



Epifaunal community associated with *Cystoseira humilis* in tide pools: Assessing the significance of depth and size as pivotal factors

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Abstract: Tide pools stand out as highly variable and distinctive ecosystems within the intertidal zone. Despite the accessibility of this environment for study, there has been a relatively limited focus on tide pools specifically. This study addresses this gap, aiming to enhance our understanding of variations among tide pools, considering factors such as depth and size. The brown algae *Cystoseira humilis* serves as the study model due to its prevalence in the tide pools of Gran Canaria. A total of 80 samples, with 10 replicates from each tide pool, were collected using a quadrat method measuring 25 cm x 25 cm in two distinct sites within each tide pool. The chosen method, though destructive, involves scraping the delimited area with a spatula to collect all material, facilitating the study of epifaunal community. Among the 6,068 organisms identified, spanning 48 species, a substantial 95% were classified as amphipods. Depth emerged as the most influential factor impacting the epifaunal community, followed closely by pool size. Notably, the study of species dissimilarity revealed that, when considering pool size, the isopod *Dynamene edwardsi* made the most significant contribution to dissimilarity. Conversely, when depth was the focal factor, the amphipods *Apholyte perieri* and *Ampithoe rubricata* stood out, with higher abundances in deep tide pools compared to shallow ones. Consequently, both size and depth emerged as pivotal factors influencing the studied epifaunal communities. For future ecological studies, it is crucial to account for the tide pool position in the intertidal zone and the level of hydrodynamic exposure at each site. Additionally, broadening the scope to include comparisons with tide pools from other regions and considering the presence of other algal species could provide further insights into differences in epifaunal communities.

Résumé : Communauté d'épifaune associée à *Cystoseira humilis* des cuvettes intertidales : évaluation du rôle de la profondeur et de la taille comme facteurs clés. Les cuvettes représentent des écosystèmes particuliers et à forte variabilité de la zone intertidale. En dépit de l'accès facile à ce milieu, l'attention portée à ces cuvettes est relativement faible. Cette étude comble ce manque en essayant d'améliorer nos connaissances des variations de ces systèmes en prenant en compte les facteurs profondeur et taille. L'algue brune *Cystoseira humilis* est utilisée comme modèle en raison de sa fréquence dans les cuvettes intertidales de la Grande Canarie. Un total

de 80 échantillons, à raison de 10 replicats par cuvette, a été réalisé à l'aide de quadrats de 25 cm x 25 cm dans deux zones distinctes de chaque cuvette. La méthode choisie, destructive, implique un grattage de la surface délimitée à l'aide d'une spatula afin de récolter tout le matériel biologique, facilitant ainsi l'étude ultérieure de la communauté épigée. Parmi les 6068 organismes identifiés dans 48 espèces, 95% étaient des amphipodes. La profondeur est apparue comme le principal facteur influençant la communauté, suivie par la taille des cuvettes. En particulier, l'analyse de dissimilarité a montré que, en considérant la taille des cuvettes, l'isopode *Dynamene edwardsi* représentaient l'espèce la plus discriminante. Au contraire, en considérant la profondeur, les amphipodes *Apothyale perieri* et *Ampithoe rubricate* ressortaient de l'analyse avec des abondances plus fortes dans les cuvettes les plus profondes. Finalement, la profondeur et la taille des cuvettes apparaissent comme les facteurs clés de structuration de ces cuvettes intertidales. À l'avenir, il est crucial de prendre en compte à la fois la position des cuvettes sur l'estran ainsi que le niveau d'exposition de chaque site. De même, élargir l'étude en incluant des comparaisons entre régions et en prenant en compte d'autres espèces d'algues permettraient de mieux caractériser les différences de communautés d'épifaune.

Keywords: Algae • Intertidal • Amphipoda • Rocky pools • Epibenthic community • Vertical dimension • Magnitude

Introduction

The intertidal zone, which can be defined as the meeting zone between the land and the ocean, is submerged during high tide and exposed during low tide (Tuya et al., 2008). This unique feature provides a natural laboratory due to its composition of open ecosystems, which naturally experience a range of abiotic stressors, biotic interactions, and biological patterns (Mendonça et al., 2018). In addition, the rocky intertidal zone is a habitat for many species, from small invertebrates to fish (Quirós et al., 2012), and many of these species are sessile or slow moving (Connell, 1972). The rocky intertidal zone constitutes a distinct microcosm, with tide pools acting as defined extensions of the perpetually submerged water. The ecological characteristics of these tide pools vary notably based on differences in their size, depth, and location within the intertidal zone (Betancor et al., 2015). These pools are formed by the tidal cycle on the rocky shore (Castellanos-Galindo et al., 2005). Furthermore, tide pools serve as dynamic habitats, providing essential functions such as feeding, nursery grounds, and refuge during emersion for a multitude of species that must adapt to their ever-changing environment (King, 2010; Riccardi et al., 2022). Despite the ongoing challenges posed by their environment, tide pools exhibit remarkable biodiversity within their microecosystems, housing marine diatoms, vascular plants, bryophytes, invertebrates, and fish (Metaxas & Scheibling, 1993; Pribadi & Kanza, 2017). Microecosystems, characterized as small-scale ecological systems, represent a subset of the biotic community and abiotic properties present in larger ecosystems (Matheson, 2008). The high variability

observed in tide pools stems from their unique nature as habitats lacking constant water circulation solely linked to the tide. Consequently, environmental factors significantly impact the characteristics of each tide pool, including temperature, pH, salinity, and oxygen saturation (Betancor et al., 2015). Temperature can fluctuate by up to 15°C, whilst salinity may vary between 5 and 25, depending on the height of the pool along the intertidal shore (Metaxas & Scheibling, 1993). Consequently, factors such as the tide pool's position within the intertidal area in relation to the sea and exposure to waves become relevant considerations. Previous studies have demonstrated that these factors influence the percentage of algal coverage (e.g., Dethier, 1982). Therefore, in tide pools, physical and biological factors intertwine to mold the specific community structure of inhabiting organisms (Metaxas & Scheibling, 1993; Mahon & Mahon, 1994; Castellanos-Galindo et al., 2005). Hence, each tide pool hosts a distinct array of organisms cohabiting within a shared habitat, influenced by the interplay of species interactions and specific abiotic factors characteristic of the tide pool environment (Metaxas & Scheibling, 1993; King, 2010). Previous studies, such as Martins et al. (2007), have highlighted the importance of various parameters, including maximum length, in shaping the composition and diversity of algae and epifauna within tide pools. For example, research has shown that the maximum length of habitat-forming algae, such as *Cystoseira humilis* Schousboe ex Kützing, 1860, directly impacts the structural complexity and availability of microhabitats within tide pools, thereby influencing the diversity and abundance of associated epifaunal communities (Vaz-Pinto et al., 2014; González-Aragón et al., 2024).

Additionally, variations in maximum length can affect the resilience of algal populations to environmental stressors, such as desiccation and wave action, further influencing the overall functioning and stability of tide pool ecosystems (e.g., Viejo, 1999). By incorporating references and examples from the literature, we aim to strengthen the support for our statement regarding the significance of maximum length in shaping tide pool ecosystem characteristics. The depth of the tide pool indeed plays a crucial role in influencing the diversity and composition of organism communities (Kooistra et al., 1989). For instance, studies have demonstrated that variations in depth create distinct microhabitats within tide pools, leading to differences in environmental conditions such as light availability, temperature, and nutrient levels. Physico-chemical conditions also affect the growth of algae and the habitat of different species (Metaxas & Scheibling, 1993; Bussell et al., 2007).

As mentioned above, tide pools are crucial refuges for many species from a range of stressful environmental conditions. These conditions include extreme temperature variations, rapid changes in salinity levels, limited oxygen availability during low tide, and potential desiccation due to exposure to air during emersion periods. Tide pools can also experience high levels of wave action, which can create turbulent conditions and dislodge organisms from their substrates. These tide pools serve as essential sanctuaries, providing protection from challenging environmental factors and offering organisms a stable habitat where they can seek refuge and persist in the intertidal zone (Metaxas & Scheibling, 1993). In light of this, ecological studies of diversity are crucial for understanding the community processes and behaviors of different species and their interactions within these changing environments (Godinho & Lotufo, 2010). By elucidating the intricate dynamics of species within tide pools, such studies contribute to our broader comprehension of how organisms adapt and thrive amidst fluctuating coastal conditions. In tide pools, epifauna are pivotal contributors to biodiversity (Chen et al., 2021; Viejo, 1999). Macrophytes serve as indispensable habitats for these organisms, which inhabit both living and non-living surfaces. Abundant within marine macroalgae and seagrasses, epifaunal organisms, including small crustaceans and gastropods, rely on these substrates for shelter and sustenance (Viejo, 1999; Chen et al., 2021). Moreover, epifauna utilize surfaces provided by other organisms such as upper layers or branches, as well as corals. While many epifaunal species are sessile filter feeders permanently attached to a substratum, herbivores that consume epiphytic algae or the host plant are the most common (Osman, 1977; Duffy & Hay, 2000).

Nonetheless, mobile epifauna are also integral, serving as primary resources for secondary consumers, such as fish and large invertebrates (Piñeiro-Corbeira et al., 2021). The spatial distribution of epifaunal organisms is intricately influenced by a multitude of physical and biological factors, including animals' physiological thresholds within the environment and interspecies dynamics (Viejo, 1999; Martins et al., 2007). Consequently, environmental factors have a direct impact on epifaunal communities, notably exemplified by prolonged periods of emersion observed in tide pools on the upper shore. Furthermore, apart from its influence on physicochemical conditions, pool size may also affect the recruitment probability (Martins et al., 2007). Additionally, the complexity of the habitat plays a significant role (Martin-Smith, 1993). In the context of epifauna, the abundance of epifaunal communities is profoundly influenced by the structural complexity of the algae hosting these organisms (Peñalver-Bravo, 2022). This complexity, determined by algae species and seasonal patterns affecting epiphyte growth, underscores the nuanced interplay between habitat structure and epifaunal abundance (Martin-Smith, 1993). The intertidal rocky zone on the Canary Islands is home to various species of brown algae, particularly the genus *Cystoseira*. One dominant species is *Cystoseira humilis*, which grows in tide pools and covers the bottom and walls (Betancor et al., 2015; Vaz-Pinto et al., 2014). The algae *C. humilis* is a monoecious species with multiple stems that can grow up to 150 cm (Pardi et al., 2000). We chose *C. humilis* because of its significance as a habitat-forming species in the tide pools of the Canary Islands (González & Afonso-Carrillo, 1990; Pardi et al., 2000; Vaz-Pinto et al., 2014), as well as the rich biodiversity of epifauna associated with this alga (González-Aragón et al., 2024). The novelty and importance of our study lie in its focused exploration of how size and depth variations in tide pools specifically affect the epifaunal community associated with *C. humilis*. While prior research has explored similar questions (Bennett & Griffiths, 1984; e.g., Anusa et al., 2012; Carvalho et al., 2021), our study's focus within this specific ecological context offers novel insights into how these environmental factors influence community structure. By elucidating the relationship between size, depth, and epifaunal communities, we contribute to understanding the ecological role of *C. humilis* in coastal ecosystems. This research highlights the importance of considering habitat-forming species like *C. humilis* in conservation and management strategies, particularly in regions with diverse marine ecosystems such as the Canary Islands.

Material and Methods

Study area and sample collection

This study was carried out in the island of Gran Canaria, Canary Islands. The island has a coastal perimeter of 236 km and a zone with a greater range of tide pools is found in the northwest between Gáldar and Arucas (Luengo Barreto, 2018). Samples were collected from eight tide pools along the Gáldar coast (Fig. 1) during March and April 2023.

When selecting tide pools, two parameters were considered: size and depth. The depth and size thresholds selected for classification derive from a combination of ecological considerations, biological zonation patterns and prior research findings (R. Riera, *unpubl. data*). The thresholds of depth and size were determined through a comprehensive consideration of various ecological factors, including the physical dimensions of tide pools, which directly influence the availability of habitat and resources for epifaunal organisms. Additionally, we considered biological

zonation patterns observed in tide pool ecosystems, where different species tend to occupy distinct vertical and horizontal zones within the intertidal area. By aligning our size and depth thresholds with these zonation patterns, we aimed to capture the full spectrum of habitat types present in the study area, thereby ensuring a representative sampling of the epifaunal community associated with *C. humilis*. Furthermore, insights gleaned from prior research, including unpublished data from the second author (RR), provided valuable context and guidance in refining our classification criteria to best suit the ecological dynamics of the study site. For example, variations in temperature, pH, and salinity levels can create distinct microhabitats within tide pools, influencing the distribution and abundance of organisms. The intervals of depth and size were established, defining deep tide pools as those with a depth exceeding 1.4 m, while shallow tide pools are characterized by a < 1.4 m depth. In addition, a tide pool was classified as large if its maximum length exceeded 5 m, while those with a < 5 m length were categorized as small. Resulting in

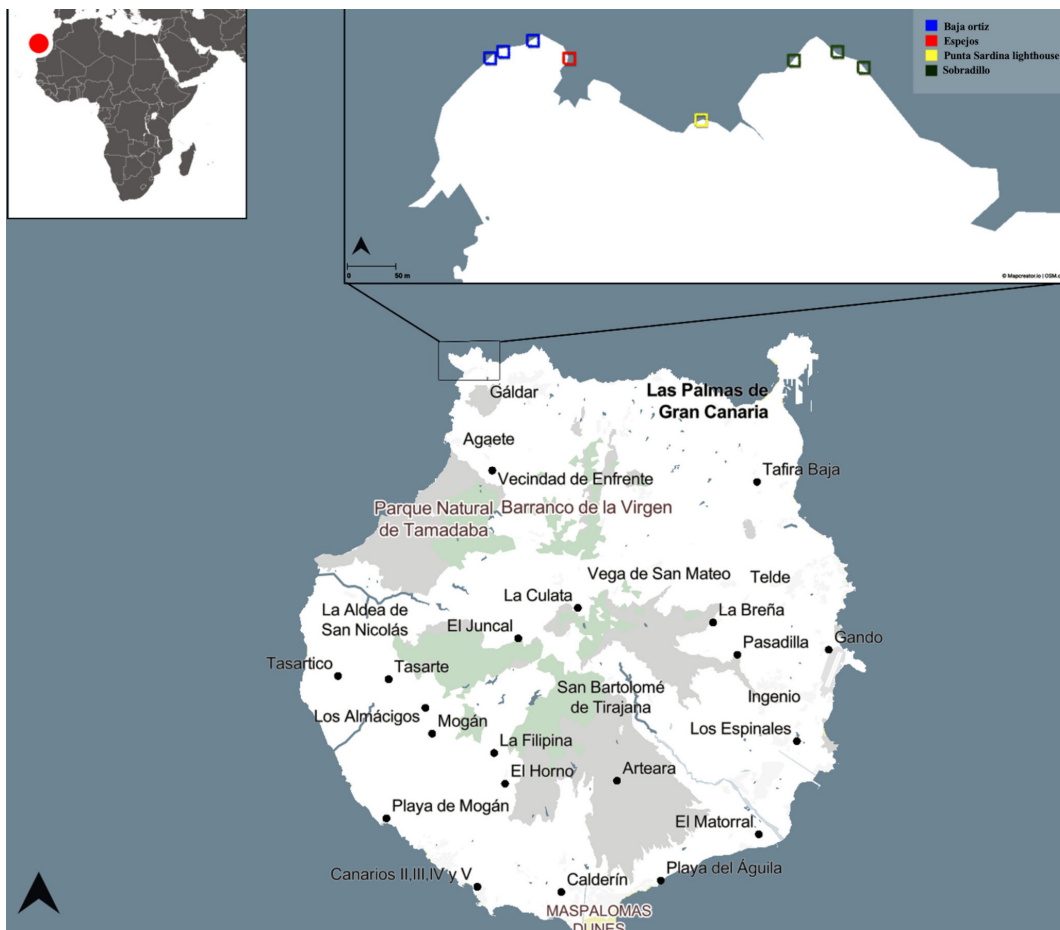


Figure 1. Sample locations on Gáldar coast (Gran Canaria, Canary Is. NE Atlantic Ocean).

Table 1. Characteristics of the studied tide pools.

Sites	Sampling date	Coordinates	Length (m)	Depth (m)
Lighthouse Punta Sardina (1)	3/12/2023	28° 9'51.92N-15°42'32.84W	5	1.0
Lighthouse Punta Sardina (2)	3/12/2023	28° 9'54.44N-15°42'25.27O	5	0.5
Baja Ortiz (3)	3/16/2023	28°10'6.70N-15°41'27.05W	4	1.5
Sobradillo (4)	3/16/2023	28° 9'52.73N-15°41'45.54W	20	1.4
Lighthouse Punta Sardina (5)	4/12/2023	28° 9'53.99N-15°42'33.97W	10	2.6
Baja Ortiz (6)	4/14/2023	28°10'8.53N-15°41'17.43W	25	0.5
Espejos (7)	4/21/2023	28° 9'56.17N-15°42'20.69O	11	1.8
Baja Ortiz (8)	4/24/2023	28°10'10.04N-15°41'20.43W	4	1.3

the following classifications: (i) deep/large, (ii) deep/small, (iii) shallow/large, and (iv) shallow/small (Table 1).

Sampling procedures

During the sampling process, a total of 8 tide pools were selected. From each tide pool, 10 samples of the studied alga (*Cystoseira humilis*) were collected, resulting in a total of 80 samples. The samples were collected using quadrats measuring 25 cm × 25 cm at two sites within each tide pool, which were chosen to ensure sampling at opposite locations within the same tidal pool. In each site, 5 samples were collected, resulting in a total of 5 samples from site A and 5 samples from site B. The minimum percentage of *C. humilis* coverage in sampled areas was carefully selected to ensure robust representation of this habitat-forming alga within the study site. Specifically, we targeted areas characterized by a minimum of 95% coverage of *C. humilis* to ensure that the

epifaunal communities sampled were associated with this algal species. Regarding the morphology of *C. humilis*, we aimed to minimize heterogeneity within samples by selecting fronds of similar size, ca. 20 cm in length. While *C. humilis* can indeed grow quite large, with fronds reaching considerable lengths, our focus on standardizing frond size within the sampled areas allowed us to mitigate potential variations in habitat structure and complexity that could confound our analysis of epifaunal communities. We used a destructive procedure, scraping the area of the quadrat (25 cm x 25 cm) with a scraper and collecting all the samples in zip bags for subsequent identification in the lab. To preserve the sample, each sample was frozen directly using seawater or preserved in 70% ethanol. Furthermore, we measured the length and depth of each tide pool.

Once in the lab, each sample of *C. humilis* underwent sorting and species identification to the lowest possible taxonomical level. Organisms were classified and counted in different vials using tweezers. To aid in the

Table 2. Results of the SIMPER analysis of dissimilarity up to 75%. Small tide pools: Average abundance in small tide pools. Large tide pools: Average abundance in large tide pools. Shallow tide pools: Average abundance in shallow tide pools. Deep tide pools: Average abundance in deep tide pools. Cumsum, Cumulative Contribution.

Species	Average small	Average large	Contribution (%)	Cumsum (%)
<i>Dynamene edwardsi</i> (Lucas, 1849)	18 925	6 350	18.3	18.3
<i>Ampithoe rubricata</i> (Montagu, 1808)	22 825	7 225	16.8	35.1
<i>Apohyale perieri</i> (Lucas, 1846)	10 775	15 300	14.9	50
<i>Sunamphitoe pelagica</i> (H. Milne Edwards, 1830)	6 800	11 375	11.9	61.9
<i>Pleonexes gammaroides</i> Spence Bate, 1857	12.55	5 100	11.7	73.6
Species	Average.Shallow	Average.Deep	Contribution (%)	Cumsum (%)
<i>Apohyale perieri</i> (Lucas, 1846)	6 500	19 575	16.3	16.3
<i>Ampithoe rubricata</i> (Montagu, 1808)	7 825	22 225	15.9	32.2
<i>Dynamene edwardsi</i> (Lucas, 1849)	17 625	7 650	15.7	47.9
<i>Pleonexes gammaroides</i> Spence Bate, 1857	2 250	15 400	13.1	61
<i>Sunamphitoe pelagica</i> (H. Milne Edwards, 1830)	11 425	6 750	11.4	72.4

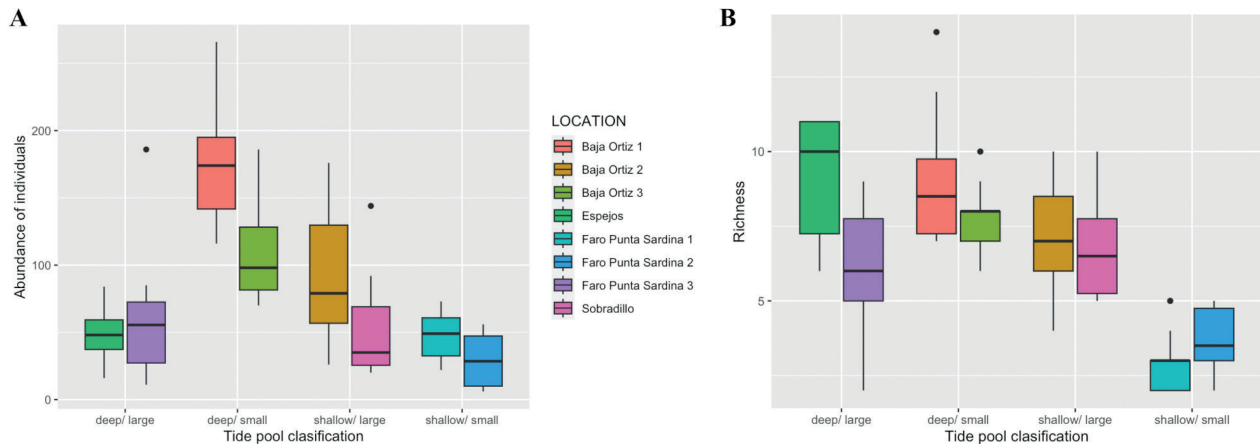


Figure 2. Abundance (A) and richness (B) of epifauna of the studied tide pools

identification process, various taxonomic identification guides and scientific articles (e.g. Lincoln, 1979; Riera et al., 2003; Abel & Riedl, 2009) were consulted. All identifiable organisms were included in further analysis to comprehensively assess the epifaunal community associated with *C. humilis*.

Data analysis

The statistical analysis was performed in R Studio. Data from the Excel database were converted to CSV format to facilitate analysis in R. Initially, a univariate description was performed, calculating the mean and standard deviation. Subsequently, the distribution of species richness and individual abundance was computed, and hypotheses were tested using either an ANOVA test or Kruskal-Wallis test, depending on the data distribution, normality and homocedasticity. Data on epifauna were not transformed. Following this, a non-metric multidimensional scaling (nMDS) analysis was employed to ordinate the sampling sites in a two-dimensional space, facilitating the observation of dissimilarities based on factors such as depth and size. Separate MDS plots were generated to depict the tide pools classified by depth and size, while an additional MDS plot visualized all groups of tide pools. However, the significance of differences between tide groups was determined using Permutational multivariate analysis of variance (PERMANOVA), allowing for the analysis of how epifaunal assemblages are distributed across groups based on factors examined in this study, i.e., pool size (large and small) and depth (deep and shallow). Lastly, a SIMPER (Similarity Percentage) test was conducted to assess the contribution of each species to the differences in epifaunal communities within the studied tide pools and determine the percentage of contribution of the species to each

classification scheme.

The statistical analyses described above were conducted using various R packages. Among these, the "vegan" package (Oksanen et al., 2022) was extensively utilized for performing MDS and PERMANOVA analyses, providing the necessary functions for these procedures. Additionally, the "ggplot2" package (Wickham, 2016) played a crucial role in facilitating the creation of diverse types of graphs. Furthermore, other packages such as tidyverse, janitor (Firke, 2023), flextable (Skintzos, 2023), and readxl (Wickham & Bryan, 2023) were employed to read the data, generate high-quality graphs, and create tables with improved formatting.

Results

Epifaunal community descriptors

A total of 6,068 organisms were collected belonging to 48 species (Table 3). Among these, the most abundant was the amphipod *Ampithoe rubricata* (Montagu, 1808) (1,202 ind., 19.72% of the total organisms across the entire study), followed by the amphipod *Aphohyalae perieri* (Lucas, 1846) (1,043 ind., 17.11%) and the isopod *Dynamene edwardsi* (Lucas, 1849) (1,011 ind., 16.59%). The rarest species found were the polychaete *Lysidice unicornis* (Grube, 1840) (2 ind., 0.03%) or the isopod *Anthurus gracilis* (Montagu, 1808) (1 ind., 0.02%).

The highest abundance was observed in the deep/small tide pools, particularly in Baja Ortiz 1 (mean \pm SD, 173.2 ± 43.44 ind.), followed by Baja Ortiz 3 (107.5 ± 36.27 ind.). The next most abundant type of tide pool was shallow/large, Baja Ortiz 2 (91.1 ± 47.66 ind.), and Sobradillo (51.9 ± 40.6 ind.). The

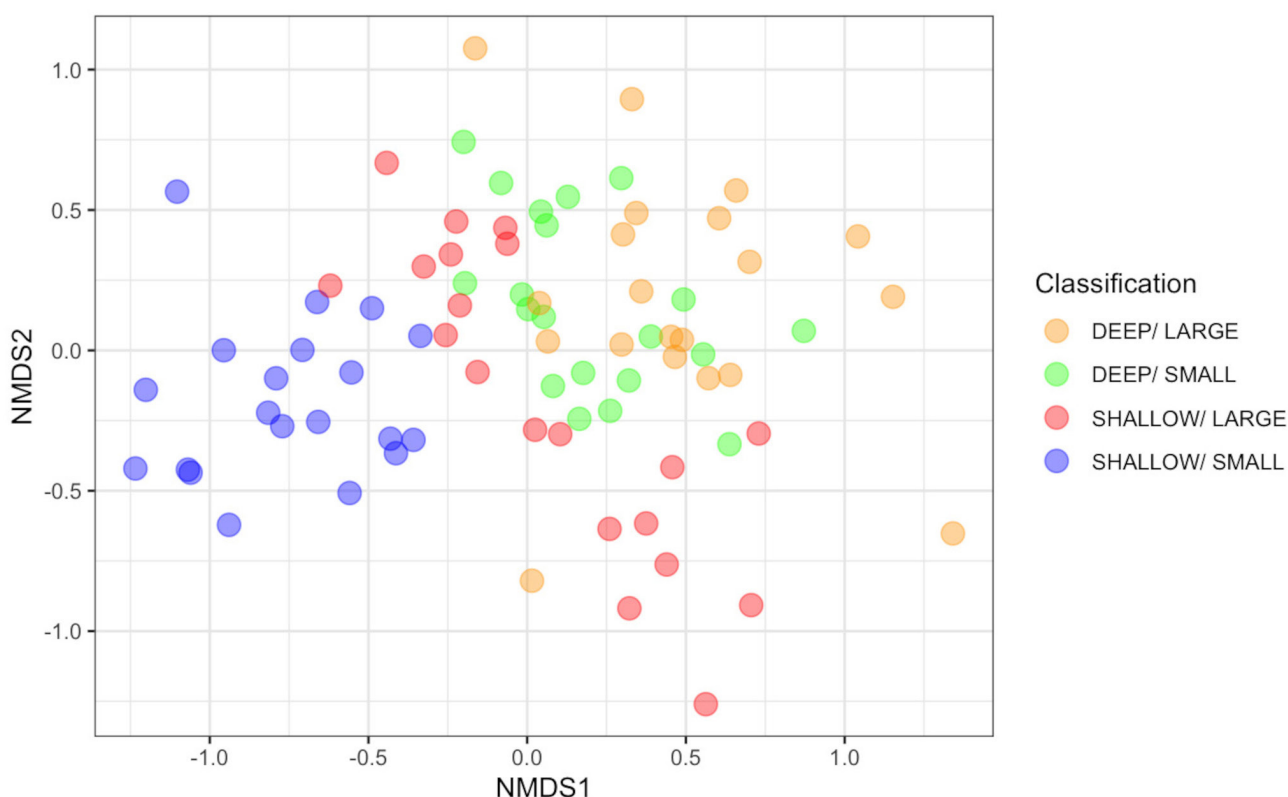


Figure 3. MDS showing tide pools classified by size and depth (deep-large, deep-small, shallow-large, shallow-small).

deep/large tide pools, Punta Sardina lighthouse 3 (61.3 ± 50.48 ind.) and Espejos (48.1 ± 21.67 ind.) had relatively lower abundance compared to the previously mentioned tide pools. In the shallow/small tide pools, the epifaunal community associated with *Cystoseira* exhibited the lowest abundance of organisms. Punta Sardina lighthouse 2 had the lowest abundance (28.8 ± 19.73 ind.), followed by Punta Sardina lighthouse 1 with 47.8 ± 17.8 ind. (Fig. 2A). The results showed significant differences between the types of tide pools in both factors, size (PERMANOVA: pseudo-F = 4.53, $p = 0.0363$) and depth (PERMANOVA: pseudo-F = 13.25, $p < 0.001$).

The deep/small and deep/large tide pools exhibited the highest species richness (Fig. 2B) with similar values. Baja Ortiz 1 and Espejos exhibited the highest species richness, with each tide pool hosting 22 species. These were followed by Baja Ortiz 3, which had 18 species, and Punta Sardina lighthouse 3, which had 14 species. Both belonged to the deep/large group. The next group in terms of species richness was the shallow/large tide pools, with 18 and 14 species, respectively. In contrast, tide pools classified as shallow/small tide pools showed the lowest species richness. Punta Sardina lighthouse 1 and Punta Sardina lighthouse 2 had 10 and 8 species,

respectively. Species richness significantly differed between large and small pools (PERMANOVA: pseudo-F = 4.74, $p = 0.0325$) and between deep and shallow ones (PERMANOVA: pseudo-F = 31.11, $p < 0.001$).

Multivariate analysis

When considering only the depth factor (Fig. 3), the MDS revealed that the epifaunal communities in deep tide pools exhibit greater similarity compared to those in shallow tide pools. The deep tide pools tended to group, indicating a higher similarity in their epifaunal communities. On the other hand, the epifauna in shallow tide pools showed more heterogeneity, suggesting a greater variation among them. In contrast, when considering only the size factor in the MDS analysis (Fig. 3), notable disparities emerge between the large and small tide pools; hence, epifauna exhibited distinct patterns of distribution. The analysis highlights notable differences among the epifaunal communities in these two size categories, indicating distinct composition and structure. The samples were distributed without any trend of grouping. However, when considering both size and depth classifications together (Fig. 3), the MDS analysis, which has a stress value of 0.22,

Table 3. List of species reported in the studied tide pools.

Phylum	Class	Order	Family	Genus	Species
Annelida	Clitellata	Enchytraeida	Enchytraeidae	Grania	<i>Grania fortunata</i> Rota & Erséus, 2003
Annelida	Polychaeta	Phyllodocida	Nereididae	Perinereis	<i>Perinereis cultrifera</i> (Grube, 1840)
Annelida	Polychaeta	Terebellida	Cirratulidae	Cirratulus	<i>Cirratulus cirratus</i> (O. F. Müller, 1776)
Annelida	Polychaeta	Terebellida	Cirratulidae	Dodecaceria	<i>Dodecaceria concharum</i> Örsted, 1843
Annelida	Polychaeta	Phyllodocida	Polynoidae	Harmothoe	<i>Harmothoe Kinberg</i> , 1856
Annelida	Polychaeta	Eunicida	Dorvilleidae	Parougia	<i>Parougia albomaculata</i> (Åkesson & Rice, 1992)
Annelida	Polychaeta	Sabellida	Sabellidae	Sabella	<i>Sabella spallanzanii</i> (Gmelin, 1791)
Annelida	Polychaeta	Phyllodocida	Syllidae	Syllis	<i>Syllis cornuta</i> Rathke, 1843
Annelida	Polychaeta	Sabellida	Sabellidae	Acromegalomma	<i>Acromegalomma vesiculosum</i> (Montagu, 1813)
Annelida	Polychaeta	Sabellida	Sabellidae	Amphiglena	<i>Amphiglena mediterranea</i> (Leydig, 1851)
Annelida	Polychaeta	Eunicida	Eunicidae	Lysidice	<i>Lysidice unicornis</i> (Grube, 1840)
Annelida	Polychaeta	Scolecida	Opheliidae	Polyopthalmus	<i>Polyopthalmus pictus</i> (Dujardin, 1839)
Annelida	Polychaeta	Phyllodocida	Syllidae	Syllis	<i>Syllis garciai</i> (Campoy, 1982)
Annelida	Polychaeta	Phyllodocida	Nereididae	Perinereis	<i>Perinereis oliveirae</i> (Horst, 1889)
Annelida	Polychaeta	Phyllodocida	Nereididae	Platynereis	<i>Platynereis dumerilii</i> (Audouin & Milne Edwards, 1833)
Arthropoda	Malacostraca	Decapoda	Diogenidae	Calcinus	<i>Calcinus tubularis</i> (Linnaeus, 1767)
Arthropoda	Malacostraca	Isopoda	Anthuridae	Anthura	<i>Anthura gracilis</i> (Montagu, 1808)
Arthropoda	Malacostraca	Decapoda	Diogenidae	Dardanus	<i>Dardanus calidus</i> (Risso, 1827)
Arthropoda	Copepoda	Harpacticoida	Harpacticidae	Harpacticus	<i>Harpacticus flexus</i> Brady & Robertson, 1873
Arthropoda	Malacostraca	Decapoda	Grapsidae	Pachygrapsus	<i>Pachygrapsus transversus</i> (Gibbes, 1850)
Arthropoda	Malacostraca	Amphipoda	Phliantidae	Pereionotus	<i>Pereionotus testudo</i> (Montagu, 1808)
Arthropoda	Malacostraca	Decapoda	Epialtidae	Pisa	<i>Pisa carinimana</i> Miers, 1879
Arthropoda	Malacostraca	Amphipoda	Liljeborgiidae	Liljeborgia	<i>Liljeborgia pallida</i> (Spence Bate, 1857)
Arthropoda	Malacostraca	Decapoda	Paguridae	Pagurus	<i>Pagurus anachoretus</i> Risso, 1827
Arthropoda	Malacostraca	Amphipoda	Podoceridae	Podocerus	<i>Podocerus variegatus</i> Leach, 1814
Arthropoda	Malacostraca	Isopoda	Sphaeromatidae	Cymodoce	<i>Cymodoce truncata</i> Leach, 1814
Arthropoda	Malacostraca	Amphipoda	Maeridae	Elasmopus	<i>Elasmopus rapax</i> A. Costa, 1853
Arthropoda	Malacostraca	Amphipoda	Hyalidae	Hyale	<i>Hyale pontica</i> Rathke, 1836
Arthropoda	Malacostraca	Amphipoda	Caprellidae	Caprella	<i>Caprella acanthifera</i> Leach, 1814
Arthropoda	Malacostraca	Tanaidacea	Tanaididae	Tanais	<i>Tanais dulongii</i> (Audouin, 1826)
Arthropoda	Malacostraca	Amphipoda	Caprellidae	Caprella	<i>Caprella cavediniae</i> Krapp-Schickel & Vader, 1998
Arthropoda	Malacostraca	Tanaidacea	Parapseudidae	Parapseudes	<i>Parapseudes latifrons</i> (Grube, 1864)
Arthropoda	Malacostraca	Amphipoda	Hyalidae	Hyale	<i>Hyale stebbingi</i> Chevreux, 1888
Arthropoda	Malacostraca	Isopoda	Idoteidae	Stenosoma	<i>Stenosoma capito</i> (Rathke, 1836)
Arthropoda	Malacostraca	Isopoda	Sphaeromatidae	Dynamene	<i>Dynamene bidentata</i> (Adams, 1800)
Arthropoda	Malacostraca	Decapoda	Diogenidae	Clibanarius	<i>Clibanarius aequabilis</i> (Dana, 1851)
Arthropoda	Malacostraca	Amphipoda	Caprellidae	Caprella	<i>Caprella equilibra</i> Say, 1818
Arthropoda	Malacostraca	Amphipoda	Dexaminidae	Dexamine	<i>Dexamine spinosa</i> (Montagu, 1813)
Arthropoda	Malacostraca	Amphipoda	Caprellidae	Caprella	<i>Caprella penantis</i> Leach, 1814
Arthropoda	Malacostraca	Amphipoda	Ampithoidae	Pleonexes	<i>Pleonexes gammaroides</i> Spence Bate, 1857

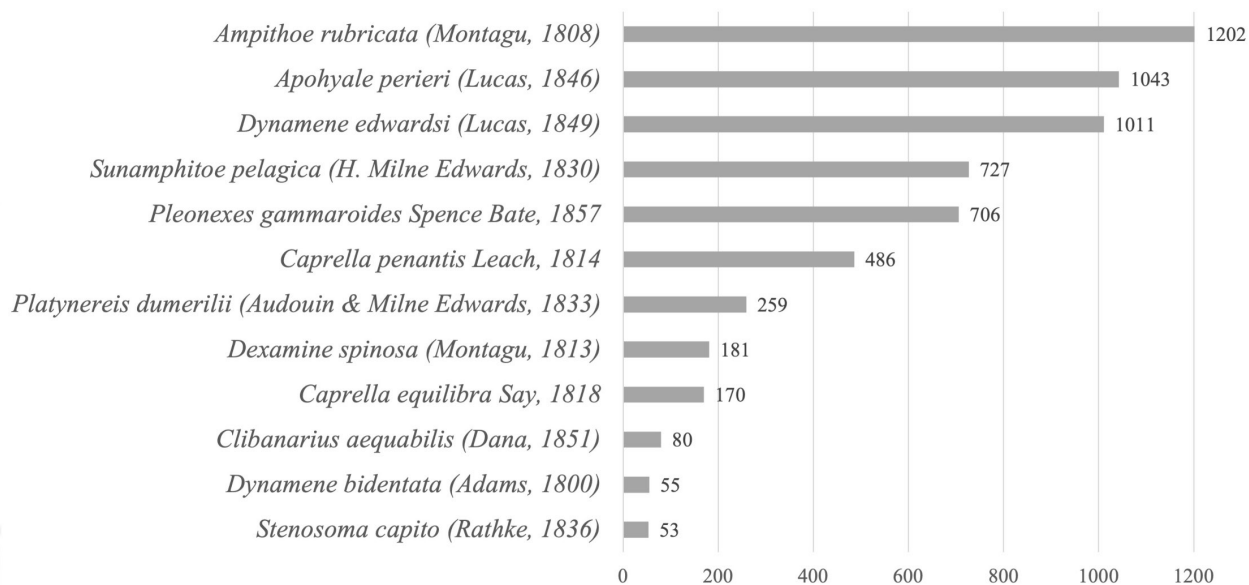
Table 3. Following

Phylum	Class	Order	Family	Genus	Species
Arthropoda	Malacostraca	Amphipoda	Ampithoidae	Sunamphitoe	<i>Sunamphitoe pelagica</i> (H. Milne Edwards, 1830)
Arthropoda	Malacostraca	Isopoda	Sphaeromatidae	Dynamene	<i>Dynamene edwardsi</i> (Lucas, 1849)
Arthropoda	Malacostraca	Amphipoda	Hyalidae	Aphoyale	<i>Aphoyale perieri</i> (Lucas, 1846)
Arthropoda	Malacostraca	Amphipoda	Ampithoidae	Ampithoe	<i>Ampithoe rubricata</i> (Montagu, 1808)
Nemertea	Nemertea				<i>Nemerthea</i> sp.
Echinodermata	Ophiuroidea	Amphilepidida	Amphiuridae	Amphipholis	<i>Amphipholis squamata</i> (Delle Chiaje, 1828)
Echinodermata	Ophiuroidea	Amphilepidida	Amphiuridae	Amphiura	<i>Amphiura chiajei</i> Forbes, 1843
Echinodermata	Ophiuroidea	Amphilepidida	Ophiotrichidae	Ophiothrix	<i>Ophiothrix fragilis</i> (Abildgaard in O.F. Müller, 1789)

revealed that the epifaunal community in shallow/small tide pools stands out as it does not overlap with the other types of tide pools. In contrast, the deep/large and deep/small tide pools exhibited greater similarity, indicating a higher degree of resemblance among their respective epifaunal communities. Lastly, focusing on shallow/large samples, we observed higher dispersion, which suggests greater heterogeneity within the epifaunal communities in these tide pools. There are significant differences among both the factors considered in this study, i.e. pool size and depth. For instance, upon considering the length of the tide pool, significant differences in community composition were observed between

small and large pools (PERMANOVA: pseudo-F = 8.43, $p < 0.001$). Similarly, when analysing tide pools by depth, significant differences were observed (pseudo-F = 8.81, $p < 0.001$) between shallow and deep pools. When both factors were considered in the PERMANOVA (pseudo-F = 15.28, $p < 0.001$), the F value was even higher compared to the individual factors of size and depth, implying that the interaction of size and depth amplifies the dissimilarity observed among the tide pool communities.

The most abundant species found in the *C. humilis* samples were the isopod *Dynamene edwardsi*, and the amphipods *Sunamphitoe pelagica*, *Ampithoe rubricata*, and *Aphoyale perieri* (Fig. 4). These species

**Figure 4.** Top 10 most abundant species in the studied samples. Total abundance is represented.

were consistently present across all tide pool groups, including deep small, deep large, shallow small, and shallow large pools. They significantly contributed to the similarity observed between tide pool groups, as indicated by the SIMPER analysis. This abundant species within certain types of tide pools had a greater influence on the overall dissimilarity between those pools compared to species with lower abundances. In shallow and large tide pools, *Dynamene edwardsi* had the highest percentage contribution (29.6 %). However, in contrast to the deep and small tide pools, *Ampithoe rubricata* emerged as the predominant species, contributing 19.7% and 22.5%, respectively. When comparing the pools based on size, it was noted that *D. edwardsi* had the highest contribution to dissimilarity, accounting for 18% and *A. rubricata* closely followed with a dissimilarity of 17%. These two species were more commonly found in small pools rather than large pools. On the other hand, *A. perieri* had a dissimilarity of 15%, and showed a higher prevalence in larger pools, with an average of 15.3%. When considering the depth factor and comparing deep and shallow pools, *A. perieri* exhibited the highest dissimilarity contribution at 16.3%. *A. rubricata* followed closely with a dissimilarity of 15.9%. Both species were more abundant in deep pools, with averages of 19.57% and 22.22%, respectively. Additionally, *D. edwardsi* and *Pleonexes gammaroides* were also present, contributing to the dissimilarity with percentages of 15.7% and 13.1% respectively (Table 2).

Discussion

In the present study, epifaunal organisms of *Cystoseira humilis* from eight tide pools were compared, focusing on their size and depth as factors. This analysis aimed to provide insights into the direct influence of these factors on epifaunal communities. Most of the organisms were arthropods, accounting for 95% of the total. We compared the similarity of the species in each type of tide pool as a function of the depth (shallow, deep) or size (small or large). It was found that *Dynamene edwardsi* was the species with the highest contribution in shallow and large pools, whilst *Ampithoe rubricata* was the dominant species in deep and small pools. Other species, such as *Pleonexes gammaroides* and *Caprella penantis*, were present in the deep tide pools, although in lower abundance. In addition, it is important to mention that when considering the dissimilarity between tide pools, the species *Apothyale perieri*, *A. rubricata*, and *D. edwardsi* were particularly influential, explaining the differences observed between tide pools. *D. edwardsi*

and *A. rubricata* exhibited greater dissimilarity in small pools, whereas *A. perieri* showed higher dissimilarity in larger pools. In the case of the dissimilarity in shallow and deep tide pools, it presents a higher contribution to the dissimilarity of the species of *A. perieri* and *A. rubricata* present in a higher average in deep tide pools. The opposite behavior was observed in *D. edwardsi*, which presents a major contribution to the shallow tide pool.

When examining the depth factor, it became evident that it was the most significant, as deep tide pools showed higher levels of richness and abundance in their epifaunal communities than in the shallow ones. Several studies have confirmed this finding (Kooistra et al., 1989), where an increase in diversity was observed in deep tide pools, with deeper pools supporting more algae and invertebrate species. Deeper pools offer a stable habitat characterized by reduced temperature fluctuations and desiccation, fostering the proliferation of algae and invertebrates by retaining nutrients and organic matter. The depth provides a refuge from predation, facilitating their growth. Additionally, wave exposure influences the abundance and diversity of algae and invertebrates (Metaxas & Scheibling, 1993). However, when considering the size factor, Bussell et al. (2007) observed that it directly affects the growth form of algae and subsequently impacts the composition of epifaunal communities. Generally, larger pools tend to have a higher density of the algae and a greater diversity of organisms, in contrast to smaller ones, which have a lower density and lower diversity (Bussell et al., 2007). This trend is consistent with the findings of our study. This supports the idea proposed by Martins et al. (2007) regarding the relationship between tide pool size and species richness. Larger pools offer more stable environmental conditions and greater niche diversity, leading to increased abundance and species richness, which can be attributed to the fluctuation of physicochemical parameters such as temperature, oxygen concentration, and pH (Metaxas & Scheibling, 1993). The surface area in shallow pools facilitates a higher rate of energy exchange between the air and water (Martins et al., 2007).

Taking into consideration the above mentioned and the present study, the lowest abundance and richness were observed in the small/shallow tide pools, as was observed by Martins et al. (2007). In contrast, we observed that the highest abundance and richness occurred in deep/small tide pools, despite not being in accordance with what was mentioned previously; considering this as a small tide pool, the depth factor may have a greater influence on community structure (Martins et al., 2007). In the case of shallow/large and deep/large tide pools, shallow/large tide pools present

a higher abundance but less richness, whereas the opposite occurs in the deep/large tide pools where there is a higher richness but fewer organisms. This high abundance in the shallow/large tide pools could be explained by the large area, which is related to the higher density of *Cystoseira humilis* and, consequently, a higher abundance (Metaxas & Scheibling, 1993).

It is important to consider that all studies based on intertidal shores have limitations when it comes to sample collection because of the high structural variability, which poses challenges for obtaining representative samples (Mendonça et al., 2018). Factors such as weather conditions and tides must be considered during sample collection, as well as, hydrodynamic conditions, i.e., periods of rough seas, and highly exposed sites, among others. In our study, we did not consider certain aspects that could have influenced the results. One major limitation was the lack of consideration of the degree of exposure to waves in each tide pool, which directly affects the epifaunal communities (Godinho & Lotufo, 2010), however, we selected tide pools with similar hydrodynamic conditions on the same coastal area of the island of Gran Canaria (North-Northwestern). Moreover, tide pools should be classified according to the height at which they enter the intertidal zone. The vertical position on the rocky shore can significantly impact the environmental conditions experienced by tide pools, potentially leading to variations in community composition and abundance. It is important to note that these factors that we did not consider in our study were considered in former studies (Metaxas & Scheibling, 1993; Bussell et al., 2007; Martins et al., 2007). These authors addressed aspects that could significantly influence the results, which may explain the discrepancies observed in our findings. Furthermore, the influence of epiphytes on *Cystoseira humilis* should also be considered. Additionally, future studies should aim to compare the epifaunal communities of tide pools from different areas of the island of Gran Canaria, as well as include other islands within the archipelago. These comparative studies should consider previous research conducted in the study area, e.g., Ocampo (2020). In addition, it would be interesting to study other algal species and explore the influence of algal complexity on epifaunal organisms to gain a better understanding of ecological interactions and community dynamics within the intertidal zone.

Acknowledgements

This piece is part of the BSc Thesis of the first author

(IC). We are also grateful to lab mates, Monica Martín, Ainara Ugalde, Juan Carlos de la Rosa and Mattia Serra for laboratory assistance.

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