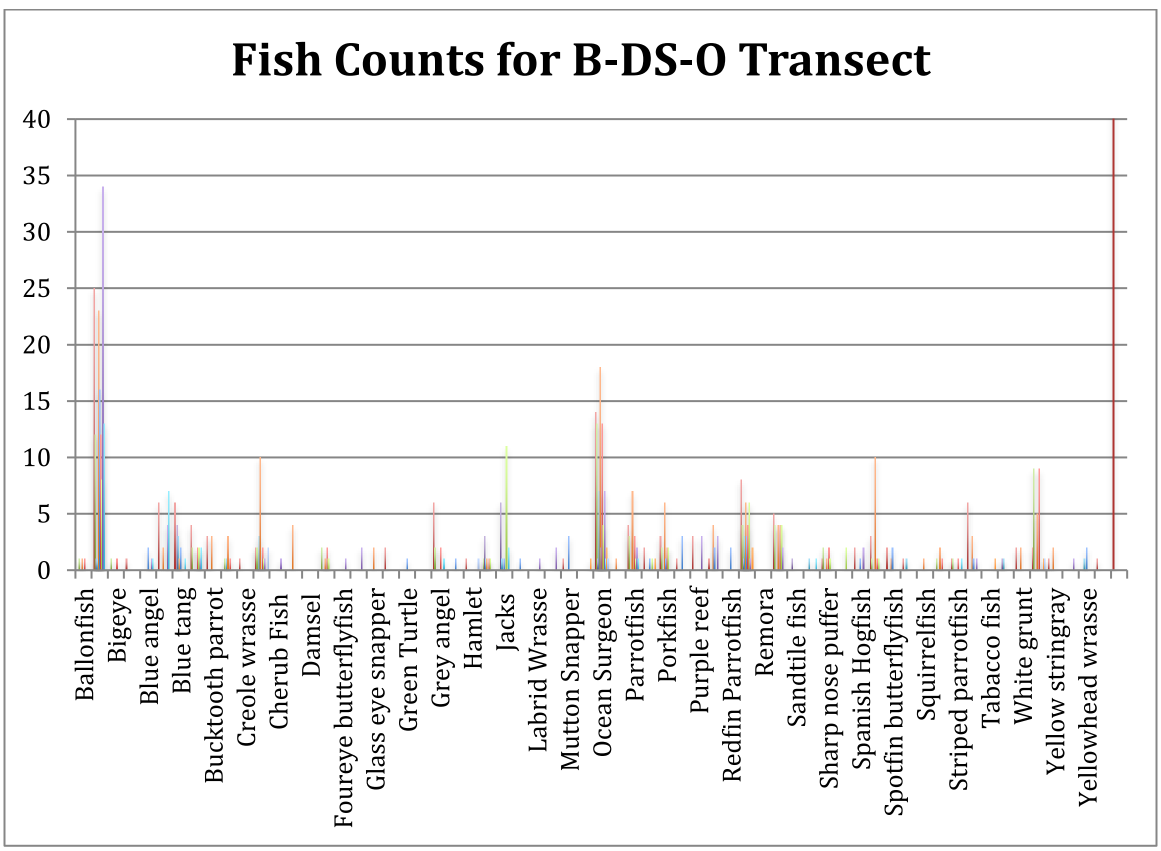
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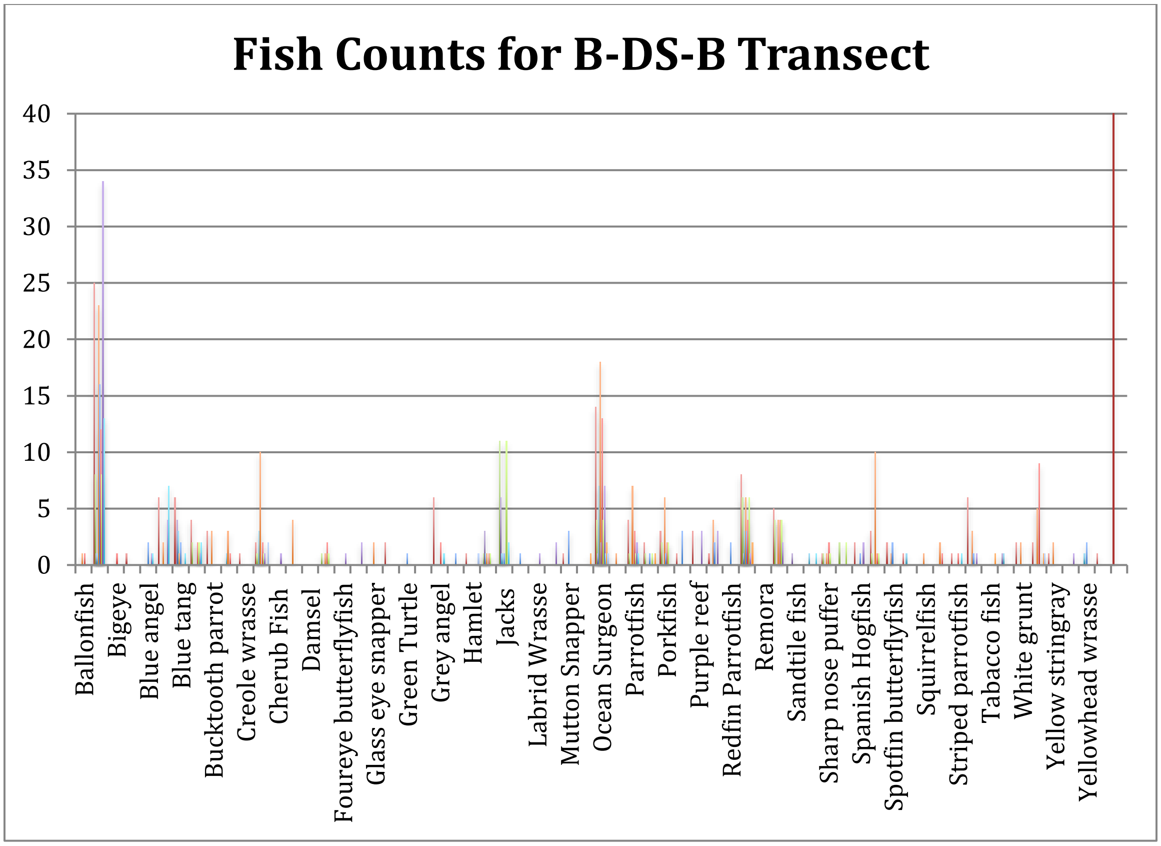
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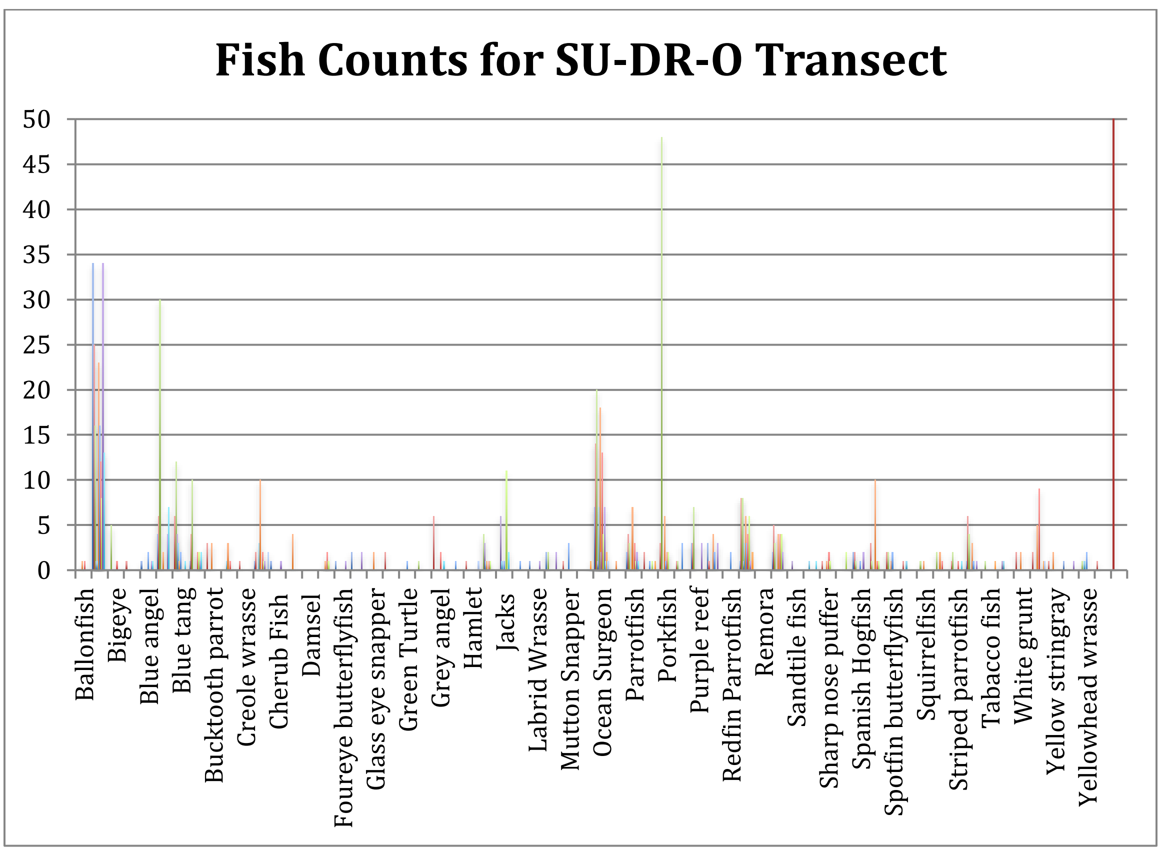
Fish Counts for SU-DR-O Transect
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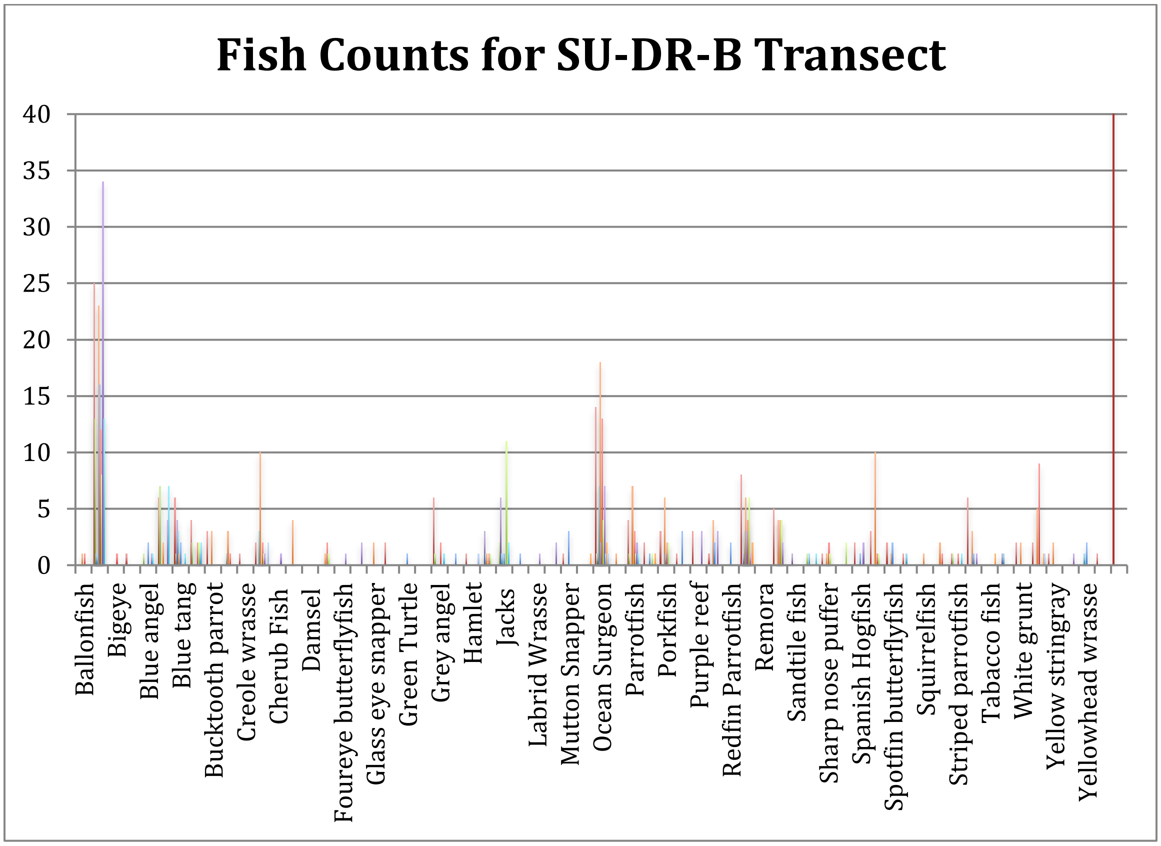
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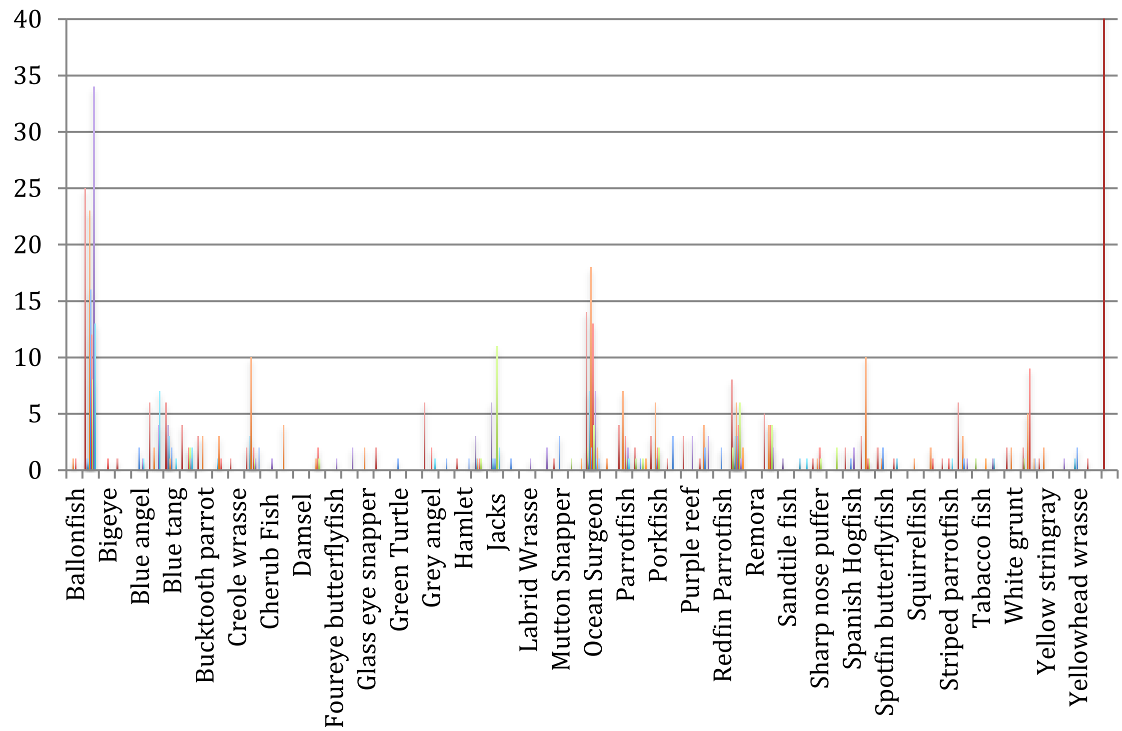


Fish Counts for SU-DS-B Transect