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Comparing Fish Species Assemblage Between Impacted and Restored Estuarine Salt Marshes within the Eel River Estuary, Humboldt Co. CA

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Applied Ecological Restoration Capstone

Department of Environmental Science & Management

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EXECUTIVE SUMMARY

This report details a study assessing the fish assemblages of a recently restored estuarine salt marsh site, and a similar unrestored and impacted site along the Eel River estuary. These two sites are located within the Eel River (Wiya't) estuary, roughly 10 miles west of the city of Fortuna, CA. This study was conducted during the months of September through October 2023. Data collection was done through seine net dragging at randomly selected sample points across both sites. Seine net hauls were recorded by species and species count, which were later computed using a diversity index to provide values representative of diversity for each haul. When testing for a significant difference in diversity between the two sites, a T-test was used to determine that there was in fact no significant difference in the fish assemblage data that was collected at the unrestored site and the restored site ($p\text{-value}<0.05$) (see Tables 1 & 2). This research and similar studies are important to understand the effects of estuarine salt marsh restoration on target fish species populations and species diversity of ecological communities.

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BACKGROUND

Estuarine salt marshes provide critical habitat for a variety of fish species. Freshwater inputs and tidal connectivity come together to create uniquely diverse aquatic ecosystems. Prior to active settlement in 1850, California contained around four million acres of coastal wetlands habitats. During settlement, wetlands were drained for agriculture, filled for development, damaged by timber harvest, and disturbed by modifications like dams and water diversions. California lost 91% of its historical wetland habitats from the late 1700s to the late 1900s, the highest rate of wetland loss across all of the United States (Dahl, 1990). Coastal wetland and estuary ecosystems are extremely important and provide numerous ecosystem services. A few of the services estuaries provide include providing homes and shelter to thousands of species, including nursery habitats for juvenile salmonids, transition zones for anadromous fish, filtering out sediment and pollutants from streams to improve water quality, contributing to flood protection, groundwater recharge, and shoreline protection (Larson, 2001). It is because of these functions and many more that we must make every effort to maintain and restore our remaining estuary systems.

Current Affected Environment

West of the town of Loleta, in Humboldt County, CA, much of the Eel River estuary has been historically impacted by agricultural land use for cattle grazing and timber harvest. Large portions of salt marsh were isolated from the tidal connection by dike construction and drained through tide gates sometime in the early 20th century, between 1916 and 1948 (GHD, 2020).

These agricultural features place the area under threat of more frequent flooding and erosion from rising sea levels. Mechanized logging was introduced to the Eel River watershed post World War II. The near absence of regulation and the accessibility of mechanized logging left devastating impacts on the fragile ecosystem (Yoshiyama, R. M., & Moyle, P. B. 2010). Ocean Ranch Unit was acquired by the CDFW in 1986 to be managed as a shallow freshwater habitat for waterfowl and native wildlife. In 1994, the CDFW discontinued management of the then-artificial freshwater wetland habitat after a breach occurred, causing several water control structure failures. Lack of maintenance allowed for most of the area to revert back to a saltwater and brackish marsh. ORU recently underwent a restoration of approximately 571 acres of salt marsh with goals to improve native fish and plant species habitat, tidal exchange, and invasive plant control (GHD, 2020). This research project was conducted at two sites within the Eel River estuary known as Cannibal Island and Ocean Ranch Unit (ORU). Cannibal Island, a site recently acquired by the California Department of Fish and Wildlife (CDFW), is an example of a site where this impact is still very prevalent.

Cannibal Island

The Cannibal Island site is a 950-acre tidal marsh (California Trout, 2023) within the 2,600-acre Eel River Wildlife Area and the ancestral land of the Wiyot people. The site lies adjacent to the mouth of the Eel River and is currently used primarily for grazing. Historically, the area would have been wooded riparian and wetland habitat, which has been diminished by the disruption of tidal and freshwater influences caused by dikes and tidal gates installed for agricultural stream control (GHD, 2020).

Ocean Ranch

The Ocean Ranch site is a restored and enhanced 571-acre tidal marsh, also located within the 2,600-acre Eel River Wildlife Area and ancestral Wiyot land. Restoration of the site included breaching of levees and dikes left behind by dairy farms reconnecting the estuary to the tides. The complexity of channel networks was improved by excavating tidal channel soil to construct habitat ridges, install ditch plugs, and curate transitional high marsh habitat (GHD, 2020).

Study Objectives

The primary objective of this study is to determine if there is a difference in fish species assemblage between Cannibal Island and Ocean Ranch Unit to help determine the efficacy of the salt marsh restoration work done at Ocean Ranch. Species assemblage refers to the ecological communities that are associated with the species that coexist in the area at the same time (Ragan, 2019). We utilized statistical analysis to see if there were differences between the sites. We also examined the potential causes for differences between the two sites to see if any significant differences existed. Data was collected on water quality metrics to be used as a factor in understanding potential differences in species assemblage diversity, as well as the richness of special status and target species for habitat restoration at the two sites. One such species found at both sites is the federally-listed endangered native Northern tidewater goby, which spends the majority of its life cycle in estuaries (CNDDB, 2023).

Eucyclogobius newberryi

The Northern tidewater goby (*Eucyclogobius newberryi),* a federally-listed endangered species, has habitat restrictions confined to the coastal brackish waters of California (Swift et al., 1989). *E. newberryi* have a unique breeding pattern that greatly depends upon certain conditions being met in their surroundings (Swift et al., 1989). Burrows are commonly found to be 10-20 cm deep in clean, coarse sand areas, to which hatched fry require aquatic vegetation to provide refuge as they develop (Swift et al., 1989). Examination of habitat features and fish assemblages using established baseline references for identified species' biological preferences is an important factor of this study. References to these preferences will be included throughout the discussion. *E. newberryi* is a species that was granted extra precaution in handling during the identification process, having considered their federally endangered status and the proximity of our study area to designated critical habitat within the Eel River estuary (GHD, 2020).

Figure 1: Tidewater Goby (*Eucyclogobius newberryi)* , Christine Fox, USFWS

Gasterosteus aculeatus

One of the most abundant fish we caught in the seine nets during all samplings was the threespine stickleback (*Gasterosteus aculeatus*). *G. aculeatus* have a vast range of aquatic habitats; populations of this fish can be found not only in marine and freshwater habitats, but there are also anadromous populations as well (Bell, 1994). Habitat of *G. aculeatus* ranges throughout the Northern hemisphere, typically found closely along the coasts of the Pacific and Atlantic oceans (Bell, 1994). Despite having various populations with far-apart ranges in between them, there are a few phenotypic features that all populations share in common (Bell, 1994). *G. aculeatus* are known for always constructing a benthic nest when breeding (Wootton, 1984). Once the males have created the nest, females will spawn their eggs inside the nest following courtship and breeding (Blouw et. al., 1990). *G. aculeatus* tend to be anadromous as they typically spawn in freshwater near where they were born and spend the majority of their lifecycle in the open ocean (Bell, 1994).

Figure 2: Threespine Stickleback (*Gasterosteus aculeatus)*, Ryan Hagerty, USFWS

<u>*Cottus asper*</u>

One of the more elusive species that we found at both sites was the prickly sculpin (*Cottus asper*). *C. asper* are known to be found in intertidal zones but typically does not spawn in intertidal zones (Krejsa, 1967). There have been records of populations of *C. asper* with their presence in streams, lakes, and other bodies of water that are far enough inland to presume that their annual seaward spawning migration takes *C. asper* to spawn in freshwater (Krejsa, 1967). This determination has been concluded by the presence of larvae, egg clusters, or pregnant female *C. asper* individuals found in inland freshwater bodies of water (Krejsa, 1967).

Figure 3: Prickly sculpin (*Cottus asper*), Dan Worth, CDFW

METHODS

Site Descriptions

Site one, Cannibal Island, is an unrestored pasture west of Loleta along the Eel River estuary (Figure 4). Pre-European contact, the area was originally populated by Sitka spruce, ferns, and other native species. Current conditions since the early 20th century (GHD, 2020) involve a conversion of estuarine salt marsh to pasture land (California Trout, 2023). Our second site, Ocean Ranch Unit, is a restored salt marsh located north of Cannibal Island near Table Bluff (Figure 4). In this study, we examined both sites' water quality metrics and fish assemblages, using ORU as a reference during our discussion when comparing the two site assemblages. Current management and future restoration efforts are the result of a partnership between the Wiyot Tribe and the California Department of Fish and Wildlife, which currently owns and manages the land. The comparison of the two sites, guided by the intentions of the tribe and state agency, will aid in the planning of future restoration and management plans at both sites.

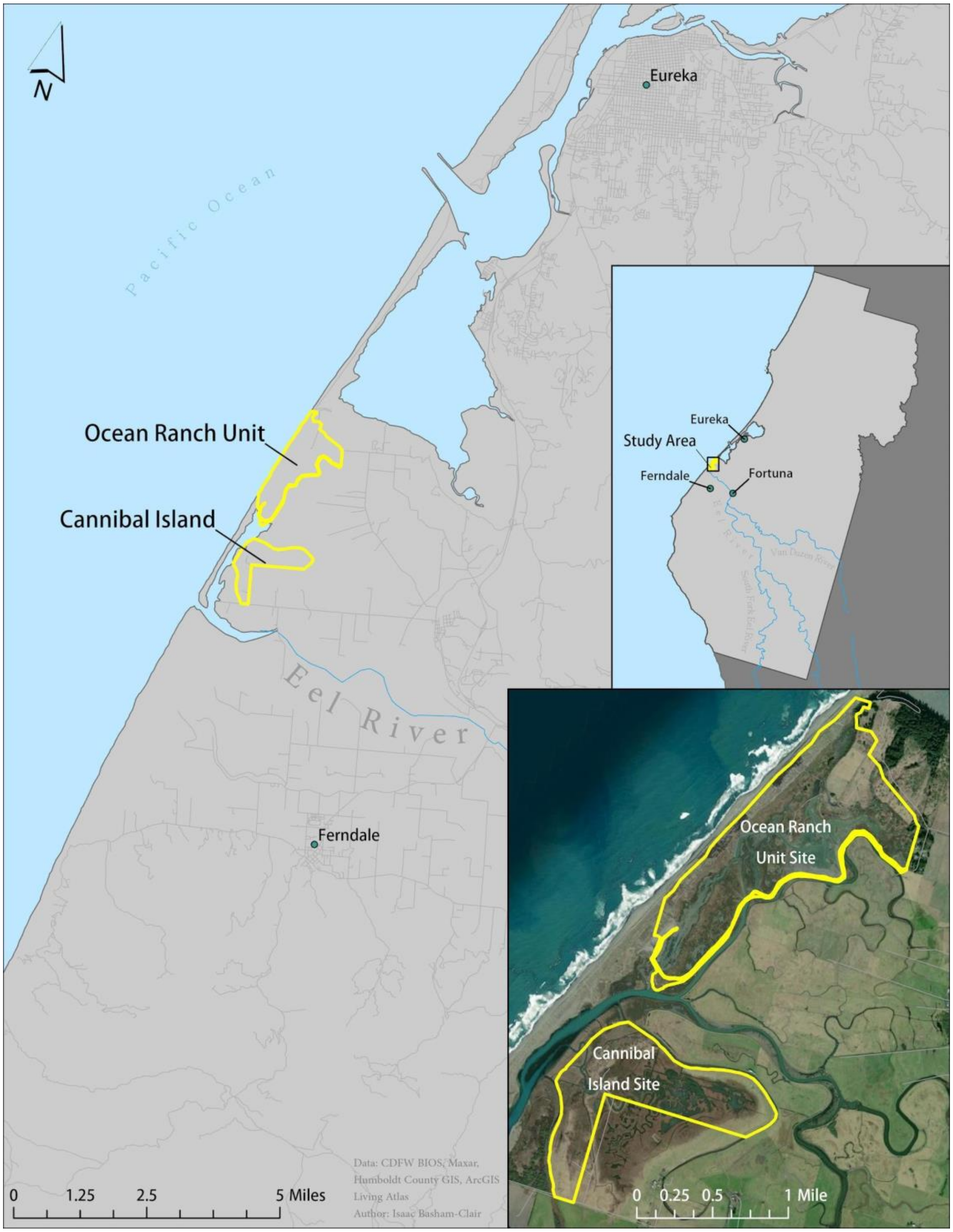

Figure 4. Location Map of Ocean Ranch Unit and Cannibal Island, Study Area relative to Humboldt County (upper right), aerial imagery of both site positions relative to each other (bottom right).

Figure 5. A visual representation of the Ocean Ranch Sampling site, the locations displayed are monitored by the CDFW and the Wiyot Tribe monthly. Map: Kaydee Boozel, CDFW

Data Sampling Procedures

Fish assemblage data was collected using seine nets at multiple randomly selected sampling points across both sites. At Ocean Ranch Unit, sample points from an existing set (Figure 5) were chosen at random using a random number generator. At the Cannibal Island site, sample points were selected on-site and the coordinates of all sample points were recorded before sampling. Each of the two sites were visited for sampling once in the month of September and again in October. Although Cannibal Island is a slightly larger site compared to ORU, there are fewer aquatic habitat features suitable for sampling at Cannibal Island. ORU was sampled 28 times at 17 different sample points. Cannibal Island was sampled 20 times at 8 different sample points. A seine net was dragged through the water at each selected sample point, with a weighted bottom line keeping the net scraping along the bottom of the sample area to most effectively capture organisms across the entire water column. Following each seine net haul, the net was brought to the edge of the water, where species identification and counts were done. Keeping the net in the water, the contents of each haul are consolidated into the middle of the net by rolling up one side while gently shaking organisms on the net towards the middle. All organisms are identified to the closest known taxonomic group, and counts for each were recorded before release. In addition, at each sample point, we measured depth and took water quality readings with a YSI sampling instrument at the bottom, middle, and top depths (where applicable), measuring temperature, dissolved oxygen, and salinity.

Data Entry and Analysis

Data was recorded and organized in a spreadsheet document where statistical analyses were performed. The analysis included calculating and comparing fish species diversity, richness, and evenness. Diversity values per haul were determined by first calculating Shannon entropy values using the Shannon-Weiner Diversity Index (Figure 6), a widely accepted ecological metric used for analyzing diversity among communities (Jost, 2006).

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Figure 6: Shannon-Wiener Equation (H'=Diversity)

The proportion of individuals per species out of the total individuals per haul (P_i) is multiplied by the natural log of P_i ($\ln P_i$). The resulting values for each species found in a single haul are summed, and the inverse of the sum is taken. Shannon entropy values represent an index of diversity, while another measure derived from Shannon entropy values known as an effective number of species (ENS), is considered to be a more comprehensive measure of diversity (Jost, 2006). ENS is calculated by raising e (≈ 2.718) to the power of the Shannon entropy value. Statistical analysis included a series of t-tests performed on a comprehensive dataset containing both Shannon entropy values and ENS from all samples done at both sites to test for a significant difference in diversity between the two sites.

RESULTS

Field Observations

Data collection included a total of 48 seine net hauls containing a wide range of fish assemblages, along with water quality measures for salinity, dissolved oxygen and temperature. Among 20 hauls at Cannibal Island and 28 hauls at Ocean Ranch Unit, there was an abundance of species richness and diversity. In visually observing haul contents, both sites had a similar abundance of fish and diversity of species. Statistical analysis was required to determine differences. All sampling was done at low tide, typically during a flow tidal stage, to have the best accessibility to sample points of various depths. *G. aculeatus* was the most abundant fish species sampled at both sites. The least abundant fish sampled was the bay pipefish (*Syngnathus leptorhynchus*) followed by *C. asper*. We noticed that the abundance of *E. newberryi* went up right after a fresh rain on September 29th when the water was less hypersaline and truer brackish.

Data Delivery

Results of species presence and count were not consistent at each sampling. During our first round of sampling at both sites, we found 10 species at Cannibal Island and 12 species at Ocean Ranch Unit (Figure 7). On our second round of sampling at both sites, we found 12 species at Cannibal Island and 15 species at Ocean Ranch Unit (Figure 7).

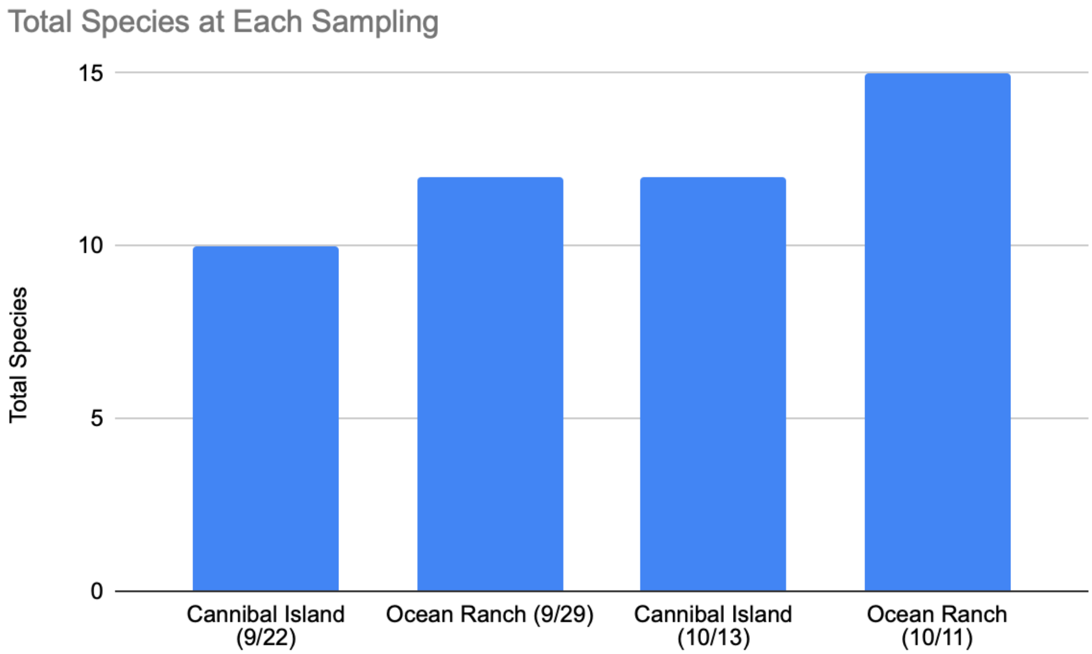

Figure 7: Bar graph illustrating the different species counts taken at each sampling

Individuals were identified by species at each haul and counts were recorded. Species makeup and totals for each sampling event were organized into tables. The dominant species at Cannibal Island on sampling event 9/22 (Table 1) was *G. aculeatus* (threespine stickleback). The dominant species at Cannibal Island on sampling event 10/13 (Table 4) was the *Crangon* species and *E. newberryi* (tidewater goby). The dominant species at Ocean Ranch Unit on sampling event 9/29 (Table 2) was *G. aculeatus* (threespine stickleback) and the *Crangon* species. The dominant species at Ocean Ranch Unit on sampling event 10/11 (Table 3) was the *Crangon* species, unidentified *Osmeridae* species, and *Atherinops affinis* (topsmelt).

Total Count	
Species	Cannibal Island (9/22)
Tidewater Goby	291
Staghorn Sculpin	27
Prickly Sculpin	26
Sculpin (spp.)	4
Stickleback	4076
Bay Pipefish	2
Topsmelt	7
Dungeness crab	2
Shore crab	11
Shrimp	175
Total:	**4621**

Table 1: Individual species counts at Cannibal Island on 9/22

Total Count	
Species	Ocean Ranch (9/29)
Tidewater Goby	11
Goby (spp.)	1
Staghorn Sculpin	23
Prickly Sculpin	41
Stickleback	2144
Topsmelt	267
Osmeridae (spp.)	5
Shore crab	1
Dungeness crab	153
Shiner perch	7
Crangon	2600
Crab (spp.)	5
Total:	**5258**

Table 2: Individual species counts at Ocean Ranch on 9/29

Total Count	
Species	Ocean Ranch (10/11)
Tidewater Goby	155
Staghorn Sculpin	30
Prickly Sculpin	13
Sculpin (spp.)	3
Stickleback	56
Bay Pipefish	4
Topsmelt	426
Day Smelt	5
Osmeridae (spp.)	554
Shore crab	2
Dungeness crab	43
Crangon	2517
Ctenophore	26
Starry Flounder	3
Herring	2
Total:	**3839**

Table 3: Individual species counts at Ocean Ranch site on 10/11

Total Count	
Species	Cannibal Island (10/13)
Tidewater Goby	247
Arrow goby	1
Staghorn Sculpin	9
Prickly Sculpin	25
Coastrange Sculpin	9
Stickleback	98
Bay Pipefish	1
Osmeridae (spp.)	34
Shore crab	8
Dungeness crab	24
Crangon	964
Saddleback Gunnel	2
Total:	**1422**

Table 4: Individual species counts at Cannibal Island site on 10/13

Entropy and T-test Analysis

Using the Shannon-Wiener Index of diversity, we calculated the entropy values for each haul to determine their general diversity. We used the general diversity to determine the effective number of species at each haul. Because we aimed to determine if the two populations are different from one another in regards to their entropy and effective species number, a two-sample two-tailed t-test (unequal and equal variance) was calculated to determine the statistical significance. Results for our t-test analysis of our entropy values yielded a p-value of .46 for our one tail analysis and .93 for our two-tail analysis (Table 6). P-values for the effective number of species analysis was 0.45 (one-tail) and 0.9 (two-tailed) (Table 7). These results were consistent in situations of assumed equal and unequal variance (Table 6 & 7).

Shannon-Wiener Entropy Values			Effective Number of Species Values		
Haul Number	Cannibal Island	Ocean Ranch Unit	Haul Number	Cannibal Island	Ocean Ranch Unit
Haul 1	0.10	1.37	Haul 1	1.11	3.92
Haul 2	0.19	0.41	Haul 2	1.21	1.51
Haul 3	0.21	0.29	Haul 3	1.24	1.34
Haul 4	1.03	0.20	Haul 4	2.81	1.22
Haul 5	0.72	0.78	Haul 5	2.05	2.18
Haul 6	1.07	0.33	Haul 6	2.90	1.39
Haul 7	0.93	0.83	Haul 7	2.53	2.30
Haul 8	0.49	0.47	Haul 8	1.63	1.60
Haul 9	0.12	0.00	Haul 9	1.12	1.00
Haul 10	0.76	0.64	Haul 10	2.13	1.89
Haul 11	0.82	1.04	Haul 11	2.28	2.83
Haul 12	0.69	0.00	Haul 12	2.00	1.00
Haul 13	1.46	0.11	Haul 13	4.30	1.12
Haul 14	0.23	0.99	Haul 14	1.26	2.68
Haul 15	0.49	0.89	Haul 15	1.63	2.44
Haul 16	0.91	0.82	Haul 16	2.49	2.27
Haul 17	1.06	0.87	Haul 17	2.88	2.39
Haul 18	0.58	0.25	Haul 18	1.79	1.28
Haul 19	0.37	0.64	Haul 19	1.44	1.89
Haul 20	0.72	0.63	Haul 20	2.06	1.88
Haul 21	-	0.81	Haul 21	-	2.24
Haul 22	-	0.74	Haul 22	-	2.10
Haul 23	-	1.29	Haul 23	-	3.62
Haul 24	-	1.43	Haul 24	-	4.16
Haul 25	-	0.67	Haul 25	-	1.96
Haul 26	-	0.69	Haul 26	-	1.98
Haul 27	-	0.84	Haul 27	-	2.33
Haul 28	-	0.40	Haul 28	-	1.49

Table 5: Results of Shannon Entropy and Effective # of Species Values Calculated From Each Haul

Two-Sample Assuming Unequal Variances (Shannon - Wiener	
t) one-tail	**0.46**
t) two-tail	**0.93**
Two-Sample Assuming Equal Variances (Shannon - Wiener	
t) one-tail	**0.46**
t) two-tail	**0.93**

Table 6: Results of Shannon Entropy t-test Analysis (P-Values Displayed)

Two-Sample Assuming Unequal Variances (Eff. # Sp.)	
t) one-tail	**0.45**
t) two-tail	**0.9**
Two-Sample Assuming Equal Variances (Eff. # Sp.)	
t) one-tail	**0.45**
t) two-tail	**0.9**

Table 7: Results of Effective # of Species t-test Analysis (P-Values Displayed)

DISCUSSION

The primary objective of our study was to determine the efficacy of salt marsh restoration at Ocean Ranch by examining whether or not there was a significant difference between fish assemblages at the restored and unrestored site. Using the entropy and ENS values we obtained in our analysis, we were able to directly compare the two sites by performing our two-sample two-tailed t-test. When examining the t-test results for the entropy values in both cases of equal and unequal variance, we obtained p-values that were above the threshold of significance (>0.05). Likewise, when comparing the ENS values using the same analysis parameters, similar results of p-values being over our threshold value were obtained. This indicates that in all 4 scenarios we ran, no significant statistical differences were found between fish assemblages at both sites. Despite these results, salt marsh restoration is still a vital goal of environmentalists for the ecosystem services these marshes provide (Larson, 2001). Our results indicate a need for more research to determine the efficacy of the restoration that took place at our site.

Implications for Future Estuarine Salt Marsh Restoration

Surprisingly, the abundance of *E. newberryi* was higher at the unrestored site (Cannibal Island) compared to the restored site (ORU). Reasons for this may include newly-introduced tidal exchange and resulting velocity changes at ebb and flow tides, perhaps exceeding the tolerance of *E. newberryi* (Swift et al., 1989). The disturbance of benthic sediments at sampling sites from recent restoration work, such as channel excavation and habitat feature construction, may also be a contributing factor to the presence of *E. newberryi* at ORU. Considering the federally endangered status of this species, some steps should be taken in order to reduce accidental take and habitat loss in future salt marsh restoration projects; many of which were in fact written into the DEIR Project Description for ORU (GHD, 2020). Intentional manipulation of tidewater exchanges should always be well-calculated to replicate pre-diked conditions. Also, areas of excavation and heavy machinery operation should be limited to only where necessary for channel excavation and habitat feature construction, excluding areas that already meet ideal *E. newberryi* habitat parameters (low velocity-nearly stagnant waters, deep and soft sediments for burrows, <1m depth) (Swift et al., 1989).

Sources of Data Variance and Error

Possible sources of variance are attributed to seasonal, environmental factors, and slight variances between sampled feature types. Our ability to sample was greatly dependent on tidal influences and weather conditions, many days being limited to the early morning and late afternoon based on tide heights and precipitation amounts. Sampling was conducted during the fall which generally hosts bigger tides during low and high events, as well as possibly influencing the species assemblage at both sites due possibly to lowered seasonal productivity within the marshes. Tide tables were examined on the days we sampled but due to scheduling limitations, we still sampled through high tides and rain, which may have contributed to the variance between hauls. On top of this, Ocean Ranch Unit itself has only been recently restored within the past year, and may not have responded to the recent restoration as of yet. Furthermore, the haul area was not kept consistent, haul paths and angles determine the total haul area but we had not developed a strict protocol for determining those two factors. Despite having predetermined sampling points and feature types, our sampling protocol was not strict in regard to haul area. Recommendations for sampling protocols will be based on best practices we found conducting our own sampling, and in reference to other similar studies.

Recommendations for Future Studies

Studies and comparisons of fish species assemblages in restored and impacted estuarine ecosystems are crucial for better understanding how aquatic communities respond to reintroduction of tidal prisms. Fish assemblage comparisons between sites would be made more accurate by expanding the number of site visits and samples per site. More samples at each site and sampling points would allow for the ability to compare points at a closer degree of scrutiny and help researchers better understand differences between the sites. Furthermore, due to the scale of both sites and considering the limitations imposed by the tides, seasonal long-term sampling over many years is a more appropriate approach to determining differences between sites rather than on a month to month basis. Seasonal sampling dates based on tide tables and weather forecasts will aid in improving the quality of data of the overall accuracy of similar monitoring projects in the future.

ACKNOWLEDGEMENTS

A special thanks to the Wiyot Tribe for allowing us to work and collaborate with them on their ancestral tribal land. We recognize the importance of both sites to the Wiyot Tribe. To repeat what our project partner and tribal representative stated at our first sampling event, we would like to thank the land for allowing us to conduct our surveys and allowing us to make use of the services it provides: Marisa McGrew. A special thanks to our partners at the California Fish and Wildlife Service for providing sampling equipment and wading gear, reference materials, and their overall guidance: Kaydee Boozel, James Ray, Travis Massey, and many more. A special thanks to the Americorps Watershed Stewards Program for providing their help with surveying. A special thanks to our Ecological Restoration Capstone course instructor, Daniel Lipe, for providing us with feedback and structure for our project.

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Appendix I

t-Test: Two-Sample Assuming Unequal Variances (Shannon-Wiener Index)			t-Test: Two-Sample Assuming Unequal Variances (Eff. # Sp.)		
	Cannibal Island	*Ocean Ranch Unit*		*Cannibal Island*	*Ocean Ranch Unit*
Mean	0.6475380909	0.6574687177	Mean	2.042992598	2.071547592
Variance	0.1384593815	0.1456182971	Variance	0.633300806	0.6720126142
Observations	20	28	Observations	20	28
Hypothesized Mean Difference	0		Hypothesized Mean Differenc	0	
df	42		df	42	
t Stat	-0.09019043006		t Stat	-0.1210288887	
P(T<=t) one-tail	0.4642823192		P(T<=t) one-tail	0.4521226652	
t Critical one-tail	1.681952357		t Critical one-tail	1.681952357	
P(T<=t) two-tail	0.9285646385		P(T<=t) two-tail	0.9042453305	
t Critical two-tail	2.018081703		t Critical two-tail	2.018081703	
t-Test: Two-Sample Assuming Equal Variances			t-Test: Two-Sample Assuming Equal Variances (Eff. # Sp.)		
	Cannibal Island	*Ocean Ranch Unit*		*Cannibal Island*	*Ocean Ranch Unit*
Mean	0.6475380909	0.6574687177	Mean	2.042992598	2.071547592
Variance	0.1384593815	0.1456182971	Variance	0.633300806	0.6720126142
Observations	20	28	Observations	20	28
Pooled Variance	0.1426613537		Pooled Variance	0.6560229543	
Hypothesized Mean Difference	0		Hypothesized Mean Differenc	0	
df	46		df	46	
t Stat	-0.08980424904		t Stat	-0.1204192572	
P(T<=t) one-tail	0.4644163282		P(T<=t) one-tail	0.4523377392	
t Critical one-tail	1.678660414		t Critical one-tail	1.678660414	
P(T<=t) two-tail	0.9288326564		P(T<=t) two-tail	0.9046754783	
t Critical two-tail	2.012895599		t Critical two-tail	2.012895599	

Appendix-Table 1: t-test Analysis results for both Shannon-Wiener Entropy Analysis and Effective #. of Species