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Sewage effects at the end of the world: impact on the intertidal macrobenthic community in the Beagle Channel

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Abstract

A first baseline assessment of low intertidal macrobenthic communities in the Beagle Channel (Ushuaia, Argentina) was conducted, focusing on the ecological effects of urban sewage discharge. A hierarchical spatial and replicate sampling design was implemented across 300 quadrats in both reference and sewage-impacted localities. Over 70,000 individuals representing 60 species were recorded. Higher species richness was observed in reference zones. Although a core group of species was shared across the environmental gradient, sensitive bioindicator taxa such as brittle stars and chitons were found primarily in reference localities, while pollution-tolerant species such as *Capitella capitata* and Nematoda were dominant in impacted ones. Beta diversity analyses indicated that species replacement prevailed in impacted localities, whereas species richness contributed more significantly in reference localities. Some localities in both conditions were identified as having a significant local contribution to overall beta diversity. Up to 60% of the variation in community structure was explained by environmental variables, notably total dissolved solids and fecal coliform concentrations. The results reveal spatially localized but ecologically significant alterations attributed to sewage discharge. Improved wastewater management in Ushuaia is recommended, along with long-term monitoring of benthic communities as a proxy for ecosystem health in this unique sub-Antarctic coastal environment.

Introduction

The planet is experiencing dramatic growth in the human population, now exceeding 8.2 billion people (Gerland et al. 2022). Globally, the number of individuals residing within 50 km of the shoreline is increasing faster than in inland areas, leading to profound impacts on coastal environments (Cosby et al. 2024). Anthropogenic impacts on coastal regions, including agriculture, nutrient runoff, engineering projects, fisheries, oil and gas production, dredging, and various forms of pollution from urbanisation, are well documented worldwide (Lotze et al. 2006; Halpern et al. 2008). Among these impacts, wastewater discharges contribute to the eutrophication of coastal environments, affecting turbidity, increasing coliform bacteria and organic matter, and reducing dissolved oxygen levels in seawater, which in turn negatively impacts marine communities in various intertidal systems around the world (Pearson and Rosenberg 1978; Terlizzi et al. 2005a; Riera et al. 2013; Cabral-Oliveira et al. 2014; Conde et al. 2020; Tuholske et al. 2021; Verga et al. 2025). Moreover, human sewage is not only an ecological health issue affecting marine life and ecosystems; this pollution also impacts human health through waterborne diseases and seafood contamination (Rangel Buitrago et al. 2024). Reconciling effective coastal ecosystem functioning and biodiversity conservation with their socio-economic benefits is fundamental for sustainable future development (Cognetti and Maltagliati 2010; Johnston et al. 2015).

Ushuaia, the southernmost city in the world located on the Beagle Channel (BC) coast, is not exempt from the aforementioned processes and their related effects. In just twelve years, population increased was 47% (rising from 58.956 in 2010 to 86.678 inhabitants in 2022), Argentinean National Institute of Statistics and Census https://www.indec.gob.ar/ftp/cuadros/poblacion/proy_1025_depto_tierra_del_fuego.xls) while the urban

area expanded by over 30% (Rodríguez and Martínez 2022). Furthermore, statistics from Fuegian Institute of Tourism (In.Fue.Tur) shows that over the last years, the number of visitors per year to Ushuaia has increased fourfold the number of residents (https://infuetur.gob.ar/estadistica/informes_de_temporada/anuarios_estadisticos). Therefore, the rapid and unplanned urban expansion and the touristic boom were not accompanied by adequate infrastructure development, such as treatment plants, submarine outfalls, incomplete sewage network and pipework (Diodato et al. 2021). In recent times, many studies have indicated that the urban watersheds of Ushuaia present good water quality upstream of urbanisation, significantly decreasing toward the coastal zone (Amin et al. 2011; Zagarola et al. 2017; Diodato et al. 2021; Diodato et al. 2022). Consequently, in the BC nearshore area around Ushuaia, pollutants such as heavy metals, organic compounds (e.g., PAHs, PCBs, pesticides), microplastics, and pharmaceutical and personal care products, have been recorded over the last few decades (e.g., Giarratano et al. 2010; Duarte et al. 2012; Perez et al. 2020). Moreover, some areas of the BC have been environmentally classified as eutrophic systems due to high values of nitrogen, phosphorus, organic matter, suspended solids, faecal coliform levels and low dissolved oxygen (Diodato et al. 2021). However, studies addressing the effects of sewage on biota are far less scarce in the BC. Macrobenthic fauna, (i.e organisms larger than 500 μm), plays a crucial role in the food web of intertidal marine habitats by connecting and transferring energy between primary producers and higher- level consumers (Wilber and Clarke 1998). Moreover, the macrobenthic community constitutes a significant portion of local richness and is frequently used to indicate environmental health (Borja et al. 2008; Muniz et al. 2013; Llanos et al. 2019). Benthic community structure reflects the effects of long- term exposure to pollution, given the sedentary characteristics and differential sensitivity of some taxa to excessive nutrients or toxic substances (Pearson and Rosenberg 1978; Borja et al. 2008; Verga et al. 2025). The magnitude, persistence, and direction of change in the macrobenthic community depend on the degree and type of perturbations (Patricio et al. 2009). Their stress response can be expressed in terms of species gains and losses, changes in abundance (biomass), and/or variability in non- impacted versus impacted zones (Chapman et al. 1995; Muniz et al. 2002). Under slightly altered conditions species diversity may not change (Díez et al. 2012), but in moderately polluted zones it can increase (Connell 1978; López-Gappa et al. 1990). Conversely, in highly contaminated areas species richness usually declines (Terlizzi et al. 2005a). This dynamic suggests that the presence and relative abundance of sensitive, tolerant, and opportunistic species could be a reliable indicator for assessing marine ecosystems exposed to pollution (Pearson and Rosenberg 1978; Culhane et al. 2019).

Sewage pollution is a significant environmental concern in rocky intertidal zones of South America (Muniz et al. 2002). Studies on the ecological impact of sewage on macrobenthic community structure along the Argentinean Atlantic coast were done mostly in Buenos Aires (López-Gappa et al. 1993; Elías et al. 2006; Llanos et al. 2019). On the other hand, this kind of studies are scarce in Patagonia despite the proximity of large urban centres to its coast (Torres and Caille 2009; Verga et al. 2025). Studies in the BC have focused on the effects of pollution on specific intertidal species (e.g., Amin et al. 1996; Duarte et al. 2011; Duarte et al. 2012). Thereby, community-level assessments of anthropogenic disturbance are

needed to generate baseline knowledge for biodiversity conservation and predictions on assembly changes in the face of human disturbance (Curelovich 2012).

There is an increasing need for ecological studies focusing on the reliable detection of environmental disturbances caused by anthropogenic influence in natural systems, considering various spatial scales (Underwood 1994). Well-designed ecological research that provides baseline output is a necessary starting point in areas where natural systems are poorly understood (Cruz-Motta 2007). Understanding the spatial scales at which assemblages associated with intertidal rocky shores vary, provides vital information to know the relative importance of different factors that may affect them (Underwood 2000; Cruz-Motta et al. 2020). A hierarchical sampling design provides a framework for quantifying variation amongst and within samples due to each relevant spatial scale (Underwood 1997). Indeed, balanced and asymmetrical After-Control/Impact ('ACI') designs have been widely used in environmental impact studies conducted in intertidal habitats, as often no data can be collected before the onset of disturbance (Archambault and Bourget 1999; Fraschetti et al. 2001; Lardicci et al. 1999; Terlizzi et al. 2002). An 'ACI' design coupled with robust statistical tools has already demonstrated valuable findings regarding main ecological patterns of macrobenthic assemblages, as well as compelling evidence on the impact effects (Terlizzi et al. 2002; Terlizzi et al. 2005a, 2005b; Fraschetti et al. 2006, but see Glasby 1997). Moreover, the simultaneous use of univariate and multivariate techniques to assess different components of diversity (alpha, beta, and gamma) has enabled a more detailed explanation of the ecological reasons behind the changes that occurred in assemblages in both impacted and reference habitats (Guerra-Castro et al. 2016; Bottero et al. 2020; Llanos et al. 2021).

The current study presents the outcomes of an initial monitoring programme assessing potential benthic impacts attributed to sewage discharge in the BC. Specifically addresses the following topics: 1) a description of the spatial patterns of rocky macrobenthic diversity in reference and impacted localities of the BC; 2) connect the ecological patterns of the macrobenthic community to environmental variables associated with sewage impact. Finally, suggestions regarding management options aimed at enhancing the sustainability of the BC coastal environment in the context of ongoing urban growth are provided, as well as the necessity for long-term monitoring of the intertidal macrobenthic community and seawater quality for future environmental assessments.

Materials and methods

Study area

Field boulders with a gentle slope characterize the beaches used for sampling. A well-developed kelp forest (*Macrocystis pyrifera*) dominates the subtidal area of the BC, where high macrobenthic biodiversity was registered (Adami and Gordillo 1999; Vanella et al. 2007; Ríos et al. 2007; Cruz-Jiménez 2019). The tidal regime has two maximum and two minimum tides per day and microtidal of 1.1 metres in Ushuaia (Servicio de Hidrografía Naval 2024, https://www.hidro.gov.ar/oceanografia/Tmareas/Form_Tmareas.asp). The surface water temperature

fluctuates throughout the year, with higher values recorded in January (9.35°C) and lower values in August (4.38°C) (Balestrini et al. 1998). The predominant winds come from the southwest and intensify in spring, particularly in November (Iturraspe et al. 1989). The general circulation pattern of sea currents in the BC, along its 240 km length and 5 km average width, flows in a west-east direction (Giesecke et al. 2021). However, the circulation within Ushuaia Bay (see Fig. 1) is modified by a counterclockwise current turn (Balestrini et al. 1998), although direction and intensity can vary according to the winds, tidal times, and seabed topography (Cucco et al. 2022).

A broad-scale study area was previously established along approximately 35 kilometers of shoreline on both sides of Ushuaia city (Fig. 1). After exploring it by foot during low tide, we chose two reference (R) *localities* (i.e. considered *a priori* non impacted). References were located into natural protected areas (R1 = Tierra del Fuego National Park, TDFNP; R2 = Larga Beach Provincial Reserve, LBPR). Three impacted (I) localities (i.e areas exposed to sewage discharges) were selected, looking for similarity concerning reference localities in the following environmental characteristics: i) availability of rocky habitat; ii) wave exposure; iii) beach slope; iv) substrate type. Habitat structure views are provided in the Supplementary Material S1.

To date, 65% of Ushuaia city is connected to the sewage system. Of this proportion, approximately 75% of the liquid waste has been redirected to a pre-treatment plant that culminates in a short coastal outfall within Golondrina Bay (Diodato et al. 2020). From this point, an average of 14,292,239 liters per day are discharged into the BC (DPOSS personal communication). An impact locality was positioned near the outfall (I1). The remaining 25% of stormwater and sewage is discharged untreated through the Arroyo Grande stream that flows into Ushuaia Bay (Diodato et al. 2020). Thus, another impact locality was established near the Arroyo Grande, but outside the low salinity effects. The remaining 35% of the city discharge is disconnected from the sewage network so it reaches the waters of the BC by runoff or independent drains (Diodato et al. 2020). Therefore, the last impact locality (I2) was placed near the urban centre.

Sampling protocol

All authors conducted pilot fieldwork to: (1) establish the future starting time for sampling by the tidal regime; (2) forecast the average number of quadrats completed per team per tidal cycle; (3) enhance intra-team homogenisation of taxonomic classification; (4) collect intertidal organisms and incorporate them into the collection; and (5) create macrofaunal digital identification cards to be used during sampling. The specimens were deposited in the biological collection of the ICPA-UNTDF. Furthermore, a series of laboratory sessions were conducted to train all fieldworkers in taxonomic identification using the specimens preserved in the collection. Sampling was undertaken by a group of eight individuals, organised into four rotating pairs. Macrobenthic fauna within each quadrat (50 cm x 50 cm) was surveyed by examining all the stones and the top 3 cm of sediments. Organisms were identified at the lowest possible taxonomic level using the least invasive techniques possible and counted. Consequently, the vast majority of taxa were classified with the naked eye *in situ*. Only those individuals that were

difficult to identify were collected for further laboratory work. Thus, for community studies, taxa determined with reliability were considered. Since mussels belonging to Mytilidae (*Aulacomya atra*, *Mytilus edulis*, *Perumytilus purpuratus*) were registered as percentage coverage, they were not considered for statistical analyses.

Three sampling sites within each locality were selected, separated by hundreds of meters from each other. Lastly, areas at least tens of metres apart, were sampled within each site using ten randomly placed quadrats (spaced a few metres apart). A total of 300 sampling units (i.e. quadrats) were collected during the austral spring (from November until the middle of December 2024). Sampling was conducted in the lowest intertidal strata during tides lower than 0.3 meters.

Environmental variables

To characterize environmental conditions and assess the potential impacts of sewage discharges, water and sediments samples were taken at site level, as well as measurements of surface water temperature, pH, salinity, turbidity and total dissolved solids (TDS) that were recorded *in situ* using a HORIBA U-50 multiparameter device and appropriate field kits.

Water samples were used to determine total and fecal coliform bacteria concentrations at each *site* using the analytical method according to APHA (1995) and the results were expressed as the most probable number per 100 mL. Six 1-liter seawater samples were collected to determine concentrations of inorganic and organic nitrogen (N) and phosphorus (P) using a QuAAtro 39 high-performance microflow analyzer, applying the ascorbic acid method for phosphates, the diazotization method for nitrites and the cadmium reduction method for nitrates, following manufacturer protocols and in alignment with APHA (2017). Sediment sampling included three random 30 g samples per site to determine total organic matter (TOM) content via the loss-on-ignition method (Heiri et al. 2001).

Data analyses

Diversity

Total and average species richness was calculated and plotted at each spatial scale using the actual sample size. Average species richness was analyzed using a one-way permutational analysis of variances (PERMANOVA) through a multifactor nested model considering four sampling-spatial scales (Anderson 2001, 2008). Factor area (n = 2, random factor) was nested within each *site* (n = 3, random factor). The site factor was nested in each *locality* (n = 5, random factor) within the *condition* factor (I vs R as a fixed factor, n = 3 for I = Impact and n = 2 for R = References levels). A matrix of Euclidean distances was estimated on untransformed data. The H0 was tested against 9,999 permutations (Anderson and ter Braak 2003), but when the number of permutations was insufficient to get a reliable p-

value, the Monte Carlo simulation was used. The relative importance of each spatial scale was calculated as the relativized square root of the pseudo-component of variation.

For comparing macrobenthic diversity in the BC and at each *locality*, rarefaction and extrapolation curves were calculated using the iNEXT (iNterpolation/EXTrapolation) from the statistical software R (Hsieh et al. 2016). Hill numbers of order q = 0 (species richness), q = 1 ("typical species"), and q = 2 ("very abundant species") were used to provide a comprehensive view of the species diversity and to assess the sample completeness (Gotelli and Chao 2013; Chao et al. 2020). Sample completeness varies from 0 to 1 and is defined as the fraction of total individuals belonging to species in the sample (Chao and Jost 2012). Hill numbers and evenness (Pielou index) was evaluated using the actual (intrapolation) and also twice (extrapolation) the size of the sample.

Beta diversity was calculated as the total variance in species composition data across areas and subsequently decomposed into its components of species replacement and richness differences (Legendre and De Cáceres 2013). The local contribution to beta diversity (LCBD) was calculated to quantify the ecological uniqueness of each area, with high values for a given area indicating strongly different species compositions compared to the mean of all other areas in the BC. LCBD was partitioned into richness difference (LCBDRich) and species replacement (LCBDRepl) components (Legendre 2014) to enhance the ecological interpretation. Beta diversity analyses were performed on a Jaccard-based index following the Podani family (Podani and Schmera 2011). All beta diversity analyses were undertaken in R (R Core Team, 2024) with the betadiv function (Legendre and Cáceres 2013) and with beta.div.comp for the LCBDRep and LCBDRich (Legendre 2014) in the adespatial package.

Community structure and environmental impact detection

A snapshot of the macrobenthos abundance distribution was created using a shaded plot. Localities were organised on the X-axis, while the Y-axis was arranged according to a factor derived from a similarity profile routine (SIMPROF, Clarke et al. 2008; Somerfield and Clarke 2013), following a cluster analysis conducted on the species data, which was standardised by total and based on the association index (Whittaker 1952).

Macrobenthic community data was transformed by a four-root to downscale the dominant species weight and a dissimilarity matrix based on Bray-Curtis was calculated (Bray and Curtis 1957). The same linear model for the species richness analyses was employed for community data. A principal coordinate analysis (PCoA, Gower 1966) was used to visualize the PERMANOVA results, which depict the *Locality* x *site* centroids in a two-dimensional plot.

The correlation and skewness for all combinations of environmental variables (n = 9) were examined using pairwise comparison and Draftsman plots. Consequently, Fec_col was chosen over Tot_col (R > 0.9) for multivariate analyses. Due to the differences in magnitude between environmental data, Fec_col and TDS were square-rooted. Besides, N and P were treated as a ratio due to its ecological significance. Because of the difference in units of measurement, the entire matrix was normalized (scaled to values

between 0 and 1) before statistical analysis. To summarize the variation between community and environmental data, a distance-based redundancy analysis (dbRDA) was used (Legendre and Anderson 1999; McArdle and Anderson 2001). dbRDA is a constrained ordination method that finds the linear combinations of environmental variables explaining the most significant variation in the biological matrix (Legendre and Anderson 1999). The dbRDA result was represented in a biplot with *sites* and those environmental variables selected according to the BIC criterion as bubbles. All multivariate statistical analyses used the PRIMER version 7 and PERMANOVA + software (Clarke and Gorley 2015).

Results

Diversity

The gamma diversity in the BC accounted for 60 species recorded across 70027 individuals in 300 quadrats. A total of 9 phyla and 12 Classes were registered. The most representative Phylum was Mollusca with 23 species that corresponded to the following Classes: Gastropoda (n=16), Bivalvia (n=3), and Polyplacophora (n=3). The second phylum in abundance was Annelida (n=11), with all species belonging to the class Polychaeta. The third most abundant phylum was Echinodermata, with 9 species (Echinoidea n=6 and Asteroidea n=3), following in order of abundance Arthropoda (Crustacea) n=5, Cnidaria (Hexacoralia) n=4, Porifera (Demospongiae) n=3, Chordata (Teleostei) n=3, Nemertea (Pilidiophora) n=1 and Nematoda n=1 (S2; Fig. 5).

The observed total richness in the 60 quadrats per *locality* was 54 in R2, 44 in R1, 45 in I1, and 37 in I2 and I3. The average species richness per quadrant was statistically higher in reference than in the impact *condition* (Table 1A, Fig. 2A). In addition, significant variability was detected at site scale but not between *localities* nested in condition (i.e average site variability per *locality*, Fig. 2B and 2C). Although the largest variation in the average species richness was found within quadrats of the same area, the overall trend was consistent with the impact gradient (Fig. 2D).

Diversity of orders q = 1 (abundant species) and q = 2 (highly abundant species) reach a fixed level in the BC and all localities, meaning that diversity estimates for these two measures satisfactorily infer true diversities. The same conclusion can be drawn for species richness (q = 0) in the BC (Fig. 3A). However, neither of the sampling curves extrapolated up to double the reference sample size stabilizes, suggesting that the asymptotic estimate of species richness represents a minimum. This is particularly evident for I1 and to a lesser extent in I2 (Fig. 3B). A more detailed description of the diversity trend is provided in Supplementary Material S3.

The total beta diversity was approximately half the maximum possible (0.55), with the replacement and richness difference components explaining almost equally in percentage (53% and 47%, respectively). However, partitioning these components by areas revealed that richness differences prevailed in reference areas while replacement predominated in impacted areas (Figure 4). Areas 1, 11, 12, 15, and 25 had LCBD values that were statistically different from the mean composition.

Community structure

In the lowest intertidal zone of the BC, a core community was composed of barnacles (Balaenus laevi and *Notobalanus flosculus*), limpets (*Siphonaria lessoni* and *Nacella*) and ribbon worms (*Parbolasia corrugatus*), Amphipoda, and Polychaeta worms (Glyceridae) were quite common although with slight variations under each environmental condition (group A in Fig. 5). Some echinoderm species (*Pseudoechinus magellanicus* and *Anasterias antarctica*), the crabs (*Halicarcinus planatus*), and some other Polychaeta worms (*Hermadion rhizoicola*), even though they were represented in both reference and impact conditions, were more abundant in the former (group B in Fig. 5). The brittle star *Ophiactis asperula*, the gastropod *Margarella violacea*, the chiton *Ischnochiton stramineus*, and the peanut worm *Golfingia margaritacea*, were components of relatively undisturbed macrobenthic assemblages (group C in Fig. 5). Certain environmental impact indicator species (*Capitella "capitata"*, Spionidae, and Nematoda) appeared only or were much more abundant within impacted localities (group D in Fig. 5).

The Significant statistical variability in community structure was detected at all spatial scales (Table 1b, p perm < 0.01). The largest source of variation was within quadrats (28%), followed by area, site and locality. Additionally, a significant statistical difference was found between reference and impact *conditions* (p MC < 0.01). Consequently, the ordination separated the centroids by area in each condition, according to environmental gradient (Fig. 6). Less dispersed areas belonging to the reference condition were positioned on the left side of the horizontal axis (except for A1 and A25). All I1 areas were arranged on the upper side of the vertical axis, while I2 and I3 areas remained clustered on the right side of the plot (except A13).

Table 1. Permutational analyses of variance to evaluate the spatial variability patterns of the average species richness (a) and the structure of the intertidal macrofauna community (b) in the Beagle Channel, based on a multifactorial linear model that includes condition, locality, site, and area. df = degrees of freedom, MS = mean square, Pseudo-F statistics, and p-perm are presented when permutations exceed 9000, while Monte Carlo permutation (PMC) is provided when lower than 100. The relative importance of each variation component is displayed as (%CV). * CV was negative and considered zero following Anderson (2008).

a) Species richness						
Source of variation	df	MS	Pseudo-F	p-perm	p MC	%CV
condition	1	2082.3	25.2		0.01	35.9
locality (condition)*	3	82.4	0.5	0.64		0
site(locality(condition))	10	146.4	3.6	0.009		17.7
area(site(locality(condition)))	15	40.3	6.4	0.0001		22.1
Residual	270	6.2				24
Total	299					100
b) Macrobenthic community						
condition	1	1.03E+05	4.5		0.006	23
locality (condition)	3	22691	2.2	0.0013		14
site(locality(condition))	10	10232	2.2	0.0001		16.3
area(site(locality(condition)))	15	4553.2	5.4	0.0001		18.7
Residual	270	833.3				28
Total	299					100

Environmental variables

The spatial trend in all environmental variables is presented as Supplementary Material (S3). The best model explained 60% of total variation of the rocky macrobenthic community structure at site scale, and after selection procedure of predictor variables based on BIC criteria was composed of TDS and Fec_col (Fig. 7). TDS contributed to the first axis of the dbRDA plot (explaining 61.3% of fitted community abundance) and Fec_Col contributed to the second axis (explaining 15.6% of fitted community abundance) (Fig. 7). Sites belonging to the impact condition were arranged on the right side of the plot which exhibited greater values of environmental variables associated with human disturbance compared to those sites within the reference condition.

Discussion

This pioneering study provides a baseline description of the low intertidal benthic community in the BC, incorporating multiple spatial scales within the context of sewage-related environmental impacts. The results revealed significant differences in macrobenthic communities between intertidal habitats exposed to sewage discharge and those not affected by pollution. Analyses indicated that community structures inhabiting beaches approximately 14 km apart on either side of the urban zone were similar to each other, yet clearly differed from those situated closer to the urban area. Aside from natural variability,

the observed differences are primarily attributable to the distance from the pollution source and suggest that the spatial extent of the environmental impact may still be relatively limited.

The observed species richness of the macrobenthic community in the BC reached 60 taxa, which according to our survey completeness assessment adequately represents the true species richness for every $q \geq 0$. This number exceeds the 33 taxa reported for Navarino Island (Ojeda et al. 2017), as well as the 18 invertebrate taxa recorded in Yendegaia Bay (Rodríguez et al. 2021), both located within the BC. Most importantly, our results reveal a significant difference in species richness between impacted and non-impacted communities across all spatial scales. This pattern is consistent with previous studies conducted in the central Argentine region (e.g., Elías et al. 2003; Vallarino 2013; Bottero et al. 2020) and northern Patagonia (Verga et al. 2025). Conversely, available evidence from other parts of the world does not show a significant decline in rocky shore biodiversity in response to sewage discharges (e.g., Terlizzi et al. 2005a, 2005b; Roberts et al. 1998) and in some cases, even the opposite trend has been reported (Vallarino et al. 2002).

Even though all localities were sampled using the same design and sampling effort (i.e., 60 quadrats), and regardless of the total number of organisms recorded in each, sample coverage varied. For all localities, coverage for richness orders ≥ 1.5 was sufficient to represent true species richness. Similarly, for orders < 0.5, richness was reasonably well estimated in the two reference localities and in one impacted locality (I3). However, extrapolations suggested an underestimation of this parameter in the other two impacted localities (I1 and I2). These localities exhibited a higher number of singleton species, which may reflect greater community instability, increased turnover of opportunistic taxa, or reduced evenness due to disturbance. Such conditions can favor the sporadic occurrence of low-abundance taxa, inflating the proportion of species represented by only a few individuals.

The decreasing richness pattern along the environmental gradient was also evident at finer spatial scales, although with some exceptions. For example, areas 1 (in site 1 at reference locality R1) and 25 (in site 13 at reference locality R2) were separated from other reference areas in the ordination plot, both exhibiting lower diversity than expected and significantly different LCBD values. In area 1, this uniqueness was primarily driven by species replacement and may be linked to specific intertidal conditions (e.g., boulder size, sediment type), as it is entirely removed from any contamination source. In contrast, area 25, the westernmost sampling point in the Provincial Reserve (i.e., closest to the city), may be receiving contaminated waters from the outfall via local oceanic currents (Balestrini et al. 1998; Cucco et al. 2022). This effect could be further intensified by nearby untreated discharges at the mouths of the Arroyo Grande. Similarly, areas 11 and 12 (within site 6 at impacted locality 1) exhibited significant LCBD values associated with low diversity and a distinct composition driven by replacement. Area 15 (in site 8 at impacted locality 2) also showed significantly high LCBD values, but explained by differences in richness. Conversely, area 22 (within site 11 in impacted locality 3) displayed higher-than-expected diversity under impacted conditions, with a non-significant LCBD value resulting from both richness and replacement effects. These findings suggest that species loss is the primary mechanism driving beta diversity patterns under impact, but also reveal that some areas reflect a range of ecological process.

Altogether, these results emphasize the importance of incorporating spatially nested replication and assessing different components of biodiversity to accurately detect and interpret the ecological impacts of human activities (Underwood 1994; Glasby and Underwood 1998).

The most significant source of spatial variation in species richness and community structure was found at the residual scale (i.e., within a few meters), followed closely by the area scale (i.e., tens of meters). This pattern aligns with previous observations of pronounced horizontal variation at fine spatial scales in high-latitude intertidal habitats of the Southern Hemisphere. For example, in the sub-Antarctic intertidal zone, local factors under extreme environmental conditions have been shown to strongly influence spatial patterns and underlying ecological processes (Rodríguez et al. 2021). Similarly, beta diversity within the mollusk community of intertidal zone in the BC varied across distances of only a few meters (Ojeda et al. 2014), a pattern also observed in benthic communities of Antarctic intertidal habitats (Valdivia et al. 2014). At small spatial scales, natural variability is likely driven by microtopographic habitat features (e.g., beach slope, tidal exposure, desiccating southerly winds) and biotic interactions (e.g., predation, recruitment, facilitation), which have been identified as major sources of variation (Connell 1961; Benedetti-Cecchi and Cinelli 1997; Benedetti-Cecchi 2001; Ojeda et al. 2017; Bertness et al. 2006; Soto et al. 2012). A review of South American rocky shore assemblages further supports this view, showing that while local-scale variation in community composition is driven by natural factors (e.g., tides, nutrient availability) and anthropogenic stressors (e.g., pollution, shipping, coastal human populations), large-scale variation is primarily influenced by gradients in sea surface temperature and productivity hotspots such as upwelling and convergence zones (Cruz-Motta et al. 2010).

The sessile components of the intertidal zone in the BC are structured along the tidal gradient. The high intertidal is dominated by the barnacle *Notochthamalus scabrosus*; the mid-intertidal is characterized by mytilid bivalves (*Perumytilus purpuratus*, *Mytilus chilensis*, and *Aulacomya atra*); while the low intertidal is primarily occupied by the barnacle *Notobalanus flosculus* (Curelovich et al. 2016, 2018). Consistently, among the core macrobenthic taxa identified in this study, barnacles of the genera *Notobalanus* and *Balanus* were well represented across all sampled localities. Gastropods were also abundant, particularly limpets of the genus *Nacella*, which are the most common grazers along the Argentine coast. *Nacella deaurata*, for instance, is frequently observed in the low intertidal and shallow subtidal zones of the BC (Morriconi and Calvo 1993). The pulmonate limpet *Siphonaria lessonii*, present along all Argentine rocky shores and often reaching high densities (Palomo et al. 2019), was also abundant in the BC. Notably, this species has been reported to increase in abundance near sewage effluents in central Argentina, suggesting a degree of tolerance to anthropogenic stress (Llanos et al. 2019).

Among the species still present but exhibiting a decline in abundance due sewage discharge with only primary treatment is *Anasterias antarctica*, which is a sea star highly sensitive to environmental stressors (Hewson et al. 2014; Bucci et al. 2017). In particular, eutrophication driven by sewage inputs leads to hypoxic conditions and altered sediment chemistry, conditions to which sea stars are especially vulnerable (Ferreira et al. 2011). As a benthic predator that primarily feeds on *Mytilus chilensis*, *Pareuthria plumbea*, and *Trophon geversianus* (Curelovich 2012), this species depends on a stable, oxygenated

substrate and a consistent supply of live prey—both of which may be negatively affected by habitat degradation and community structure changes induced by sewage pollution. The ecological consequences of this decline could be significant. Reduced predation pressure from sea stars may allow prey species, such as mussels, to proliferate. Moreover, the crab *Halicarcinus planatus* also showed a marked decrease in abundance in impacted localities. This species has been previously identified as sensitive to both natural stressors and anthropogenic effluents in the BC (Diodato et al. 2021). Other echinoderm classes, such as Echinoidea and Ophiuroidea, were mostly observed in reference areas, as expected given their known sensitivity to pollution (Borja et al. 2000; Garcês and Pires 2022). Conversely, in sewage-impacted areas, opportunistic polychaetes such as Capitella “capitata” and members of the family Spionidae colonized the organically enriched interstitial sediments. The former has been consistently reported in environments degraded by organic over-enrichment across a variety of impacted habitats in Latin America and is perhaps the only taxon that could be considered a universal indicator of organic pollution (Elías et al. 2021 and reference therein). The latter group has also been described as characteristic and highly abundant in sewage-enriched zones (Pearson and Rosenberg 1978). Lastly, nematode specimens, presumably mycophagous and bacteriophagous, were found exclusively in impacted areas, in agreement with previous findings (Lambshead 1986; Nanajkar and Ingole2010; Weiss and Larink 1991).

Environmental parameters associated with sewage—namely turbidity, total organic matter, fecal coliforms, and total dissolved solids—showed a general increasing trend in the impacted localities. A similar pattern was observed in previous comprehensive water quality assessments conducted in the same study area. Several studies have demonstrated that the downstream sections of freshwater courses in Ushuaia receive substantial nutrient loads from untreated sewage discharges (Torres et al. 2009; Diodato et al. 2022; Albizzi et al. 2021; Granitto et al. 2021). The lower reaches of the watersheds draining into the Beagle Channel—particularly Arroyo Grande—are the most disturbed and present the worst environmental issues, coinciding with the most densely populated areas. Diodato et al. (2020) concluded that total coliforms and fecal coliforms were the most indicative parameters of urban inputs in Ushuaia’s watersheds. In line with this, both fecal coliforms and total dissolved solids were significantly associated with changes in the structure of the intertidal macrobenthic communities along the sampled beaches of the BC.

Collectively, these findings demonstrate that urban development and sewage discharges alter benthic communities by favoring pollution-tolerant species while reducing the abundance of those dependent on cleaner environments. These biological shifts, coupled with changes in key environmental variables, underscore the negative impact of sewage contamination on Ushuaia’s coastal ecosystems and highlight the urgent need for improved sanitation infrastructure and monitoring strategies to safeguard biodiversity and ecosystem health (Diodato et al. 2022). To mitigate these impacts and remediate already affected areas—despite the city’s continued population growth and urban expansion—the completion of the wastewater treatment plant at Arroyo Grande (I3) is of critical importance. Additionally, the construction of a new submarine outfall discharging into deeper waters of the BC, rather than into the current shallow subtidal habitats (I1), is under evaluation (DPOSS, personal

communication). Submarine outfalls are intended to dilute effluents, improve water quality, and reduce associated ecological and human health risks. In central Argentina, such modifications have led to the recovery of epilithic communities dominated by the mussel *Brachidontes rodriguezii*, displaying characteristics of either unimpacted or moderately impacted assemblages (Cuello et al. 2019). However, in the BC, the final placement of any future submarine outfall must be planned with caution. It should be informed by hydrodynamic plume dispersion models that account for local marine currents and guided by robust ecological baseline studies assessing the state of subtidal macrofaunal communities prior to discharge. Moreover, the BC is not an open marine system; it includes several islands that support important bird and marine mammal populations (Schiavini and Raya Rey 2001; Raya Rey et al. 2014), which are valuable both from a conservation and ecotourism perspective. Despite the diluting effects of ocean outfalls, the associated organic enrichment and contaminants may still produce localized impacts on the marine environment near the discharge point (Andrew-Priestley et al. 2022).

Conclusion

In conclusion, localized but ecologically significant changes in intertidal benthic communities were observed as a result of sewage discharges in the Beagle Channel. To achieve a more comprehensive ecological assessment of the region, future studies should be designed to incorporate seasonal replication and to extend sampling to higher intertidal zones and subtidal habitats, such as kelp holdfasts macrobenthic community. Most importantly, the need for improved wastewater treatment and long-term monitoring is emphasized to ensure the preservation of biodiversity and the maintenance of ecosystem functioning in this sub-Antarctic coastal system.

Declarations

Author Contribution Statement

FML, SMD, JS, LH, CPA, AH, MP, and SP conceived and conceptualized the idea. FML, SMD, JS, LH, CPA, AH, and MP were involved in data curation and methodology. FML, SMD, JS, and SP acquired the funding. SMD, JS, and MP wrote the original draft. AH contributed to writing, and FML and SP performed the writing – review and editing. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

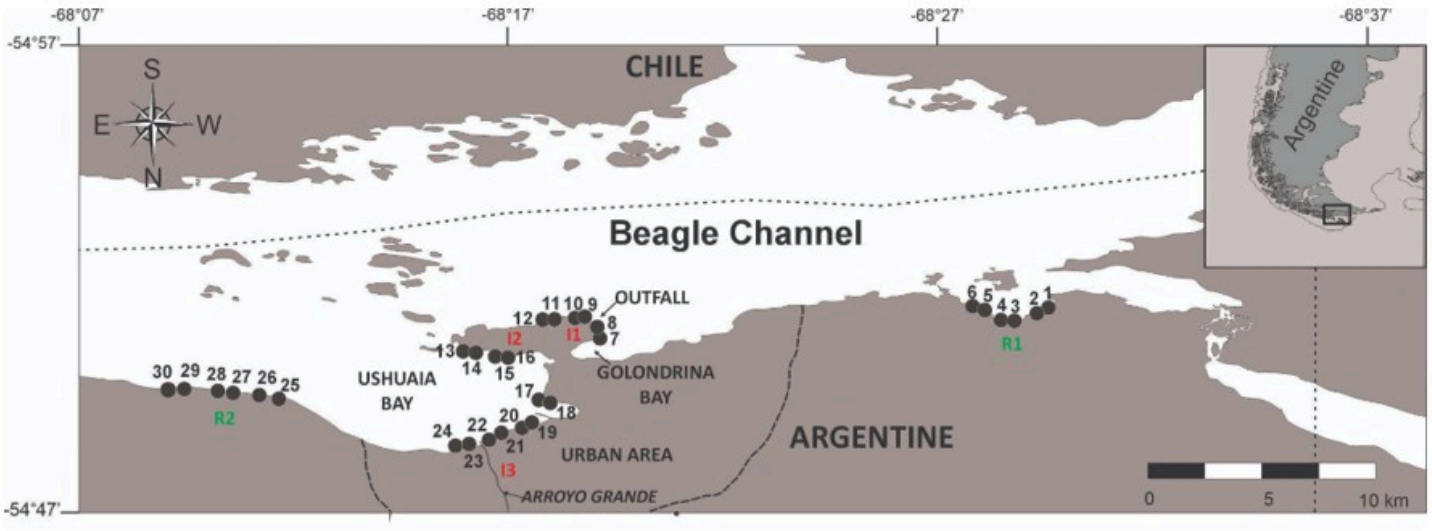


Figure 1

Map of study areas showing reference (R) and impact (I) localities along the northern shoreline of the Beagle Channel. R1 = TDFNP: R2 = LBPR: I1 = outfall: I2 = Ushuaia center: I3 = Arroyo Grande creek. Black dots indicate sampling sites. Dashed line represents the Ushuaia city area.

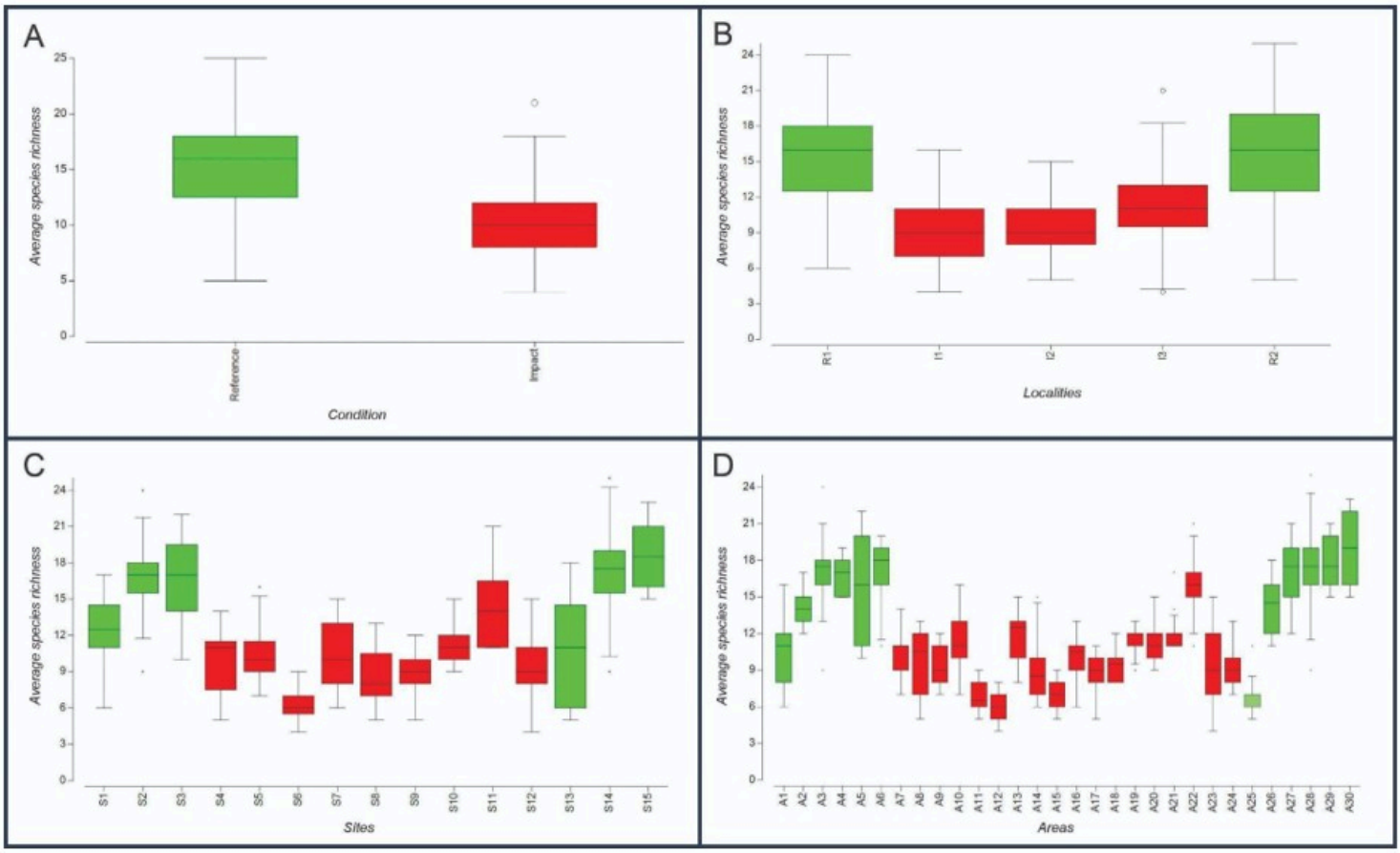


Figure 2

Average richness per quadrats at condition (A), localities (B), sites (C) and areas (D) scales. Reference (green) and impacted box in (red).

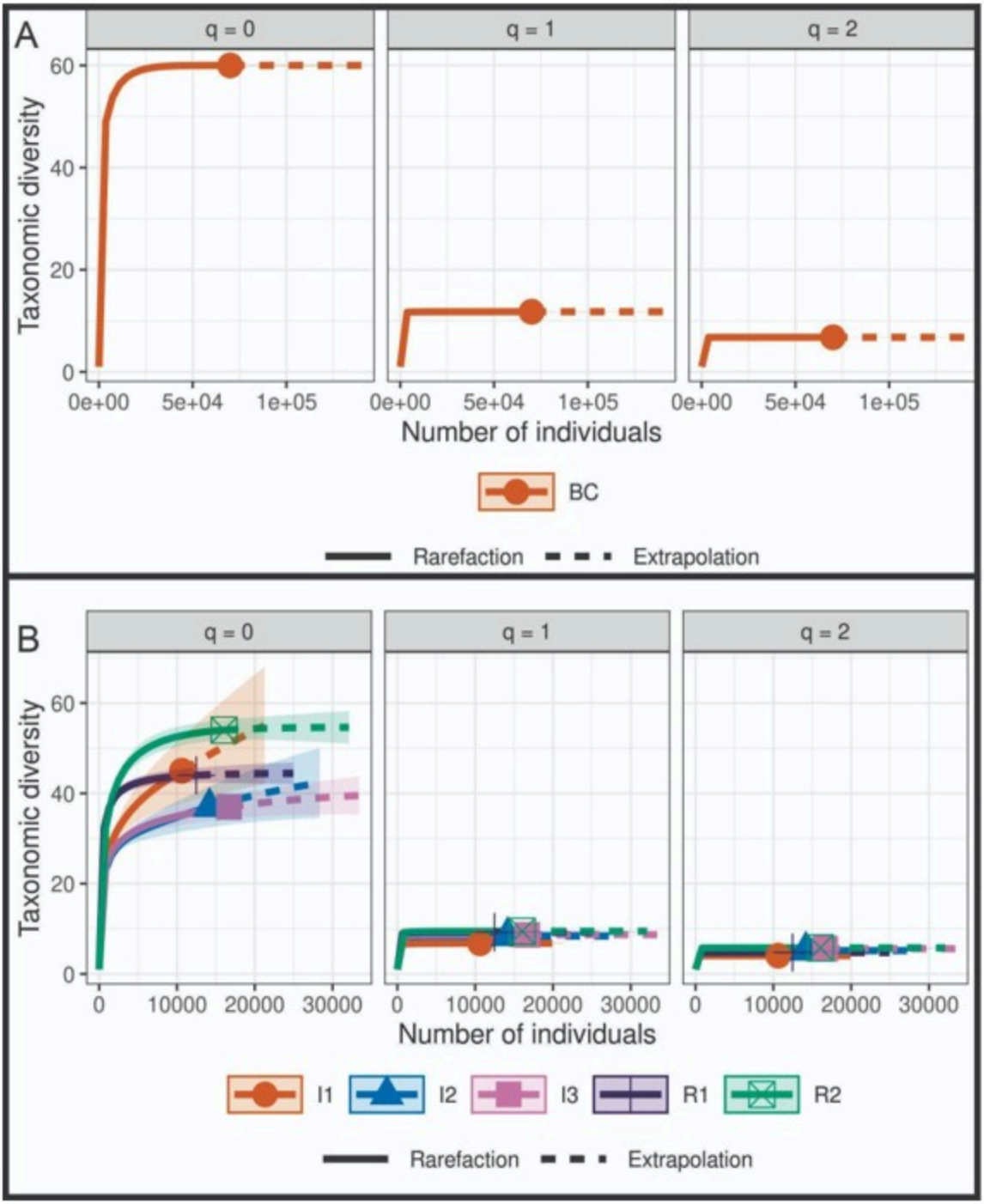


Figure 3

Size-based rarefaction (solid lines) and extrapolation curves (dashed lines) for the Beagle Channel (A) and at each locality (B). BC = Beagle Channel, I1 = impact locality 1 (brown), I2 = impact locality 2 (blue), I3 = impact locality 3 (pink), R1 = reference locality 1 (violet), R2 = reference locality 2 (green). Y axis refer to the estimated asymptotic diversities

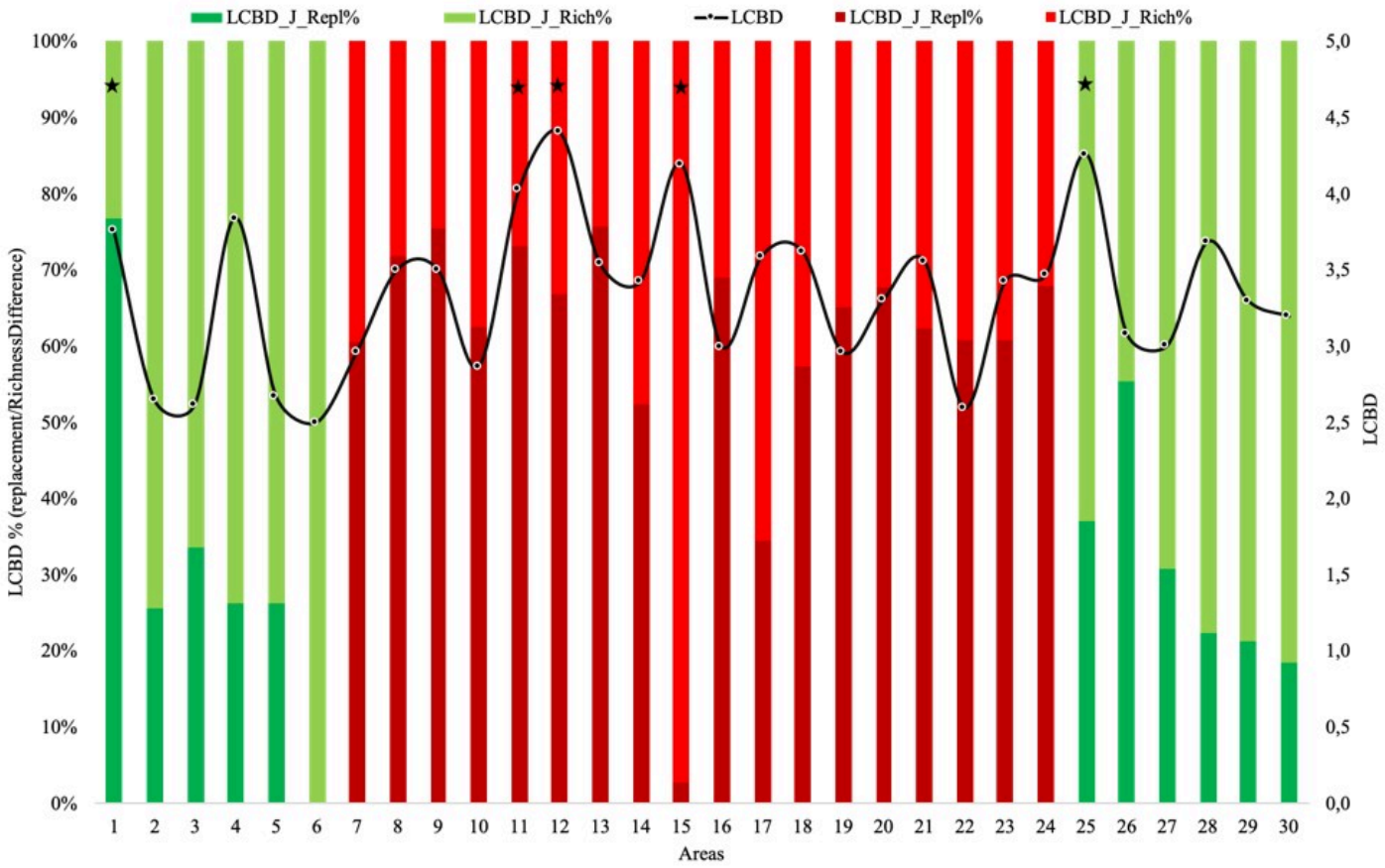


Figure 4

Local component of beta diversity (LCBD) based on Jaccard dissimilarity (J) per areas (continuous line). Replacement (Repl) and richness difference (Rich) components are expressed in percentage (%) both in reference (green) and impacted (red) conditions. Starts identified those areas with significantly different LCBD values.

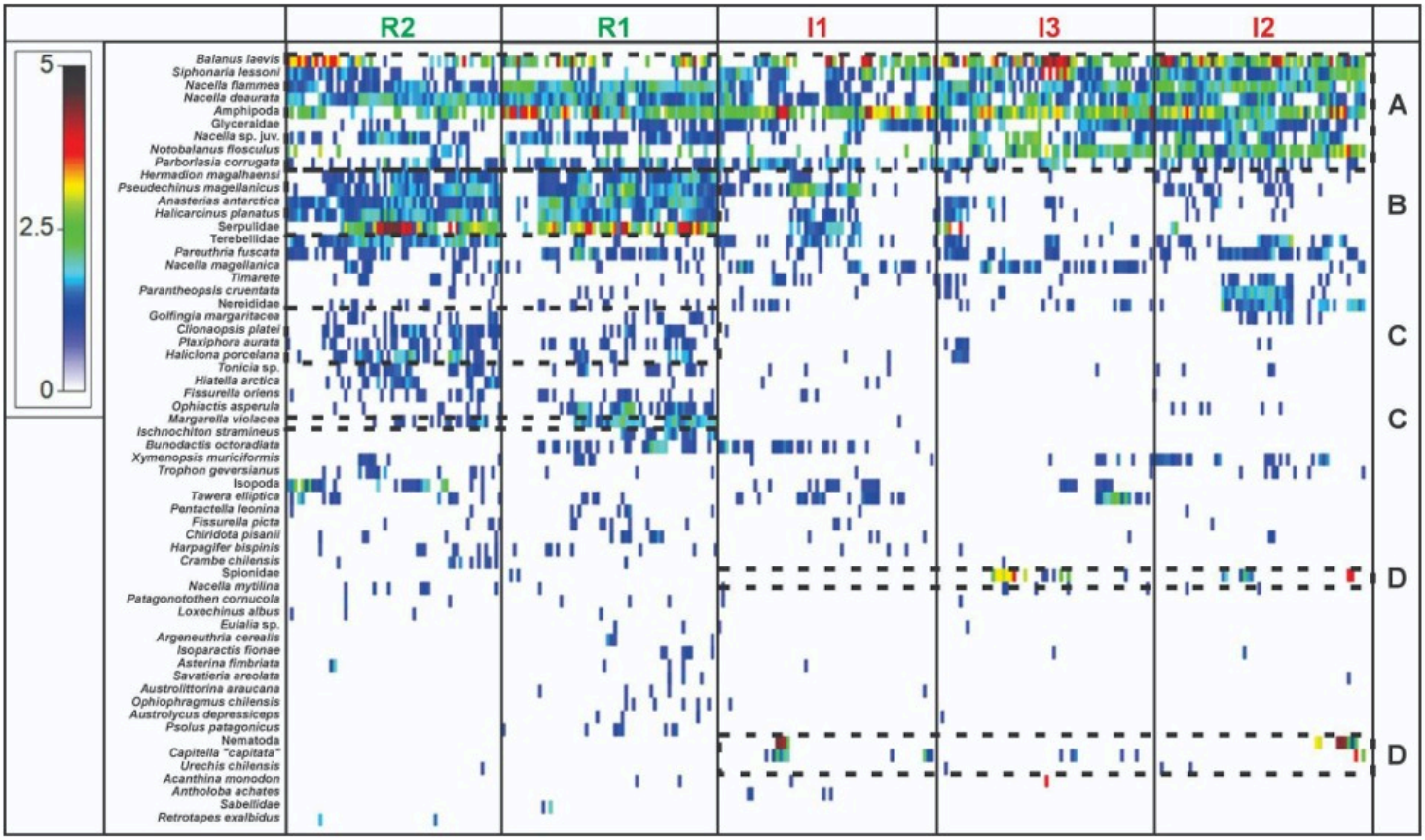


Figure 5

Shade plot of transformed abundance taxa by localities. R1 and R2 = References localities; I1 , I2, I3 = Impact localities. Dashed area A represents the core macrobenthic community. Dashed area B indicates taxa with greater abundance in reference localities than in impacted ones. Dashed area C indicates those taxa present in non-polluted localities. Dashed area D denotes taxa that are only present or are more abundant in impacted localities.

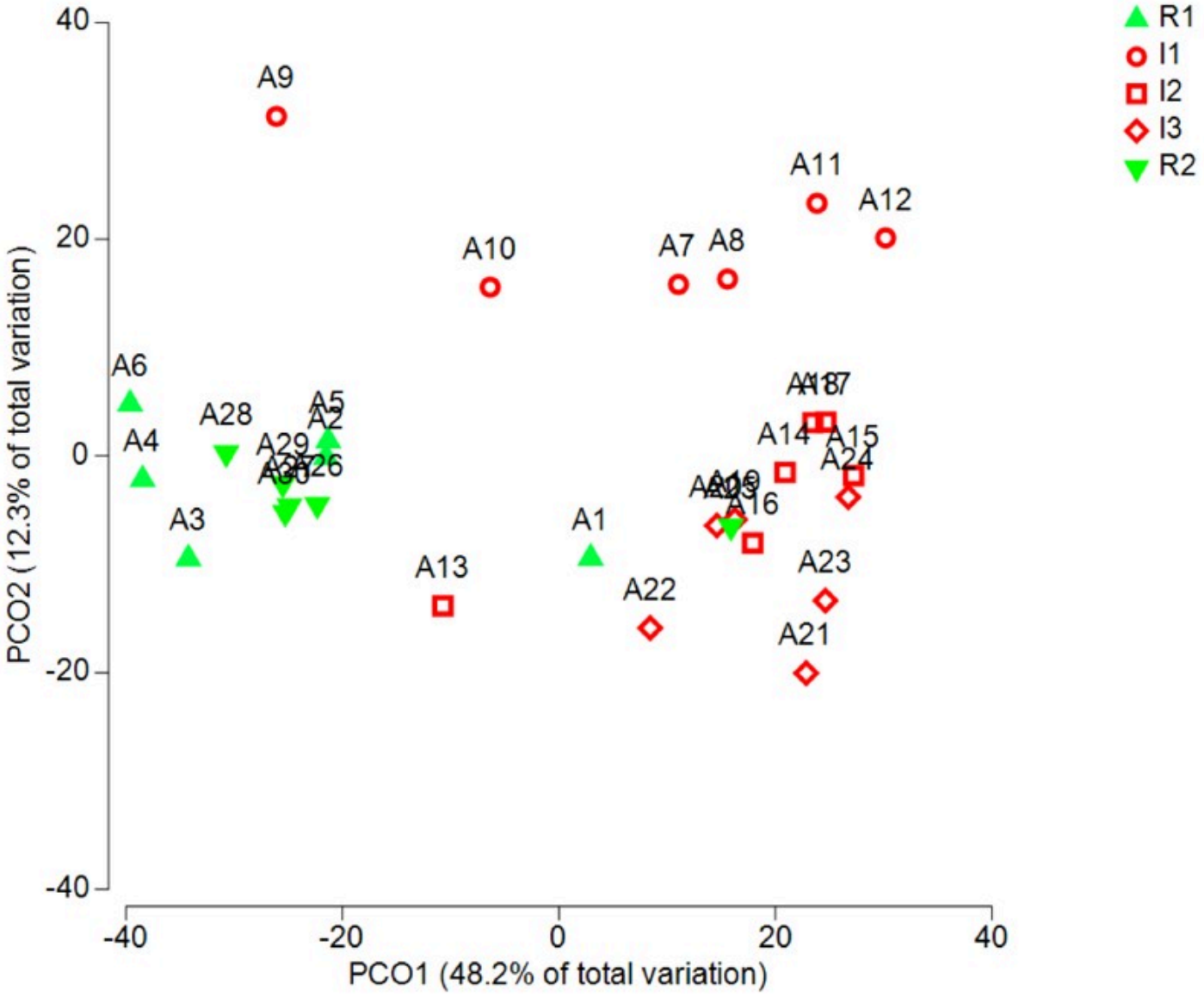


Figure 6

Ordination of centroids by areas (A) in each condition (Green = Reference, Red = Impact) of the rocky macrobenthic community. R1 = National Park (Upper triangles), R2 = Larga Beach-Estancia Túnel (Lower triangles), I1 = Outfall (open circles), I2 = Ushuaia centre (open squares), I3 = Arroyo Grande (open diamonds).

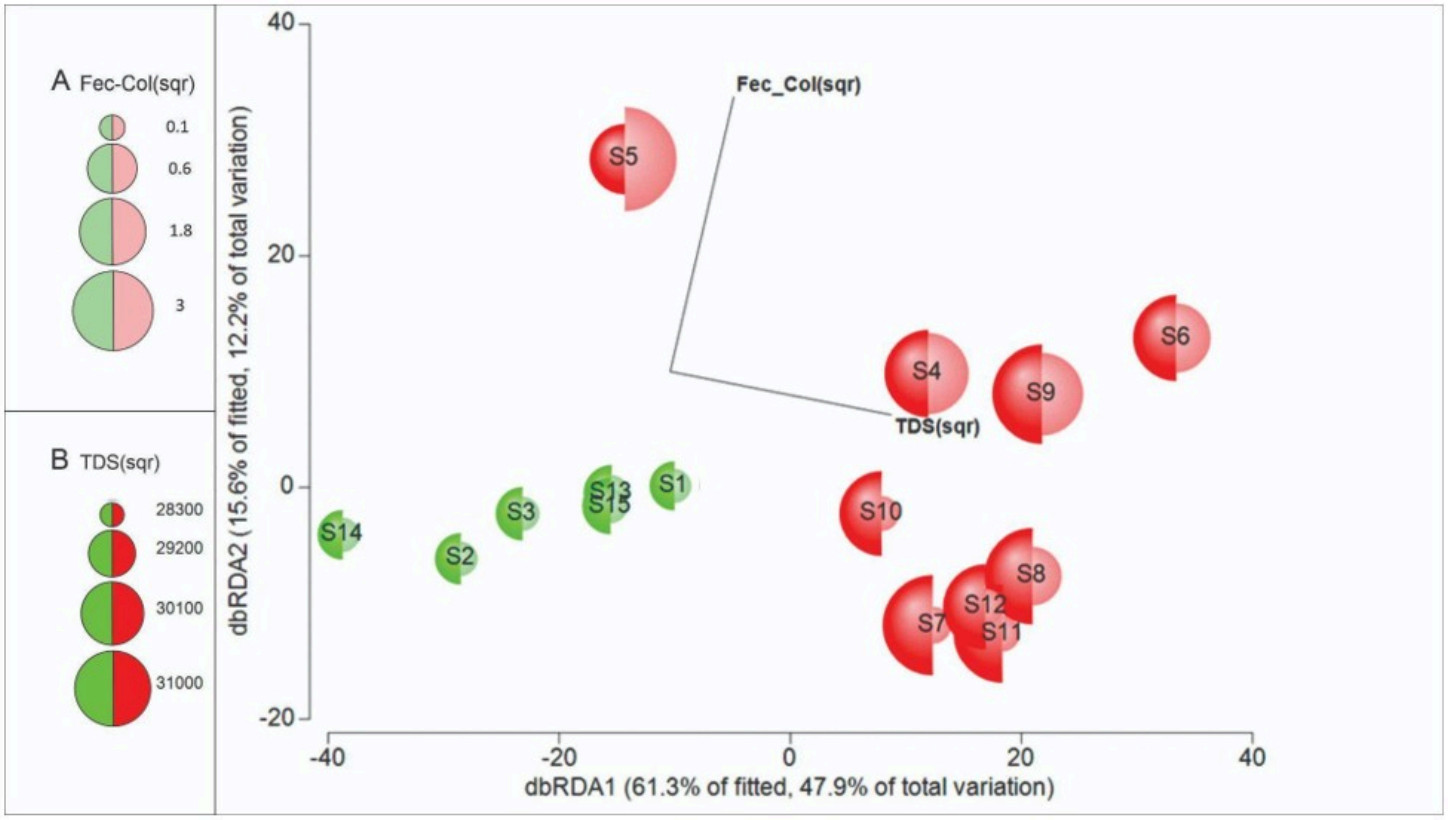


Figure 7

Distance-based redundancy analysis (dbRDA) plot of the best model based on significant predictor variables fitted to the macrobenthic community inhabiting the Beagle Channel. Vectors indicate the direction of the effect of the variables, and bubbles represent sizes (square root transformed). (A) Fecal Total bacteria (Fec_Tot): light green bubbles = reference sites; pink bubbles = impact sites. (B) Total Dissolved Solids (TDS): dark green bubbles = reference sites; red bubbles = impact sites.

Supplementary Files

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