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Epifauna-associated community of tide pools dominated by *Cystoseira humilis* from the NE Atlantic Ocean

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Abstract: Tide pools are common in the intertidal regions. They show high heterogeneity regarding their inherent characteristics and associated communities. Epifaunal tide pool communities have been overlooked in coastal studies. Here, we describe the epifaunal macroinvertebrate communities of six tide pools dominated by *Cystoseira humilis* on northwest coast of Tenerife (Canary Islands) and attempt to unravel the variability of these habitats to determine the effect of different variables on these communities. The epifaunal community in the studied tide pools comprised 74 species, mostly mollusks (24 species), annelids (23 species), and crustaceans (18 species). In terms of epifaunal richness and algal coverage the tide pools exhibited a parallel trend. The same tide pools boasting higher species richness also display high algal coverage, with both variables declining in the same order along the different tide pools. Tide pools with a high human presence showed the highest abundance, species richness, and algal coverage. A statistically significant effect of human presence and algal cover on the tidepool epifaunal community was observed. Unfortunately, the variability remained too high to unravel the effects of different variables on the epifaunal invertebrate community.

Résumé : *Communauté d'épifaune associée aux cuvettes dominées par* Cystoseira humilis *de l'Atlantique nord-est.* Les cuvettes sont courantes dans les zones intertidales. Elles présentent une grande hétérogénéité en ce qui concerne leurs caractéristiques intrinsèques et les communautés qui y sont associées. Les communautés d'épifaune des cuvettes intertidales ont été négligées dans les études côtières. Nous décrivons ici les communautés d'épifaune macrobenthique de six cuvettes dominées par *Cystoseira humilis* sur la côte nord-ouest de Tenerife (îles Canaries) et tentons d'estimer la variabilité de ces habitats afin de déterminer l'effet de différentes variables sur ces communautés. La communauté d'épifaune des cuvettes étudiées comprenait 74 espèces, principalement des mollusques (24 espèces), des annélides (23 espèces) et des crustacés (18 espèces). En termes de richesse de l'épifaune et de couverture algale, les cuvettes ont montré une tendance parallèle. Celles présentant une plus

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grande richesse en espèces présentent également une couverture algale élevée, les deux variables diminuant dans le même ordre le long des différentes mares. Les cuvettes où la présence humaine est importante présentent la plus grande abondance, la plus grande richesse en espèces et la plus grande couverture algale. Un effet statistiquement significatif de la présence humaine et de la couverture algale sur la communauté d'épifaune a été observé. Malheureusement, la variabilité est restée trop élevée pour que l'on puisse déterminer les effets des différentes variables sur la communauté d'épifaune.

Keywords: Tide pools ● Epifaunal community ● *Cystoseira humilis* ● Canary Islands

Introduction

Tide pools are common in rocky reefs, characterized by vertically, spatially, and temporally fluctuating environmental conditions profoundly influenced by hydrodynamics, such as waves and tides (Raffaelli & Hawkins, 2012). The environmental factors within tide pools are affected in a different way depending on the volume, shape, depth, surface area, location on the coast, degree of shade, drainage, and exposure to waves (Martins et al., 2007). The complexity and high heterogeneity among tide pools may explain the challenges in conducting ecological studies, leading to a lack of information on the key processes driving the structure and functioning of tide pool communities (Astles, 1993; Metaxas & Scheibling, 1993; Firth et al., 2014).

The presence of species in tide pools is regulated by their ability to colonize and tolerate physical gradients and variations in environmental factors such as desiccation, sun exposure, and hydrodynamics, together with interactions between organisms (Dethier, 1982; Thompson et al., 2002). In addition, tide pool characteristics greatly influence the associated communities. For example, depth strongly affects the diversity and composition of communities, with higher algae and invertebrate richness in deep tide pools than in shallow pools (Kooistra et al., 1989; Fairweather & Underwood, 1991). In contrast, tide pool area has been previously shown to have little influence on the diversity of tide pool-associated species (Martins et al., 2007).

Tide pools are subjected to multiple human-induced disturbances, such as pollution, invasive species, habitat loss, and fragmentation (Thompson et al., 2002; Martins et al., 2008). However, distinguishing between anthropogenic impacts and natural environmental variations is challenging (Vieira et al., 2017). Nonetheless, the destruction of habitats due to coastal structures, such as promenades, breakwaters, and groins, is one of the most important anthropogenic disturbances that directly affect tide pools

Oceanic islands are hotspots with a high number of species and a high number of endemism (Myers et al., 2000; Martín et al., 2010). However, the community structure of rocky coasts and their tide pools on temperate oceanic islands has received less attention than the adjacent continental coasts (Hawkins et al., 2000; Martins et al., 2008). The present study was conducted in the Canary Islands, an archipelago that has experienced a sharp increase in tourists, from < 10,000 in 1960 to over 13 million in 2015, with a massive coastal expansion of resorts and tourism-related infrastructures, e.g. marinas, pipelines, and promenades (Galán, 2008; Hernández et al., 2017). Artificial substrates, i.e. groins, breakwaters, and jetties constitute approximately 157 km of the coast, representing approximately 9% of the perimeter of the archipelago (Riera et al., 2014). Most of the larger tide pools in the Canary Islands are subject to different types of disturbances, e.g. many of them are used as natural pools and recreational zones, however, there is less information available on epifaunal tide pool communities.

Tide pools formed in the Canary Islands harbor diverse ecosystems, providing habitats for numerous invertebrates including mollusks, echinoderms, crustaceans, annelids, zoanthids, and fish (Ramirez et al., 2008). These species have been widely studied over the years (Brito, 1991; Pérez Sánchez & Moreno Batet, 1991; González Pérez, 1995; Gómez Rodríguez & Pérez Sánchez, 1997; González-Delgado et al., 2018), but always on a species basis, not at the community level. In tide pools, the interaction between invertebrates and the algae they inhabit is close and varies in species richness and diversity based on the species of algae and the structural complexity (Delgado & Fraga, 1997). Tide pools provide refuge for algae less resistant to wave exposure or dehydration, and the genus *Cystoseira* is one of the most representative in midlittoral tide pools in Canary Islands (Pinedo & Carrillo, 1994; Ramirez et al., 2008). *Cystoseira* species play an important role as canopy-forming algae hosting diverse communities, with a study

Figure 1. On the left: Los Chochos tide pool in northern Tenerife, Canary Islands. On the right: the alga *Cystoseira humilis* in the same tide pool.

revealing 597 associated taxa across different spatial scales (Piazzi et al., 2018).

Herein, we focused on tide pools dominated by the native alga *Cystoseira humilis* Schousboe ex Kützing, 1860 because it is one of the most common algae in Canarian tide pools and harbors a rich and diverse epifaunal community that is highly sensitive to human disturbances (Fig. 1; Delgado & Fraga, 1997; Tuya & Haroun, 2006; Veiga et al., 2014). *Cystoseira humilis* is a bushy eulittoral algae in moderately wave-exposed situations (Garreta, 2000). *Cystoseira humilis* has recently been widely studied, as it has been displaced by invasive species (i.e. *Sargassum muticum* (Yendo) Fensholt) and was also included in the list of endangered and threatened species at the Barcelona Convention (Mediterranean Action Plan of the United Nations) (Engelen & Santos, 2009; UNEP, 2009; Vaz-Pinto et al., 2014).

The aim of the present study was to describe the epifaunal macroinvertebrate community of tide pools dominated by *C. humilis*, and to unravel the variability of these habitats to determine the effect of different variables on these communities. Our hypothesis suggests that the abundance and the diversity of the *C. humilis* community will exhibit variability according to changes in algal cover and human presence within these tide pool habitats.

Material and Methods

Study location

The study was conducted along the northwest coast of Tenerife (Canary Islands). Six mid-intertidal tide pools, characterized by the prevailing presence of the dominant alga *C. humilis*, were selected (Fig. 2 & Table 1). Our selection on a singular geographical area, the same mid-intertidal zone, and one dominant alga was to mitigate large variations in oceanographic parameters, physical factors, and structural complexity of the habitat.

Sample collection

The tide pools were sampled during low tide from October to November 2017. To examine the effect of wave exposure in the epifaunal community, three zones inside the tide pools were selected. These zones were selected based on their proximity to the sea, forming a gradient ranging from the outermost ('exposed') to the innermost ('sheltered') areas of the tide pool. Additionally, an intermediate zone was included between these extremes. Within each zone, three quadrats measuring 25 cm x 25 cm were sampled, and photographic documentation was utilized to estimate algal coverage within each quadrat.

The maximum depth of the tide pools was calculated. An average depth was chosen when collecting epifauna samples from *C. humilis*. A separation of at least 30 cm from both the surface and bottom to prevent dehydration or contamination by deposited sediments. Each quadrat of *C. humilis* was meticulously scraped, and the contents were carefully stored in individually coded plastic bags. Subsequently, the samples were preserved in 4% formaldehyde for maximum 72 h and processed at the Benthos Laboratory of the University of La Laguna. All epifaunal specimens were identified

Table 1. Sampled tide pools with their abbreviations and characteristics.

Tide pools	**Viento (CV)**	**Chochos (CS)**	**Buenavista 1 (CB1)**	**Buenavista 2 (CB2)**	**Coloradas 1 (CC1)**	**Coloradas 2 (CC2)**
Coordinates	28°24’03.6”N 16°40’29.6”W	28°22’52.8”N 16°48’52.3”W	28°23’32.9”N 16°50’02.7”W	28°23’34.7”N 16°49’51.4”W	28°22’55.9”N 16°43’57.2”W	28°22’55.9”N 16°44’03.1W
Maximum depth (m)	1.1	1.3	2.1	1.5	1.2	1.6
Pool area (m²)	85.47	84.33	178.87	194.19	303.54	136.1
Accessibility	High	High	Medium	Medium	Low	Low
	(Stairs)	(Stairs)	(Trail)	(Trail)	(Rocky coast)	(Rocky coast)
Human presence (individuals)	High	High	Medium	Medium	Low	Low
	(6-20)	(6-20)	(1-5)	(1-5)	0	0
Trash presence	Yes	Yes	No	No	No	No

to the species level whenever possible. Percentages of coverage were determined through photographic analysis of each quadrat, adhering to the methodology outlined by Murray (2001). In this methodology, each photograph of every quadrat is divided into four smaller squares. A grid is then superimposed on these sub squares, further dividing them into smaller units. The number of these smaller units occupied by algae is calculated, and subsequently scaled to a percentage relative to the entire 25 cm x 25 cm quadrat.

The measurement of the tide pool area, distance to an urban center (as a proxy of sewage pollution), and distance to a banana plantation (as a proxy of eutrophication by water-soluble fertilizers) was conducted using QGIS 3.6. Moreover, to obtain a proxy for human pressure in each tide pool, the factors “accessibility”, “human presence” and “trash presence” were collected. All three variables contributed equally to assessing human pressure. Three categories were defined within the accessibility

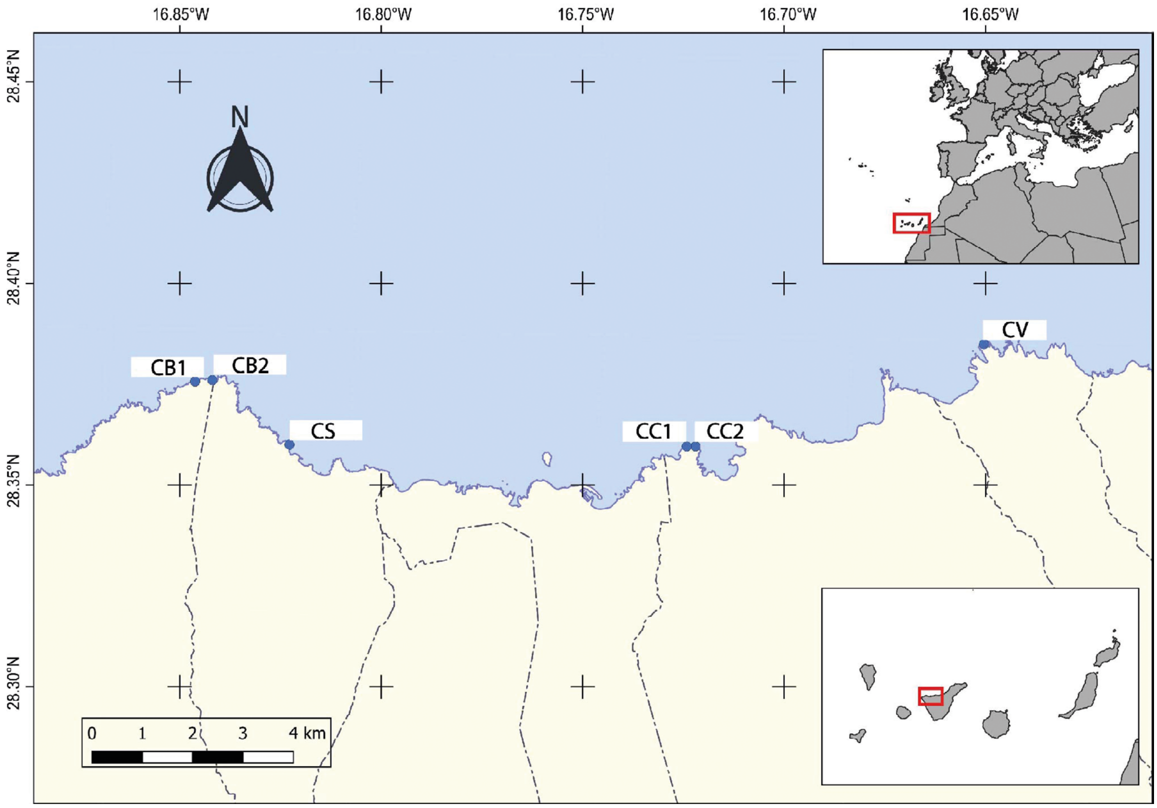

Figure 2. Map of the six tide pools sampled in the north of Tenerife, Canary Islands. CB1 = Buenavista 1, CB2 = Buenavista 2, CS = Chochos, CC1 = Coloradas 1, CC2 = Coloradas 2, and CV = Viento.

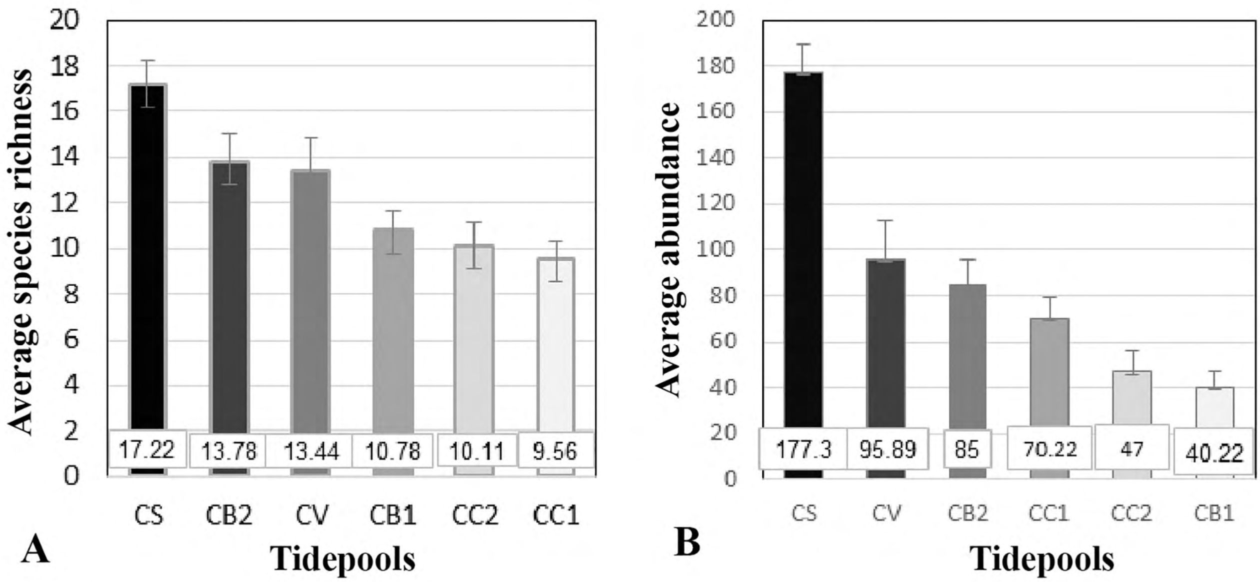

Figure 3. A. Average epifaunal species richness with standard error of each sample in the six tide pools. **B.** Average epifaunal abundance with standard error of each sample in the six tidepools. Chochos (CS), Viento (CV), Buenavista 1 (CB1), Buenavista 2 (CB2), Coloradas 1 (CC1) and Coloradas 2 (CC2).

factor: high (corresponding to the presence of stairs), medium (presence of trail tracks), and low (no tracks). Three categories were defined within the factor of human presence: high (6-20 ind.), medium (1-5 ind.), and low (no presence). Two categories were defined within the factor trash presence: the presence of trash in the tide pools and the surroundings areas, or no trash observed. Visualization of the parameters “human presence” and “trash presence” was carried out during the summer and autumn of 2017 up to five times throughout this period.

Data analysis

The analyses were performed using PRIMER v6 + PERMANOVA software (Gorley & Clarke, 2006). Overall abundance and species richness were calculated for the six tide pools. Data were square root transformed and resemblances were based on Bray-Curtis similarity. To examine the effects of different variables on the epifaunal community, i.e., tide pool, tide pool area, wave exposure, algal coverage, distance to an urban center, distance to a banana plantation, and human presence, a PERMANOVA analysis was used. All variables were considered as fixed factors in the statistical analysis (Anderson, 2001). P-values were calculated from 9,999 permutations, and a P-value of 0.01 was used to avoid an increase in type I error (Underwood, 1991). PERMDISP analysis was performed *a posteriori*, and no results were significant; thus, it was not included herein. The contribution of the abovementioned variables to explain variations in the epifaunal community was tested using a distance-based redundancy analysis (db-RDA) (Legendre & Anderson, 1999). Multivariate multiple regression was used, using the adjusted R^2 selection criterion for all DistLM procedures, to retain variables with good explanatory power.

Results

A total of 4,641 individuals were identified, belonging to 7 phyla, 56 families and 74 species (24 mollusks, 23 polychaetes, 20 arthropods (18 crustaceans), 3 echinoderms, 2 nemerteans, 1 sipunculid, and 1 flatworm) (Appendix). The most abundant group was crustaceans (1,940 individuals, 41.8% of the overall abundance), followed by polychaetes (1,092 ind., 23.53%), and echinoderms (638 ind., 13.75%). The most abundant species were the amphipod *Sunamphitoe pelagica* (938 ind., 20.21%), followed by the echinoderm *Amphipholis squamata* (627 ind., 13.51%), and the polychaete *Amphiglena mediterranea* (425 ind., 9.16%).

Chochos tide pool (CS) showed the highest species richness on average per sample, followed by Buenavista 2 (CB2), Viento (CV), Buenavista 1 (CB1), Coloradas 1 (CC1), and Coloradas 2 (CC2) (Fig. 3). In terms of abundance, tide pools exhibited a distinct trend compared to richness; notably, CS showed the highest abundance, significantly diverging from the other tide pools (Fig. 3). The average algal cover of *C. humilis* in tide pools ranged from 96% to 68 % (CS: 96.67 ± 1.44%, CB2: 93.89 ± 2.98%, CV: 92.22 ± 3.74%, CB1: 83.33 ± 4.41%, CC2: 77.78 ± 4.79% and CC1: 68.89 ± 4.06%). The trend in algal cover, from highest to lowest, mirrored exactly the pattern observed in the richness data of the epifaunal community (Fig. 3).

High variability in major taxonomic groups was observed among the studied tide pools (Fig. 4). Chochos (CS) stands out, where the highest species richness and abundance were obtained, with an overwhelming dominance of crustaceans (66.12%). The abundances of different taxas were very similar in Buenavista 1 (CB1) and Buenavista 2 (CB2). The annelids had greater importance in Viento (CV), Buenavista 1 (CB1), and Coloradas 1 (CC1). In terms of mollusks, their highest representation was found in CV, with notably lower percentages observed in CB1 and CC1. It is noteworthy that a large percentage of dipteran larvae was reported in Coloradas 1 (CC1).

The statistical analysis revealed significant differences in the epifaunal community among the six tide pools (Table 2). Both algal coverage and human presence exhibited statistically significant effects on the epifaunal community. Conversely, wave exposure did not demonstrate a significant effect in the analysis.

The first two axes from db-RDA explain ca. 32.7% of the variation in the epifaunal community (Fig. 5). In the biplot of the first two db-RDA axes, the distance to the banana plantation was positively correlated with both axes. The first axis (21.6%) was positively correlated with tide pool area, and the second axis (11.1%) was positively correlated with human presence. Coloradas 1 (CC1) and Viento (CV) tide pools showed the highest similarity (Fig. 5).

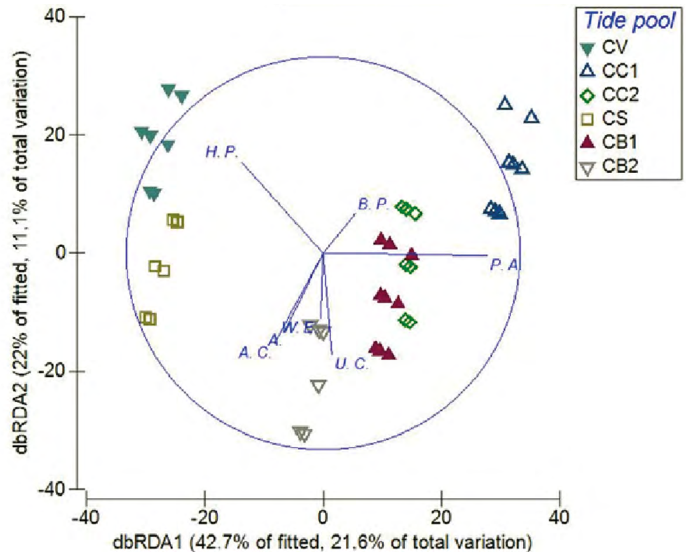

Figure 5. Distance-based redundancy analysis (db-RDA) biplot of first and second axes relating the variables: **A. C.** (Algal coverage), **A.** (Accessibility), **W. E.** (Wave exposure), **U. C.** (Distance to an urban center), **P. A.** (Tide pool area), B. P. (Distance to a banana plantation) and **H. P.** (Human presence) (Table 1). The samples are distributed by the tidepool.

Table 2. PERMANOVA values between tidepools with the variables epifaunal community, algal coverage, wave exposure and human presence. Significant values are marked with *

Variable	Pseudo-F	P-value
Epifaunal community	8.183	0.0001*
Algal coverage	1.623	0.0006*
Wave exposure	1.486	0,085
Human presence	9.378	0.0001*

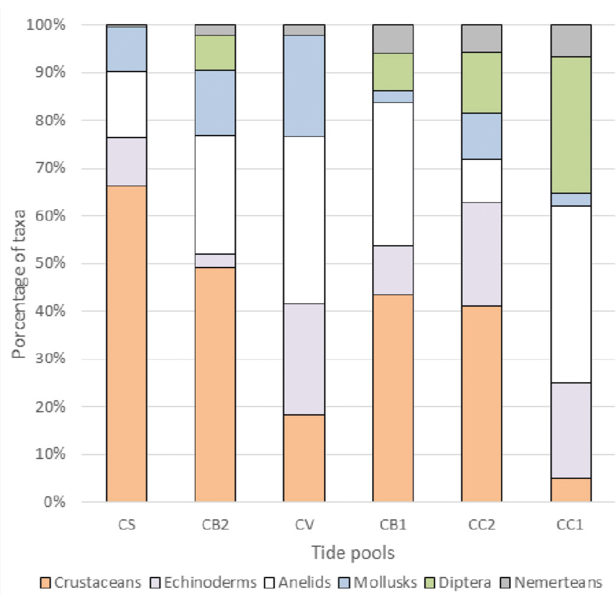

Figure 4. Percentage of major taxonomic groups abundance of the six tide pools. Chochos (CS), Viento (CV), Buenavista 1 (CB1), Buenavista 2 (CB2), Coloradas 1 (CC1) and Coloradas 2 (CC2).

Discussion

The epifaunal community of *C. humilis* tide pools in the north of Tenerife was described. These community comprised 74 invertebrate species, mostly mollusks (24 species), annelids (23 spp.) and crustaceans (18 spp.). All species identified in these tide pools are consistent with those previously documented for the Canary Islands.

Tide pools displayed a consistent trend where higher richness corresponded to higher algal coverage, and conversely, lower richness correlated with lower algal coverage. In tide pools, algae-associated invertebrates play a significant role in these habitats, influencing diversity and growth of algae species (Bracken, 2004; Bracken & Nielsen, 2004; Bracken et al., 2007). This observation aligns with the statistically significant impact of algal coverage on the epifaunal community, leading us to conclude that algal coverage emerges as the most influential variable affecting the community dynamics.

Tide pools with high human presence exhibited the highest abundance, species richness, and algal coverage, and they were positively correlated in the dbRDA. This suggests that a certain degree of human presence may favor greater diversity. Tide pools with low human presence had the lowest average abundance, species richness, and algal coverage. This seems to be explained by the fact that human presence favors a higher percentage of algal coverage, which entails a greater number of habitable spaces or niches. This fits with the ecological theory of the Intermediate Disturbance Hypothesis, which states that diversity should be maximized at intermediate frequencies and/or intensities of disturbance or environmental change (Connell, 1972; Fox, 2013). This study suggests that human presence may act as an intermediate disturbance in structuring tide pool communities. However, due to high habitat variability and a small sample size, further research is necessary to confirm these findings.

Two main natural stress gradients affect organisms in the intertidal zone: (i) vertical, which wasn't a factor in our study as all tide pools were in the middle intertidal, and (ii) horizontal due to wave action differences (Crowe et al., 2000; Raffaelli & Hawkins, 2012). However, data analysis showed no significant differences in the epifaunal community. This result also suggests that there was no difference found among the three zones sampled within the same tide pools. Comparative studies among epifaunal tide pool communities have not been previously addressed in an integrative way, as high variability exists among studies, i.e. sampling technique, sampled area, targeted species, tide pools with different features and species composition, e.g. algae, differences in intertidal height, among others (Metaxas & Scheibling, 1993; Bracken et al., 2007; Martins et al., 2007).

High presence of dipteran larvae greatly differed in low human presence tide pools, and yet they were not found in pools with high human presence. Dipteran larvae have exhibited considerable temporal variability in seasonal abundance in previous studies (Garbary et al., 2009). Hence, the present results need to be interpreted with caution, although dipteran larvae showed a preference for undisturbed tide pools.

A plethora of studies have previously used intertidal communities and/or taxonomic groups as indicators of human-driven perturbations, such as crustaceans, polychaetes, and mollusks (Bellan et al., 1988; Sánchez-Moyano & García-Gómez, 1998; Sánchez-Moyano et al., 2000; Fraschetti et al., 2006; Dean, 2008; Haroun et al., 2010). However, its prevalence in tide pools in the Canary Islands remains understudied. The present study constitutes a first step in this direction, involving epifaunal tide pool communities. However, it will be necessary to develop a thorough study with seasonal surveys, a larger number of tide pools in different locations and islands, and the identification of epifaunal communities on other intertidal algae to accurately identify the environmental status of tide pools considering epifauna as a proxy for human-driven perturbations in the coastal realm. In addition, it needs to be taken into consideration that we focused on tide pools dominated by the alga *Cystoseira humilis* because it is one of the most common algae in Canarian tide pools and harbors a rich and diverse epifaunal community (Delgado & Fraga, 1997; Veiga et al., 2014), but studies involving a higher number of alga species need to be considered for future ecological studies.

In short, the study concludes that the variability remained too high to unravel the effects of the different variables on the epifaunal community of *Cystoseira humilis*. However, human presence was shown to be a disturbance of intermediate intensity, favoring greater algal cover and consequently greater species richness and abundance among the studied tide pools.

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