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On the Rocks, Below the Rocks: A study of intertidal life in the low, middle and high zones of the Puerto Cabuyal – Punta San Clemente Marine Reserve during an El Niño event

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Ophiocoma aethiops in the Puerto Cabuyal-Punta San Clemente Marine Reserve. Author: Juliana Ferrer

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Abstract
The rocky intertidal zone is one of the most difficult environments for organisms to survive in due to its harsh biotic and abiotic conditions. As such, it is also one of the best indicators of climate change and an important barrier to beach erosion. This study looked at the rocky intertidal zone of the Puerto Cabuyal-Punta San Clemente Marine Reserve in the Manabí province of Ecuador, with a focus on observing and identifying the organisms found in the area. Data was collected along 30 m transects at varying elevations along the shore using quadrants of two sizes: 50x50 cm for sessile organisms and algae and 1x1 m for macroinvertebrates. That data was then compared with other studies along coastal Ecuador where it was determined that Puerto Cabuyal does hold a similar level of species richness and diversity as well as similar species. A total of 56 species were identified from the following phyla: Mollusca (20 species), Arthopoda (12), Ochrophyta (6), Echinodermata (5), Rhodophyta (5), Chlorophyta (2), Chordata (2), Cnidaria (2), and Platyhelminthes (2). The low transects, measured at the level closest to the water, were shown to have the highest species richness. The data collected in this study can be used in the future to understand changes in the diversity of the intertidal zone of this marine reserve and for comparisons along coastal Ecuador.

Resumen
La zona intermareal rocosa es uno de los ecosistemas más difíciles para que los organismos sobrevivan debido a las condiciones bióticas y abióticas. Como tal, también es uno de los mejores indicadores del cambio climático y un bloqueo importante de la erosión de las playas. Esta investigación se centró en la zona intermareal rocosa de la Reserva Marina Puerto Cabuyal-Punta San Clemente en la provincia de Manabí, Ecuador, con el objetivo principal a observar e identificar los organismos que se encuentran en la zona. Los datos fueron colectados en transectos de 30 m en diferentes elevaciones en la playa utilizando cuadrantes de dos tamaños: 50x50 cm para organismos sésiles y algas y 1x1 m para macroinvertebrados. Esos datos se compararon con otros estudios de la costa de Ecuador, donde se determinó que Puerto Cabuyal tiene un nivel similar de riqueza y diversidad de especies, así como especies similares. Se identificaron un total de 56 especies de los siguientes filos: Mollusca (20 species), Arthopoda (12), Ochrophyta (6), Echinodermata (5), Rhodophyta (5), Chlorophyta (2), Chordata (2), Cnidaria (2), and Platyhelminthes (2). Los transectos bajos, medidos en el nivel más cerca al agua, mostraron la mayor riqueza de especies. Los datos colectados en este estudio pueden ser utilzados en el futuro para comprender los cambios en la diversidad de la zona intermareal de esta reserva marina y para comparaciones en el resto de la costa ecuatoriana.

Acknowledgements
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Introduction

The rocky intertidal zone combines terrestrial and marine environments and faces a diverse range of living conditions that make calling it home difficult for most organisms. Each day, the tides will rise and recede significantly, exposing organisms to both air and aquatic life (Newell, 2013). For organisms to survive in the intertidal zone, they must be able to handle biotic and abiotic stressors, including tidal action, temperature changes, predation, and food competition (Vinuganesh et al., 2022; Mendoza et al., 2023). Algae species in the intertidal have adapted to the strong solar radiation and changing salinity by regulating cellular mechanisms like metabolic processes and osmotic pressure changes as well as growing in specific zones within the intertidal that are tolerable for the species (Vinuganesh et al., 2022). Species such as *Padina pavonica* have adapted to accumulate heavy metals in the water, thus playing an interesting but vital role in the ecosystem as bioindicators of pollution (Ameen et al., 2022). Ecologically, the rocky intertidal provides habitats for many species that are adapted to the conditions of the zone, forming the base for a complex food chain. Additionally, the rocky intertidal serves as a protective barrier for the shoreline by blocking intensive tidal action, hence aiding in the prevention of beach erosion (Martinez, 2021). Moreover, the intertidal zone is known to be an ideal system for measuring impacts of climate change for a multitude of reasons including but not limited to: organisms are exposed to steep gradients of stressors, many organisms in upper zones of the intertidal live close to their thermal tolerance limits, and the limits of upper zones are thought to be set by stressors including temperature (Szathmary et al., 2009). Therefore, studies focusing on the species and overall health of the rocky intertidal zone are vital for protecting species diversity as well as understanding ecological roles of the area, including allowing for a visualization of climate change.

Animals living in the intertidal must alter their methods of feeding to both get the energy they need to live and to survive the harsh conditions of the intertidal. The predatory snail species *Acanthina* has adapted its foraging period to search for prey during the low tide. Since it cannot consume its prey in one low tide period, it will restrict movement during high tide and use that time for feeding on the previously discovered prey (Menge, 1974). Another species with adaptations for feeding is the crab *Pachygrapsus transversus*, which lives on an omnivore diet. This allows for it to adapt its diet to whatever is available, whether it is sessile invertebrates or algae (Christofoletti et al, 2010). Similar to *P. transversus*, herbivores such as sea slugs in the genus *Elysia* have adapted to use an opportunistic strategy for feeding (Aguilera, 2011; Paul and Van Alstyne, 1988). Conversely, sea hares in the genus *Dolabrifera* have adapted to have noticeably short and precise foraging patterns to avoid the predators of the high tide (Himstead and Wright, 2018).

Additionally, organisms in the intertidal adapt to live in certain zones as the conditions are incredibly different. Organisms that live in the high intertidal at the high tide line are exposed to air and strong solar radiation and are rarely underwater. It is common for fewer species to be found there as a result of the specific adaptations needed to survive (Newell, 2013). Species of algae are subject to dehydration from lack of water, and this causes reduced productivity therefore fewer species can thrive. There is then less competition between intertidal algae and certain species will either dominate or coexist (Jorgensen, 2009). High intertidal animals such as the limpet *Lottia digitalis* have a high tolerance to desiccation and are able to survive the temporary loss of body water content (Leeuwis & Gamperl, 2022). However, the primary adaptation of high intertidal animals is to reduce or avoid the loss of water by minimizing exposed body surfaces. Sessile animals such as bivalves close their shells and members of the phylum Cnidaria retract their feeding appendages. Mobile animals will find hiding places in tidepools, under rocks, or in burrows (Leeuwis & Gamperl, 2022).

Organisms living at the mid tide level are underwater for about half of the day and out of the water for the rest of the day, so they must be able to live both underwater and in air (Newell, 2013). This level is mainly inhabited by species of the phyla Mollusca and Arthropoda which can live in a wide range of salinities as well as both in and out of the water for periods of time (Womersley & Thomas, 1976; Berger & Kharazova, 1997). Mollusks are able to burrow into sediments or close their shells when the tide recedes and then open their shells or look for food when the tide rises back. Arthropods such as crabs will hide under rocks and then go and scavenge when the tide recedes (Berger & Kharazova, 1997; Jorgensen, 2009). Many rocky intertidal zones are covered by mussels, barnacles, and types of brown algae, all of which are adapted to withstand the rise and fall of the tides (Jorgensen, 2009). While more organisms can survive in the area, that also means that there is more competition for space and food (Jorgensen, 2009).

At the lowest point of the intertidal, organisms are underwater for the vast majority of time and therefore must be well adapted to aquatic life (Newell, 2013). Competition between intertidal algae species is much more intense than other levels because many more species are able to survive. Many species of algae will first claim space by encrusting the rocks, sometimes producing a very thin crust so they can grow faster (Jorgensen, 2009; Keats et al., 1994). The low intertidal is the most common area to find echinoderms as many species either cannot breathe out of water or cannot be out of water very long (Leeuwis & Gamperl, 2022). Animals commonly found in the area cannot handle the conditions of the high intertidal and experience high mortality rates with water loss less than 10% (Leeuvis & Gamperl, 2022). At the low intertidal, many grazers are commonly found such as gastropods and sea urchins, which can affect the distribution of algae but only has a significant effect if the grazer populations are significantly large (Jorgensen, 2009).

Intertidal communities are also affected by El Niño Southern Oscillation (ENSO) events. An El Niño event began in the summer of 2023, reached moderate strength in September 2023 and is expected to continue until April 2024 (World Meteorological Organization, 2023). A study done in Chile by Espinoza et al (2023) over the course of 15 years found that during El Niño years, populations of intertidal fish showed significant differences in what species were most abundant. During strong El Niño events, fish that had high tolerances to elevated water temperatures, such as *G. laevifrons*, were much more abundant than those with low tolerances (Espinoza et al., 2023). Another study in Chile found that recruitment rates were lower overall for mussel species during the 1997-1998 El Niño year (Navarrete et al., 2002). A study by Barry et al (1995) found that a warming in temperature from ENSO led to an overall increase in the abundance of southern intertidal species but an overall decrease in the abundance of northern intertidal species. In Peru, it was found that El Niño potentially caused a turnover of intertidal species, especially in the transition zone where the highest sea surface temperatures occurred (Valqui et al., 2021). A study done in the Galapagos after the 1997-98 El Niño event found that most algae were either scarce or the population generally declined (Vinueza et al., 2006). Relating to temperature change in general, a study done on the Pacific coast of Costa Rica found that an increase in temperature led to increased red algae cover while a decrease in temperature led to increased green algae cover (Sibaja-Cordero & Cortes, 2009). ENSO events have strongly varying effects based on year and location so potential disruptions cannot fully be predicted for the current event (World Meteorological Organization, 2023).

The coast and waters of Ecuador are home a total of eight marine reserves (Ministerio del Ambiente, Agua y Transición Ecológica, 2021). Studying the organisms that reside within marine reserves is important to properly understand what is being protected. However, few studies have been conducted on the life in the intertidal zones within these reserves. A study by Martinez (2021) in the El Pelado and Cantagallo-Machalilla Marine Reserves focused on monitoring the biodiversity of rocky intertidal sites at slightly different elevations, specifically looking at sessile organisms and macroinvertebrates. Additionally, a study conducted by Cardenas-Calle et al. (2020) looked at the diversity of marine invertebrates and algae within coastal Ecuador sites, including the Galeras San Fransisco and El Pelado Marine Reserves. However, the Puerto Cabuyal-Punta San Clemente Marine Reserve, created in 2021, and the rocky intertidal zone of the area have not yet been extensively explored scientifically (Ministerio del Ambiente, Agua y Transición Ecológica, 2021).

The purpose of this investigation was to perform surveys of the intertidal region and answer the following questions:

- What are the dominant organisms of the intertidal regions of the Puerto Cabuyal-Punta San Clemente Marine Reserve?
- Does the diversity of the region differ based on shore elevation?
- Does the region demonstrate similar or different levels of diversity in comparison to the El Pelado and Cantagallo-Machalilla Marine Reserves?

The answers to these questions will be useful for the community of Puerto Cabuyal to have a baseline of data about the organisms in the area and their importance for both the environment and the people.

Materials and Methods

Site Descriptions and Data Collection:
All data was collected from the intertidal zone of Puerto Cabuyal within the Puerto Cabuyal-Punta San Clemente Marine Reserve in the province of Manabí, Ecuador. The town of Puerto Cabuyal is a secluded fishing community, with little tourism and human impacts in comparison to many other cities along coastal Ecuador (Figure 1, 2). The marine reserve has been defined as the area from Punta Ballena to Punta San Clemente (Figure 1) (Ministerio del Ambiente, Agua y Transición Ecológica, 2021). Site 1 was located close to the first point of the beach south of the town Puerto Cabuyal (Figure 2, 3a). Site 2 was located the farthest from any human settlement (Figure 2, 3b). Site 3 was located closest to human settlement (Figure 2, 3c).

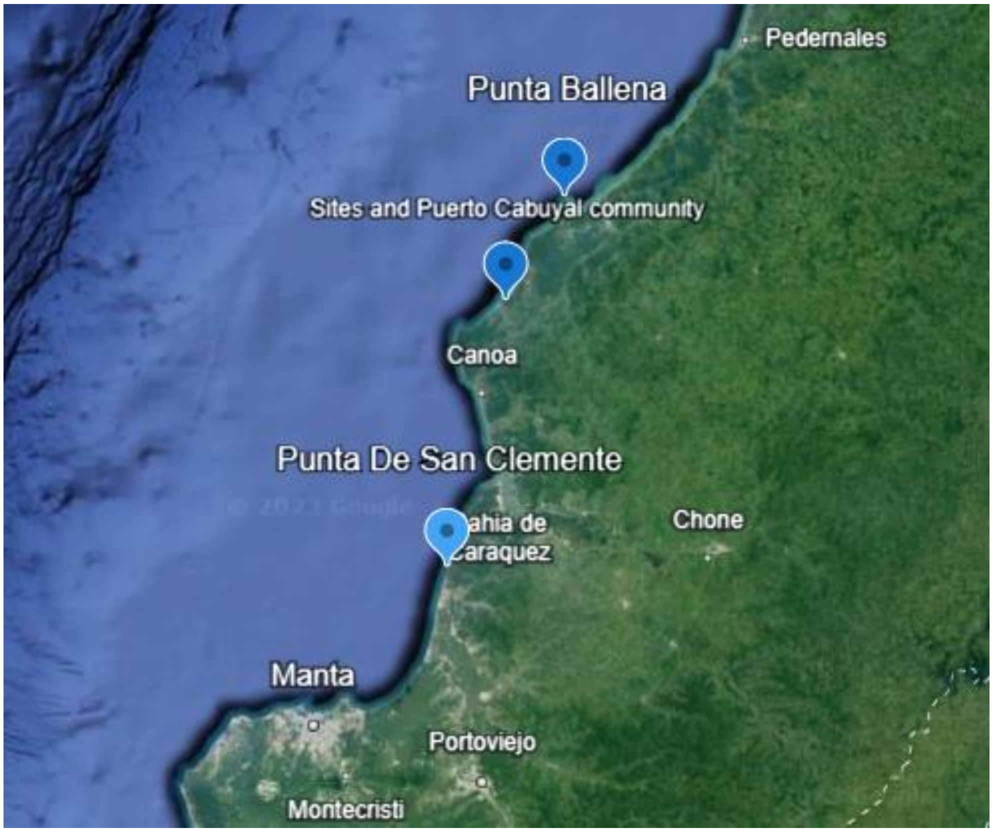


Figure 1. A map showing the Puerto Cabuyal-Punta San Clemente Marine Reserve. Author: Google (n.d.)

Figure 2. Locations of field sites and the community in Puerto Cabuyal. Author: Google (n.d)

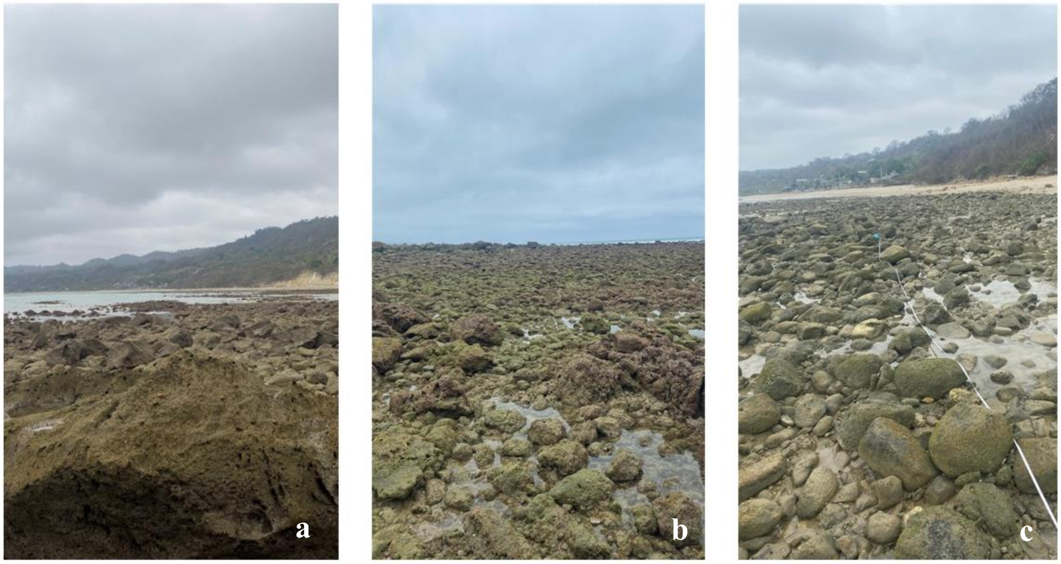


Figure 3. a) Site 1. b) Site 2. c) Site 3

Table 1. Coordinates of each site in Puerto Cabuyal.

Site	Coordinates
Site 1	*0°18’47.9” S, 80°24’49.2” W*
Site 2	*0°18’47.9” S, 80°24’55.3” W*
Site 3	*0°18’38.2” S, 80°24’31.6” W*

Methods were replicated from the previously mentioned study by Martinez (2021) so the most accurate comparisons could be made. Research was conducted during the new moon and during the lowest low tides to allow for samples to be taken throughout the maximum possible area of the intertidal zone. The lowest low tides during data collection were at 9:18 am, 9:57 am, and 10:35 am (Tide Times and Charts for Puerto Cabuyal, Manabí and Weather Forecast for Fishing in Puerto Cabuyal in 2023, 2023). A total of 3 sites were used, and all were located within the Puerto Cabuyal-Punta San Clemente Marine Reserve. At each site, 3 transects, each 30 m in length, were measured out parallel to the coastline at varying distances from the shore. The first was at the point where the exposed rocks meet the water at the low tide line (low transect), the second was in the middle of the exposed zone (mid transect), and the third was at the rocks closest to the shore (high transect). Sessile organisms and algae were measured together while macroinvertebrates were measured separately in accordance with the methodology used by Martinez (2021).

To study sessile organisms, a total of 10 quadrants were placed along each transect, with one placed every 3 meters. Each quadrant was measured out with a 50 cm x 50 cm square of PVC pipe. String was placed in lines along the pipe to create squares with a total of 81 intersections. Every organism at an intersection was noted, photographed, and later identified so that the percent cover of sessile organisms in each quadrant could be calculated (Figure 4a, 4b).

To study macroinvertebrates, a total of 6 quadrants were placed along each transect, with one placed every 5 meters. Each quadrant was measured out with a 1.0 m x 1.0 m square of PVC pipe. The area of the quadrant was then carefully observed, and all non-sessile organisms were noted, photographed, and later identified (Figure 4c).

The methods used allowed for consistency in measuring each quadrant.

Figure 4. a). A sessile quadrant. b). Researcher Juliana Ferrer recording data for sessile quadrants. c). A quadrant used for observing macroinvertebrates.

Data Analysis:
Data was analyzed using the coding language R version 4.3.2 using the packages "ggplot2" (Wickham, 2016), "tidyverse" (Wickham et al., 2019) and "rstudioapi" (RStudio Team, 2023) as well as Excel. Relative abundance graphs were made for both sessile and macroinvertebrate taxa. Abundance of sessile organisms and algae is demonstrated through a series of charts. The analysis of relative abundance allows not only for a measure of diversity but also an idea of how species are distributed in the environment and how populations change over time. Relative abundance calculations were made separately for sessile and macroinvertebrate species as the data was collected differently. In addition, graphs were made for species richness and for the number of individuals of a species found in a quadrant. Understanding species richness is necessary for understanding biodiversity as well as monitoring changes over time and comparing the number of species in different locations.

Finally, three indices were used: the Shannon Diversity Index to measure species diversity, the Shannon Equitability Index to measure evenness of species, and the Jaccard Similarity Index to measure similarity between sites. The Shannon Diversity Index is calculated with the following formula: $\mathbf{H = -\Sigma pi * ln(pi)}$ where **pi** is the proportion of the entire community made up of a species. A higher number indicates higher diversity (Gorelick, 2006). Using this index is important as a method of combining relative abundance and species richness to further assess the diversity of a community.

The Shannon Equitability Index measures evenness of species and is calculated with the following formula: $\boldsymbol{E_H = H / ln(S)}$ where H is the Shannon Diversity Index and S is the total number of unique species. The scores range from 0-1 with 1 meaning the abundances of all species at the site are the same (Hossain et al., 2017). Measuring evenness through this index enables a deeper comprehension of the balance of the populations in an ecosystem.

The Jaccard Similarity Index is calculated with the following formula: **(number of species simultaneously present in sites)/(number of species not simultaneously present in sites).** The score ranges from 0-1 with 1 meaning the sites compared are the same (Ivchenko & Honov, 1998). This index is useful for this study as it does not take data set size into account and is also a simple way to accurately measure the similarity between sites.

Ethics
All fieldwork was completed without any adverse effect on the environment, including all species involved in the work as well as researchers.

Results

Relative Abundance:
Looking at the macroinvertebrates, Mollusca had 19 species, 161 individuals, and a relative abundance of 0.521 while Platyhelminthes had 2 species, 4 individuals and a relative abundance of 0.013 (Figure 5, Table 2). For sessile organisms and algae species, Chlorophyta was the most abundant phylum, with 2 species and 48.5% coverage of sessile quadrants while Mollusca was the least abundant, with 1 species and 0.04% coverage of sessile quadrants (Figure 6, 10). The most dominant phylum looking only at sessile organisms and not algae was Arthropoda with 2 species and 3% coverage of sessile quadrants (Figure 6, 10).

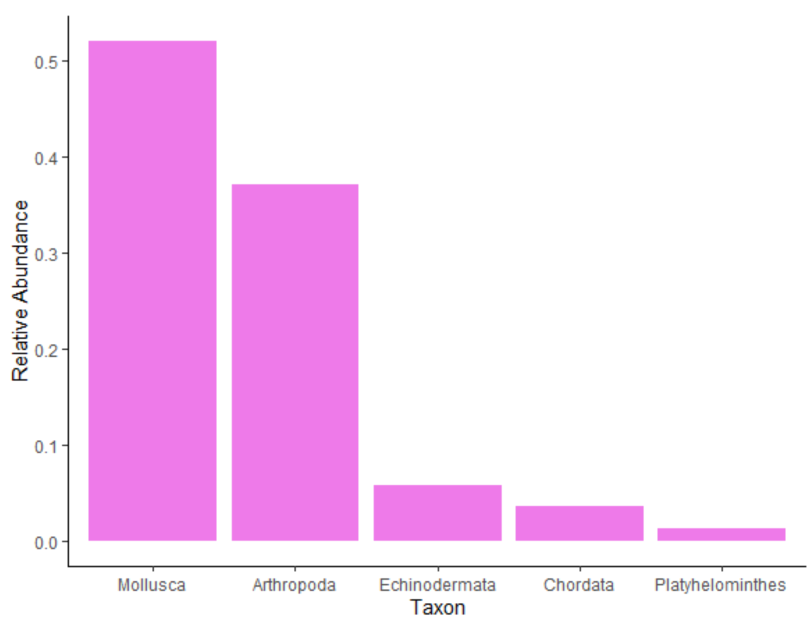


Figure 5. Relative abundance of macroinvertebrate taxa in Puerto Cabuyal.

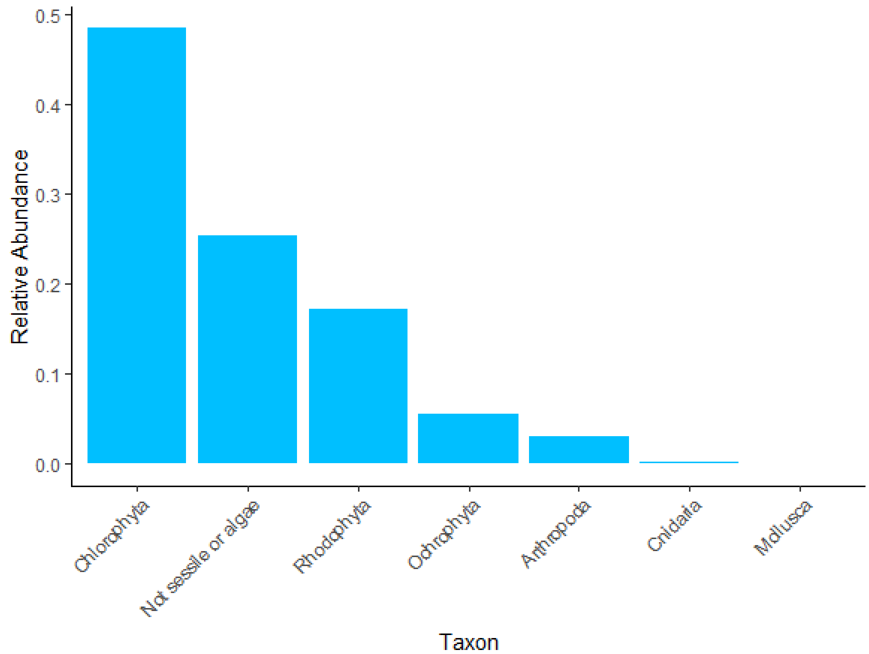


Figure 6. Depictions showing the relative abundance of sessile organisms and algae in Puerto Cabuyal.

Species richness and individuals of a species per quadrant:

Data was analyzed by the level of the shore from which it was observed as well as by the site from which it was taken (Figure 9a, 9b). Species richness was highest for macroinvertebrates in Site 1 with 26 species and for sessile organisms and algae in Site 2 with 17 species (Figure 7a). Samples from low sites had the highest level of species richness in both macroinvertebrates with 23 species and sessile organisms and algae with 16 species (Figure 7b). The data was also analyzed to view the average number of individuals of a species per quadrant. On average, the most macroinvertebrate individuals were found in Site 2 at an average of 8.66 individuals and at the mid-level quadrants on average at 5.19 individuals (Figure 8a, 8b). Meanwhile, the most sessile organisms and algae individuals were found in Site 3 at an average of 5.78 individuals and at the high-level quadrants at an average of 6.61 individuals (Figure 8a, 8b).

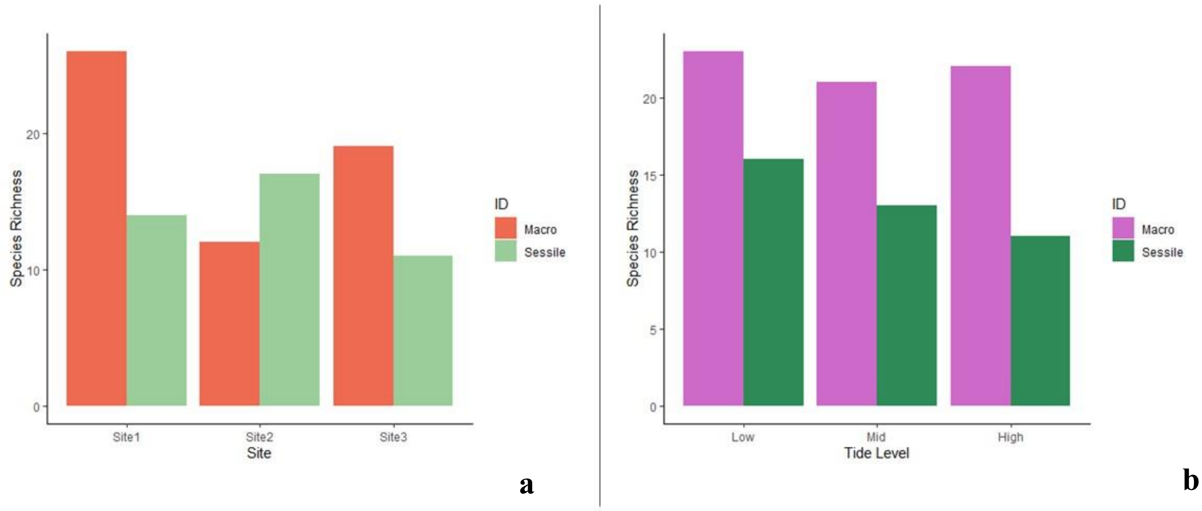


Figure 7. Species richness in Puerto Cabuyal by site. b) Species richness in Puerto Cabuyal by tide level. N=3

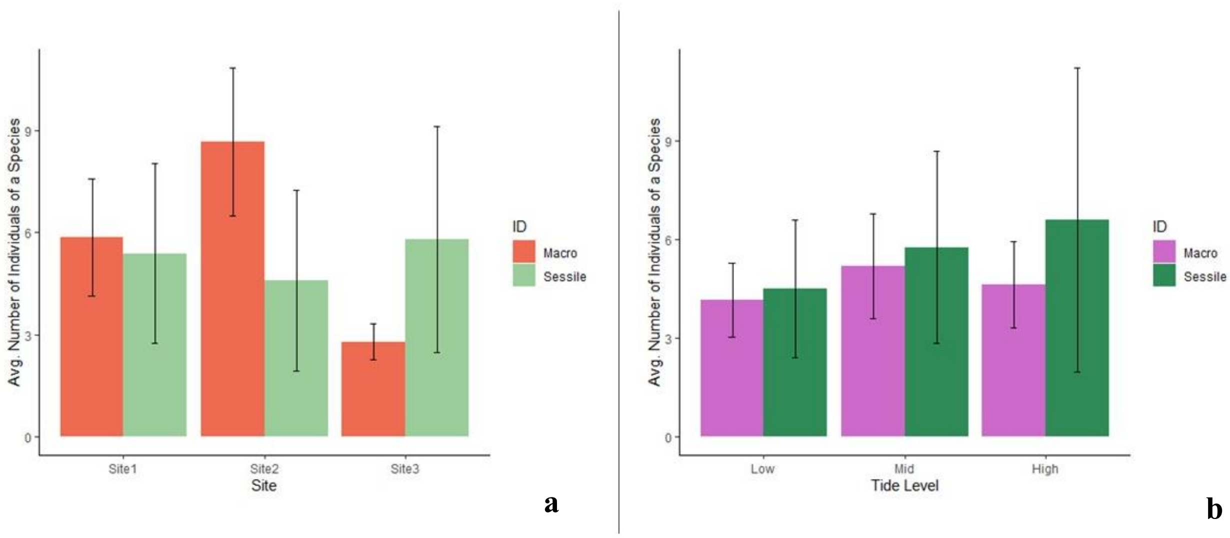
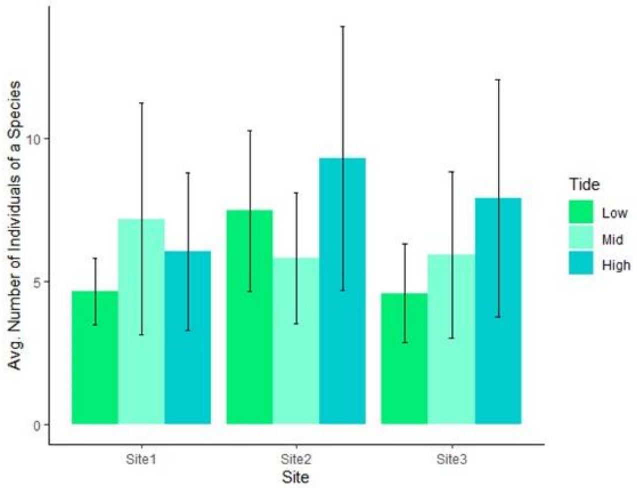


Figure 8. a) Average number of individuals of a species in a quadrant based on site. b) Average number of individuals of a species based on tide level. N=3, mean +/- se



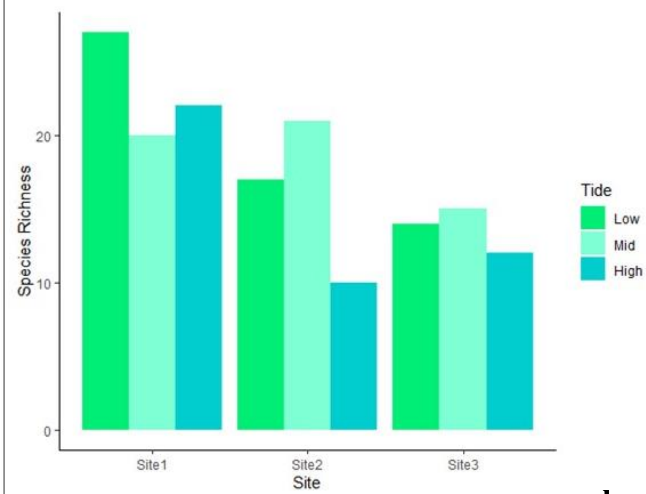


Figure 9. a) Average number of individuals of a species in a quadrant by site and elevation on the beach, N=3, mean +/- se. b) Comparing species richness using both site location and elevation on the beach.

Species found:
A total of 56 species were identified between macroinvertebrates (38), sessile organisms (5) and algae (13). The species belong to the follow phyla: Mollusca (20), Arthropoda (12), Ochrophyta (6), Echinodermata (5), Rhodophyta (5), Chlorophyta (2), Chordata (2), Cnidaria (2), and Platyhelminthes (2).

Sessile organisms and algae

The sessile organisms belong to the phyla Mollusca (1), Arthropoda (2), and Cnidaria (2). The algae belong to the phyla Ochrophyta (6), Rhodophyta (5) and Chlorophyta (2) (Figure 10, 11).

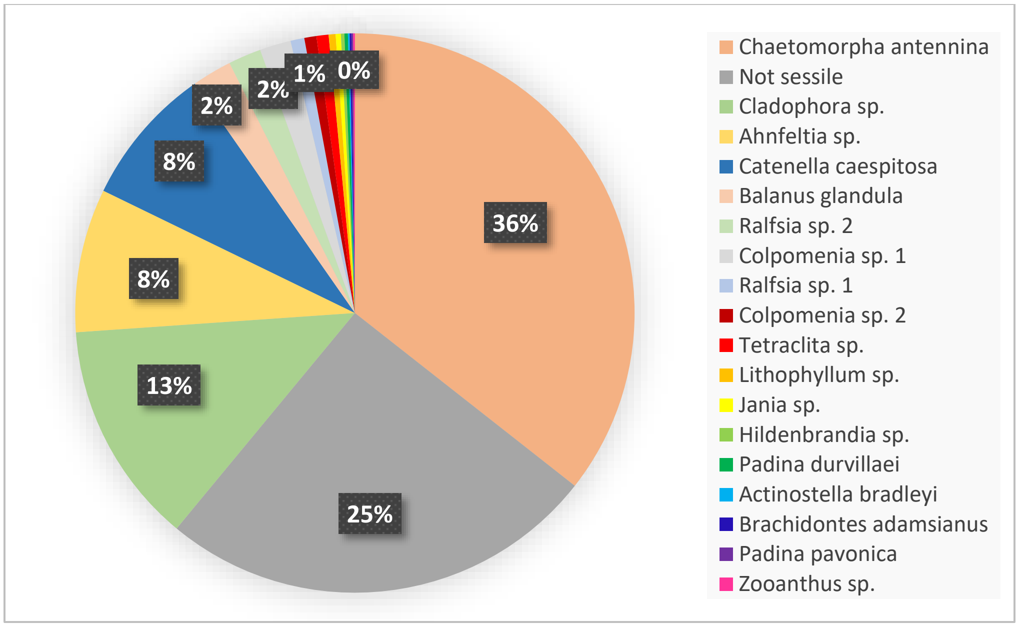


Figure 10. Depictions showing the percent cover of species in all sites.

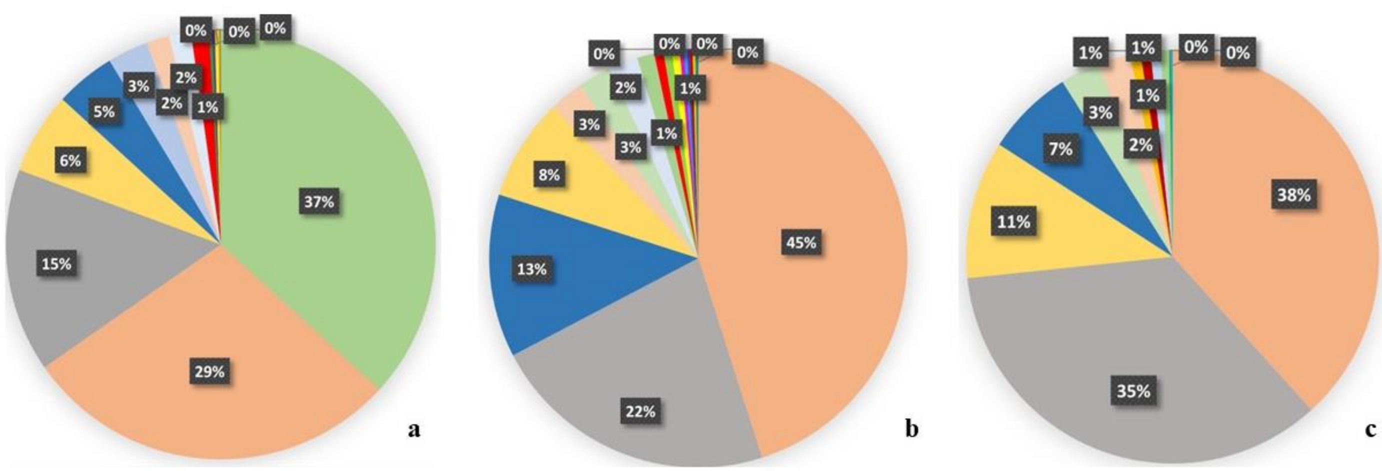


Figure 11. a) Percent coverage of species in Site 1. b) Percent coverage of species in Site 2. c) Percent coverage of species in Site 3.

Note: the figure legend from Figure 10 is used here

Macroinvertebrates
The macroinvertebrate phyla include Mollusca (19), Arthropoda (10), Echinodermata (5), Chordata (2), and Platyhelminthes (2) (Table 2).

Table 2. Macroinvertebrate species found at each site.

Site	Phylum	Organism	Individuals
Site 1	**Arthropoda**	*Calcinus sp.*	17
		Grapsus grapsus	1
		Isopoda sp. 1	5
		Pachygrapsus transversus	44
	Chordata	*Entomacrodus sp.*	5
	Echinodermata	*Echinometra vanbrunti*	5
		Eucidaris thouarsii	1
		Heliaster cumingii	1
		Ophiactis savignyi	2
		Ophiocoma aethiops	1
	Mollusca	*Acanthina brevidentata*	5
		Acanthochitona hirudiniformis	3
		Acanthopleura sp.	3
		Cerithium sp.	10
		Chiton stokesii	4
		Dolabrifera sp.	9
		Elysia diomedea	8
		Felimare sp.	1
		Gemophos gemmatus	1
		Limaria sp.	2
		Nerita sp.	12
		Polyplacophora sp. 1	1
		Siphonaria sp.	1
		Vasula melones	7
		Zonulispira zonulata	2
	Platyhelminthes	*Platyhelominthes sp. 1*	1
Site 2	**Arthropoda**	*Brachyura sp. 2*	1
		Calcinus sp.	14
		Caridea sp. 1	12
		Coenobita compressus	1
		Pachygrapsus transversus	8
	Echinodermata	*Echinometra vanbrunti*	1
	Mollusca	*Anachis rugulosa*	22
		Elysia diomedea	22
		Gemophos gemmatus	4
		Stramonita biserialis	7
		Vasula melones	9
	Platyhelminthes	*Polycladida sp.*	3
Site 3	**Arthropoda**	*Brachyura sp. 1*	1
		Brachyura sp. 3	1
		Brachyura sp. 4	1
		Calcinus sp.	3
		Caridea sp. 1	2
		Coenobita compressus	3
		Pachygrapsus transversus	1
	Chordata	*Entomacrodus sp.*	5
		Osteichthyes sp. 1	1
	Echinodermata	*Echinometra vanbrunti*	2
		Heliaster cumingii	1
		Ophiactis savignyi	1
		Ophiocoma aethiops	3
	Mollusca	*Anachis rugulosa*	3
		Elysia diomedea	5
		Gemophos gemmatus	3
		Littorinidae sp.	10
		Polyplacophora sp. 2	1
		Vasula melones	6
Total:			**309**

Note: The most abundant species were *Pachygrapsus transversus* in Site 1, *Anachis rugulosa and Elysia diomedea* in Site 2, and *Littorinidae sp* in Site 3.

Table 3. Species found at different elevations of Site 1.

Elevation Level	Phylum	Organism	Individuals
Low	**Arthropoda**	*Calcinus sp.*	1
		Isopoda sp. 1	2
		Pachygrapsus transversus	8
	Chordata	*Entomacrodus sp.*	5
	Echinodermata	*Echinometra vanbrunti*	1
		Ophiactis savignyi	1
	Mollusca	*Acanthochitona hirudiniformis*	3
		Acanthopleura sp.	3
		Cerithium sp.	1
		Chiton stokeii	3
		Elysia diomedea	6
		Nerita sp.	4
		Vasula melones	6
		Zonulispira zonulata	1
Mid	**Arthropoda**	*Calcinus sp.*	4
		Isopoda sp. 1	1
		Pachygrapsus transversus	31
	Echinodermata	*Echinometra vanbrunti*	2
		Eucidaris thousarsii	1
		Heliaster cumingii	1
		Ophiactis savignyi	1
	Mollusca	*Acanthina brevidentata*	5
		Chiton stokeii	1
		Dolabrifera sp.	4
		Elysia diomedea	1
		Gemophos gemmatus	1
		Nerita sp.	6
		Polyplacophora sp. 1	1
High	**Arthropoda**	*Calcinus sp.*	12
		Grapsus grapsus	1
		Isopoda sp. 1	2
	Echinodermata	*Echinometra vanbrunti*	2
		Ophiocoma aethiops	1
	Mollusca	*Cerithium sp.*	9
		Dolabrifera sp.	1
		Elysia diomedea	1
		Felimare sp.	1
		Limaria sp.	2
		Nerita sp.	2
		Siphonaria sp.	1
		Vasula melones	1
		Zonulispira zonulata	1
	Platyhelminthes	*Platyhelpminthes sp. 1*	1
Total:			**148**

Note: The most abundant species were *Pachygrapsus transversus* at the low and mid transects and *Calcinus sp.* at the high transect.

Table 4. Species found at different elevations of Site 2.

Elevation Level	Phylum	Organism	Individuals
Low	**Arthropoda**	*Brachyura sp. 2*	1
		Pachygrapsus transversus	1
	Mollusca	*Anachis rugulosa*	13
		Elysia diomedea	16
		Gemophos gemmatus	3
		Stramonita biserialis	1
		Vasula melones	4
	Platyhelminthes	*Polycladida sp.*	2
Mid	**Arthropoda**	*Coenobita compressus*	1
		Pachygrapsus transversus	2
	Echinodermata	*Echinometra vanbrunti*	1
	Mollusca	*Anachis rugulosa*	9
		Elysia diomedea	6
		Gemophos gemmatus	1
		Stramonita biserialis	6
		Vasula melones	5
	Platyhelminthes	*Polycladida sp.*	1
High	**Arthropoda**	*Calcinus sp.*	14
		Caridea sp. 1	12
		Pachygrapsus transversus	5
Total:			**104**

Note: The most abundant species were *Elysia diomedea* at the low transect, *Anachis rugulosa* at the mid transect, and *Calcinus sp.* at the high transect.

Table 5. Species found at different elevations of Site 3.

Elevation Level	Phylum	Organism	Individuals
Low	**Arthropoda**	*Brachyura sp. 3*	1
		Caridea sp. 1	1
	Chordata	*Osteichthyes sp. 1*	1
	Echinodermata	*Ophiocoma aethiops*	1
	Mollusca	*Anachis rugulosa*	3
		Gemophos gemmatus	3
Mid	**Arthropoda**	*Brachyura sp. 1*	1
		Calcinus sp.	2
		Pachygrapsus transversus	1
	Echinodermata	*Echinometra vanbrunti*	2
		Heliaster cumingii	1
		Ophiocoma aethiops	2
	Mollusca	*Elysia diomedea*	5
		Polyplacophora sp. 2	1
		Vasula melones	4
High	**Arthropoda**	*Brachyura sp. 4*	1
		Calcinus sp.	1
		Caridea sp. 1	1
		Coenobita compressus	3
	Chordata	*Entomacrodus sp.*	5
	Echinodermata	*Ophiactis savignyi*	1
	Mollusca	*Littorinidae sp.*	10
		Vasula melones	2
Total:			**53**

Note: The most abundant species were *Anachis rugulosa* and *Gemophos gemmatus* at the low transect, *Elysia diomedea* at the mid transect, and *Littorinidae sp.* at the high transect.

Diversity and similarity indices:

The Shannon Diversity Index was applied to the data and produced scores of 2.94202 for macroinvertebrates and 1.47357 for sessile organisms (Table 6a). The Shannon Equitability Index was applied to the data and produced scores of 0.809 for macroinvertebrates and 0.501 for sessile organisms (Table 6a). The Jaccard Similarity Index was calculated for the data and gave the following results: the overall similarity between sites was 0.375, sites 1 and 2 scored a 0.613, sites 2 and 3 scored a 0.905, and sites 1 and 3 scored a 0.679 (Table 6b).

Table 6. a) Results from the Shannon Diversity Index and Shannon Equitability Index. b) Results from the Jaccard Similarity Index.

a.

Shannon Diversity	Shannon Equitability	ID	# of Species
1.47357	0.501	Sessile	18
2.94202	0.809	Macro	38

b.

Jaccard	Sites Compared
0.375	All Sites
0.613	Sites 1 & 2
0.905	Sites 2 & 3
0.679	Sites 1 & 3

Analysis and Discussion

Looking first at macroinvertebrates, Mollusca was found to be the most abundant phylum with a relative abundance of 0.521 (Figure 5). The most abundant members of the phylum were *Elysia diomedea* (35), *Anachis rugulosa* (25), *Vasula melones* (22), *Nerita sp.* (12), *Cerithium sp.* (10), and *Littorinidae* (10) (Table 1). The next most abundant phylum was Arthropoda with a relative abundance of 0.372 (Figure 1). The most abundant members of the phylum were *Pachygrapsus transversus* (53), *Calcinus sp.* (34), *Caridea sp. 1* (14), and *Entomacrodus sp.* (10) (Table 2). All the most abundant maroinvertebreates found in this study, with the exceptions of *A. rugulosa, Caridea sp., and Entomacrodus sp,* were also found in the study by Martinez (2021). The most abundant macroinvertebrates in Martinez (2021) were *Echinometra vanbrunti* (Figure 12) and *Ophiocoma aethiops*, and they were found mostly in the low and mid transects. Both species were also found in this study in the low and mid transects, however they were not nearly as abundant (Table 3).

It is possible they were not as abundant due to a lack of plentiful food sources. One study on *Echinometra* species found that when individuals were removed, there was a significant increase in the growth of brown algae, including *Padina* species (Mcclanahan and Muthiga, 2013). This indicates that *E. vanbrunti* might prefer species of brown algae, which were not abundant in the sites observed in this study (Figure 6). However, brown algae species were not abundant in the study by Martinez (2021) while *E. vanbrunti* was very abundant, thus pointing to another reason for the low numbers in Puerto Cabuyal. It is possible that as the sites in this study only went to the edge of the rocks where there was still not much water, the area surveyed was not the ideal habitat for *E. vanbrunti.* A similar logic might follow for *O. aethiops* because as both species are echinoderms, they cannot survive for long out of the water but thrive when completely submerged (Leeuwis & Gamperl, 2022).

Figure 12. *Echinometra vanbrunti* individual

In this study, the crab *P. transversus*, the most abundant macroinvertebrate, was most commonly found in the mid-level transect (Table 3). In the study by Martinez (2021), looking at the data from their site in Puerto Lopez, *P. transversus* was also most abundant in the mid-level. The elevated presence of *P. transversus* (Figure 13) in the sites in Puerto Cabuyal indicates that the species is common along the coast of Ecuador as it was also commonly found in the study by Martinez (2021) and in a study of Ecuador's coasts by Cardenas-Calle et al. (2020). In terms of their effect on the environment, a study by Christofoletti et al. (2010) found that *P. transversus* has a strong consumer effect on sessile invertebrates. In one area of the study, the rocks of the intertidal were dominated by macroalgae. When the presence of *P. transversus* was removed, a rapid increase in sponge, ascidian, and bivalve, including *Brachidontes sp.,* coverage occurred (Christofoletti et al, 2010). In Puerto Cabuyal, there was a presence of *Brachidontes sp.*, but it was extremely minimal and covered less than 1% of total quadrants (Figure 11b). Figure 10 demonstrates how there was only 5% coverage in sessile quadrants by sessile organisms; the majority of the quadrants were completely covered by macroalgae species. This implies that a high population of *P. transversus* predates upon the sessile invertebrates in Puerto Cabuyal, thus causing an increase in macroalgae growth.

Figure 13. *Pachygrapsus transversus* individual

Chlorophyta was found to be the most abundant phylum looking at sessile organisms and algae, with 48.5% coverage of sessile quadrants and 2 species (Figure 6, 10). The most abundant species of the phylum were *Chaetomorpha antennina* (35.6% total coverage) and *Cladophora sp.* (12.9% total coverage) (Figure 10). Rhodophyta was the next most abundant phylum, with 17.3% coverage of sessile quadrants and 5 species (Figure 6, 10). The most abundant species of the phylum were *Ahnfeltia sp.* (8.3% total coverage) and *Catenella caespitosa* (8.1% total coverage) (Figure 10). The most dominant phylum looking only at sessile organisms was Arthropoda with 3% coverage of sessile quadrants and 2 species (Figure 6, 10). One of the species commonly found in the study by Martinez (2021) was *Caulerpa racemosa*, which they noted as an invasive species of algae. That alga was not found in this study in Puerto Cabuyal.

Something to note is that while Ochrophyta had the highest level of species richness out of the algae with 6 species, it had the lowest abundance at 0.055 or 5.5% coverage of sessile quadrants (Figure 6, 10). Additionally, Chlorophyta had the highest abundance at 0.48 or 48.5% coverage of sessile quadrants, but it had exceptionally low species richness (Figure 6, 10). This lack of species richness in the Chlorophyta phylum but overall abundance might indicate something about the dominance of certain species of green algae or its ability to survive in the intertidal zone. As stated previously, *C. antennina* was found to be the most abundant alga with a total coverage of 35.6% (Figure 10). One study done on green algae and seaweed species including *C. antennina* found that the maximum abundance was either before or after the rainy season, but not during (Adsul et al., 2019). The study also found that salinity corresponds negatively with abundance of *C. antennina* (Adsul et al., 2019). This could possibly be a factor in the growth of *C. antennina* (Figure 14) in Puerto Cabuyal, however salinity was not measured so that conclusion requires further study. Vinueza et al. (2006) found that *C. antennina* was a dominating algae species leading up to and all the way through the 1997-98 ENSO event in the Galapagos. When temperatures decreased after the event, *C. antennina* was dominated by other algae species (Vinueza et al., 2006). It is possible that *C. antennina* is dominating in Puerto Cabuyal due to changes in temperature from the current El Niño event, but since temperatures were not measured, more studies would need to be done to confirm this theory.

Figure 14. *Chaetomorpha antennina*

Species diversity was assessed with the Shannon Diversity Index and results found that macroinvertebrate diversity was 2.94202 while sessile was lower at 1.47357 (Table 6a). These results demonstrate that overall macroinvertebrate diversity was higher than the overall sessile diversity. Species evenness was assessed with the Shannon Equitability Index and it was found that macroinvertebrates had a high level of evenness at 0.809 while sessile organisms had a mid-level of evenness at 0.501 (Table 6a). These levels of evenness imply that the populations of macroinvertebrates are relatively balanced while sessile populations are a little less so. Results from the Jaccard Similarity Index showed that overall, the three sites tested were not particularity similar with a score of 0.375. But one interesting result is that Sites 2 and 3, the furthest apart, were found to be very similar with a score of 0.905 while Sites 1 and 2, the closest together, were less similar at 0.613 (Table 6b). One possible explanation is that Sites 2 and 3 had fewer species than Site 1, therefore the comparison is between fewer species.

Species richness was highest for macroinvertebrates in Site 1 (Figure 7a) and for sessile organisms and algae in Site 2 (Figure 7a). Conversely, species richness was the lowest at Site 2 for macroinvertebrates and Site 3 for sessile organisms and algae. The greatest species richness overall was found at Site 1 (Figure 9b). The lowest species richness overall was found at Site 3 (Figure 9b). One interesting point to note is that Site 3 was the closest to human settlement and was the only site to have macroplastics found in quadrants (Figure 15). Since there were macroplastics found in a site, it is important to think about the probability and possible issues of microplastics in the intertidal as well. One study looked at microplastics in the intertidal zone and found that in the rocky intertidal, microplastics have the potential to embed into the rocks, a phenomenon known as “plastiglomerate”, and then act as vectors for contaminants and pathogens (Wu et al., 2022). This could prove to be problematic for both the organisms living in the intertidal but also for the people living in the community. The study also described the rocky intertidal as a sort of ‘temporary sink” for microplastics as they get funneled out into the ocean (Wu et al., 2022).

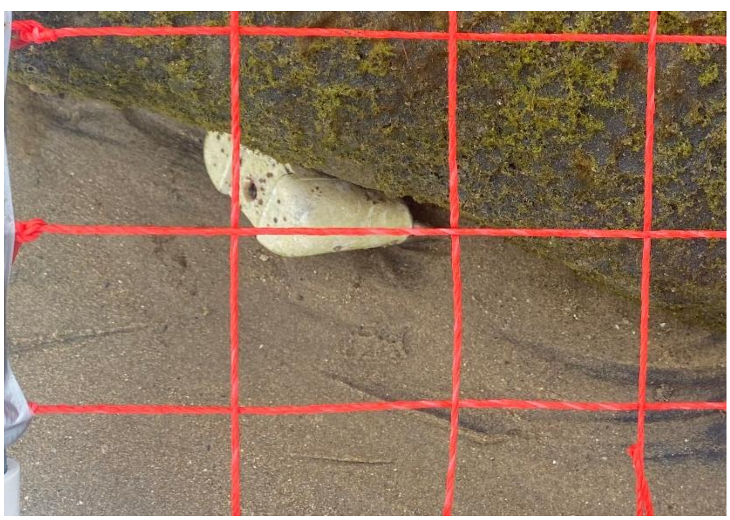


Figure 15. Macroplastic piece found in a quadrant of Site 3

It was found that samples taken from low sites had the highest level of species richness while the high sites had the lowest species richness in both macroinvertebrates and sessile organisms and algae (Figure 9b). This concurs with the data from Martinez (2021) as they had many sites where very little or nothing was found in the high transect. These results are most likely due to the harsher conditions of the higher intertidal, as fewer species are adapted to thrive in that environment. Newell (2013) discusses how organisms living at the high-water mark are exposed to air and direct sunlight for a majority of the time while organisms living at the low tide mark are almost always underwater.

Data was also analyzed to view the average number of individuals of a species found in a quadrant. Site 2 averaged the most macroinvertebrate individuals per quadrant (Figure 8a) while Site 3 averaged the most sessile organisms and algae per quadrant (Figure 8a). These results are interesting because Site 2 is the farthest site from human settlement while Site 3 is the closest. There could be a correlation to the amount of human impact, as macroinvertebrates are able to move away from potential disruptions or threats as opposed to sessile organisms that are anchored to one place. However, the species richness for macroinvertebrates was lowest at Site 2 (Figure 7a). It may be the case that a select few species have moved farther from humans, but there was a very high similarity score from the Jaccard index between species of Sites 2 and 3 (Table 6b). Therefore, another cause is more likely and should be further studied.

On average based on tide level, the most macroinvertebrate individuals per quadrant were found in the mid quadrants while the most sessile and algae coverage per quadrant was found in the high quadrants (Figure 8b). Looking at all the data, the greatest average number of individuals of a species were found in Site 2 at the high level transect (Figure 9a). This is interesting to consider as the lowest species richness was found at the high transect (Figure 9b). One reason for these results could be that the data was taken during low tide, so more organisms were out scavenging in the mid and high levels (Jorgenson, 2009). The significant coverage of algae in the high quadrants is mostly due to a few species dominating the area and being adapted well to grow in the area (Jorgenson, 2009).

Overall, the error bars for these graphs (Figure 7, 8, 9a) were rather large, indicating a significant range in the number of individuals of a species per quadrant. In Figures 8b and 9a, there were not significant differences in each bar for macroinvertebrates indicating the range of individuals of species was similar. Figure 8b depicts the sessile organisms in the high sites with a notable sized error bar, thus indicating high standard error due to a low sampling size as well as a dominance in *C. antennina* (Figure 10). Conversely, the macroinvertebrates in Site 3 have a low standard error, which is due to the very few individuals found in quadrants at that site (Figure 8a, Table 5).

The presence of certain species of algae can be important to understand as some species are either especially helpful to the environment or have human uses. The most abundant alga found in Puerto Cabuyal was *C. antennina* (Figure 10, 11, 14), which studies have found to have properties useful for humans. One study on *C. antennina* in the rocky intertidal of India found that in the post monsoon season, *C. antennina* had elevated levels of antioxidants and then during the moonson season had high levels of sugars and fatty acids (Vinuganesh et al., 2022). These elevated levels give *C. antennina* a commercial purpose for most of the year (Vinuganesh et al., 2022). Another algae found in this study was *Padina pavonica* (Figure 10, 16), which is also known to have human use. A study done by Ameen et al. (2022) found that species of macroalgae, especially the green alga *Ulva* sp. and the brown alga *Padina pavonica*, can accumulate heavy metals in the water in addition to nutrients. Heavy metals in the environment are incredibly problematic as they can remain in the bodies of animals such as fish who breathe the chemicals in from the water and bioaccumulate up the food chain up to humans (Araújo and

Cedeño-Macias., 2016). It is possible that species of macroalgae could serve as bioindicators of heavy metal pollution in an ecosystem (Ameen et al., 2022). Araújo and Cedeño-Macias (2016) studied the presence of heavy metals in commercial fish species caught in Manta, Ecuador, and found that half their samples had levels of metals above the limits considered safe for human consumption. As Puerto Cabuyal is a fishing community, understanding *P. pavonica* populations and looking for increases could be a useful method to monitor possible heavy metal accumulation in the fish species.

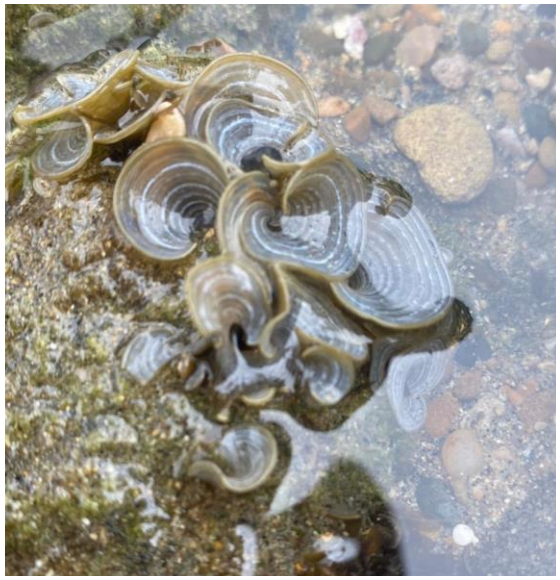


Figure 16. *Padina pavonica*

Data collection was done both during the dry season in Ecuador and at the start of an ENSO event. As the data for this investigation was collected during an El Niño year (World Meteorological Organization, 2023), it is vital to consider the possible effects on the ecosystem. ENSO events are known to bring warmer temperatures and fewer nutrients to the coast of South America (Cai et al., 2020; Vinueza et al., 2006). The nutrient differences in the water and the warmer temperatures could have effects on the dominance or presence of specific types of algae. One study in the Galapagos found that a temperature increase of 1.45°C led to a significant decrease in the biomass of intertidal seaweed as well as a decrease in the abundance, richness, and diversity of invertebrates in the area (Harris, n.d.). Vinueza et al. (2006) found that in the heart of an ENSO event, herbivores did not have a strong influence on the cover of algae, but they did on the size of the algae. The study also found that many of the grazers had a loss in population, and most algae still declined in population even without many predators (Vinueza et al., 2006). Although the effects of El Niño are known to vary from year to year (World Meteorological Organization, 2023), there is a strong possibility that populations of the intertidal zone in Puerto Cabuyal will be affected. It is likely that species adapted well to temperature or salinity changes will dominate, such as those in the high intertidal (Newell, 2013).

Given that this study was conducted at the beginning of an ENSO event (World Meteorological Organization, 2023), the effects of the event are not strongly present in the data. Therefore, one future direction would be looking at the same sites after the event and comparing the results to see what the effect was. The same could be done before and after La Niña events. Observing the same sites during the rainy season in Ecuador would also be interesting to compare what species are most dominant when there is a greater amount of fresh water. One issue encountered during the analysis was difficulty in identifying algae species due to a lack of studies on marine algae in coastal Ecuador, so a productive direction to take this study would be to create a database of algae found along the coast. Another direction this work could be taken in would be looking at the possible presence of microplastics in the sites surveyed as it is now known that macroplastics are present in the area (Figure 15). Looking at a different type of pollution, studies could determine whether pollution by heavy metals is present along coastal Ecuador and if so, if algae species such as *P. pavonica* and other brown algae have a high presence in the area.

Conclusion

Many sections of the rocky intertidal zone of coastal Ecuador are now protected as marine reserves. This is essential to protecting their biodiversity, but it is equally as important to understand what species are found in those areas. The sites tested in Puerto Cabuyal were found to be diverse and minimally impacted by humans, with a high abundance of both macroinvertebrates and algae species. The most abundant phyla found in Puerto Cabuyal were Mollusca for macroinvertebrates, Chlorophyta for algae, and Arthropoda for sessile animals. The low transects demonstrated the highest level of species richness in sessile organisms, algae and macroinvertebrates. It was confirmed with this study that the intertidal zone of Puerto Cabuyal does have many of the same species as other intertidal zones along coastal Ecuador. The observations and identifications of the intertidal species in the area allow for the community to compare species in the future as well as have a greater understanding of the importance of the area. The discovery of macroplastics in one site prompts further research into the possibility of microplastics. Additionally, the presence of certain species that have known human uses indicates that further study of those species would be useful to determine if some could be used without damaging the ecosystem. The intertidal zone is a vital part of the ocean ecosystem as a barrier to erosion as well as an indicator of climate change and is therefore necessary to protect, as it currently is in the Puerto Cabuyal-Punta San Clemente Marine Reserve.

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Appendix

Appendix A: Identified macroinvertebrates

Figure 17. a). *Zonulispira zonulata* individual. b). *Vasula melones* individual

Figure 18. a). Individual of the genus *Nerita.* b). *Ophiactis savignyi* individual

Figure 19. *Elysia diomedea*

Figure 20. a). *Heliaster cumingii* individual b). Individual of genus *Polycladida*

Appendix B: Identified algae species and sessile animals

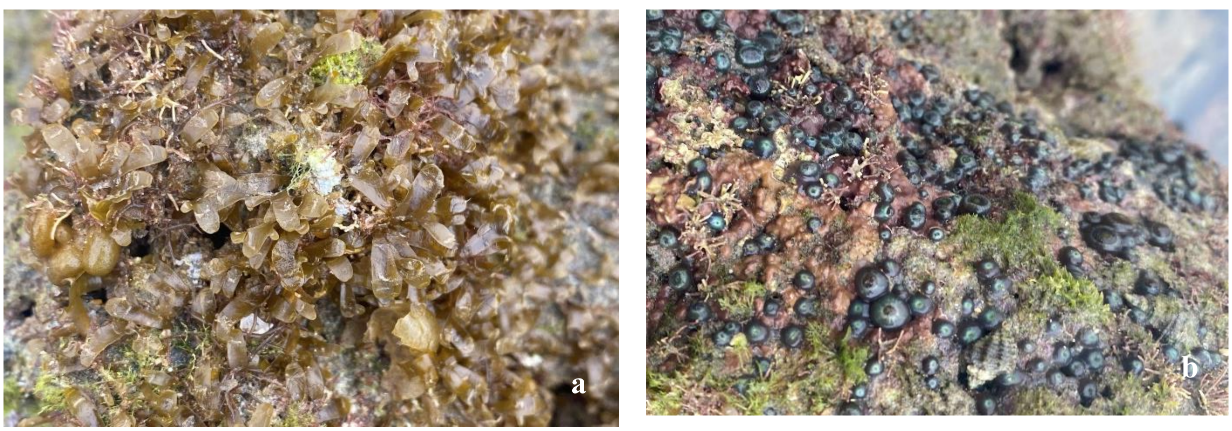

Figure 21. a). *Catenella caespitosa*. b). Members of the genus *Zooanthus*

Figure 22. a). *Colpomenia* sp. 2. b). *Actinostella bradleyi* individual

Figure 23. *a). Tetraclita sp.* b). *Padina durvillaei*

Figure 24. a). *Balanus glandula.* b). *Ahnfeltia sp.*

Figure 25. *Lithophyllum* sp.