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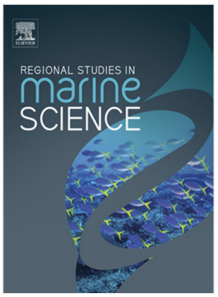

Seasonal composition and activity of the intertidal macrobenthic community of Caleta Valdés (Patagonia, Argentina) applying *in situ* and *ex situ* experimental protocols

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HIGHLIGHTS

- The seasonal variation of the macrobenthic community of Caleta Valdés were reported
- Highest abundance were found in Spring-Summer with a predominance of biodiffusors
- A deeper advective sediment transport was demonstrated in Spring-Summer
- *Ex situ* and *in situ* sediment reworking measurements showed similar results
- The experimental procedure for *ex situ* ecotoxicological studies was validated

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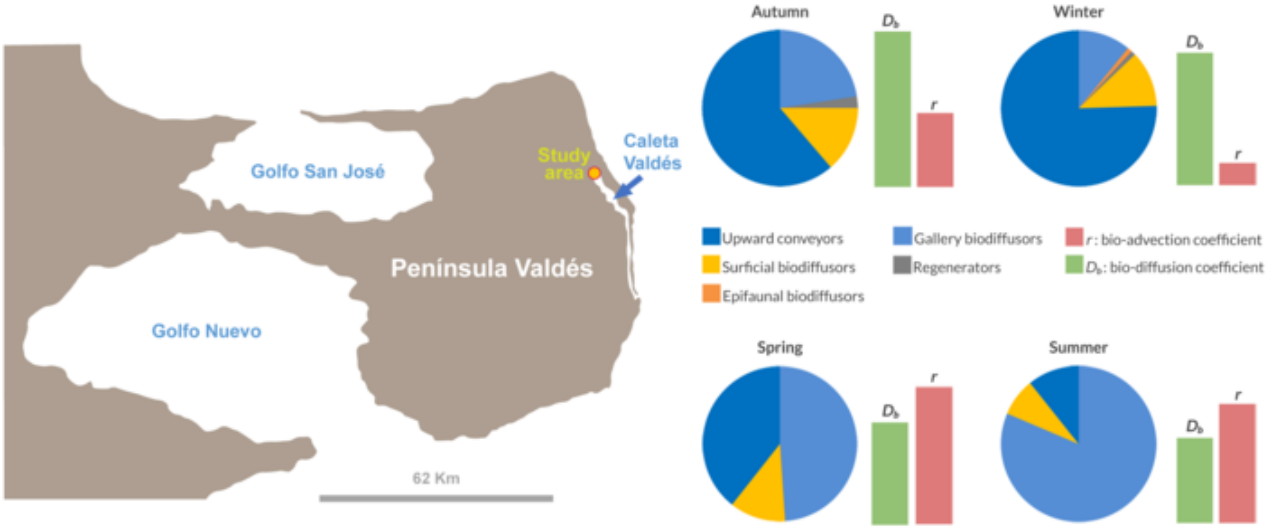

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Seasonal composition and activity of the intertidal macrobenthic community of Caleta Valdés (Patagonia, Argentina) applying *in situ* and *ex situ* experimental protocols

Abstract

Caleta Valdés (CV) is a coastal lagoon of the Patagonian Atlantic coast located in the Península de Valdés declared as Humanity Mundial Patrimony due to its remarkable biodiversity, ecosystemic richness, and pristine state. Marine mammal populations are well documented in this area but few studies have been carried out on the local macrobenthic communities. The goals of this study were (a) to evaluate for the first time the seasonal variation of the structure and activity (i.e., sediment reworking) of the intertidal macrobenthic communities of CV, and (b) to validate an *ex situ* experimental protocol for future ecotoxicological studies. To do so, sedimentary (granulometry, water content, and organic matter) and biological (macrobenthic assemblages, biodiffusive-like and advective sediment reworking components) parameters were analyzed using a combination of *in situ* and *ex situ* measurements. Overall, polychaete and crustacean dominated the macrobenthic community. The highest abundances were found in Spring-Summer along with a predominance of biodiffusors versus conveyors. *Ex situ* and *in situ* measurements demonstrated similar results, thus allowing validation of an *ex situ* experimental procedure for macrobenthic community and functioning studies. In addition, these results provide a first baseline of benthic information on CV that will be helpful to monitor the effects of potential pollution in Patagonian coastal systems.

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Keywords

Biodiversity, bioturbation, sedimentary matrix, soft-bottom macrofauna, protected areas.

1. Introduction

The Patagonian coast is about 3000 kilometers long with a high value in terms of biodiversity (Esteves and Arhex, 2009). This coast is used by migratory birds and marine mammals to rest, feeding and mating (Yorio, 2009). Moreover, it includes zones of reproduction and breeding of fish, crustaceans, and mollusks sustaining one of the most productive temperate marine ecosystems in the world (Vázquez, 2004). As in other marine coastal environments, the food webs are mainly sustained by phytoplankton (Smetacek, 1999). Particularly, in these coasts, upwelling phenomena increase primary production (Acha et al., 2004). Due to its remarkable biodiversity and ecosystemic richness, big areas of this coast have a special protection status (e.g. Península de Valdés was established as Humanity Mundial Patrimony in 1999, North Zone San Jorge gulf was defined as Patagonian Austral Inter-jurisdictional Marine Coastal Park in 2007 and Punta Tombo as Natural Protected Area in 1979, among others). Despite these protection measures, there are diverse and growing anthropogenic activities constituting a direct threat to the ecosystem. Therefore, studies about the seasonal dynamics of the macrobenthic communities constituting a particular interest at ecological level but also for integrated coastal zone management purposes.

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Indeed, the soft-bottom benthic organisms play a key role in nutrient cycles, pollutant metabolism, dispersion, and burial of the organic matter and secondary production processes (Hopkinson et al., 1999; Snelgrove, 1998). These organisms have reduced mobility (Pearson and Rosenberg, 1978; Teixeira et al., 2012) and therefore they are highly sensitive to the physical and chemical changes of the sediment matrix (Dauvin et al., 2010; Muniz et al., 2005). For this reason, this group of organisms is useful as an indicator of ecological state in impacted sites and/or in monitoring programs (e.g. Teixeira et al., 2012). Through the excavation, burrow ventilation, and/or mud and organic matter ingestion (Taghon and Greene, 1992), they induce the transport of particles (sediment reworking) and fluids also known as bioturbation (Kristensen et al., 2012). Community bioturbation is a good integrator of macrofaunal functioning as it incorporates various aspects of behavior (e.g. feeding, locomotion, burrow building) and may vary depending on the community structure and local heterogeneity (Solan et al., 2019). Differences in bioturbation over time may also reflect the abiotic and/or biotic changes of not only the benthic but also the pelagic ecosystem. Particularly, bioturbation affects processes such denitrification (Gilbert et al., 2003), nitrification (Aller et al., 1998), sulfate reduction (Canavan et al., 2006), benthic fluxes (Aller and Aller, 1998; Mermillod-Blondin et al., 2005; Michaud et al., 2005; Mortimer et al., 1999) and microbe dynamics (Goñi-Urriza et al., 1999; Papaspyrou et al., 2006).

Reworking organisms can be classified into functional groups based on the different ways in which the organisms behave and on the resulting transport of

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particles within the sedimentary column. According to Kristensen et al. (2012), these organisms can be differentiated into biodiffusors, upwards and downward conveyors, and regenerators. Biodiffusors are species whose activities produce a local, constant, and random mixture of sediment over short distances. Upwards and downward conveyors are species vertically distributed that respectively feed head down and deposit its dregs in the surface of the sediment or inversely, and finally the regenerators, excavator species that transfer the sediment from the bottom to the surface. In ecosystems, the complex communities are then able to generate various patterns and intensities of sediment reworking depending on their functional composition (e.g. Duport et al., 2007). Moreover, it can be modulated by the environmental conditions via changes in organisms behavior. For example, the burrowing polychaetes *Hediste diversicolor* can switch from deposit-feeding to filter-feeding if the phytoplankton concentration is high enough (Riisgård, 1994) and *Alitta virens* has been shown to have a temperature dependence of sediment reworking activity (Ouellette et al., 2004).

The goals of this study were (a) to evaluate for the first time the seasonal variation of the structure and the activity (i.e., sediment reworking) of the intertidal macrobenthic communities of Caleta Valdés and, (b) to validate an *ex situ* experimental protocol for future ecotoxicological studies.

2. Materials and methods

2.1. Study area

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Caleta Valdés (CV) is a north-south-oriented coastal lagoon located at the eastern side of Península Valdés. This is 30 km long and has its mouth at the southern end where the water exchange is produced at two and four nudes of velocity. A gravel bank limits the lagoon, generating a channel of 200 m width and a mean depth of 5 m. The enclosed north zone presents a marsh that cover a 25% of the total surficial of the system (Esteves et al., 1993). The tidal regimen is semidiurnal with a mean high of 5 m. The sampling site for the experimentation was chosen in the muddy north continental zone of CV (42°15′53″ S, 63°40′50″ W; Figure 1) due to its macrofauna richness and the absence of anthropogenic pollution. Currently, this natural reserve is protected of human activities so that strict control is carried out on touristic and smaller productive activities, which are developed in the area.

2.2. Sampling

Sediment cores were sampled by hand in autumn (April 2011), winter (July 2011), spring (November 2011), and summer (February 2012) using 10 cm diameter and 25 cm length PVC cores, in agreement with previous bioturbation studies (Ferrando et al., 2015; Hedman et al., 2011; Quintana et al., 2007; Timmermann et al., 2002). At each season, at low tide, eight cores were vertically and randomly pushed down to 20 cm sediment depth approximately. Then, for *ex situ* experiments, four of them were immediately collected by hand and transported to the laboratory. The other four cores were embedded in the sediments for *in situ* assessments. In addition to sediment sampling, 60 L of seawater were also collected in plastic containers to be used for the *ex situ* experiments. This procedure was applied for each studied season.

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2.3. Incubation conditions

2.3.1. *In situ* experiments

In order to assess biological reworking activity, in each sediment core, 4 g of luminophores (inert fluorescent particles, Duport et al., 2007) of two sizes and colors (pink: 65-125 µm; green: 125-355 µm) were homogeneously spread at the sediment surface at initial time (T=0 day). Luminophores sizes were selected according to the two major size distribution modes of sedimentary particles in the studied site (Sturla Lompré et al., 2018). After 13 days of *in situ* incubation, the cores were withdrawn and carried out to the laboratory. Then, the sediment cores were sliced to provide 0.5 cm thick sediment layers from the surface to 2 cm depth and 1 cm thick sediment layers from 2 to 17 cm depth. Each sediment slice was separated in four equal parts that were randomly distributed to perform the different analyses.

2.3.2. *Ex situ* experiments

Similarly, for each season, the four cores containing the sediments sampled at CV were distributed in a 56 L tank that was then filled until the cores were totally submerged with the seawater collected the same day in the same sampling station. To assess biological reworking activity in a no water flow system where no hydrodynamic loss of tracers could occur, in each sediment column, 2 g of the two types of luminophores (pink: 65-125 µm and green: 125-355 µm) were homogeneously spread at the sediment surface at the initial time (T=0 day). The system was kept continuously aerated (air bubbling). Sediment cores were incubated for 13 days at a similar ambient temperature to the *in situ*

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assessment. Then, they were manually collected and processed identically as for the *in situ* experiments.

2.4. Analytical procedures

2.4.1. Sediment characterization

A quarter of each sediment slice was used to determine sediment granulometry (only in autumn 2011) by laser beam diffraction (Partica LA-950; HORIBA Instruments, Inc.), and for the four seasons, water content and organic matter by oven drying at 105 °C during 24 h and muffle furnace ignition at 450 °C during 4 h, respectively. Eight granulometry fractions were obtained: fine silt < 6 µm, medium silt 6-20 µm, coarse silt 20-60 µm, very fine sand 60-125 µm, fine sand 125-200 µm, medium sand 200-600 µm, coarse sand 600-1000 µm, and very coarse sand 1000-2000 µm.

2.4.2. Macrobenthic communities

The remaining three quarters of each sediment slice were fixed and dyed with a neutralized 4% formaldehyde solution and Rose Bengal colorant, respectively. Then, the samples were sieved, first with a 500 µm mesh and then with a 44 µm mesh. The sediment retained in the first mesh was preserved in ethanol 70% for the identification and counting of the macrobenthic organisms. The sorting of organisms (for the four seasons) was done to the lowest possible taxonomic level using stereoscopic and optic microscopes in the laboratory with reference material and dichotomous keys (Banse and Hobson, 1974; Blake and Ruff, 2007; Hartman, 1968, 1969; Orensanz et al., unpublished data, among others). Only the whole organisms or anterior fragments of each taxon were recorded.

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2.4.3. Bioturbation

The sediments retained in the 44 µm mesh were used (see above) to quantify sediment reworking. The sediments were homogenized and subsampled to quantify the luminophores percentage using a microplate reader (Biotek, Synergy Mx) at λex/λem: 460/500 and 565/602 nm for the pink and green luminophores, respectively (Majdi et al., 2014). For each sediment column at the four seasons, we obtained the vertical luminophores profile at the experimental final time (13 days) from the percentage of luminophores found in each layer concerning the total amount in the core. The quantification of sediment reworking was then evaluated from the distributions of luminophores by the gallery-diffusor model (François et al., 2002). This model allows describing both the biodiffusion-like transport (D_b coefficient) due to the continuous displacement of the tracers and the nonlocal advective displacement of the tracers (*r* coefficient). The best fit between observed and modeled tracer distribution with depth (i.e., producing the best D_b and *r* coefficients couple) was estimated by the least-squares method (Gilbert et al., 2007).

2.4.4. Statistical data analysis

Variation between seasons (autumn, winter, spring and summer) and experimental conditions (I: *in situ* and E: *ex situ*) of water content and organic matter (OM) were evaluated by core through two-way ANOVA considering seasons and conditions as fixed factors (n=32) (Statistica, version 7). A square root transformation was applied to satisfy the homogeneity of variances

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assumption. The differences between pairs of seasons and conditions were tested through Tukey test for multiple mean comparisons (Statistica, version 7).

A comparison of the mean abundance by core of dominant taxa between seasons and I and E experimental conditions were evaluated through two-way ANOVA considering seasons and conditions as fixed factors (n=32) (Statistica, version 7). A square root transformation was applied to satisfy the homogeneity of variances assumption. The differences between pairs of seasons and conditions were tested through Tukey test for multiple mean comparisons (Statistica, version 7).

Biological data analyses were performed using the software package PRIMER 7.0 (Plymouth Marine Laboratory, UK). The sorting using the total abundance of each taxa by core recorded in the I and E experiments at the four seasons was carried out with a nonmetric multidimensional scaling (nMDS) analysis (Bray-Curtis index; group average link; square root). In addition, a Permutational Multivariate Analysis of Variance (PERMANOVA) was applied to assess the effect of the seasons, I and E experimental conditions, and the interaction between both factors (Bray-Curtis index; fourth root and 9999 permutations) (PRIMER v7). A Pair-wise PERMANOVA was applied to analyze the significant differences between seasons and conditions (Bray-Curtis index; fourth root and 9999 permutations) (Anderson et al. 2008). Moreover, Specific richness (S), total abundance (N), and the Shannon diversity index (H′) were calculated by layer and core (I and E) at the four seasons. Variation of each biological parameter between seasons and conditions was evaluated through two-way

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ANOVA considering seasons and conditions as fixed factors (n=32) (Statistica, version 7). The differences between pairs of seasons and conditions were tested through Tukey test for multiple mean comparisons.

A comparison of the depth distributions of the luminophores for the two particles range sizes (pink and green) and the bio-diffusion (D_b) and bio-advection (r) coefficients calculated for each experimental condition (I and E) was performed through the non-parametric Kruskal-Wallis one-way analysis of variance by ranks (Kruskal and Wallis, 1952) (Statistica, version 7). Moreover, a one-way ANOVA was applied to test the differences between seasons (n=16). The differences between pairs of seasons were tested through Fisher LSD test for multiple mean comparisons (Statistica, version 7). A Kruskal-Wallis and Pair-wise Multiple Comparisons analysis were carried out to test the differences as a non-parametric alternative due to lack of homogeneity of variances of data in some cases.

3. Results

3.1. Characterization of the sediment

The granulometry profiles were similar for the *in situ* and the *ex situ* sediments showing a predominance of medium sand (200-600 µm) (Figure 2). Moreover, the fine fractions (< 200 µm) were highest at the first two centimeters of the sedimentary columns. Regards *in situ* sediments, the maximum percentages of water content (58.5 ± 8.2%) and organic matter (OM) (1.7 ± 0.1%) were recorded in the first layer (0-0.5 cm) in winter and autumn, respectively (Online Resource 1). In spring, the maximum percentages of water content (48.5 ±

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19.6%) and OM (1.3 ± 0.2%) were found in the fourth layer (1.5-2 cm) and the first layer (0-0.5 cm), respectively. In summer, the maximum percentages of water content (33.3 ± 0.8%) and OM (0.9 ± 0.0%) were recorded in the second (0.5-1 cm) and second and third layers (0.5-1.5 cm), respectively. These patterns were similar to those recorded in *ex situ* conditions (Online Resource 1). Comparing seasons and experimental conditions (I and E), percentages of water content did not show significant effect of the interaction of these factors (Two-way ANOVA, $p = 0.15$, n = 32) but highly significant effect of each factor ($p < 0.01$, n = 16). Percentages of water content recorded in I and E conditions for winter and summer were similar (Tukey test $p = 0.23$ and $p = 0.06$; n = 8, respectively). Contrary, in autumn and spring, this parameter was significantly higher I than E (Tukey test $p < 0.01$; n = 8) (Figure 3). Moreover, in I conditions, water content showed not significant differences (Tukey test $p > 0.05$; n = 16) among autumn, winter and spring, but did (Tukey test $p < 0.01$; n = 16) in summer compared to other seasons. By contrast, in E conditions, in winter water content was significantly higher (Tukey test $p < 0.01$; n = 16) than in other seasons (Figure 3). Percentages of OM showed significant effect of the interaction season and experimental condition (Two-way ANOVA, $p = 0.01$, n = 32) and just significant effect of season ($p < 0.01$, n = 16). Only significant differences between I and E conditions were recorded in spring ($p = 0.026$, n = 8). Comparing seasons, at both conditions, the highest values were recorded in autumn with significant differences (Tukey test $p < 0.01$; n = 16) from the rest seasons. At I conditions, OM significant differences ($p < 0.01$; n = 16) were between winter and spring from summer; conversely, E conditions presented

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significant differences (p < 0.01, n = 16) between winter from spring and summer (Figure 3).

3.2. Macrobenthic communities

A total of 27 taxa (I and E conditions) were found in CV including sixteen taxa of crustaceans, nine taxa of polychaetes, and one taxon of oligochaetes and nemerteans (Table 1). Mean abundance of the six most dominant taxa (> 4.8%) are shown in Figure 4. All taxa except *Axiothella* sp. were more abundant in the spring-summer period. This was pronounced in summer for the polychaete *Exogone molesta* (Syllidae), the most abundant species (37.4% of total macroinfauna). In addition, the two-way ANOVA showed only for *Scoloplos* sp. and *E. molesta* highly significant (p = 0.00, n = 32) and significant (p = 0.02, n = 32) effect of the interaction (season and experimental condition) and I and E factor, respectively. On the contrary, all the dominant taxa showed a highly significant effect (p = 0.00, n = 32) of the season factor. Particularly, the differences between pairs of seasons showed not significant differences (Tukey test p > 0.05; n = 32) for *Axiothella* sp. and between autumn and winter all the cases (Tukey test p > 0.05; n = 32). Moreover, abundance recorded in these seasons showed highly significant differences (Tukey test p < 0.01; n = 32) with spring and summer, which were similar in most cases (Figure 4). Finally, only for *E. molesta* in spring were detected significant differences (Tukey test p < 0.05; n = 32) between experimental conditions.

The nMDS showed a good representation (stress = 0.15) and grouped spring and summer samples, which were located on the opposite side of winter

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samples. Autumn samples were in an intermediate position close to spring-summer it. In addition, I and E faunistic composition were similar all the seasons (Figure 5). Moreover, the interaction between the factors season and experimental condition (I and E) were not significant (PERMANOVA, $p > 0.01$) meanwhile the effect of each factor was it ($p < 0.01$) (Table 2). The Pair-wise PERMANOVA showed highly significant differences ($p < 0.01$) between all the seasons in both experimental conditions (I and E) meanwhile the differences between I and E were highly significant ($p < 0.01$) only in spring.

Vertical profiles of the community parameters (S, N and H´) showed a similar trend for the four seasons with maximum values in the first five centimeters of the sedimentary columns and a decrease with depth (Figure 6). The two-way ANOVA for each community parameter showed only significant effect of the interaction (season and experimental condition) for H´, meanwhile the effect of both factors were significant for S and H´ ($p < 0.01$, n = 32) and just factor season for N ($p < 0.11$, n = 32). Regarding the differences between experimental conditions (I and E), significantly higher values I than E were recorded in spring H´ (Tukey test $p = 0.023$, n = 8, respectively). Particularly, at I condition, the highest S and N values were recorded in spring and summer while H´ values were relatively constant between seasons (Figure 7). Moreover, S and N showed strong differences between autumn and winter versus spring and summer (Tukey test $p = 0.00$ n =16), and were no differences between autumn versus winter (Tukey test $p = 0.00$; n = 18). Similarly, N showed strong differences between all the seasons (Tukey test $p = 0.00$; n = 16) except between autumn versus winter (Tukey test $p = 0.15$; n = 16). Regard to E

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condition, S had no significant differences among autumn, winter and spring (Tukey test $p > 0.05$, n = 16) but did between winter and summer (Tukey test $p = 0.029$, n = 16). Total abundance were similar between autumn versus winter (Tukey test $p = 0.17$, n = 16) and spring (Tukey test $p = 0.06$, n = 16), and in summer was significantly higher than the other season (Tukey test $p < 0.01$, n = 16). Likewise, H´ in autumn, spring and summer were similar (Tukey test $p = 0.99$, n = 16), but in winter showed differences compared to spring and autumn (Tukey test $p = 0.006$ and $p = 0.016$; n = 16, respectively).

Overall, the macrobenthic community was functionally composed of biodiffusors (surficial biodiffusors, epifaunal biodiffusors and gallery-biodiffusors), regenerators, and upward conveyors (Table 1). Upward conveyors predominated in autumn (61%) and winter (76%). Spring showed more balanced repartition between upward conveyors and biodiffusors, and this latter finally represented 81% of the functional groups in summer (Figure 8).

3.3. Bioturbation

The depth distributions of the luminophores for the two particles range sizes (pink and green) were similar (Kruskal-Wallis test $p > 0.05$; n = 32). Although the main bioturbation activity was recorded until 2 cm depth, both tracers were found buried down to 13 to 17 cm depth within the different sediment cores and seasons (Figure 9). The shapes of the tracer's distribution were also the same whatever the tracers and seasons showing a combination between a biodiffusive-like distribution from the surface completed by the presence of tracers deeper down. Thus, all tracers (pink and green) data were pooled

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together and the gallery-diffusor model (François et al., 2002) was used to calculate the apparent bio-diffusion (D_b) and bio-advection (r) coefficients within each core. Like previously observed for the majority of physical, chemical, and biological parameters, whatever the season the calculated coefficients did not show differences between the sediments incubated *in situ* and *ex situ* conditions (Kruskal-Wallis test $p > 0.05$; n = 16). In I conditions, nor *Db* nor *r* showed season effect (Kruskal-Wallis test $p = 0.5$ and one-way ANOVA $p = 0.05$; n = 16, respectively). Contrary, in E conditions, highly significant differences were found for the D_b coefficient between autumn versus spring and summer versus autumn and winter (Fisher LSD test $p < 0.01$; n = 16). Moreover, the r coefficient showed significant differences between spring versus winter (Multiple comparisons test $p < 0.01$; n = 16) (Figure 10). Particularly, the D_b highest average values were found in autumn and winter (1.71 ± 0.14 and 1.49 ± 0.31 cm^2 year^{-1}, respectively). Nevertheless, the results were highly variable in spring and summer. Finally, maximum values of *r* were recorded in spring and summer (Figure 10).

4. Discussion

The seasonal composition and functioning (sediment reworking activity) of the intertidal macrobenthic community of Caleta Valdés (CV) applying an *in situ* and *ex situ* experimental protocol were evaluated. The macrofaunal invertebrates (> 0.5 mm) occupy almost every trophic level in marine ecosystems and influence the physical, chemical, and biological structure surroundings (Lenihan and Micheli, 2001). Moreover, it is recognized that soft-sediment ecosystems are driven by complex interactions between water column processes, organic

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matter inputs and their utilization by benthic populations, and the hydrodynamic and sedimentary conditions (Barry and Dayton, 1991; Snelgrove and Butman, 1994). The coastal sediments of shallow environments play a key role in nutrient recycling because they can provide even greater fluxes to the water column than those from the continent (Clavero et al., 2000; Niencheski and Jahnke, 2002). Moreover, numerous studies highlight the importance of physical processes such as the velocity and intensity currents as the main environmental factors that influence the benthic systems (Pastor de Ward, 2000). These factors determine the granulometry of the sediment (Brown and McLachlan, 2010; Dauvin et al., 2004; Muniz and Pires, 2000, among others) and food availability (Brown and McLachlan, 2010; McLachlan and Dorvlo, 2005), modifying the composition of the communities. In the present study, the superficial layers (0-2 cm) were enriched in fine-grained particles with a relatively high percentage of water content and organic matter, meanwhile in the sandy deeper sediment, these parameters decreased to lower and almost uniform values. Similar results were recorded in sediments from Caleta Sara (San Jorge gulf, Patagonia Argentina), applying a similar methodology (Romanut, 2019). Particularly, the benthic organisms are able to change the local geochemical conditions through the reworking of sediment particles and irrigation during feeding, excretion, and locomotion within and through different sediment layers (Flach and Heip, 1996; Kristensen and Holmer, 2001; Venturini et al., 2011). Therefore, it is not surprising that in our study the bioturbation activity was concentrated mainly in the first centimeters of the sedimentary columns, i.e., 50% of the luminophores were finally retained (Gambi et al., 1998; Gambi and Bussotti, 1999; Jorein, 1999; Venturini et al., 2011) where the

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higher densities of macroorganisms were observed. In fact, the water-sediment interface, considered as a large sink of organic matter and oxygen, is an area of intense biological activity and remineralization (Nixon, 1981). Particularly, the combined effect of particulate and fluid transport on sediment biogeochemical processes is reflected in the vertical color transition (from brown to olive green/black) of the sediment profile (Lyle, 1983). This color transition is dictated by the change from iron (oxyhydr)oxides at the surface to black sulphidic phases at depth (Statham et al., 2017) correlating with a variety of environmental drivers (Solan et al., 2019). Moreover, depending on the biogeochemical transformations developed in this zone by the benthic communities, the sediment may be source of some nutrients and sink for others (Cabrita and Brotas, 2000).

Macrobenthic fauna is a key element of many marine and estuarine monitoring programs but those usually do not (or cannot) take into account distribution patterns at different spatial-temporal scales (Ysebaert and Herman, 2002). In the present study, the abundance of the dominant taxa and the bioturbation activity (especially in depth) were globally higher in spring-summer (D_b = 1.04 ± 0.59 cm^{-2} y^{-1} and r = 12.60 ± 3.22 y^{-1}) than in the rest of the seasons (D_b = 1.59 ± 0.26 cm^{-2} y^{-1} and r = 4.81 ± 5.85 y^{-1}). The D_b and r values recorded at CV were in the range of those measured in Mediterranean Sea lagoons, the Thau lagoon, and the Carteau Cove by Duport et al. (2007) and Gilbert et al. (2015), respectively. Nevertheless, we can go no further in the comparison between sites because the sediment mixing intensity is mainly driven by population biomass (Matisoff, 1982; Reible et al., 1996), density (Duport et al., 2006;

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Ingalls et al., 2000; Mermillod-Blondin et al., 2001; Sun et al., 1999), and interspecific variability within functional groups (François et al., 1999). Moreover, it can be modulated by environmental conditions (e.g. Gilbert et al., 2021; Hollertz and Duchêne, 2001; Maire et al., 2007; Nogaro et al., 2008; Orvain and Sauriau, 2002; Ouellette et al., 2004).

The maximum of macrofauna abundance recorded in spring-summer in this study were similar to those found at Cerro Avanzado (Golfo Nuevo, Patagonia Argentina) which was associated with higher juvenile occurrence and primary productivity (Lizarralde et al., unpublished data). This increase could be also influenced by the closeness to the Península Valdés frontal system, which is one of the best-known tidal fronts on the northern Patagonian continental shelf (Derisio et al., 2014). These fronts are generated usually within the same water mass where the boundary between stratified (offshore) and coastal vertically mixed water is found (Sabatini and Martos, 2002). In temperate climates, seasonal thermoclines are established near the surface during late spring and summer. This structure is maintained until autumn when stratification breaks down (Acha et al., 2004; Carreto et al., 1986; Glorioso, 1987). The fronts are zones where the lateral and vertical mixes are increased producing an increase of the primary and secondary production (Mann and Lazier, 2005; Olson and Backus, 1985; Yamamoto et al., 2000). The vertical circulation not only promotes the fertilization by nutrients in the surface (Mann and Lazier, 2005) but also the sinking water with the exportation of particles and organisms to the deepest strata facilitating the persistence of big invertebrates and vertebrates benthic populations (Sournia, 1994; Turley et al., 2000). The highest

concentrations of *Zygochlamys patagonica* found in the region have also been related to the formation of this front (Bogazzi et al., 2005). Viñas and Ramírez (1996) have reported previously eggs and nauplii of copepods peaking in frontal waters off Península Valdés at highest chlorophyll “a” concentration zones. The importance of the fronts to the benthic communities lies not only on the high primary production but also by vertical fluxes that transport food particles to the bottom and by the weakening of the vertical stratification that allowing increasing the particulate material sink rate. This material rich in labile OM is consumed and incorporated into the benthic biomass. Particularly, all dominant taxa recorded in CV except *Axiothella* sp. were more abundant in the spring-summer period. The feeding modes are not defined specifically for *Exogone molesta* (Syllidae), *Cerathocephale* sp. (Nereididae), *Scoloplos* sp. (Orbiniidae), Oligochaeta sp. (Oligochaeta), and *Leuroleberis poulseni* (Crustacea). Nevertheless, the members of the studied taxa generally are identifying as carnivores, filters, selective or no selective sediment feeders and scavengers, respectively. Therefore, these taxa could tend to move from the surface to the most depth zone of the sedimentary column, producing an increase of the bioturbation activity in the whole column at this period.

From a functional point of view, the overall high activity of the spring-summer community (i.e., increased D_b and r coefficients) produced a higher deep repartition of particles than those recorded in autumn and winter, even when in summer the community was dominated by biodiffusors meanwhile in autumn and winter predominated upwards conveyors. This clearly demonstrates that the repartition of particles within sediments is strongly dependent of the

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interactions between the different functional groups rather than the presence of a dominant group and that we cannot simply assume that the dominance of one functional group automatically produces a dominant sediment reworking process within a community. In spring-summer period in CV by example, the increase of subsurface biodiffusive transport may have feed the deeper advective process resulting in a global deeper burying of surface particles by the community.

Regarding *in situ* and *ex situ* comparing, in general terms, the community parameters profiles did not differ between the sediment incubated in field conditions versus those incubated in the laboratory. Moreover, there was a high similarity in the faunistic composition in both experiments. Consistently, there were no differences in the bioturbation activity of macrobenthic organisms for both experimental conditions. Thus allowing validation of an *ex situ* experimental protocol for macrobenthic community and functioning studies. To the best of our knowledge, there are no previous studies allowing such protocol to be validated. These results are particularly useful as a reliable alternative for ecotoxicological studies in protected environments where *in situ* addition of pollutants to the sedimentary matrix won't be feasible (see Ferrando et al., 2015, 2019; Sturla Lompré et al., 2018; Romanut, 2019; among others).

5. Conclusions

The seasonal composition and functioning of the intertidal macrobenthic community of CV were reported for the first time, contributing to the knowledge of this vulnerable ecosystem threatened by anthropogenic activities. In addition,

ex situ and *in situ* measurements demonstrated similar results, thus allowing validation of an *ex situ* experimental protocol for macrobenthic community and functioning studies. The information generated will allow assessing pollutant effects on macrobenthic communities inhabiting sediments from a natural environment without disturbing the ecosystem. In addition, these results provide a first baseline of benthic information on CV that will be helpful to monitor the effects of potential pollution in Patagonian coastal systems.

6. Author contributions

Agustina Ferrando: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, **Julieta Sturla Lompré:** Investigation, Writing - Review & Editing, **Emilia Gonzalez:** Investigation, **Marcos Franco:** Investigation, **Marta Commendatore:** Methodology, Investigation, Writing - Original Draft, Funding acquisition, **Marina Nievas:** Investigation, Writing - Review & Editing, Funding acquisition, **Cécile Militon:** Investigation, **Georges Stora:** Supervision, Funding acquisition, **José Luis Esteves:** Conceptualization, Methodology, Investigation, Writing - Original Draft, Project administration, Funding acquisition, **Philippe Cuny:** Investigation, Writing - Review & Editing, Project administration, Funding acquisition, **Franck Gilbert:** Conceptualization, Methodology, Investigation, Writing - Review & Editing, Project administration, Funding acquisition.

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872 **Tables**

873 **Table 1** Macrobenthic species mean abundance (n=4) in *in situ* and *ex situ*

874 sediments for the four seasons. I: *In situ*; E: *Ex situ*

		Autumn		Winter		Spring		Summer	
	F.G.[a]	I	E	I	E	I	E	I	E
Polychaeta									
Exogone molesta	GB	13.8	14.0	3.3	0.3	47.5	1.8	210.5	198.0
Ceratocephale sp.	GB	1.8	1.5			41.5	19.5	34.3	29.3
Axiothella sp.	UC	16.3	21.8	13.5	17.3	18.8	19.0	6.5	9.0
Scoloplos sp.	GB	0.5	2.3	0.3		31.0	19.8	4.8	5.3
Levinsenia gracilis	SB	1.5	5.8	0.3		0.8			0.3
Pionosyllis sp.	GB					2.3		4.8	1.5
Capitella "capitata"	UC			2.5	5.8				
Chone mollis	SB	0.8	2.5			1.3	0.3	0.8	0.8
Brania sp.	GB	0.3							
Oligochaeta									
Oligochaeta sp.	GB	15.5	16.0	1.0	6.5	28.5	32.5	37.5	58.8
Crustacea									
Leuroleberis poulseni	SB	8.3	10.8	0.8	0.5	31.5	23.0	26.5	34.5
Ostracoda sp. 1	SB	2.8	2.5	17.8	8.0	15.5	8.0	6.0	5.3
Phoxocephalopsis sp.	SB	1.3	2.8	4.8	2.3		1.5	0.8	0.8
Ostracoda sp. 2	SB			5.8	2.5		0.5	0.5	4.0
Anacalliax argentinensis	GB	1.5	2.8	3.0	0.8	0.8	0.3	2.0	0.8
Paranthura sp.	SB	0.5	0.8					2.0	2.5
Gammaridae sp. 1	SB	3.3		0.3			0.3	0.3	
Paranebalia sp.	GB			1.8	0.8				1.3
Caprella scaura	SB	0.5	0.5	1.0					
Pseudocumatidae sp.	SB				0.8		1.0		
Cyrtograpsus angulatus	R	0.8			0.3				
Cirolanidae sp.	SB			0.3		0.3	0.3	0.3	
Gammaridae sp. 2	SB		0.5		0.3				

Corophioidea sp.	SB				0.5
Leptostraca sp.	EB	0.3			
Anthuroidea sp.	SB			0.3	
Nemertea					
Nemertea sp.	GB		1.5	0.5	

[a] F.G.: Functional group; Biodiffusors species presented in three sub-groups as EB: Epifaunal biodiffusors; SB: Surficial biodiffusors and GB: Gallery biodiffusors; R: Regenerators; UC: Upward conveyors. For a complete description of the different functional groups, see Kristensen et al. (2012).

Table 2 Summary PERMANOVA. Res: Residuals; df: Degrees of freedom; SS: Sum of squares; MS: Middle squares; mc: Monte Carlo simulation; ** *p* (mc) < 0.01

Source	df	SS	MS	Pseudo-F	*p* (mc)
Experimental condition	1	2013.5	2013.5	5.9166	0.001**
Season	3	15226	5075.4	14.914	0.0001**
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Total	31	26648			

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Figures captions

Figure 1. Sampling site in Caleta Valdés (CV; Península de Valdés, Patagonia Argentina). *Ex situ* cores sampled and *in situ* cores embedded in the intertidal zone.

Figure 2. Mean contribution percentage of each granulometry fraction with depth (n = 4) in Caleta Valdés sediments

Figure 3. Water content and organic matter (OM) by core (mean ± SD; n = 4) measured in Caleta Valdés sediments for the four different seasons. I: *In situ*; E: *Ex situ*. Significant differences ($p < 0.05$) tested between conditions are represented with different letters (uppercase for *in situ*, and lowercase for *ex situ*). For each season, significant differences ($p < 0.05$) between *in situ* and *ex situ* are represented with an asterisk

Figure 4. Abundance of the dominant species by core (mean + SD; n = 4) in Caleta Valdés sediments for the four seasons. I: *In situ*; E: *Ex situ*. Significant differences ($p < 0.05$) tested between conditions are represented with different letters (uppercase for *in situ*, and lowercase for *ex situ*). For each season, significant differences ($p < 0.05$) between *in situ* and *ex situ* are represented with an asterisk

Figure 5. Nonmetric multidimensional scaling (nMDS) analysis (Bray-Curtis index; group average link; square root) of total abundance by core (n = 4) in Caleta Valdés sediments for the different four seasons. I: *In situ*; E: *Ex situ*

Figure 6. Macrobenthic community parameters by layer (mean + SD; n = 4) in the *in situ* and *ex situ* Caleta Valdés sediments for the four seasons. S: Specific richness; N: Total abundance; H´: Shannon diversity I: *In situ*; E: *Ex situ*.

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Figure 7. Macrobenthic community parameters by core (mean + SD; n = 4) in Caleta Valdés sediments for the four seasons. I: *In situ*; E: *Ex situ*. Significant differences ($p < 0.05$) tested between conditions are represented with different letters (uppercase for *in situ*, and lowercase for *ex situ*). For each season, significant differences ($p < 0.05$) between *in situ* and *ex situ* are represented with an asterisk

Figure 8. Functional groups in Caleta Valdés sediments (pooled data between *in situ* and *ex situ* sediments) (n = 8) for the four seasons. Biodiffusors species presented in three sub-groups as EB: Epifaunal biodiffusors; SB: Surficial biodiffusors and GB: Gallery biodiffusors; R: Regenerators; UC: Upward conveyors. For a complete description of the different functional groups, see Kristensen et al. (2012)

Figure 9. Luminophores percentage (pooled data between both size of luminophores particles) in each layer (mean + SD; n = 8) for the *in situ* and *ex situ* Caleta Valdés sediments by season

Figure 10. Bio-diffusion (D_b) and bio-advection (r) coefficients in Caleta Valdés sediments (pooled data between both size of luminophores particles) by season and experimental condition (mean ± SD; n = 8) Significant differences ($p < 0.05$) tested between conditions are represented with different letters (uppercase for *in situ*, and lowercase for *ex situ*). For each season, significant differences ($p < 0.05$) between *in situ* and *ex situ* are represented with an asterisk

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Seasonal composition and activity of the intertidal macrobenthic community of Caleta Valdés (Patagonia, Argentina) applying *in situ* and *ex situ* experimental protocols

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Figure 1

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Figure 2

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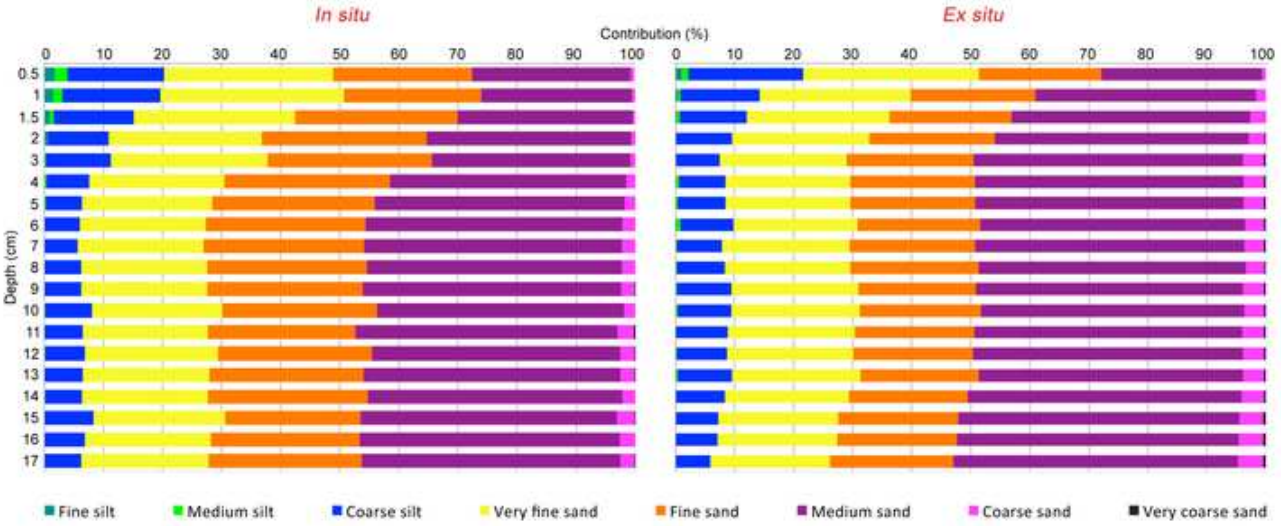

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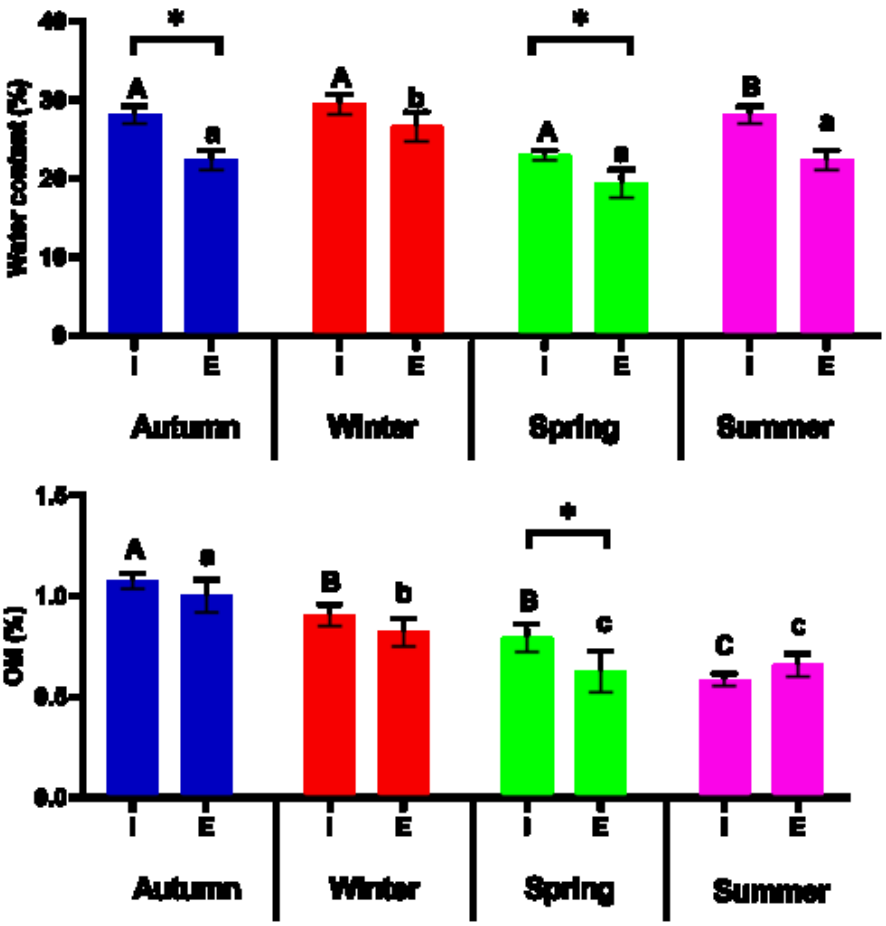

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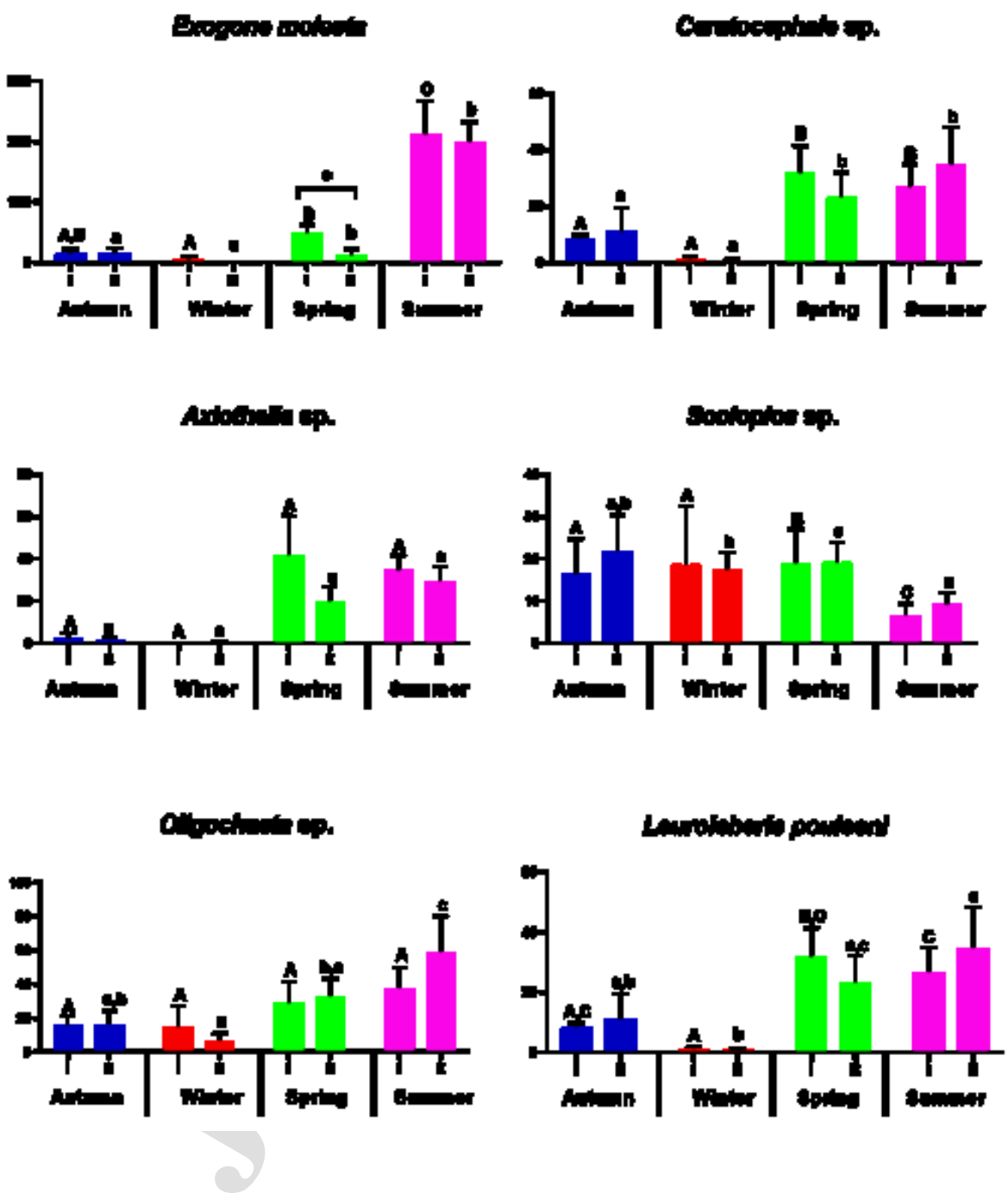

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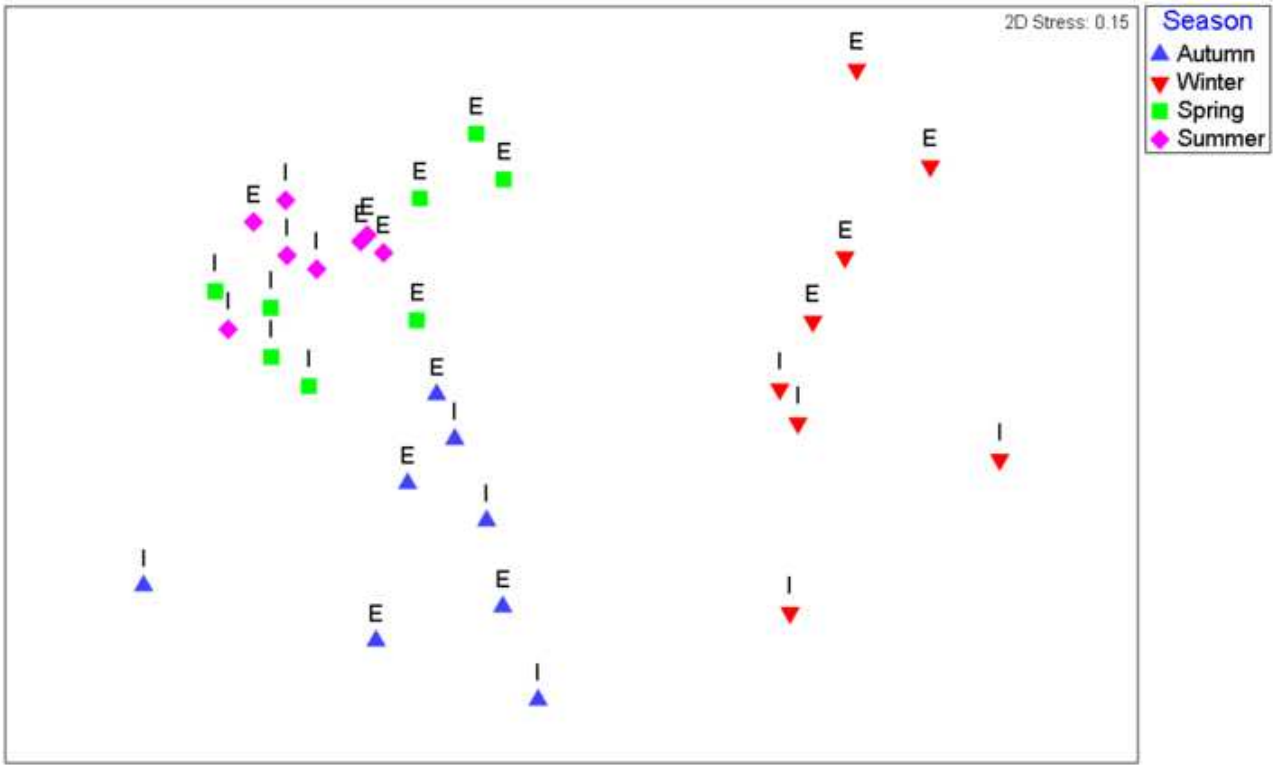

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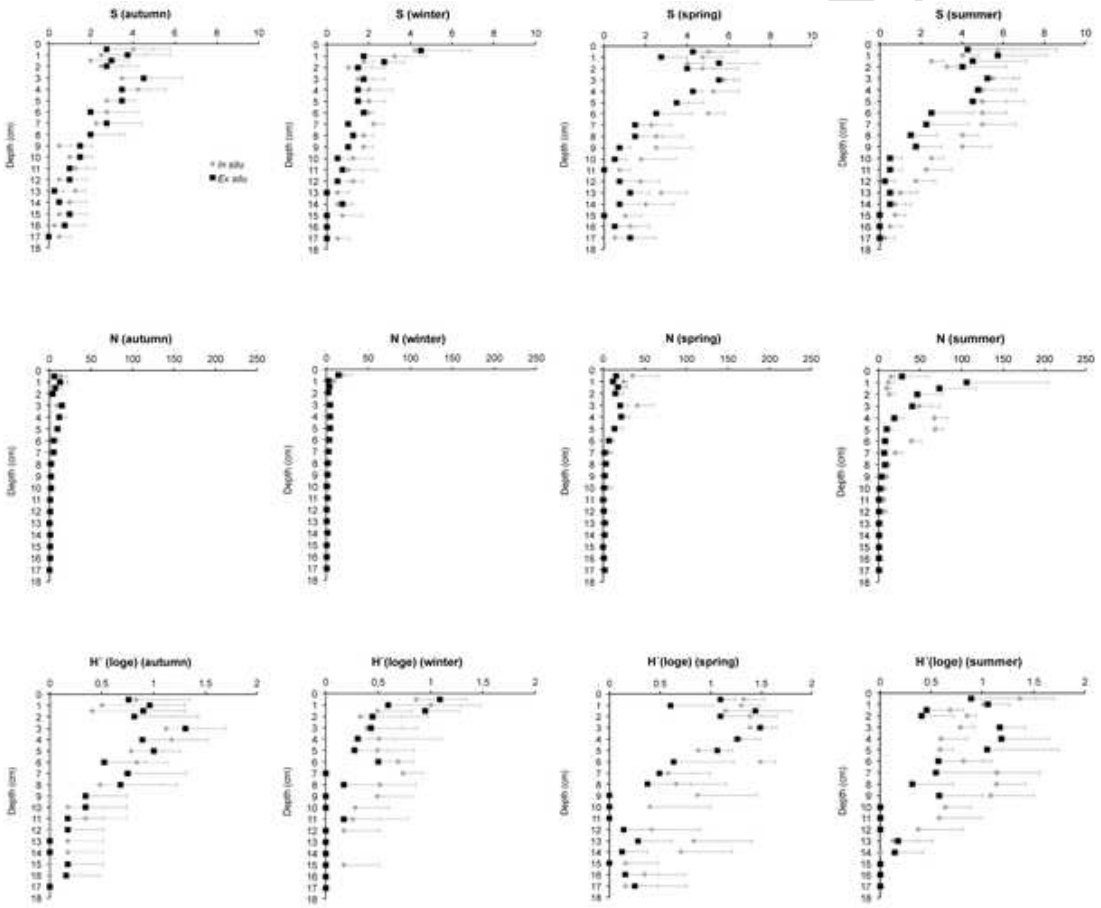

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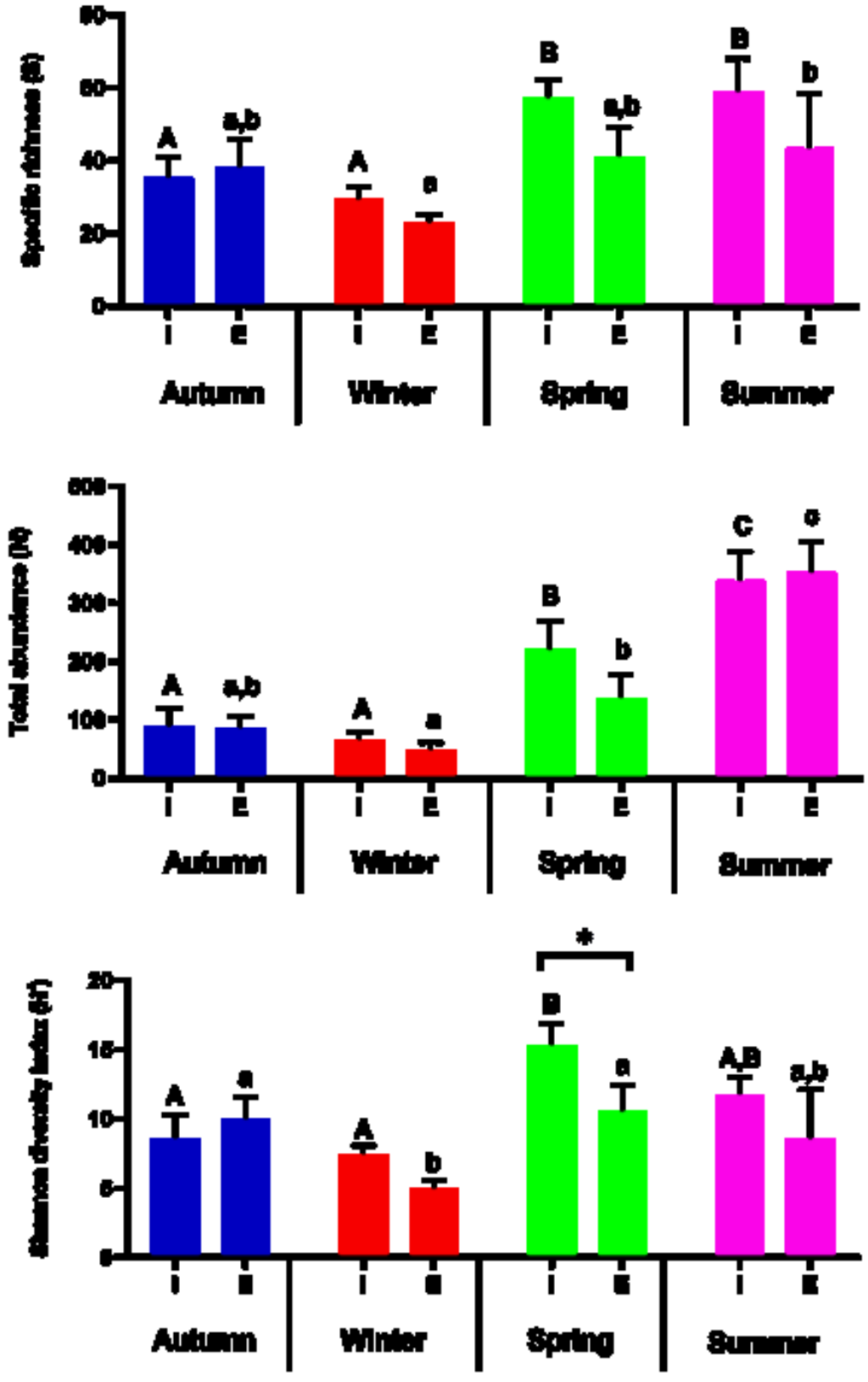

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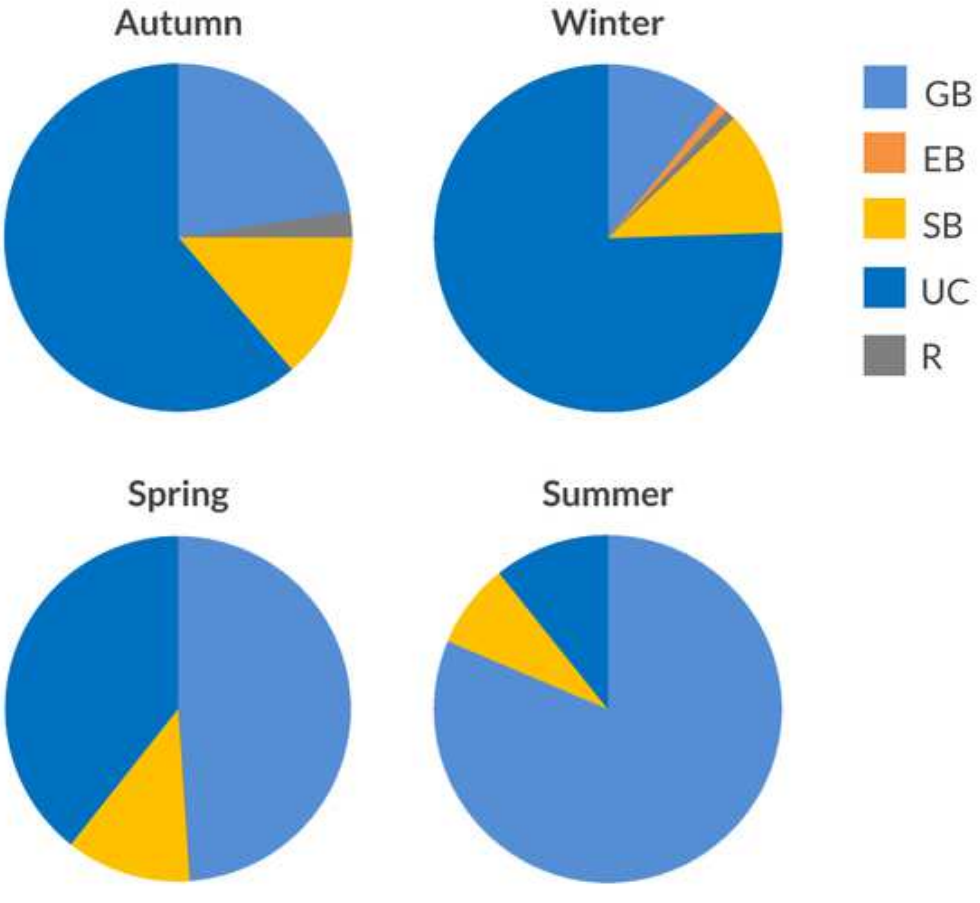

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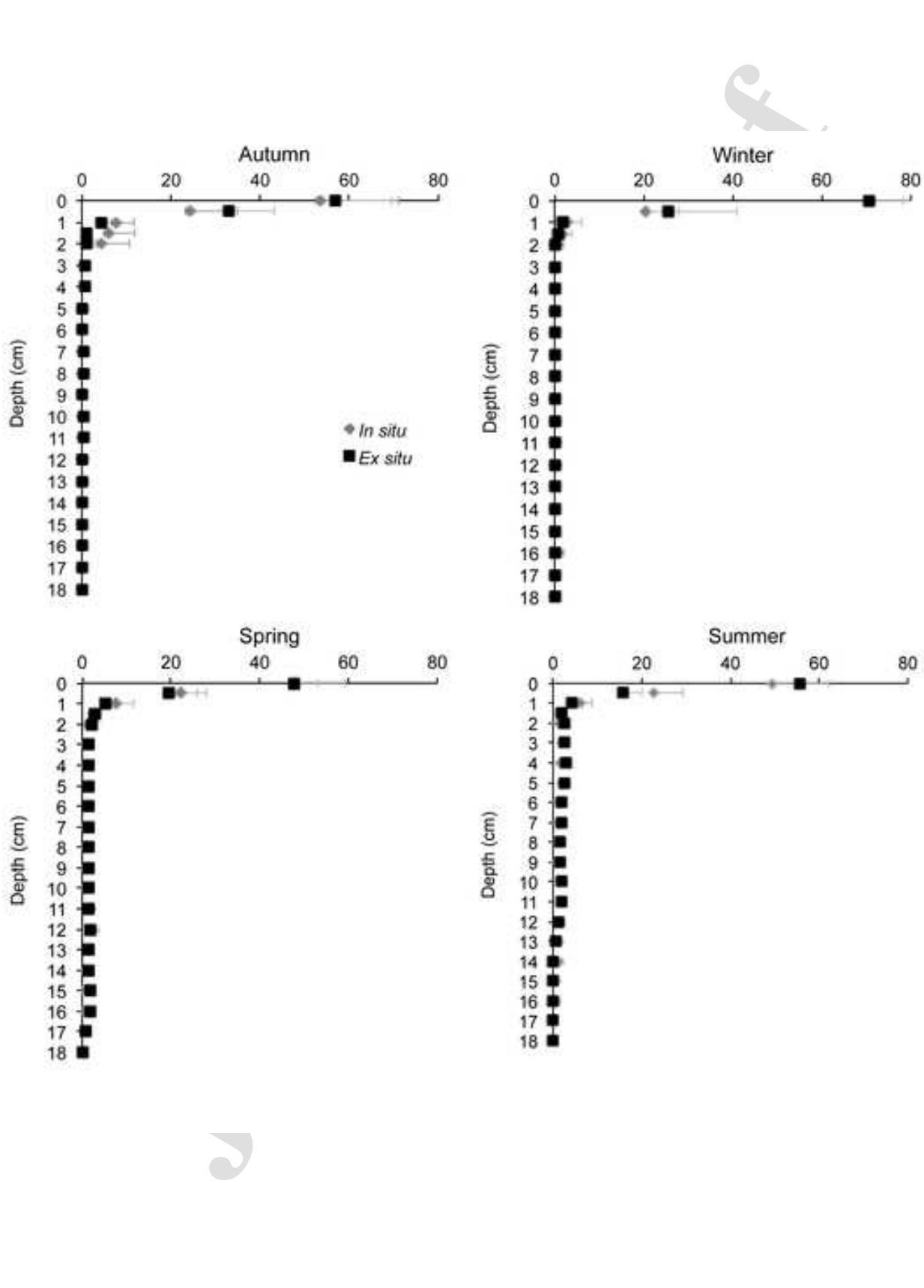

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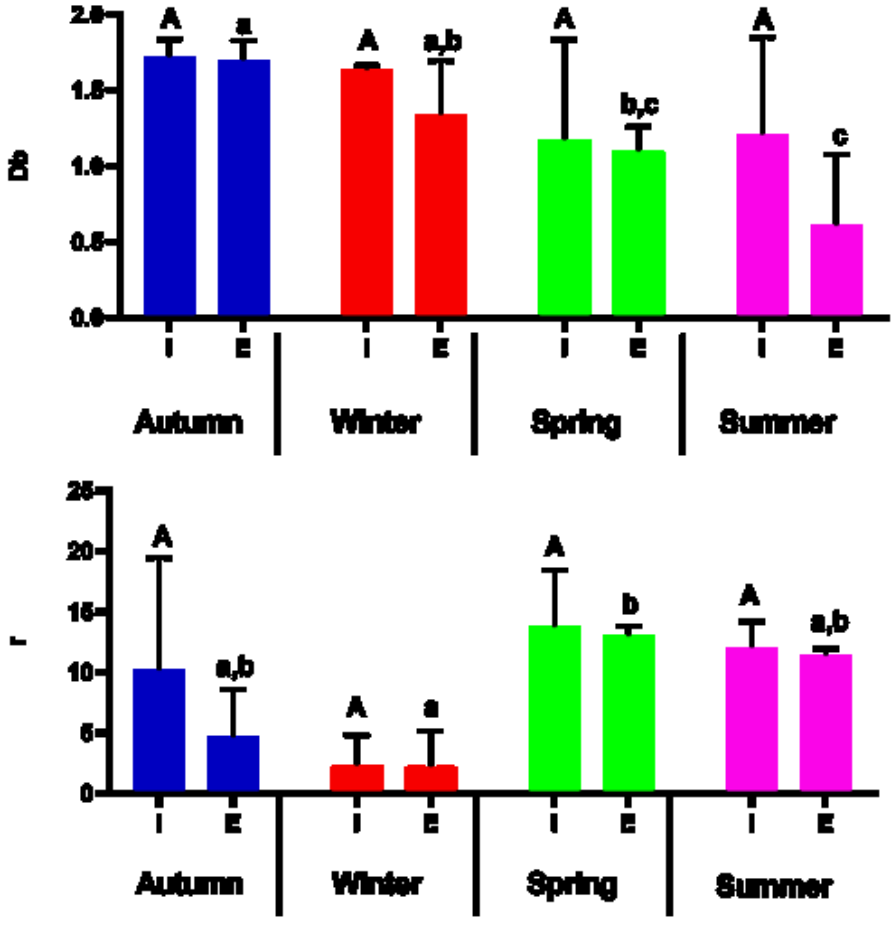

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Tables

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Pseudocumatidae sp.	SB				0.8		1.0		
Cyrtograpsus angulatus	R	0.8			0.3				
Cirolanidae sp.	SB			0.3		0.3	0.3	0.3	

Gammaridae sp. 2	SB	0.5		0.3		
Corophioidea sp.	SB					0.5
Leptostraca sp.	EB		0.3			
Anthuroidea sp.	SB				0.3	
Nemertea						
Nemertea sp.	GB			1.5	0.5	

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Author Statement

Author contributions

Agustina Ferrando: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, **Julieta Sturla Lompré:** Investigation, Writing - Review & Editing, **Emilia Gonzalez:** Investigation, **Marcos Franco:** Investigation, **Marta Commendatore:** Methodology, Investigation, Writing - Original Draft, Funding acquisition, **Marina Nievas:** Investigation, Writing - Review & Editing, Funding acquisition, **Cécile Militon:** Investigation, **Georges Stora:** Supervision, Funding acquisition, **José Luis Esteves:** Conceptualization, Methodology, Investigation, Writing - Original Draft, Project administration, Funding acquisition, **Philippe Cuny:** Investigation, Writing - Review & Editing, Project administration, Funding acquisition, **Franck Gilbert:** Conceptualization, Methodology, Investigation, Writing - Review & Editing, Project administration, Funding acquisition.

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Conflict of interest

The Authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria, educational grants, participation in speakers' bureaus, membership, employment, consultancies, stock ownership, or other equity interest, and expert testimony or patent-licensing arrangements), or nonfinancial / interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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