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Effects of surface seawater temperature and oxygen availability on molluscan taxonomic diversity in Cyprus



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Abstract

The Mediterranean Sea, a semi-enclosed basin, hosts a rich marine biodiversity, with most of the region's native species being of temperate origin. Climate change poses a significant threat, with sea surface temperatures increasing at a rate 20% faster than the global average. Projections suggest further warming by 2100, exacerbating ocean acidification and threatening calcifying organisms. Moreover, local anthropogenic stressors have worsened in recent decades, compounded by an ever-increasing population density. Lessepsian species pose additional ecological threats by altering native biodiversity patterns. To better understand these dynamics, this paper examines the impacts on mollusks by utilizing living and death assemblages. It compares two locations along the southern coast of Cyprus, Akrotiri and Cape Greco, which have sea surface temperature differences of up to 3°C in the summer. Additionally, it compares two habitats, rocky substrates and *Posidonia oceanica* meadows, to gain insight into the effects of local oxygen availability.

Results reveal a live-dead match, suggesting a relatively healthy ecosystem compared to other areas in the Eastern Mediterranean. However, radiocarbon dating results indicate young death assemblages at all location/habitat subsets, rendering them insufficient to capture all anthropogenic impacts. Akrotiri appears to be a refugium for endemic species due to its higher relative species richness compared to Cape Greco. The most drastic live-dead mismatch in species richness was at Cape Greco's *Posidonia* meadow, highlighting that the benefits of oxygen super-saturation are not infinite, and many endemic species may have already been pushed beyond their thermal tolerance limits. Finally, non-indigenous species abundance increased at all four sample subsets, but their ecological ramifications likely differ. In conclusion, this study highlights the need for the extension of conservation efforts in the Mediterranean, especially for key habitats like *Posidonia oceanica* meadows and rocky substrates.

1. Introduction

1.1. History of Mediterranean

The Mediterranean Sea is a semi-enclosed basin bordering Europe, Africa, and Asia. It hosts over 17,000 marine species [1] and its natural assets are estimated to be worth at least 4.7 trillion euros [2]. Native Mediterranean species are primarily of temperate origin due to the biotic interchange from the Atlantic to the basin following the Messinian salinity crisis [3]. Furthermore, climatic oscillations during the Pleistocene altered species compositions to favor cold-or-warm adapted species during Earth's last glacial-interglacial cycle [4].

Recent decades have introduced multiple anthropogenic stressors into the region, including tourism [5,6], industrial fishing [7], and pollution [8,9]. Population density has increased by 1.35% annually, with numbers predicted to reach 529 million by 2025 [10]. With about a third of people living on the coast, this makes nearshore environments especially vulnerable to land-based pollution.

Moreover, the Mediterranean is a hotspot for climate change, with warming being about 20% faster than the global average [11-14]. From 1982 to 2016, sea surface temperatures (SSTs) in the region increased by 0.36°C per decade [13]. By 2100, SSTs could rise by another 1.8 to 3.5°C with hotspots including Spain and the eastern Mediterranean [10].

With the increase in CO₂ emissions, ocean acidification has also increased. This decreases aqueous carbonate ion concentrations and makes it more difficult for calcifiers such as corals and mollusks to form calcareous skeletons [15-17].

1.2. Non-Indigenous Species

The construction of the Suez Canal in 1869 initiated a unidirectional migration of Red Sea species into the Mediterranean, termed the Lessepsian migration [18]. Today, there are ~1000 non-indigenous species in the region, primarily due to this phenomenon, but also linked to other human activities such as shipping and aquaculture [19]. There are spatial differences in native species biodiversity, with the overall richness declining on a north-west to south-east gradient, with the opposite occurring for non-native species [19]. Though few non-indigenous

species become successful invaders, they have been ranked as a major driver for biodiversity loss, second only to habitat destruction [20].

1.3. Living and Death Assemblages

A living assemblage (LA) is defined as a collection of organisms or species that are alive and inhabit a specific ecological or environmental niche at a particular site, habitat, or ecosystem [21]. These species represent the composition and diversity of species in a given area at the time of collection. A death assemblage (DA) is the “taxonomically identifiable, dead or discarded organic remains encountered in a landscape or seabed” [21]. Live-dead comparisons can give insights into the discordance between the species diversity, composition, and distribution in modern and past ecosystems.

A critical obstacle in accurately assessing live-dead comparisons is time-averaging: a natural process by which remains of organisms that lived at different times accumulate into a single assemblage [22]. A single DA can contain skeletons that range from 10s to 1000s of years old. Several key factors that control the scale of time-averaging include the rate of 1) skeletal destruction, 2) sedimentation rate, 3) depth and rate of sediment mixing, and 4) rate of skeleton production. Notably, most DAs encompass young shells, and typically follow an exponential distribution, with some molluscan DAs being as old as 10,000 years [23]. This means that the DA could illustrate a pristine ecosystem before anthropogenic impact.

The uppermost part of surface sediments is known as the taphonomically active zone (TAZ), where the sediment is frequently mixed by bioturbators and currents. Typically, the TAZ is the top 20 cm of the sediment, but this largely depends on the habitat, environmental conditions, bioturbation rates, and depth of sampling. Underneath lies the sequestration zone, where it is assumed that mixing no longer occurs, thereby burying the shells deep within the sediment.

It is important to acknowledge that the DA typically surpasses the LA in both abundance and diversity, even under pristine conditions. This is because the LA offers only a small snapshot of currently living organisms, whereas DAs represent a broader timespan, spanning from decades to millennia of past living organisms. Furthermore, DAs tend to change at a significantly

slower rate than LAs, a phenomenon termed "taphonomic inertia." Finally, the age of DAs varies by habitat. The DAs on rocky substrate environments and *Posidonia oceanica* meadows are very young compared to soft substrates [24]. In this study, we believe the differences between the LA and DA are primarily due to an ecological change in response to an anthropogenic impact [25]. However, time-averaging and taphonomic biases likely also play a role.

1.4. Applicability of Mollusks in Live-Dead Studies

Mollusca is a ubiquitous, diverse phylum found in various terrestrial and aquatic environments worldwide. These organisms play crucial roles as ecosystem engineers, providing protection, food, and habitat for other marine taxa [26]. Their calcareous shells contribute to an extensive fossil record, often spanning centuries, making them valuable proxies for understanding past and current ocean conditions [27]. Moreover, many mollusk species have relatively short life spans; for instance, *Bittium latreilli* and *Bittium reticulatum* typically live for around 18 months. Finally, adult benthic mollusks tend to remain within a limited radius throughout their lives. Their extensive fossil record and lack of mobility make them the ideal organisms for live-dead comparisons.

1.5. Temperature Variations in the Mediterranean

The eastern Mediterranean is the warmest sector of the region due to a north-west to south-east gradient of SSTs [14], with the highest temperatures typically recorded in July and August (Figure 1). However, a unique area in the Mediterranean is located at the southwestern coast of Cyprus where northerly winds in the summer lead to upwelling and cause temperature variations of up to 3°C (Figure 2).

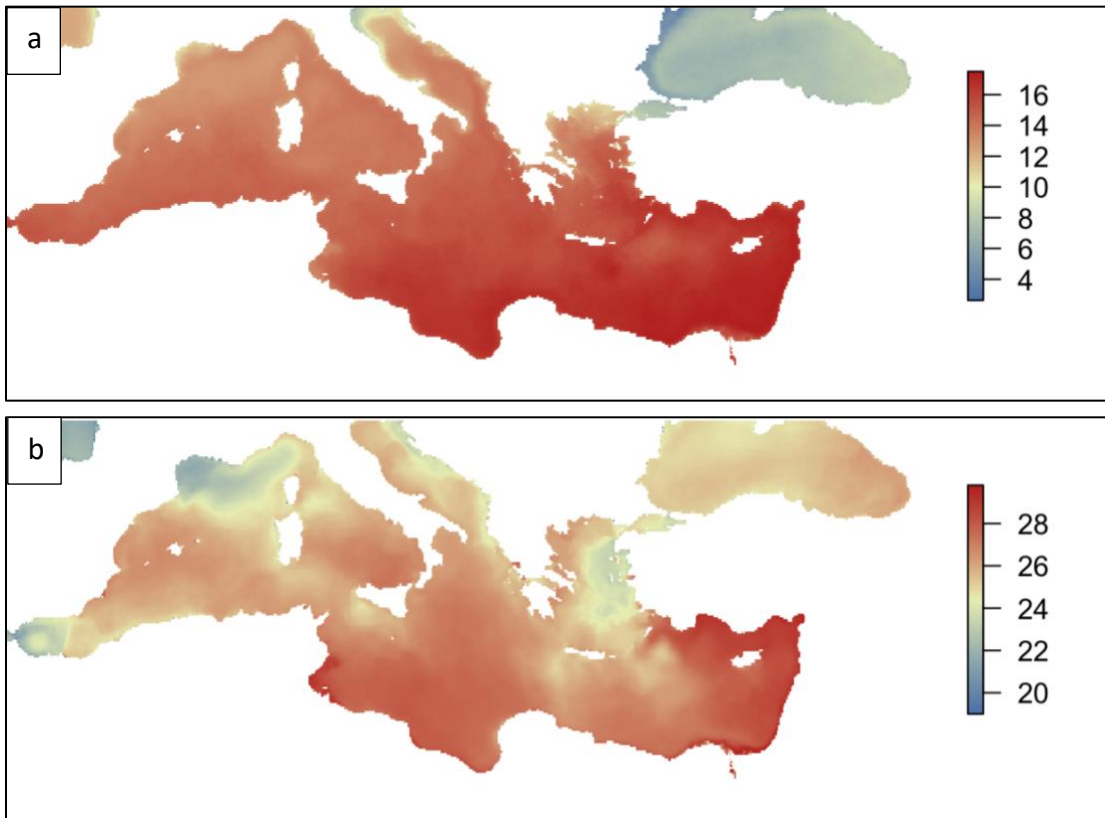


Figure 1: (a) Spatial distribution of average SST ($^{\circ}\text{C}$) of Mediterranean region from 2000-2014 during February. Most of the basin is between 14-16 $^{\circ}\text{C}$. (b) Spatial distribution of average SST ($^{\circ}\text{C}$) of Mediterranean region in 2000-2014 during August. There is a clear north-west to south-east gradient of SST, with the highest SSTs recorded in the E. Mediterranean.

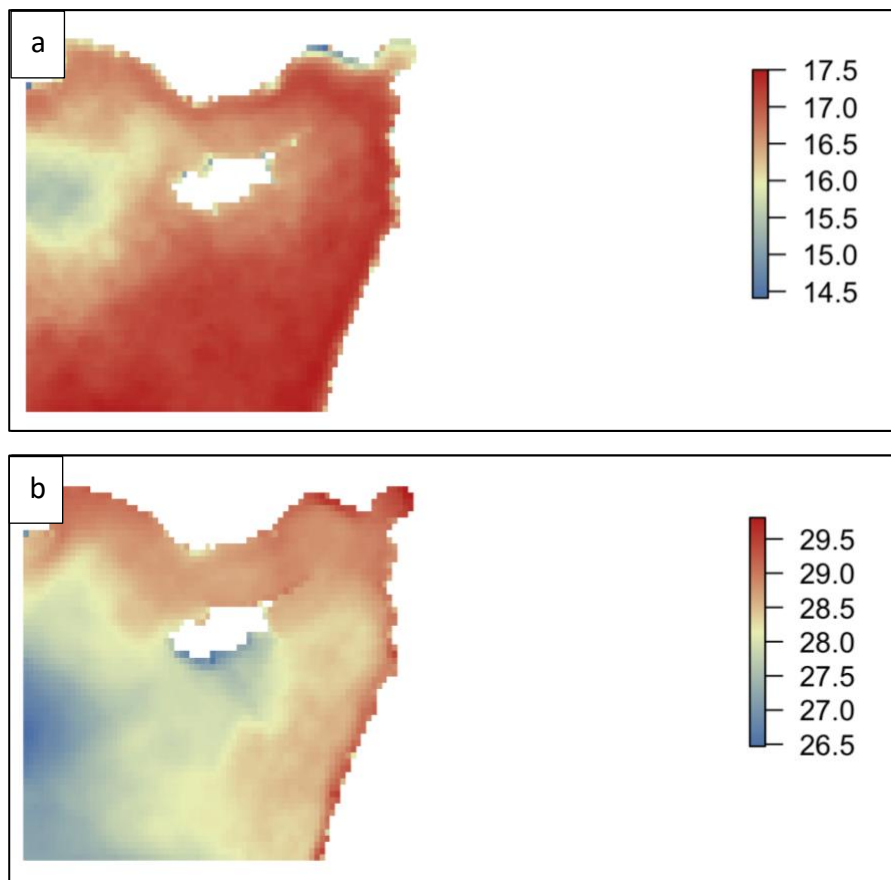


Figure 2: (a) Average SST ($^{\circ}\text{C}$) of Cyprus in February 2000-2014. No distinguishable temperature differences around the island. (b) Average SST ($^{\circ}\text{C}$) of Cyprus in August 2000-2014. Summer northerly winds create upwelling in southwestern Cyprus, causing temperature differences of 2-3 $^{\circ}\text{C}$ compared to regions without upwelling. The lower SSTs could contribute to a regional refugium for endemic species in Cyprus.

1.6. Thermal Tolerance under Hyperoxia

Marine species have been shown to be more resilient to thermal stress when exposed to oxygen super-saturation with the lethal temperature increasing by 1 $^{\circ}$ to 4 $^{\circ}\text{C}$ [28]. This has pertinent applications to oceans facing climate change threats.

Posidonia oceanica, an endemic seagrass species, is widespread throughout the Mediterranean and is present at almost a quarter of the region's total coastlines [29]. It has been postulated as a refugium for thermally stressed native species due to the increased oxygen availability. Moreover, the cumulative impact of alien species on *Posidonia oceanica* meadows is lessened, likely due to increased survivability for endemic species [30].

1.7. Recent Loss of Molluscan Biodiversity

Recent studies paint a grim future for the future of Mediterranean native biodiversity. Albano et al., 2021 found a near-total collapse of the native molluscan taxonomic diversity on the Israeli shelf with only 12% and 5% of previously documented native species present on shallow subtidal hard and soft substrates [31]. Additionally, 60% of those had not reached reproductive size. It is likely that multiple stressors are at play but is primarily due to the increased SSTs and the successful invasion of Lessepsian species.

1.8. Refugia

Refugia typically denotes areas where species sought sanctuary during the last glacial period, but its usage has expanded to include 'climate change refugia'. Unlike interglacial refugia, which emerged during cooler periods, climate change refugia are facing higher global average temperatures and its species must cope with fragmented landscapes [32]. Given the temperate origins of most endemic Mediterranean species, they prefer cooler conditions. Although climate change refugia are not immune to the impacts of rising temperatures, they serve as a crucial "slow lane" by buffering the adverse effects of climate change, particularly for endemic species [33].

1.9. Aim

The primary focus of this paper is to investigate an area along the southwestern coast of Cyprus as a potential refugium for endemic Mediterranean species. Additionally, the island's coast is home to *Posidonia oceanica* meadows, which may act as a refugium at a habitat scale due to the increased oxygen availability.

2. Hypotheses & Research Questions

2.1. Research Questions

- How does seawater temperature affect the taxonomic diversity and composition of molluscan assemblages in Cyprus?
- What is the biodiversity at each location/habitat at 15-meters depth?
- Does biodiversity change from spring to autumn?
- What is the diversity and abundance of native and non-indigenous species?
- What are the differences in biodiversity in the living and death assemblage?

2.2. Hypotheses

- The highest biodiversity is expected at Akrotiri's *Posidonia oceanica* meadows due to lower summer SSTs and increased oxygen availability.
- The lowest biodiversity is expected at Cape Greco's rocky substrates due to higher summer SSTs and decreased oxygen availability.
- Biodiversity will decrease from spring to autumn at both a habitat (rocky substrate vs. *Posidonia oceanica* meadow) and regional (Cape Greco vs. Akrotiri) scale due to increased thermal stress in the summer months.

3. Methodology

3.1. Study Site

Cyprus is the third largest island in the Mediterranean Sea and the closest to the Suez Canal. Its economy relies heavily on tourism; of five popular touristic EU islands, it ranked the most sensitive to marine deterioration due to SST increases and Lessepsian species invasions [34]. The surface waters around Cyprus are ultra-oligotrophic due to their high temperature and salinity levels and therefore have very low productivity and nutrient levels.

Cyprus has implemented legislation for the protection and conservation of its natural environments. Marine protected areas (MPAs) make up ~20% of the island's waters, including seven artificial reef MPAs. Notably, 29 of 31 designated Special Protection Areas (SPAs) include a coastal MPA up to 1.5 km or up to the 30-m bathymetric contour. This prevents any netting or anchoring to protect *Posidonia oceanica* meadows [35].

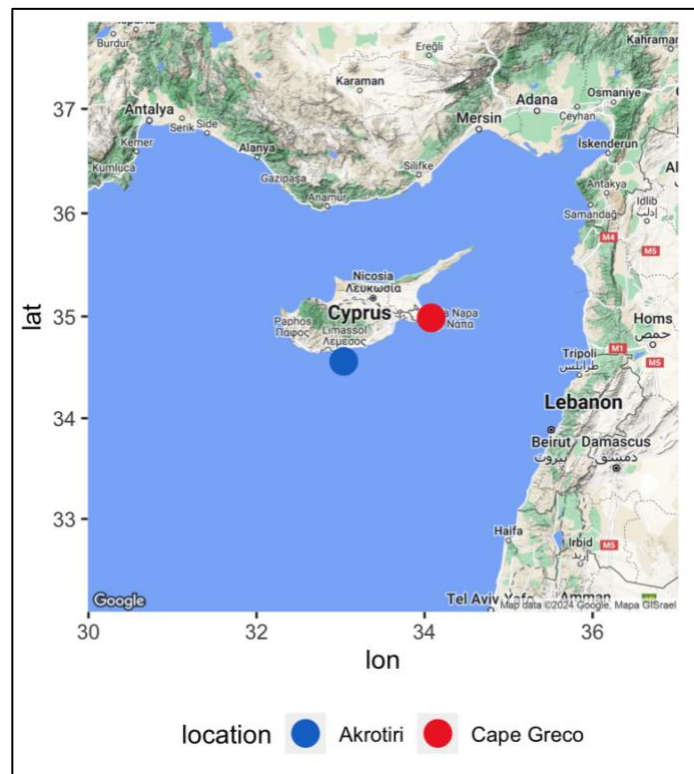


Figure 3: Map of two sampling locations in Cyprus.

3.2. Sample Collection

All sampling was conducted at two locations in Cyprus: Akrotiri and Cape Greco (Figure 3) in spring and autumn 2022. The samples were collected by SCUBA divers using an air-lift suction sample sampler and net (

Figure 4). At each location, transects were completed at 5, 10-, 15-, 20-, and 30-meters depth at a *Posidonia oceanica* meadow and rocky substrate area. The leaves and the rhizome were collected at the *Posidonia* meadow. Three replicates for each sample were collected during each season. Each replicate encompassed an area of 1 m². Therefore, each depth level is comprised of 24 samples (eight samples, three replicates). It is important to note that this study only includes data from the 15-meter depth transect.

All samples were air-dried on site. They were sieved into three fractions: fine (0.5-1 mm), medium (1-5 mm), and large (>5 mm) (F, M, and L). Residue <0.5 mm was discarded. Ziploc bags were used to store the sediments and labelled accordingly (

Table 1).

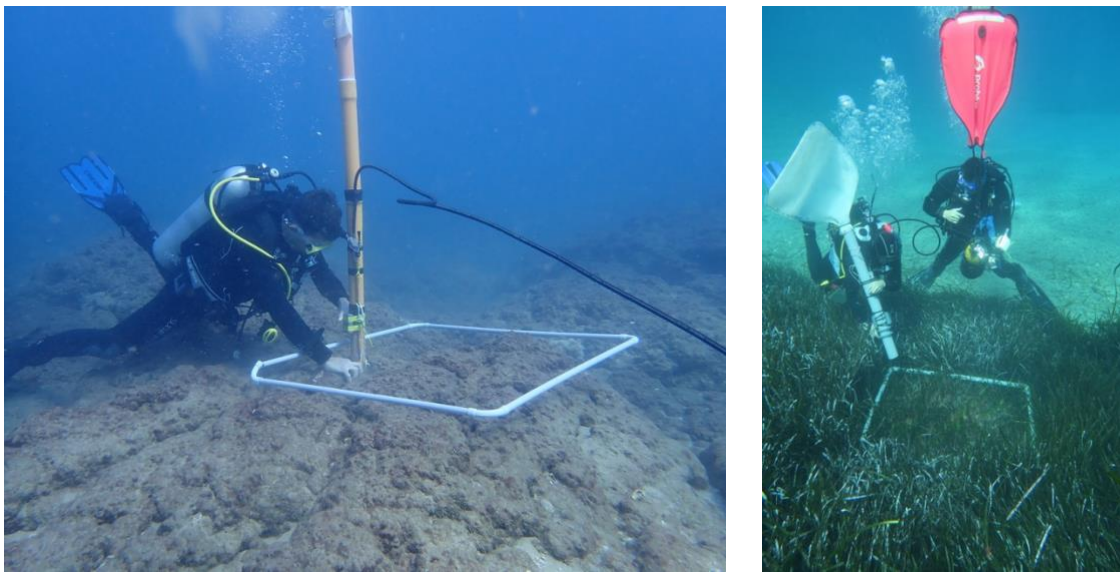


Figure 4: SCUBA divers collecting samples at two habitats: rocky substrate (left) and *Posidonia oceanica* meadow (right).

Table 1: Abbreviations for samples.

GR	Cape Greco, Rocky substrate
GPos	Cape Greco, <i>Posidonia oceanica</i> meadow
AR	Akrotiri, Rocky substrate
APos	Akrotiri, <i>Posidonia oceanica</i> meadow

3.3. Laboratory Methods

A Leica EZ4 microscope was used to analyze all sediment samples. The LA and DA compared species within the phylum Mollusca (classes Gastropoda and Bivalvia). Although the discrimination between live-collected and dead molluscs can be difficult for novices, clear signs of death include a missing operculum (Figure 5), predation holes (Figure 6), significant fractures/cracks, or the presence of sand or debris deep within the shell. More arbitrary criteria include the luminosity of the shell and overall shell disintegration. After picking live-collected mollusks, they were sorted into taxonomic units and recognized as native or non-indigenous.



Figure 5: Example of a missing operculum in *Tricolia speciosa*: living (left) and dead (right).



Figure 6: Examples of predation holes in *Jujubinus exasperatus* (left) and *Bittium reticulatum* (right).

3.4. Data analysis

Rarified species richness (S_{rar}) and species evenness (J') were calculated using the ecological software PAST [36]. S_{rar} was considered a better index than observed species richness (S_{obs}) for species diversity due to the large discrepancy in sample sizes in the LAs and DAs. Additionally, total species abundance (n) of non-indigenous species was not used to assess the

presence of non-indigenous species; instead, non-indigenous S_{rar} was compared to the native S_{rar} .

All other data analysis was conducted in RStudio [37]. Various statistical analyses were used to assess the live-dead discordance. Mismatch between LAs and DAs were assessed by measuring similarity in taxonomic composition (Jaccard-Chao), rank-order agreement in species relative abundance (Spearman rho), species richness (delta-S), and species evenness (delta-PIE).

3.4.1. Taxonomic similarity

Taxonomic similarity is a measure of similarity between two or more taxonomic communities based on the presence or absence of species (or taxa) within those communities. This was based on Chao et al.'s (2005) abundance based Jaccard index to compensate for differences in living and dead sample sizes [38]. The index ranges from 0 (no shared species) to 1 (all species occur both alive and dead).

3.4.2. Rank-order agreement

The rank order in species relative abundance is dependent on the proportion of the species present in the DA and LA. The correlation coefficient rho of the non-parametric Spearman rank-order test was used [39]. The values range from -1.0 to 1.0 with negative values indicating opposite abundance rankings between the DA and the LA and positive values indicating similar rankings between the DA and the LA.

3.4.3. Delta-S

Species richness is the number of taxa in an assemblage. To calculate delta-S, the difference between logarithmic values of species richness was rarefied to the living assemblages sample size: $\text{delta-S} = \log_{10}(\text{dead species richness}) - \log_{10}(\text{living species richness})$ [40]. Delta-S is 0 when the species richness of DA and LA is the same. Values closer to 1 indicate a more species-rich DA, while values closer to -1 indicate a more species-rich LA.

3.4.4. Delta-PIE

Species evenness is the description of the abundance across the species in a community. It is highest when all the species in a sample have the same abundance. To compute

the differences between the DA and the LA within each sample, $\text{delta-PIE} = \text{LA species evenness} - \text{DA species evenness}$ [40]. The values range from -1.0 to 1.0, with more negative values indicating that the DA is less even than the LA and more positive values when the DA is more even.

3.4.5. NMDS ordination/PERMANOVA

Non-metric multidimensional scaling (NMDS) ordinations [41] were used to visualize differences between the living and death assemblages and the four primary sample groups (GR, GPos, AR, and APos). A subsequent PERMANOVA was used to test the statistical significance of the ordination.

3.4.6. K-dominance curves

K-dominance curves were computed to visualize differences in the species rank of the LA and DA in the four primary groups and to assess what species made up 80 percent of the cumulative abundance [42].

3.4.7. Top 10 species rank

Top 10 species were ranked in total abundance to compare the most dominant species' prevalence in the location/habitat's DA and LA.

4. Results

4.1. Spring vs. autumn

There are no significant differences between the spring and autumn datasets (Appendix 5-12).

4.2. Quantification of species richness and evenness

Akrotiri has approximately double the species richness compared to Cape Greco in both the DA and LA. Current molluscan species richness across all samples does not surpass 52% of historical richness ($p < 0.001$) (Figure 7; Appendix 1; Appendix 2). Figure 7The most significant decline occurred in the Cape Greco/Posidonia meadow, with only 29% of its historical richness

remaining in the LA. Most samples saw a decrease in species evenness from their DA to LA (APos = -0.04; AR = -0.06; GPos = -0.02) (Figure 8; Appendix 3). Contrarily, GR exhibited an increase in species evenness in its LA compared to its DA (+0.06).

4.3. Live-dead metrics

4.3.1. Taxonomic similarity & rank order

Taxonomic similarity and rank order were used to assess the status of both locations and habitats (Figure 9) (Appendix 4). For taxonomic similarity, values ranging from 0.5 to 1 were deemed pristine, whereas for rank order agreement, values spanning from 0 to 1 were regarded as pristine. Cape Greco/rocky substrate exhibited the highest taxonomic similarity, scoring between 0.95 to 0.96, while Akrotiri/Posidonia had the highest rank order agreement, with values ranging from 0.17 to 0.42. Conversely, Akrotiri/rocky substrate showed signs of greater impact, with taxonomic similarity values ranging from 0.57 to 0.72 and rank order agreement values from -0.16 to -0.30.

4.3.2. DeltaS & deltaPIE

DeltaS and deltaPIE were used to compare the differences in species richness and species in the DA and LA across all location/habitats (Figure 10) (Appendix 4). Both metrics range from -1.0 to 1.0; values between 0 and 1 are expected, whereas those between 0 and -1.0 are unexpected. GPos exhibited the highest DeltaS values, ranging from 0.49 to 0.56, while APos showed the lowest, spanning from 0.29 to 0.33. Conversely, GR demonstrated the lowest deltaPIE values, indicating unexpected findings, ranging from -0.11 to -0.04.

4.4. NMDS ordination

The NMDS ordination (Figure 11) shows the discrepancies between the four samples and their respective LAs and DAs. These discrepancies were caused by their differences in species composition and abundance, with Akrotiri/rocky substrate having the largest difference between its DA and LA. Stress (=0.06) indicates that the ordination is a very good fit for the data and accurately represents the dissimilarities. The PERMANOVA results ($F_d = 6.0317$, $R^2 =$

0.21518, $p = 0.002$) further denote that there is a statistically significant mismatch between the DAs and LAs.

4.5. K-dominance

The k-dominance curves (Figure 12; Figure 13; Figure 14; Figure 15) show distinct taxonomic differences in the DA and LAs of all samples. While it is important to note that the sample sizes of the death assemblages were much larger, there are indications of a community shift in the molluskan assemblage. Both Akrotiri samples have more diverse DAs and LAs. The Cape Greco/rocky substrate DA (Figure 15a) indicates that ~80% of the entire assemblage was *Bittium reticulatum*. However, the LA (Figure 15b) includes *Cerithium scabridum*, an alien species. The Cape Greco/Posidonia meadow sample had a major decrease in diversity. The DA (Figure 14a) includes eight species that make up ~80% of the cumulative abundance. Notably, 2 species, *Septifer cumingii* and *Cerithium scabridum*, are non-indigenous. The LA (Figure 14b), however, is dominated by one species, *Bittium latreillii*.

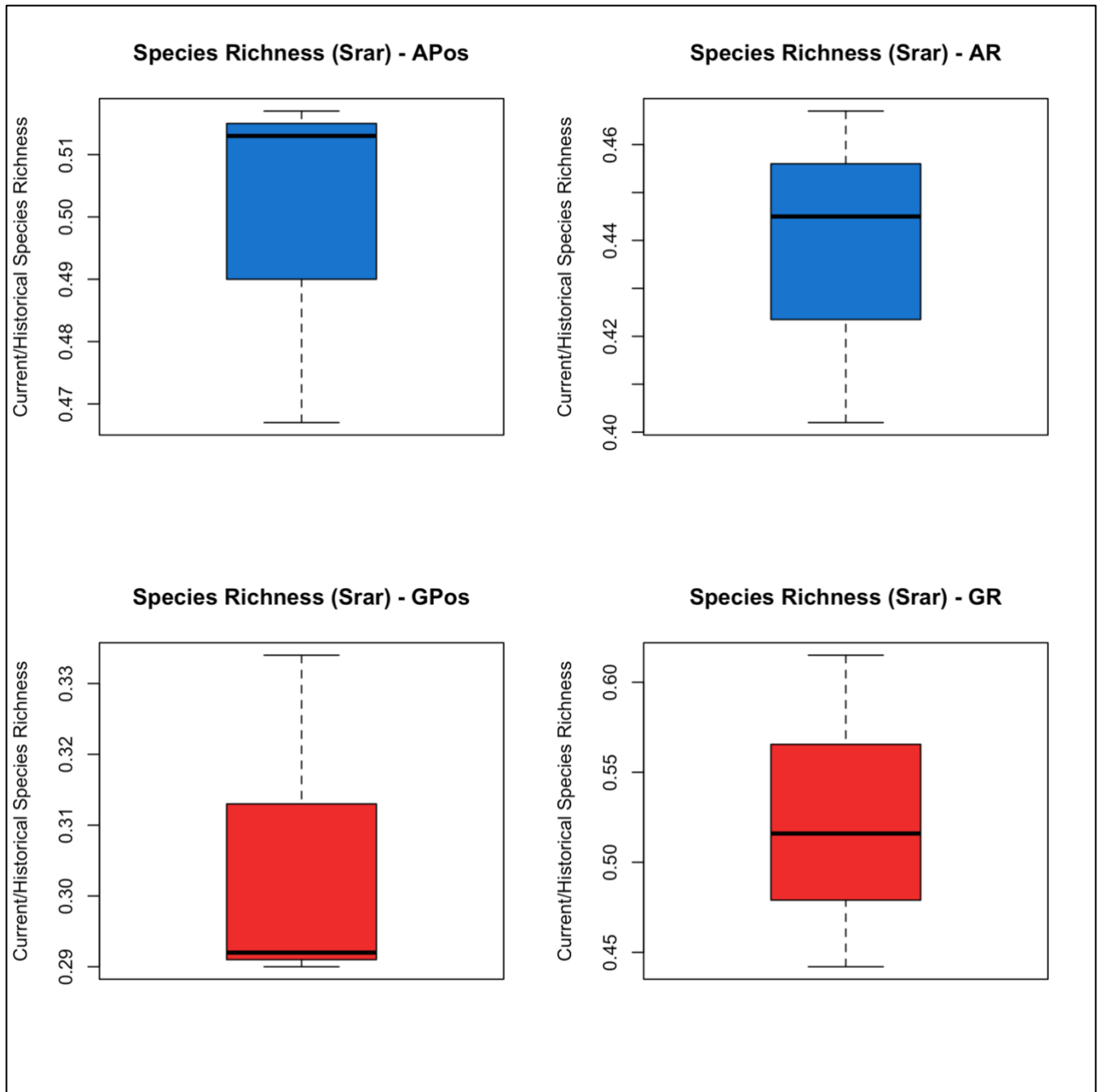


Figure 7: Boxplots indicating the proportion of current/historical species richness (S_{rar}) of four sample subsets. APos, AR, and GR, had about half of species in both their DA and LA. GPos had a marked decrease in species richness, with only 29% of species present in both the DA and LA.

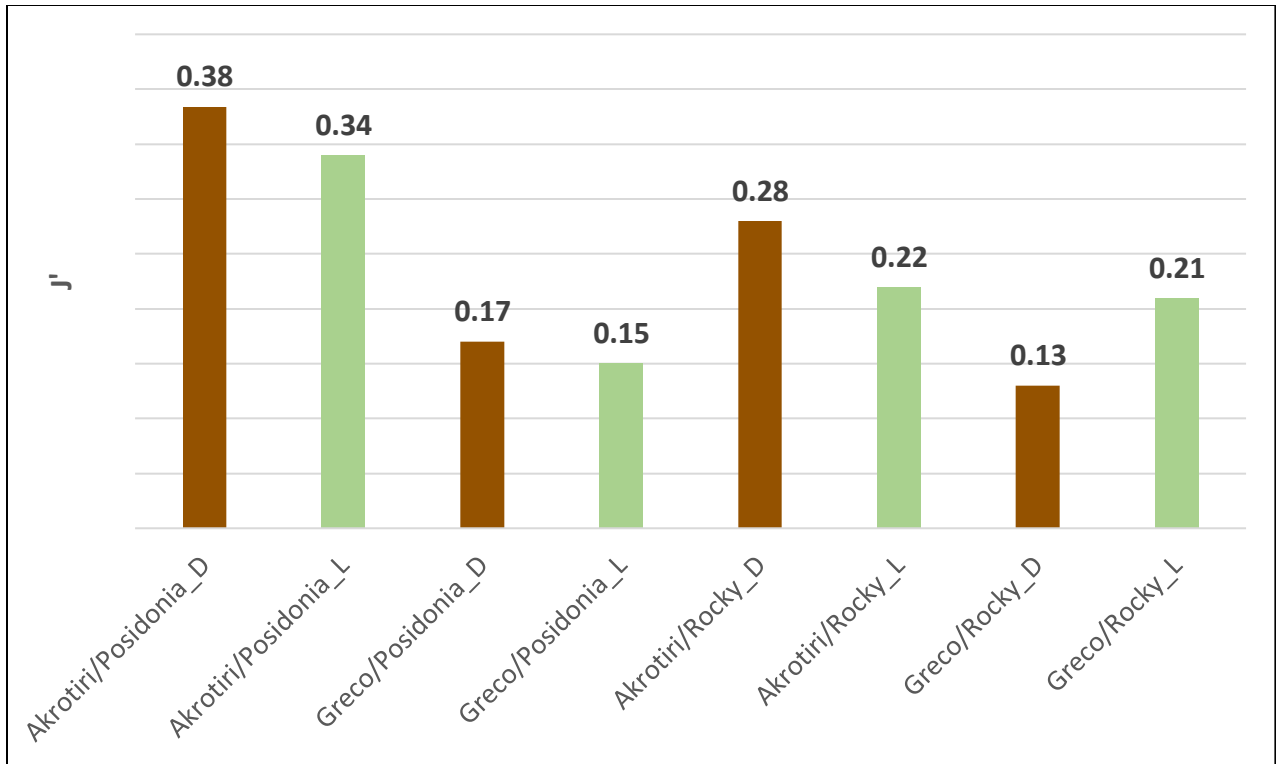


Figure 8: Species evenness (J') of all location/habitat subsets in the death assemblage (D) and living assemblage (L). The J' values for the living assemblage are the average of the three respective replicates. All location/habitat subsets had a decrease in species evenness from DA to LA except Cape Greco/Rocky substrate.

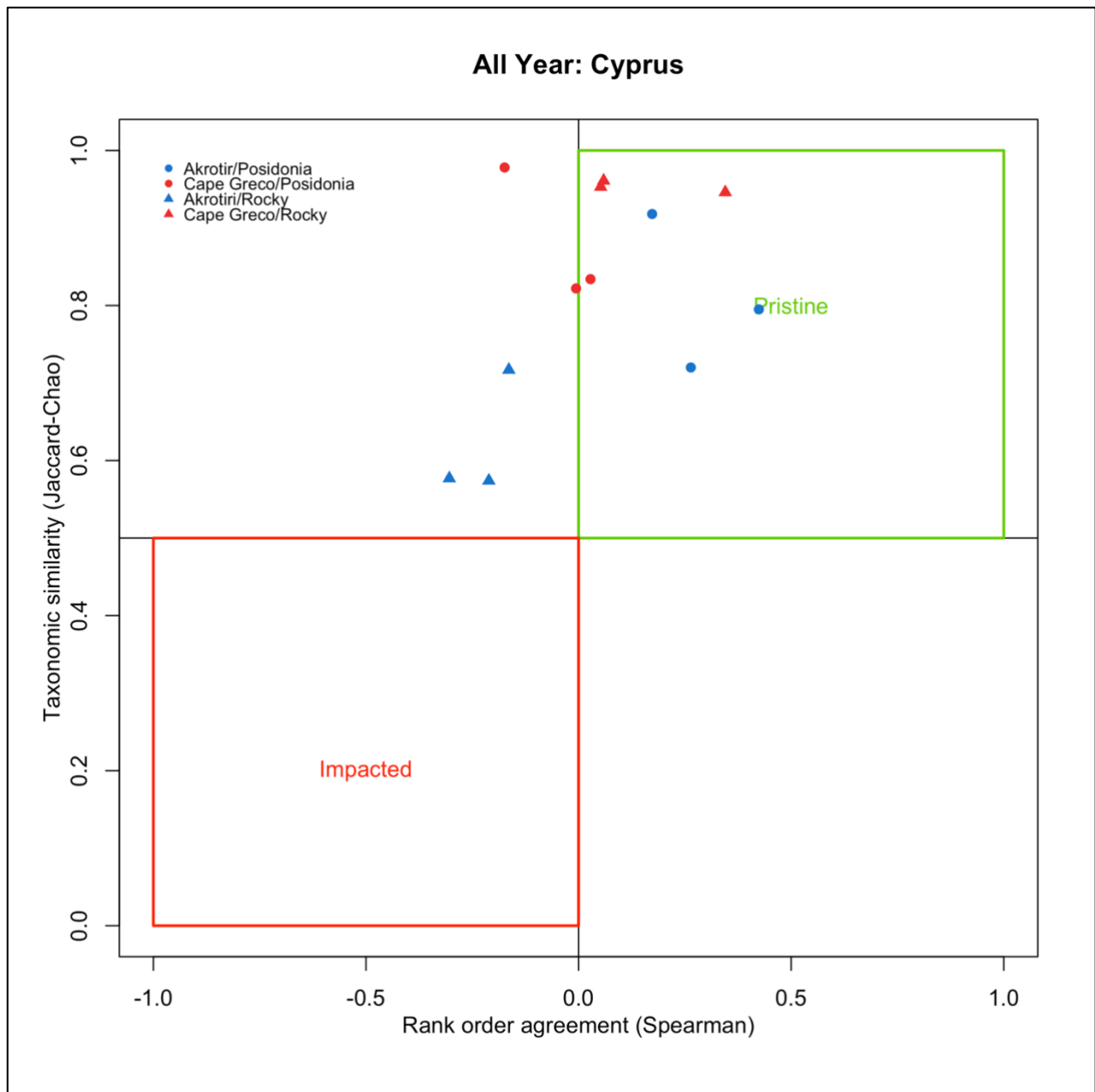


Figure 9: Cross plot of live-dead metrics (Jaccard-Chao taxonomic similarity index and the Spearman rank order agreement) of all four samples and their 3 respective replicates. Akrotiri/Posidonia meadow had the highest values for rank order agreement, ranging from 0.17 to 0.42; Cape Greco/rocky substrate had the highest values for taxonomic similarity ranging from 0.95 to 0.96. Akrotiri/rocky substrate was considered with the most impacted, with the lowest values for taxonomic similarity (0.57 to 0.71) and rank order agreement (-0.30 to -0.16).

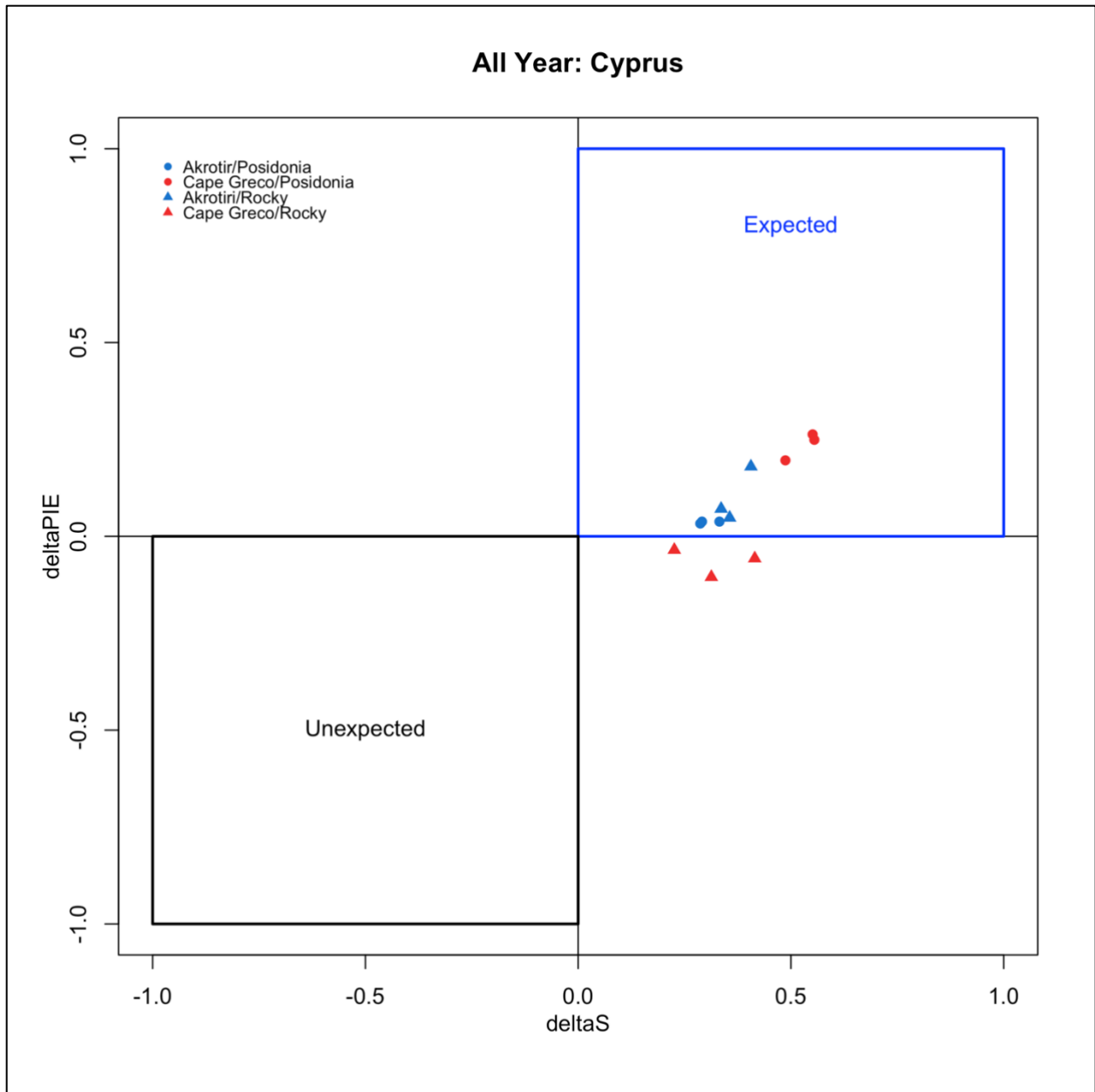


Figure 10: Cross plot of live-dead metrics (difference in species richness, ΔS , and the difference in evenness, ΔPIE) of all four samples and their respective replicates. No significant differences in species richness between any of the location/habitats, ranging from 0.23 (GR) to 0.56 (GPos). GR had ΔPIE values that were considered unexpected, ranging from -0.11 to -0.04.

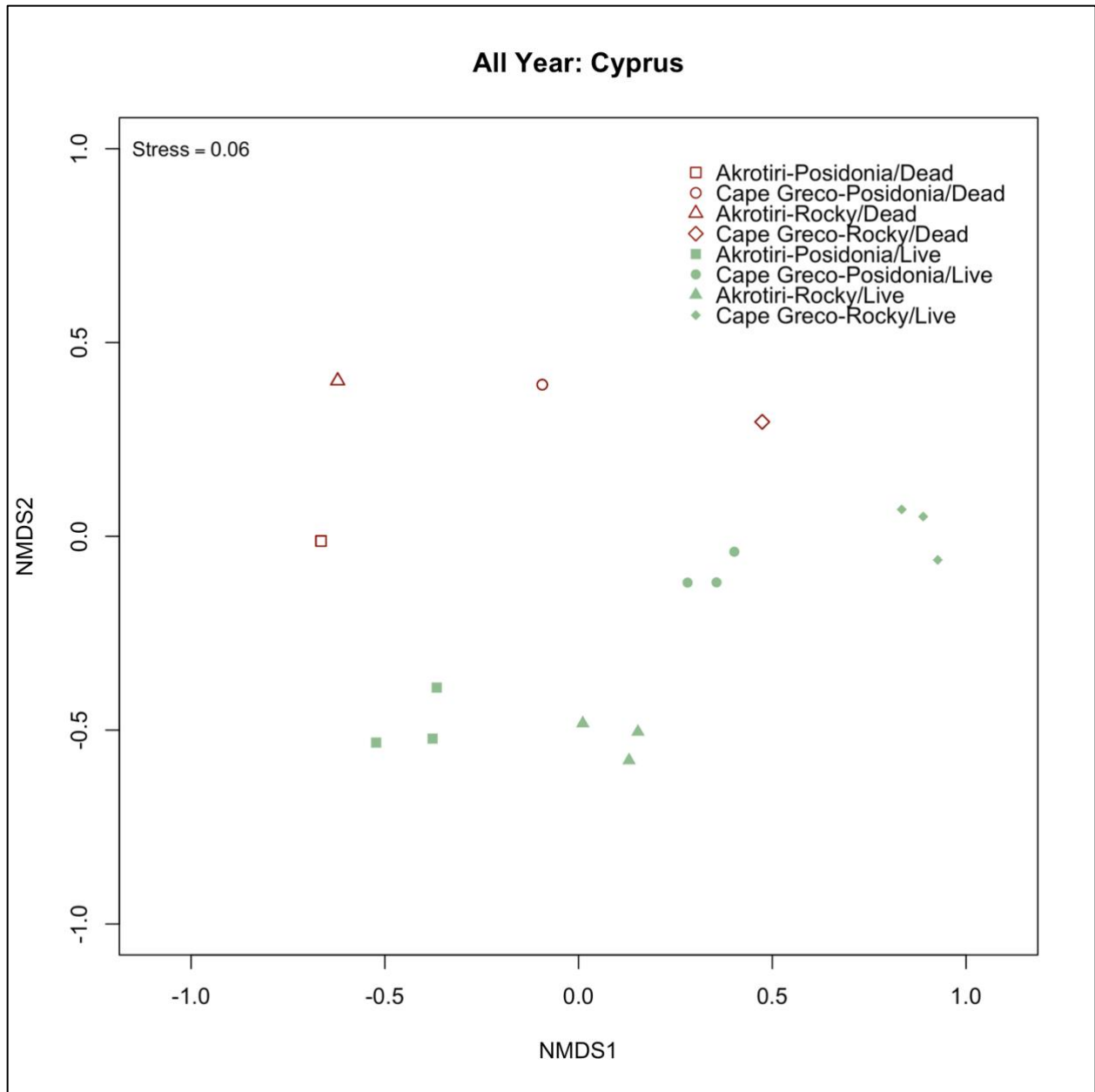


Figure 11: NMDS ordination representing the dissimilarity structure of molluscan living and death assemblages in Akrotiri & Cape Greco, Cyprus. Each point represents a sample replicate (three for each LA). The molluscan assemblages differ between the location/habitat subsets and the living and death assemblages. The largest discrepancy between the DA and LA is at Akrotiri/Rocky substrate.

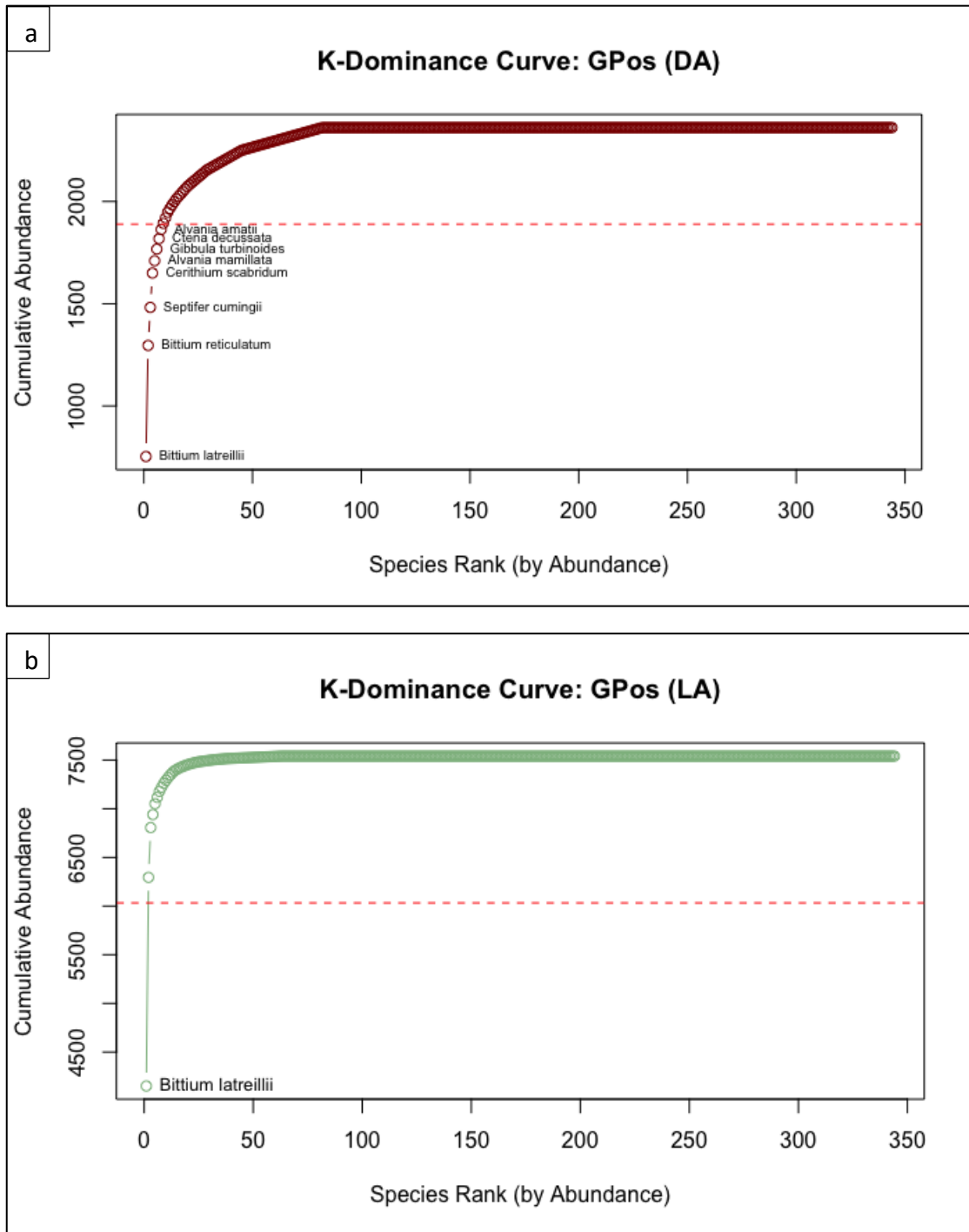


Figure 14: K-dominance curves of the DA and LA of the Cape Greco/Posidonia meadow sample. Each point represents a different species. The red dotted line indicates the point where species abundance reaches 80% of the total community abundance. (a) K-dominance curve of the DA; eight species constitute 80% of the total community abundance. (b) K-dominance curve of the LA; *Bittium latreillii*, an endemic micrograzer, constitutes over 80% of the total community abundance.

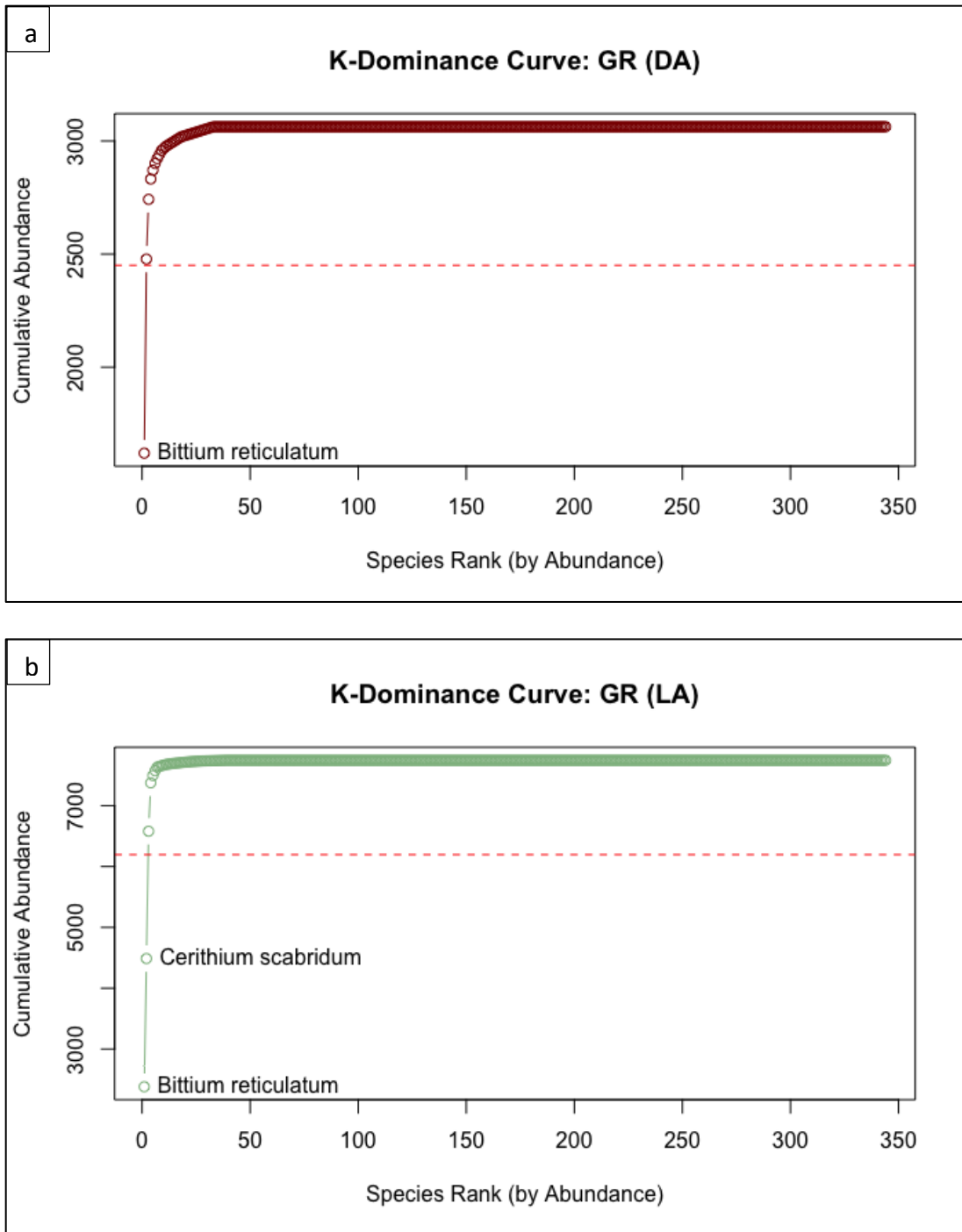


Figure 15: K-dominance curves of the DA and LA of the Cape Greco/Rocky substrate sample. Each point represents a different species. The red dotted line indicates the point where species abundance reaches 80% of the total community abundance. (a) K-dominance curve of the DA; *Bittium reticulatum*, an endemic micrograzer, constitutes 80% of the total community abundance. (b) K-dominance curve of the LA; *Bittium reticulatum*, an endemic micrograzer, and *Cerithium scabridum*, an invasive micrograzer, constitute 80% of the total community abundance.

4.6. Radiocarbon dating results

Ten shells from 5-and-20-meters depth were used for radiocarbon dating. The year 1850 was used as the benchmark because it marks the beginning of global industrialization and the associated increase in CO₂ emissions in addition to more comprehensive data collection [43]. Results indicate that the median ages of all DAs are very young and do not precede 1850 nor the beginning of Lessepsian migration in 1869 (Figure 16; Table 2).

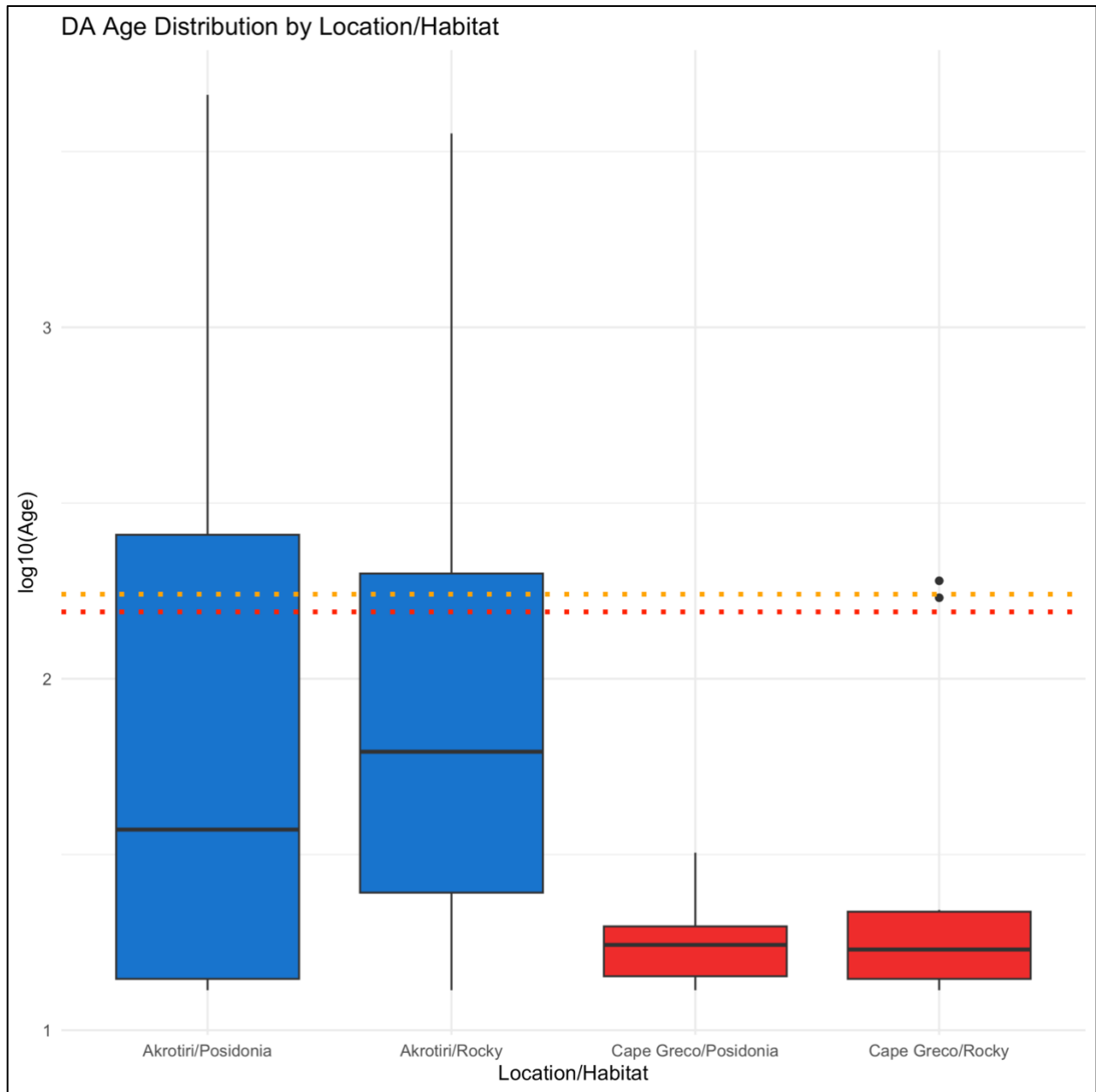


Figure 16: Results of radiocarbon dating. The age of the shells is on a log scale. The orange dotted line indicates year 1850, marking the approximate beginning of global industrialization. The red dotted line indicates year 1869, marking the opening of the Suez Canal and initiation of the Lessepsian migration. Akrotiri's median DA age is markedly older than Cape Greco's but does not precede 1850 nor 1869.

Table 2: Oldest, youngest, and median-aged shell of all 4 location/habitat subsets.

	APos	AR	GPos	GR
Oldest shell (years old)	4586	3561	32	190
Youngest shell (years old)	13	13	13	13
Median age	56.5	62	17.5	17

4.7. Non-indigenous species

The prevalence of non-indigenous species varies across the different location/habitats. Cape Greco/Posidonia meadow saw a 12% increase in non-indigenous species richness (S_{rar}) from its DA to LA (Figure 18; Appendix 15), while Akrotiri/Posidonia meadow saw a markedly lower 3% increase in non-indigenous species richness from its DA to LA (Figure 20). Cape Greco/rocky substrate had the highest proportion of non-indigenous species, both in its DA and LA (DA = 9%; LA = 17%) (Figure 17). Finally, Akrotiri/Rocky substrate had no non-indigenous species in its DA but saw an eight percent increase in richness in its LA (Figure 19).

The two most abundant non-indigenous species were *Cerithium scabridum* and *Septifer cumingii*. *Septifer cumingii*'s total abundance increased at Akrotiri/Posidonia meadow, ranking second in the LA compared to third in the DA (Figure 21). Similarly, *Cerithium scabridum*'s abundance increased at Cape Greco/Rocky substrate, becoming the second most abundant species in the LA compared to third most abundant in the DA (Figure 22).

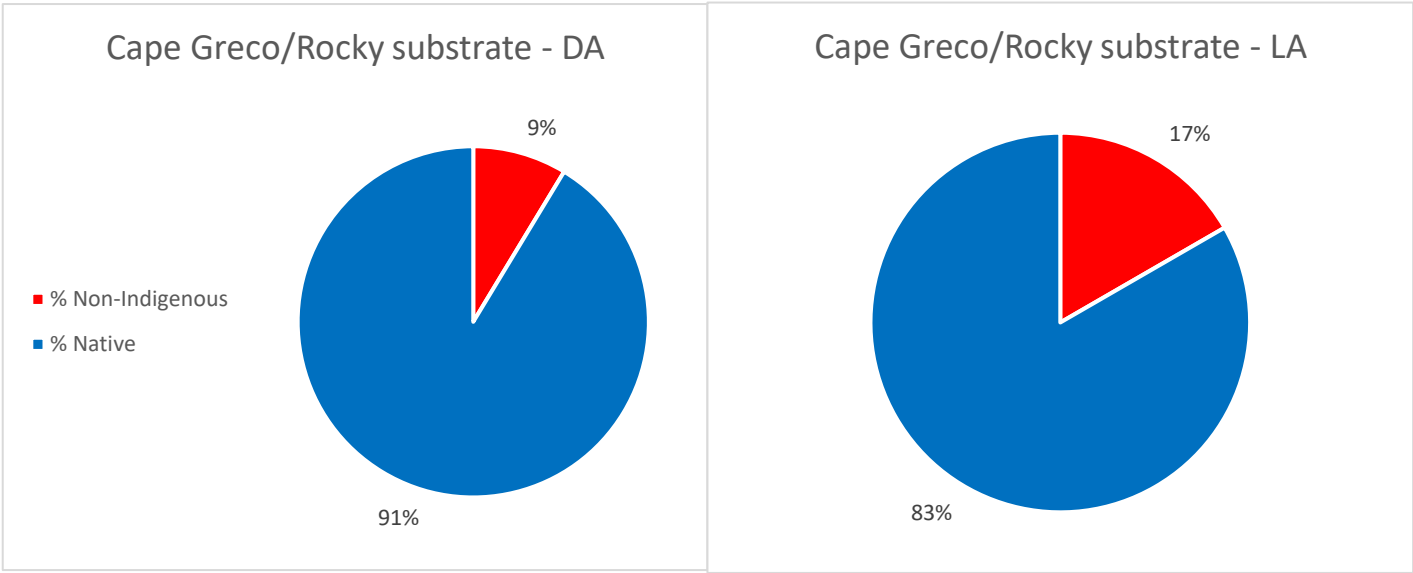


Figure 17: Percentage of non-indigenous & native species richness (S_{rar}) in the Cape Greco/Rocky substrate DA and LA.

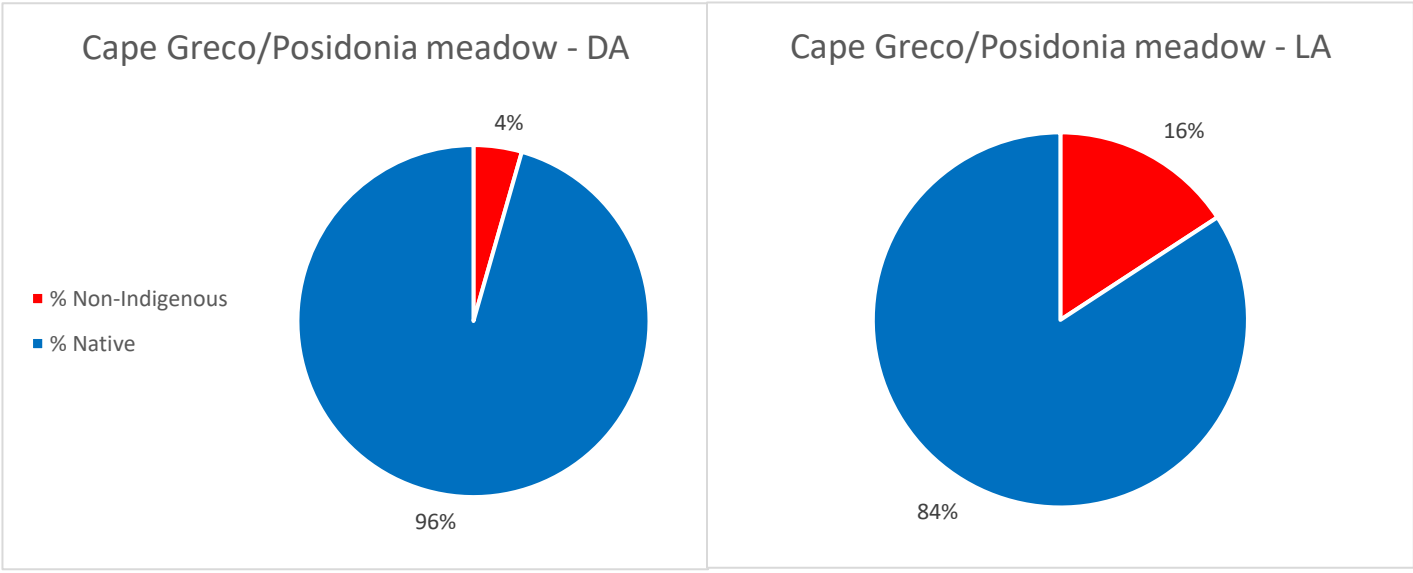


Figure 18: Percentage of non-indigenous & native species richness (S_{rar}) in the Cape Greco/Posidonia meadow DA and LA.

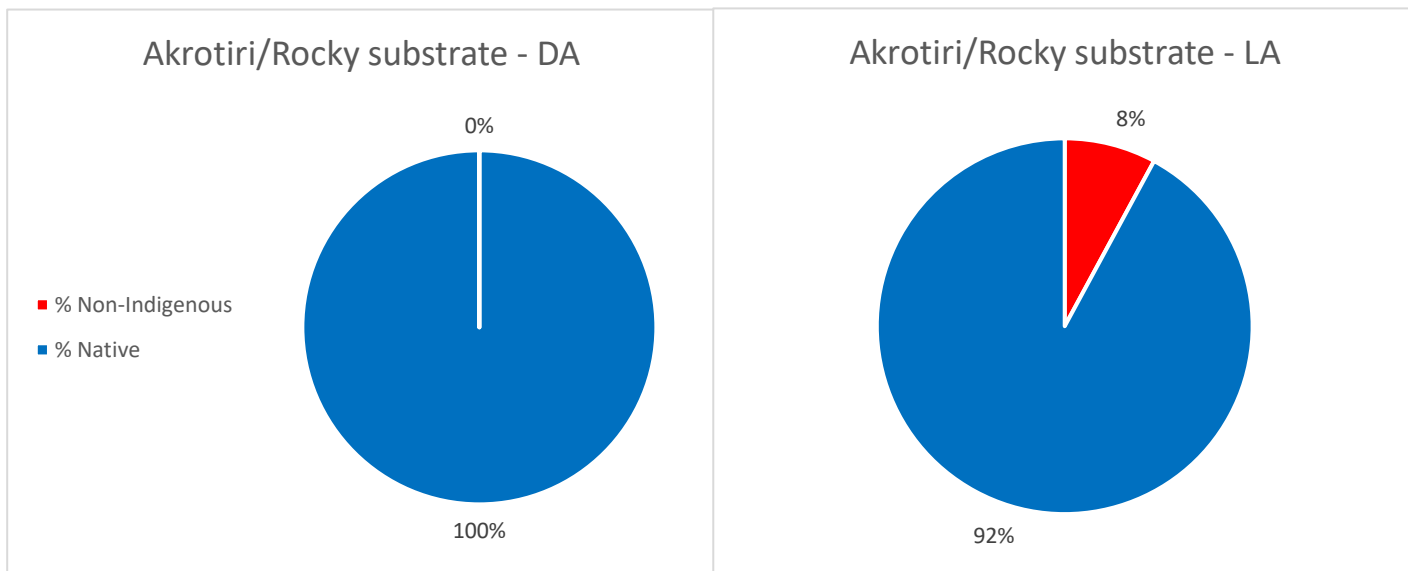


Figure 19: Percentage of non-indigenous & native species richness (S_{rar}) in the Akrotiri/Rocky substrate DA and LA.

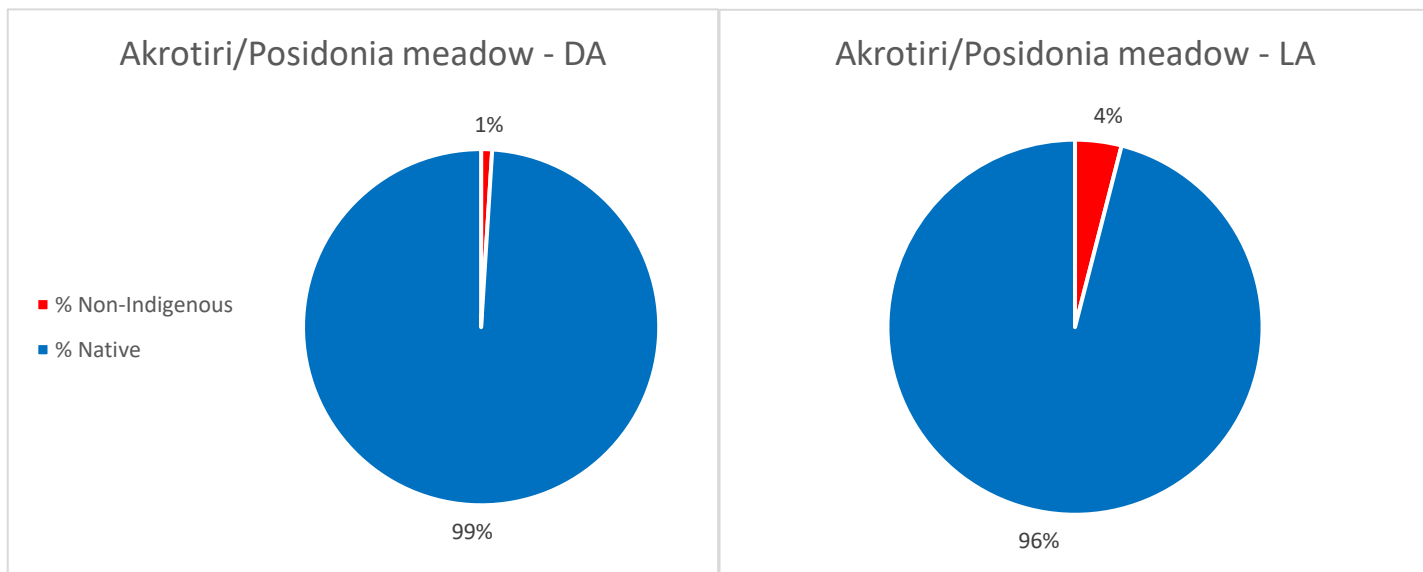


Figure 20: Percentage of non-indigenous & native species richness (S_{rar}) in the Akrotiri/Posidonia meadow DA and LA.

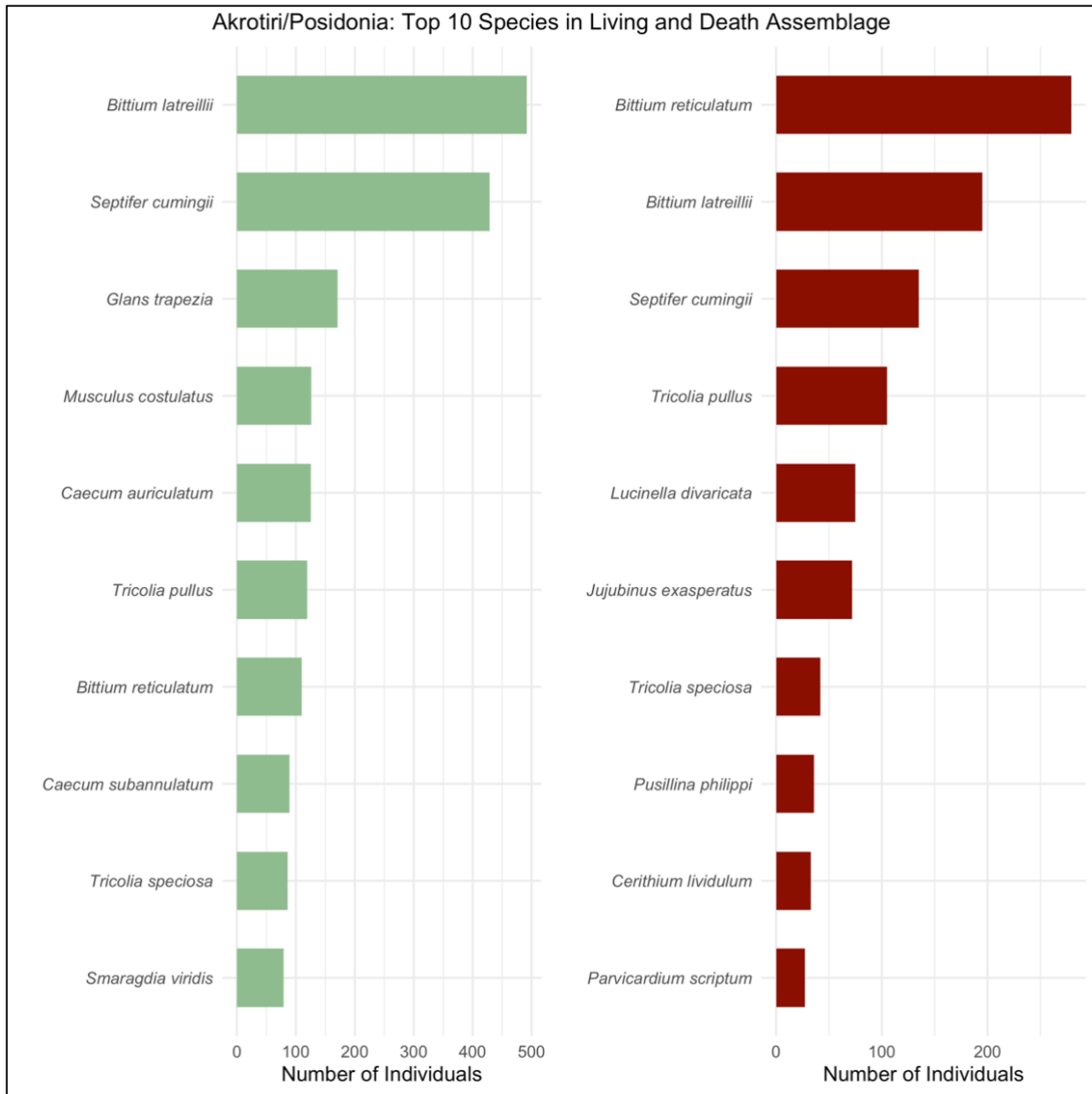


Figure 21 Absolute abundance of top 10 species in Akrotiri/Posidonia living and death assemblage. *Septifer cumingii*, an invasive species, ranked 2nd in abundance in the LA, compared to 3rd in the DA. *Bittium reticulatum*, an endemic micrograzer, ranked most abundant in the DA compared to 7th in the LA.

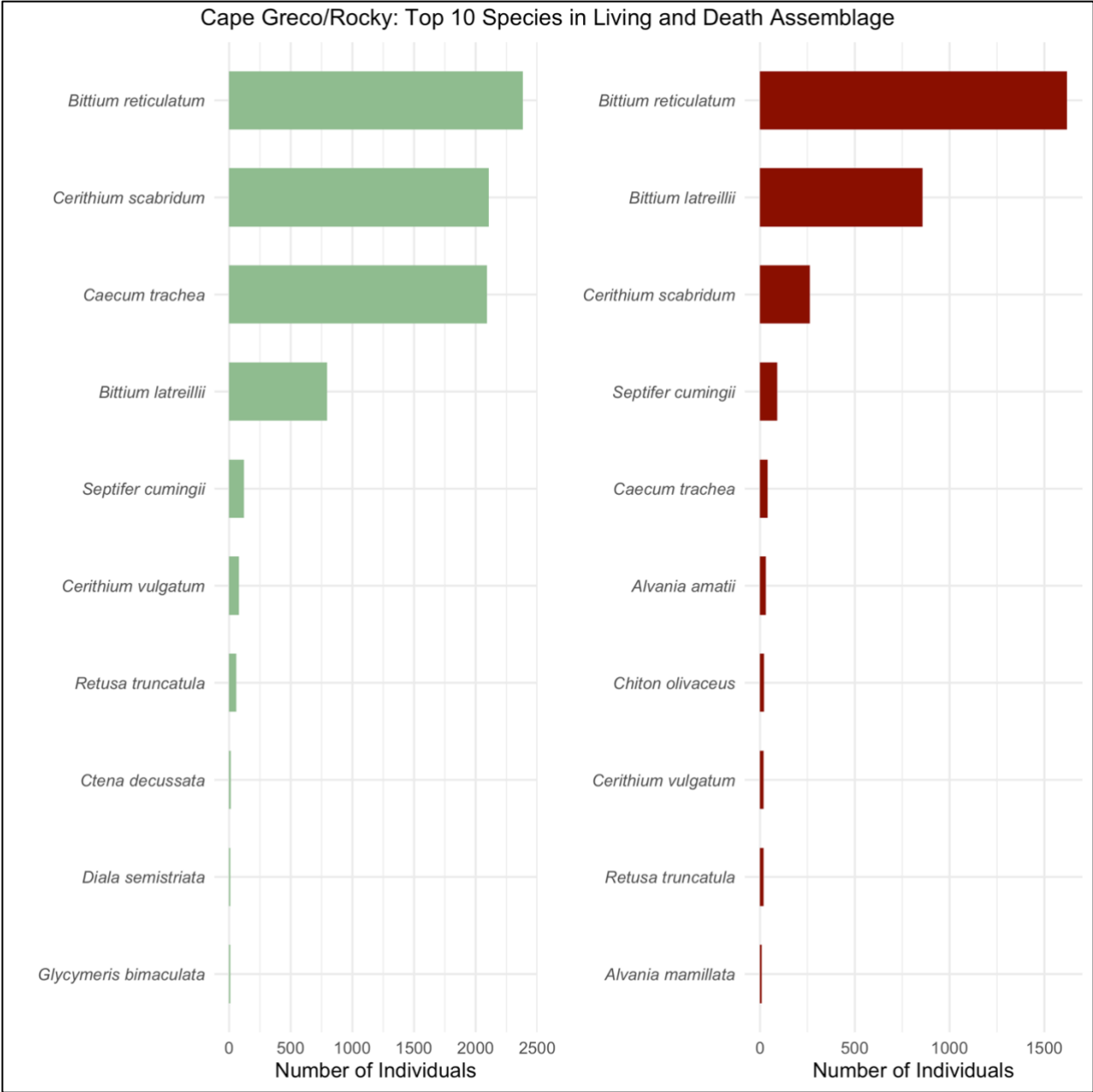


Figure 22: Absolute abundance of top 10 species in Cape Greco/rocky substrate living and death assemblage. *Cerithium scabridum*, an invasive species, ranked 2nd in abundance in the LA, compared to 3rd in the DA. *Bittium latreillii*, an endemic micrograzer, ranked 2nd in abundance in the DA compared to 4th in the LA.

5. Discussion

5.1. Species richness & evenness

The decrease in S_{rar} in all samples was expected due to taphonomic inertia and time-averaging. However, the marked decrease in species richness at Cape Greco/Posidonia meadow was unexpected with only 29% of historically documented species present in the LA (Figure 7). This is in stark comparison to its rocky substrate counterpart (GR), where 48% of historically recorded species persisted. This is because Cape Greco/rocky substrate has an overall low species richness, with only 24 species documented in the DA and 13 in the LA. In contrast, Cape Greco/Posidonia meadow witnessed a stark decline from 69 species in the DA to 21 in the LA. Despite this, Cape Greco/Posidonia meadow still maintains a greater species richness than its rocky substrate counterpart.

The increase in species evenness at Cape Greco's rocky substrate can be attributed to the concurrent increase in the abundance of *Caecum trachea* and *Cerithium scabridum*. While *Bittium reticulatum* dominated in the DA, the LA had more comparable abundances among *Bittium reticulatum*, *Cerithium scabridum*, and *Caecum trachea* (Figure 15; Figure 22).

5.2. Age of Death Assemblages

Two important aspects of DAs are their age and degree of time-averaging. The median age of DAs on rocky substrates and *Posidonia oceanica* meadows have been shown to be extremely young: 18-56 years and 9-15 years, respectively [24]. The young DA ages on rocky substrates can be attributed to strong hydrodynamic processes that can transport shells far from the area of production, thereby hindering sedimentation and burial. The rocky substrate sites only had small, shallow pockets of sediment, rendering it impossible to retrieve older shells which are typically found deeper within the sediment. In *Posidonia* meadows, the mat, a dense structure made of the root and rhizome system, grows between 0.06-0.41 cm a⁻¹. Shells can become entangled and buried very quickly into the plant's root network. Our sampling methods did not include collection of the entire root and therefore, the DA at the *Posidonia* meadows is very superficial.

The overall results suggest a relatively healthy ecosystem compared to other areas in the Eastern Mediterranean. However, the baseline is too young to establish what a pre-

anthropogenic ecosystem looked like, and this is why there is a good match between the LA and DA. Establishing a baseline predating anthropogenic influences is crucial to avoid underestimating the true extent of environmental effects. Earth's temperature has increased by $\sim 0.06^{\circ}\text{C}$ per decade since 1850, and the pace of warming has surged threefold from 1982 onwards, reaching about 0.20°C per decade [44]. This may also explain why Akrotiri/Rocky substrate had the largest discrepancies in taxonomic similarity and rank order between its LA and DA (Figure 9; Figure 11). Its median DA age was the oldest ($=62$ years old) (Figure 16; Table 2), which predates the accelerated warming observed since 1982. Though it does not capture the full extent of post-Industrial Revolution anthropogenic impacts, it provides a better baseline than the DAs at Cape Greco. Contrarily, the discrepancies between the DA and LA in both Cape Greco samples are much smaller due to their very young median DA age (DA GPos = 17 years; DA GR = 17.5 years).

Additionally, molluscan shells can be preserved in sediments for decades to millennia depending on the local sedimentation rate and the species' shell durability [45]. This could heavily impact the DA which can contain species that were not observed at all in the LA. For example, *Alvania fractospira* ranked 8th in total abundance in the Akrotiri/Rocky substrate DA; it was not identified at all in the LA (Figure 13).

5.3. Prevalence of non-indigenous species

The exact drivers for marine invasions differ, but literature has tried to explore why some non-indigenous species become successful invaders, while others do not. Two main hypotheses have garnered attention and debate.

First proposed by Elton (1958), the biotic resistance hypothesis states that species-rich communities possess greater resilience against invasions due to their more efficient resource utilization, which fosters heightened interspecific competition [46]. Additionally, diverse ecosystems have a broader spectrum of ecological niches occupied by native species, thereby impeding the colonization and invasion of non-indigenous species.

Conversely, other studies have found that species-rich assemblages are more prone to invasion compared to their less diverse counterparts [47]. This could be due to the invasional meltdown hypothesis, first postulated by Simberloff & Von Holle, 1999 [48]. In short, it is a

process by which non-indigenous species develop facilitative relationships to promote each other's invasion potential by increasing the likelihood of survival and/or overall impact on the endemic community. This could further promote the invasion of new non-indigenous species. Notably, there is limited research on invasional meltdowns in marine systems [49].

While opposing, both viewpoints have broad support in the literature and are supported by empirical evidence from a range of different communities facing biological invasions [50]. However, many studies have questioned whether if relationships are indeed driven by diversity and resource availability or by other abiotic factors such as spatial heterogeneity [51] and environmental stress gradients [52].

Our overall results suggest that the biotic resistance hypothesis holds greater relevance, as evidenced by the differential non-indigenous species richness at Akrotiri/Posidonia and Cape Greco/rocky substrate. Akrotiri's *Posidonia* meadows offer abundant resources, fostering interspecies competition and limiting non-indigenous species' ability to colonize. *Septifer cumingii* has become the second most abundant species at Akrotiri/Posidonia meadow, but its impacts on native species abundance do not appear drastic due to the sample having the highest rank order agreement. Conversely, the significant increase in abundance of *Cerithium scabridum* in the LA compared to its DA at Cape Greco/rocky substrate in only a few decades highlight that its impacts likely have a greater impact on endemic species.

5.4. Environmental Protection in the Mediterranean

Mediterranean countries have taken several steps to adopt legal frameworks aimed at safeguarding ecosystems and advancing sustainable development. In 1975, the Mediterranean Action Plan of the UN Environment Program was established as the first regional cooperation platform. At present, there are 22 contracting parties, comprised of 21 Mediterranean countries and the European Union [59]. Furthermore, the Barcelona Convention, formally known as the Convention for the Protection of the Mediterranean Sea Against Pollution, was ratified in 1976 [60]. Contracting parties committed to implementing all necessary measures to prevent and combat pollution to safeguard the marine environment. Despite these endeavors, the Mediterranean continues to suffer due to land-based stressors such as pollution and unprecedented increases in SSTs.

At present, only 8.6% of Cyprus's total marine area is protected and none of these areas have been officially assessed [61], leaving uncertainty regarding their effectiveness. Notably, all designated Marine Protected Areas (MPAs) are situated on the western coast of the island, potentially having distal effects on Akrotiri. Although various protected zones have been established around Cape Greco, they represent a mere fraction (~52 km²) of the extensive protected area totaling 8,460 km² [61].

5.5. *Posidonia oceanica* meadows

Posidonia oceanica, an endemic seagrass species in the Mediterranean, grows between 0 to 45 meters depth [53]. Recognized as a habitat of significant community importance by the EU, *Posidonia* meadows play a pivotal role in numerous ecosystems. Among the 64 documented seagrass species, it is the most proficient in carbon storage and functions as a carbon sink [54-56] Additionally, *Posidonia* meadows provide essential ecosystem services such as sediment stabilization and wave attenuation [57]. Finally, they provide mollusks, particularly micrograzers, with sustenance and shelter.

However, *Posidonia* is highly susceptible to various anthropogenic pressures, including eutrophication, erosion, dredging, invasive species, and the impacts of climate change [53]. Over recent decades, *Posidonia* meadows have experienced a significant decline, with total biomass diminishing at a rate of ~6.9% per year from 1960 to 2010. Amidst this, Cyprus has implemented legislation to prevent anchoring in *Posidonia* meadows [35]. Additionally, it is obligated to protect its endangered marine species under the Barcelona Convention, which includes *Posidonia oceanica* [58]. These protections coupled with the ecosystem services likely contribute to the observed higher species richness at Akrotiri's *Posidonia* meadow compared to its rocky substrate counterpart.

5.6. Future for the Mediterranean

Similar studies conducted on the Israeli shelf found a significant loss of molluscan diversity, described as the largest climate-driven regional-scale marine diversity loss to date; this occurred within just a few decades [31]. Although the median age of the data analyzed in our study is relatively young, the results suggest that Cyprus is faring considerably better, with

approximately half of its historical species richness still present across most of its locations and habitats. Yet, the ongoing rise in SSTs poses a significant threat to endemic species. The notable decrease in species richness from the DA to the LA observed at Cape Greco/Posidonia meadow underscores that the beneficial effects of increased oxygen availability on species survival have limitations. Giomi et al. (2019) found that the lethal temperature of *Modiolus* sp. (Mollusca) increased by 2.6°C under hyperoxia [28]. Considering the temperature differential between Akrotiri and Cape Greco is 2-3°C in the summer, the lethal temperature thresholds for many endemic species at Cape Greco may have already been exceeded.

Unprecedented global warming is inducing shifts in species distributions, prompting native species to seek refuge in higher-latitude areas within their thermal tolerance ranges [62]. However, endemic species in the Mediterranean are constrained by oceanographic barriers and land masses, leaving them effectively trapped. Moreover, the proliferation of Lessepsian species is exacerbated by their higher thermal tolerance limits. The synchronous decline in endemic species richness and rise in SSTs may be creating 'niche opportunities' for non-indigenous species to colonize [63,64].

Our results suggest that Akrotiri does serve as a refugium for native species, as evidenced by its comparatively higher species richness relative to Cape Greco. However, projections under the intermediate climate scenario (SSP2-4.5) predict a global temperature surge of another 2.7°C by 2100 [43], with SSTs increases in the Mediterranean potentially reaching 3.5°C [10]. This could force many endemic species beyond their thermal tolerances and potentially facilitate the colonization of tropical Atlantic species through climate connectivity [65]. This could exacerbate the current 'tropicalization' trend within the basin, ultimately resulting in ecosystems beyond recognition [66].

5.7. Limitations & Recommendations for Future Studies

It is important to note that these results do not provide a holistic assessment of molluscan assemblages in Cyprus as it only discusses the 15-meter transect. The deepest depth transect at 30-m will likely have higher species richness due to the decreased impacts of land-based stressors, while the 5-m transect could show opposite trends due to the increased risk of pollution effects.

Additionally, the high abundance of benthic foraminifera found at Cape Greco/rocky substrate could have been problematic. Benthic foraminifera, a type of calcareous protist, are highly resistant to decay. Due to their high volume in the sediment, the absolute abundance of mollusks in the Cape Greco/rocky substrate's DA and LA could have been diluted.

Future studies should explore interspecies relationships between dominant endemic species and invasive species by conducting a functional trait study. For example, *Cerithium scabridum* appears to be effectively occupying the ecological niche of the native micrograzer, *Bittium latreillii* at Cape Greco/Rocky substrate (Figure 22).

Additionally, to ensure the best chances for Akrotiri to remain a refugium, local land-based stressors should be mitigated to protect nearshore ecosystems. Finally, Cyprus should implement further legislation to protect their most biodiverse marine environments, specifically *Posidonia* meadows.

5.8. Shifting Baselines

A term first coined by Daniel Pauly (1995), 'shifting baselines' refers to a psychological and sociological phenomenon that causes each generation to accept the situation in which they grew up in as normal [67,68]. CO₂ emissions have increased from ~280 ppm pre-Industrial Revolution to ~420 ppm today [43]. This drastic increase has led to global climate change and has been one of the fastest climatic transitions in Earth's history. Amidst these unprecedented changes, establishing baselines is crucial both ecologically and psychologically. This study stresses the importance of establishing pre-anthropogenic baselines, i.e. death assemblages, to assess the true extent of recent human impacts and their effects on modern ecosystems.

Furthermore, maintaining a proactive stance is imperative. Scientists should seek to understand ecosystems before human-induced disturbance to guide future conservation efforts. While addressing global warming requires a global effort, by mitigating land-based stressors and implementing legislation to preserve vital ecosystems such as seagrass meadows and rocky substrates, we can alleviate the effects of climate change.

6. Conclusion

Mediterranean ecosystems are facing a breadth of anthropogenic stressors and are experiencing warming 20% faster than the global average. This study focused on the impacts of summer SSTs and oxygen availability on molluscan taxonomic diversity in Cyprus by comparing two sites and two habitats and using their respective living and death assemblages. Our hypotheses were partially correct; Akrotiri's *Posidonia* meadow was the most species-rich assemblage, while Cape Greco was the least species-rich. However, we did not find any significant differences between the spring and autumn datasets. Overall results indicate that Cyprus' molluscan assemblages are faring better than those in other parts in the Eastern Mediterranean. However, the DA is very young, with a median age of 17-62 years, thereby limiting our ability to assess the true extent of anthropogenic impacts, which were exacerbated at ~1850.

The impacts of non-indigenous species are unclear, with scientists still debating about what drives successful invasions and what endemic ecosystems are best adept to resist them. Our study reflects the biotic resistance hypothesis, as evidenced by Akrotiri's *Posidonia* meadows, which hosts a higher relative native species richness compared to Cape Greco's rocky substrate. Thus, they are most likely to resist future invasions.

Cape Greco's *Posidonia* meadow exemplify that the benefits of increased oxygen availability are not limitless. It had the most drastic live-dead mismatch in species richness and with the median DA age of only 17 years, it shows that the decrease in biodiversity likely occurred within the last two decades. Nevertheless, it retains a greater species richness compared to its rocky substrate counterpart, underscoring the urgent need for enhanced protection measures aimed at preserving *Posidonia* meadows.

While local mitigation efforts hold potential, curbing carbon emissions is imperative. Projections under the intermediate climate scenario indicate another increase in global average temperatures by 2.7°C by century's end. This could further proliferate the tropicalization of the Mediterranean, pushing endemic species beyond their thermal thresholds and enabling the colonization of non-indigenous species. Hence, concerted global action aimed at slowing down climate change is paramount.

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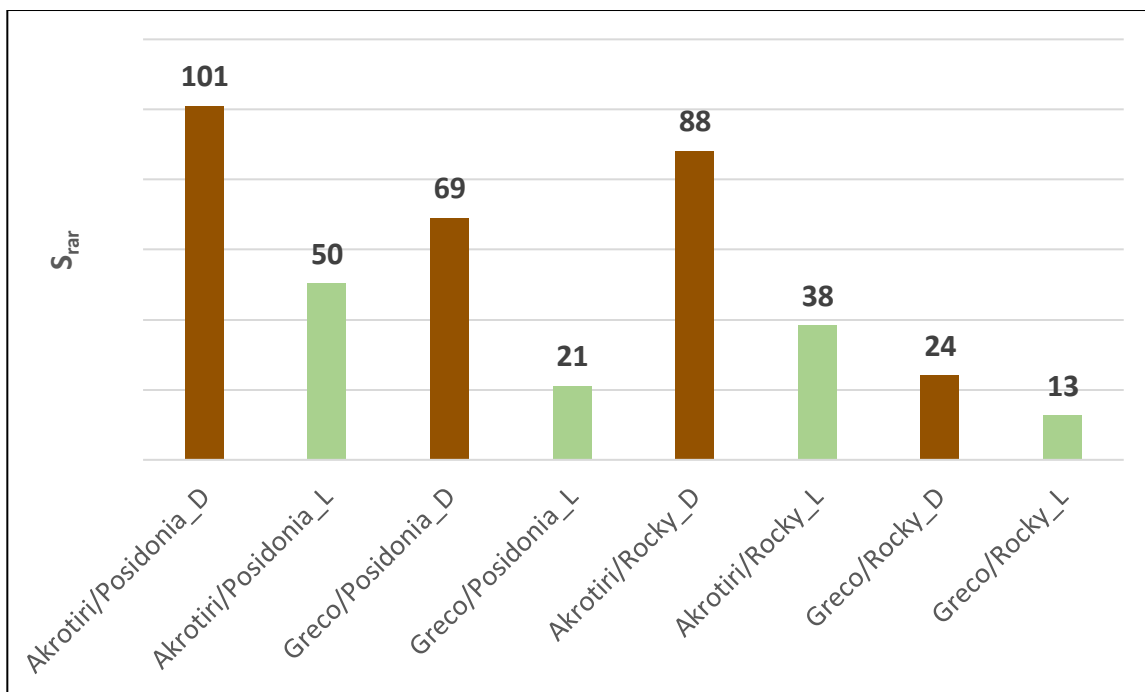
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Appendix

Appendix 1: Raw values of current/historical species richness of each location/habitat. Proportions are a ratio between the respective DA and each LA replicate.

	S _{rar}	Proportion of current/historical richness
APos15_D	101	
GPos15_D	69	
AR15_D	88	
GR15_D	24	
APos15_L1	52	0.52
APos15_L2	47	0.47
APos15_L3	52	0.51
GPos15_L1	23	0.33
GPos15_L2	20	0.29
GPos15_L3	20	0.29
AR15_L1	41	0.47
AR15_L2	39	0.44
AR15_L3	35	0.40
GR15_L1	12	0.52
GR15_L2	11	0.44
GR15_L3	15	0.62



Appendix 2: Rarified species richness (S_{rar}) of all location/habitat subsets in the death assemblage (D) and living assemblage (L) for the annual dataset. The S_{rar} values for the living assemblage are the average of the three respective replicates. All location/habitat subsets had a decrease in S_{rar} from the DA to the LA.

Appendix 3: Raw values of species evenness (J') of each location/habitat's DA and its respective LA.

	J'
APos15_D	0.38
GPos15_D	0.17
AR15_D	0.28
GR15_D	0.13
APos15_L1	0.31
APos15_L2	0.34
APos15_L3	0.37
GPos15_L1	0.09
GPos15_L2	0.22
GPos15_L3	0.13
AR15_L1	0.24
AR15_L2	0.23
AR15_L3	0.20
GR15_L1	0.16
GR15_L2	0.32
GR15_L3	0.16

Raw values of live-dead metrics: Annual, Spring & Autumn

Appendix 4: Raw values of live-dead metrics of all samples of the annual dataset. The three LA replicates of each location/habitat are compared to its respective DA.

	Spearman_rho	Spearman_p	Jaccard-Chao	deltaS	deltaPIE
APos15_D	0.173	0.053	0.918	0.287	0.033
APos15_D.1	0.264	0.004	0.72	0.332	0.038
APos15_D.2	0.424	0	0.795	0.291	0.038
GPos15_D	-0.174	0.078	0.978	0.487	0.196
GPos15_D.1	-0.006	0.957	0.822	0.551	0.263
GPos15_D.2	0.028	0.787	0.834	0.555	0.249
AR15_D	-0.164	0.083	0.717	0.336	0.071
AR15_D.1	-0.304	0.001	0.577	0.356	0.048
AR15_D.2	-0.211	0.027	0.574	0.406	0.18
GR15_D	0.059	0.688	0.961	0.313	-0.105
GR15_D.1	0.345	0.037	0.946	0.415	-0.057
GR15_D.2	0.052	0.73	0.953	0.226	-0.035

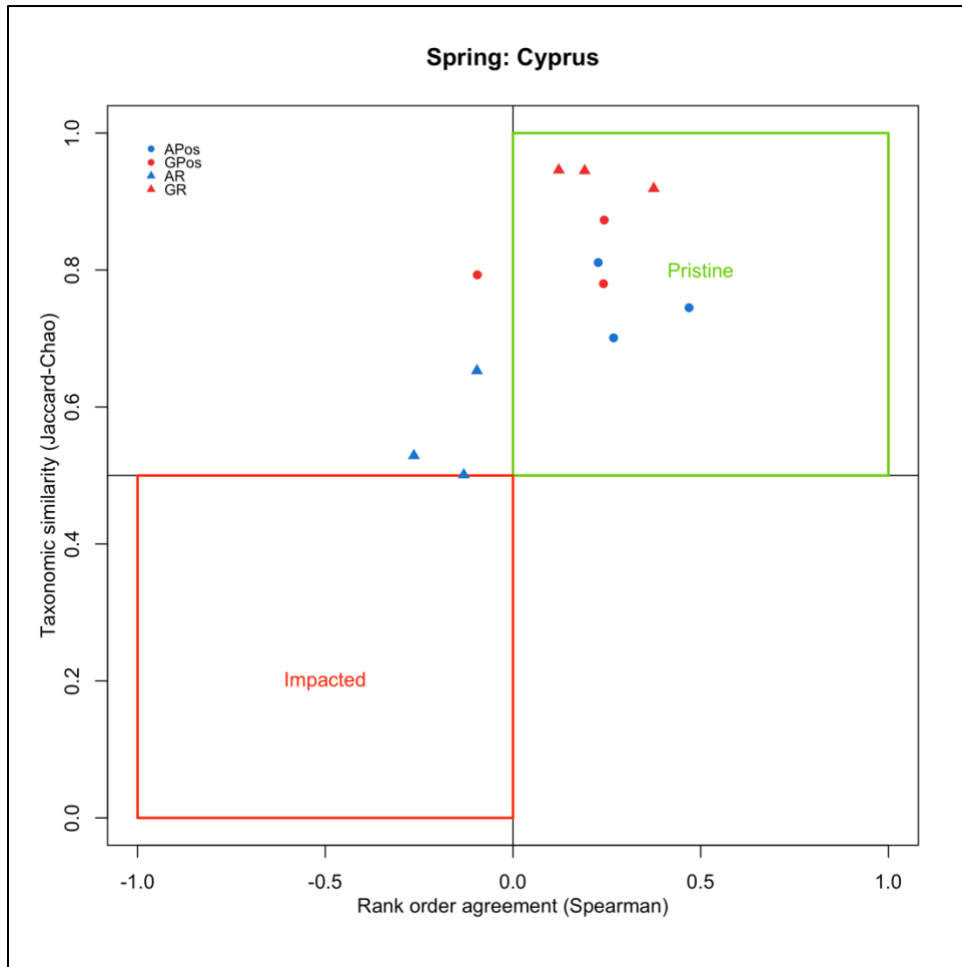
Appendix 5: Raw values of live-dead metrics of all samples of the spring dataset. The three LA replicates of each location/habitat are compared to its respective DA.

	Spearman_rho	Spearman_p	Jaccard-Chao	deltaS	deltaPIE
APos15_D	0.227	0.015	0.811	0.239	0.037
APos15_D.1	0.268	0.004	0.701	0.33	0.044
APos15_D.2	0.469	0	0.745	0.328	0.037
GPos15_D	-0.095	0.347	0.793	0.245	0.113
GPos15_D.1	0.243	0.027	0.873	0.273	0.024
GPos15_D.2	0.241	0.026	0.78	0.486	0.358
AR15_D	-0.096	0.329	0.653	0.301	0.041
AR15_D.1	-0.264	0.006	0.529	0.371	0.077
AR15_D.2	-0.131	0.19	0.501	0.277	0.03
GR15_D	0.191	0.224	0.945	0.173	-0.06
GR15_D.1	0.375	0.024	0.919	0.139	-0.047
GR15_D.2	0.122	0.435	0.946	0.241	0.205

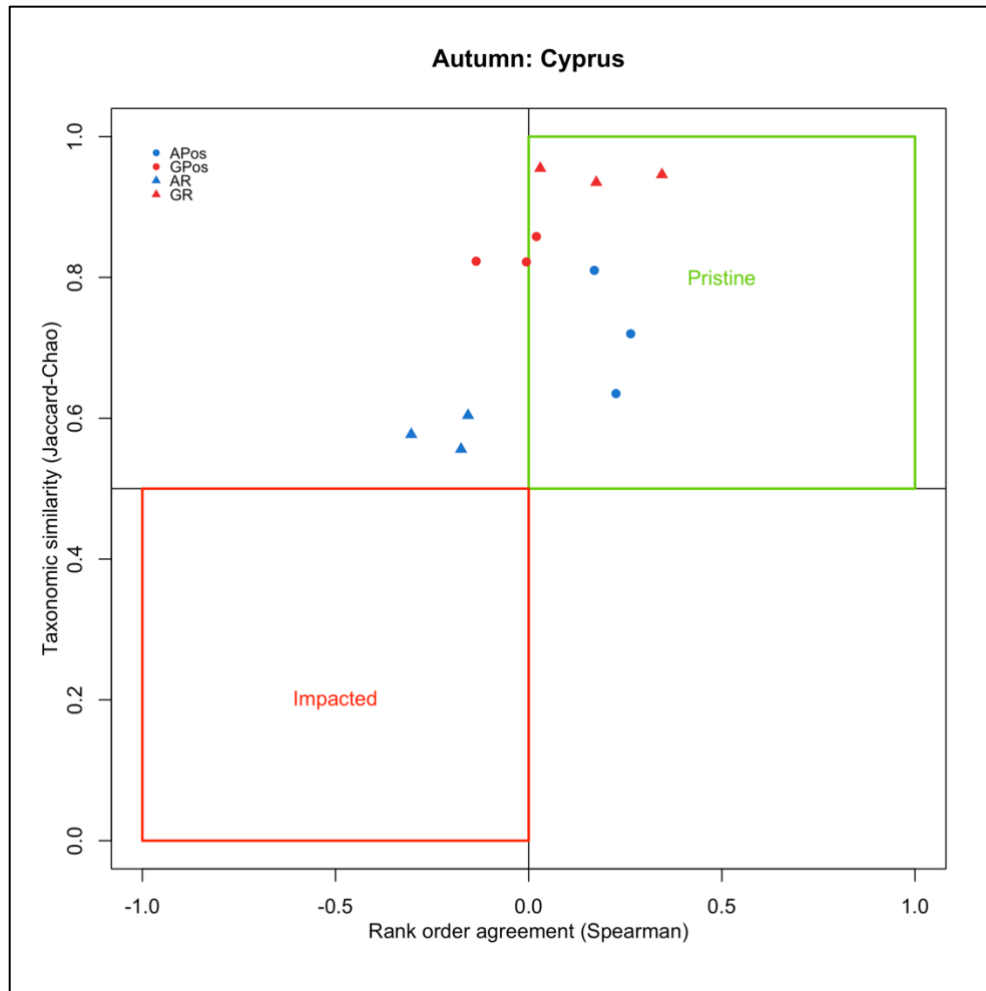
Appendix 6: Raw values of live-dead metrics of all samples of the autumn dataset. The three LA replicates of each location/habitat are compared to its respective DA.

	Spearman_rho	Spearman_p	Jaccard-Chao	deltaS	deltaPIE
APos15_D	0.17	0.065	0.81	0.34	0.051
APos15_D.1	0.264	0.004	0.72	0.332	0.038
APos15_D.2	0.226	0.017	0.635	0.238	0.055
GPos15_D	-0.136	0.189	0.823	0.656	0.276
GPos15_D.1	-0.006	0.957	0.822	0.551	0.263
GPos15_D.2	0.02	0.851	0.858	0.578	0.284
AR15_D	-0.175	0.072	0.556	0.462	0.097
AR15_D.1	-0.304	0.001	0.577	0.356	0.048
AR15_D.2	-0.157	0.108	0.604	0.455	0.222
GR15_D	0.03	0.844	0.955	0.413	-0.067
GR15_D.1	0.345	0.037	0.946	0.415	-0.057
GR15_D.2	0.175	0.286	0.935	0.34	-0.092

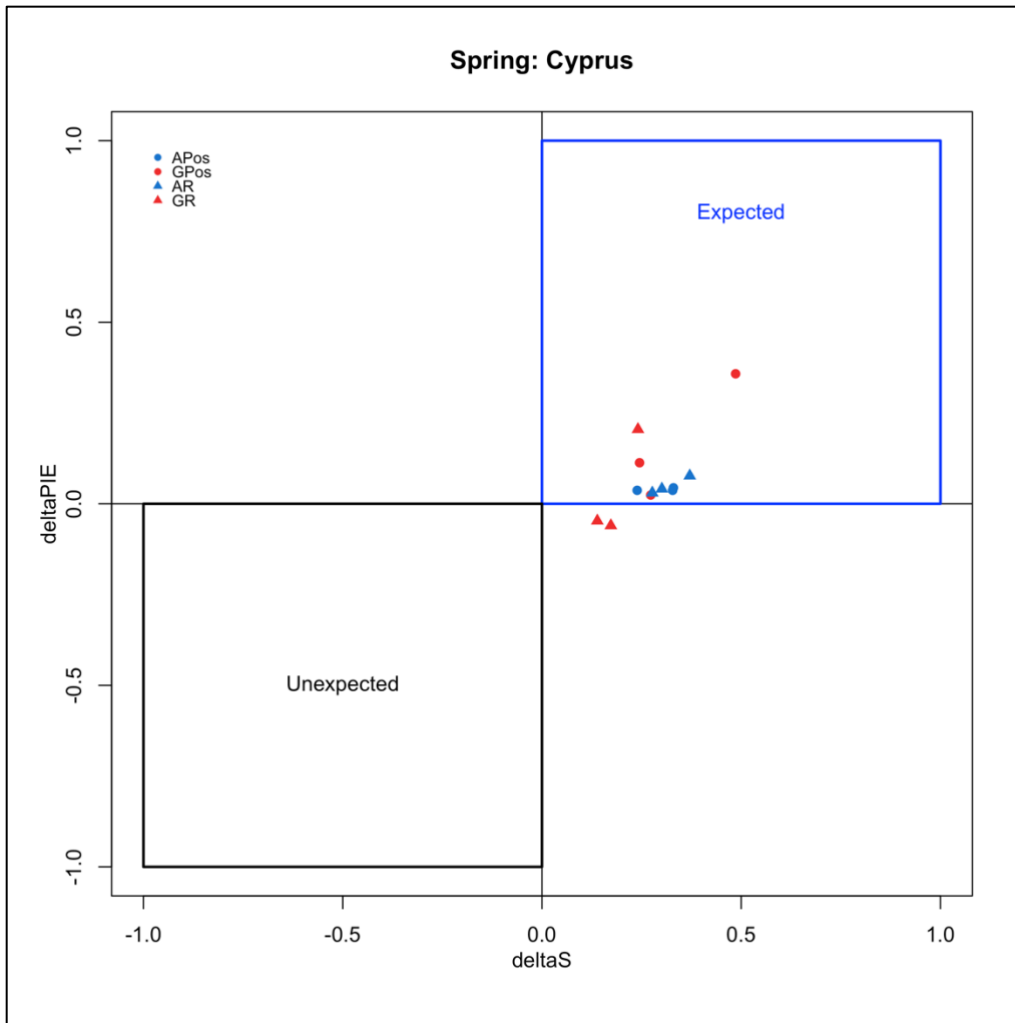
Live-dead metrics: Spring & Autumn



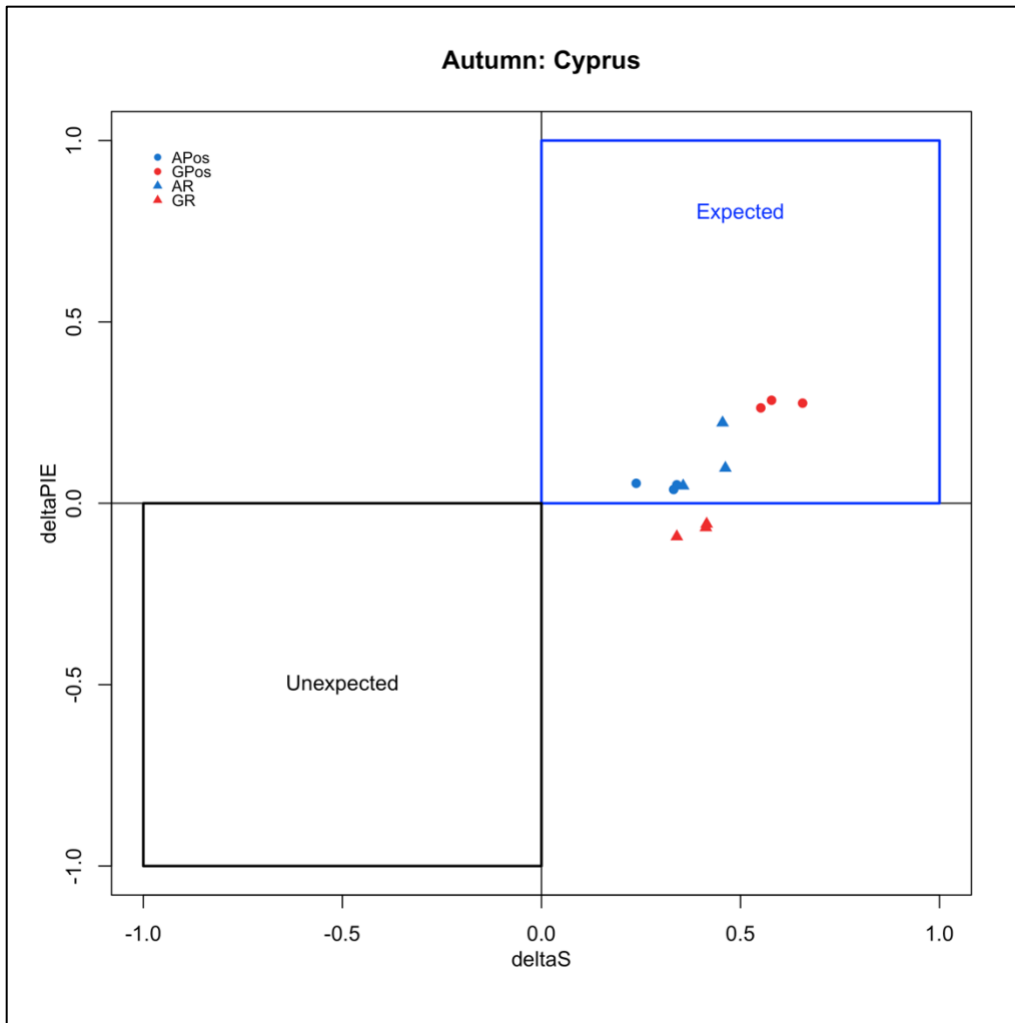
Appendix 7: (Spring) Live-dead metrics (Jaccard-Chao taxonomic similarity index and the Spearman rank order agreement) of all 4 samples and their respective replicates.



Appendix 8: (Autumn) Live-dead metrics (Jaccard-Chao taxonomic similarity index and the Spearman rank order agreement) of all 4 samples and their respective replicates.

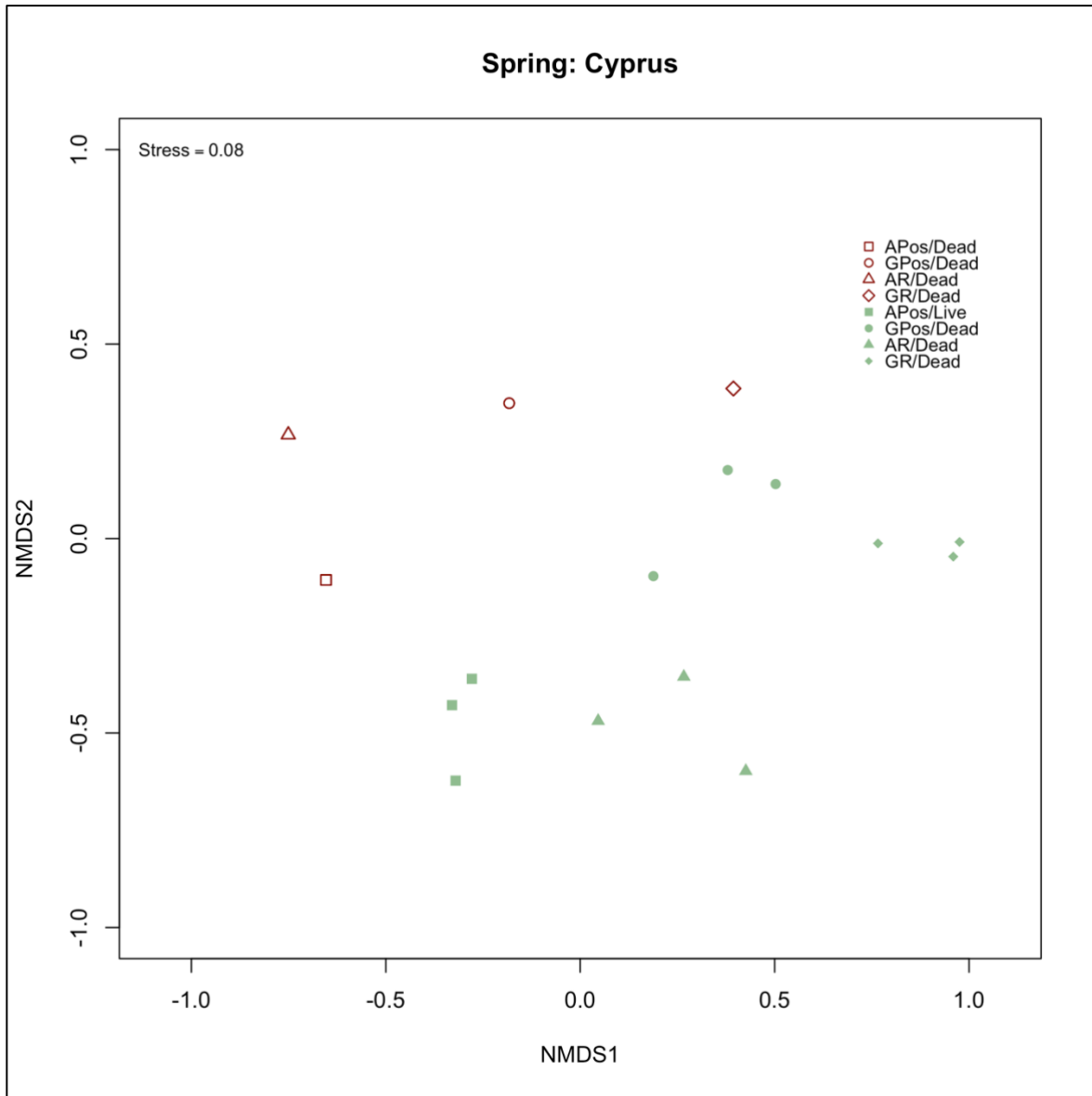


Appendix 9: (Spring) Live-dead metrics (difference in species richness, deltaS, and the difference in evenness, deltaPIE) of all 4 samples and their respective replicates.

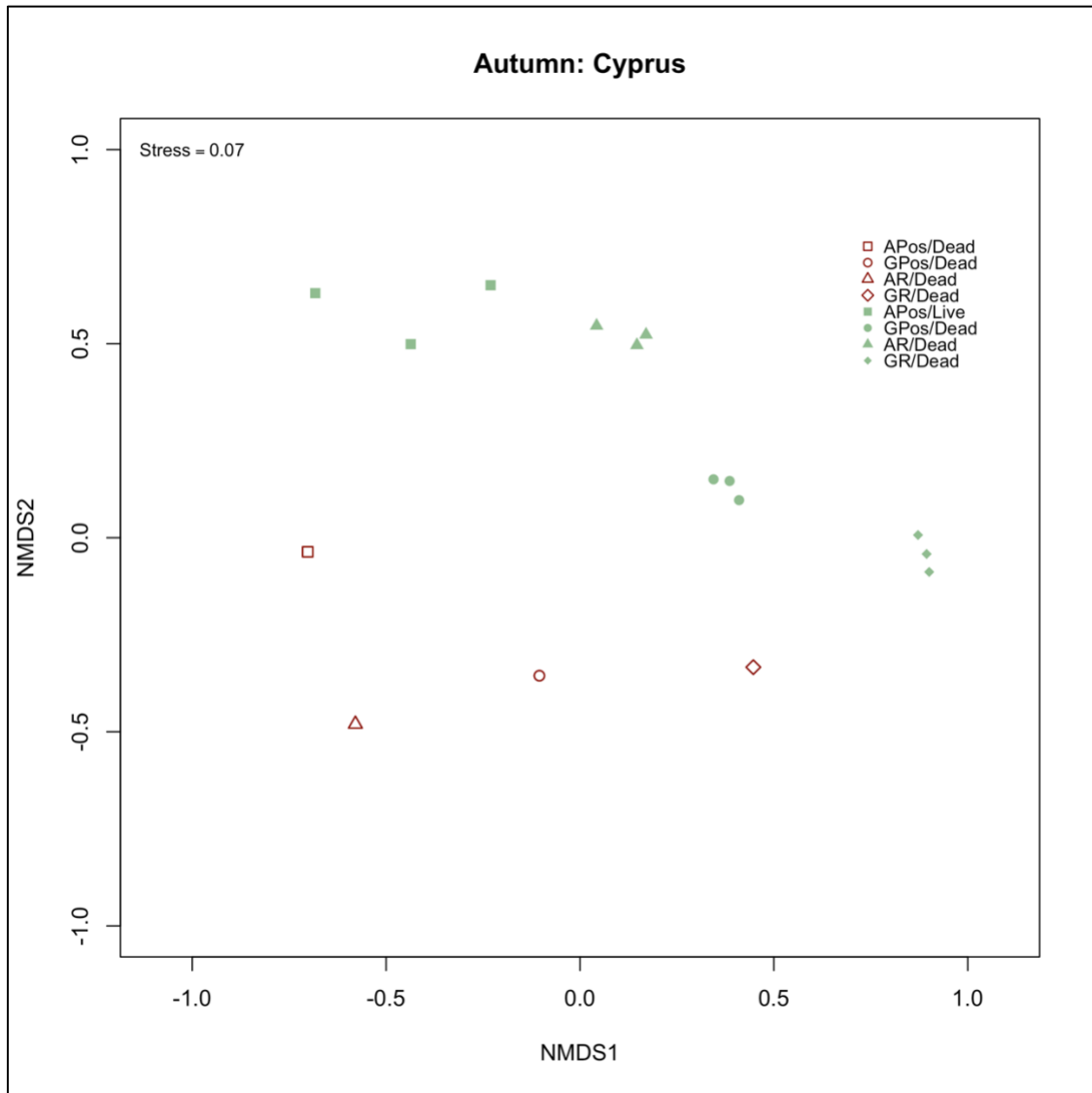


Appendix 10: (Autumn) Live-dead metrics (difference in species richness, ΔS , and the difference in evenness, ΔPIE) of all 4 samples and their respective replicates.

NMDS ordinations: Spring & Autumn

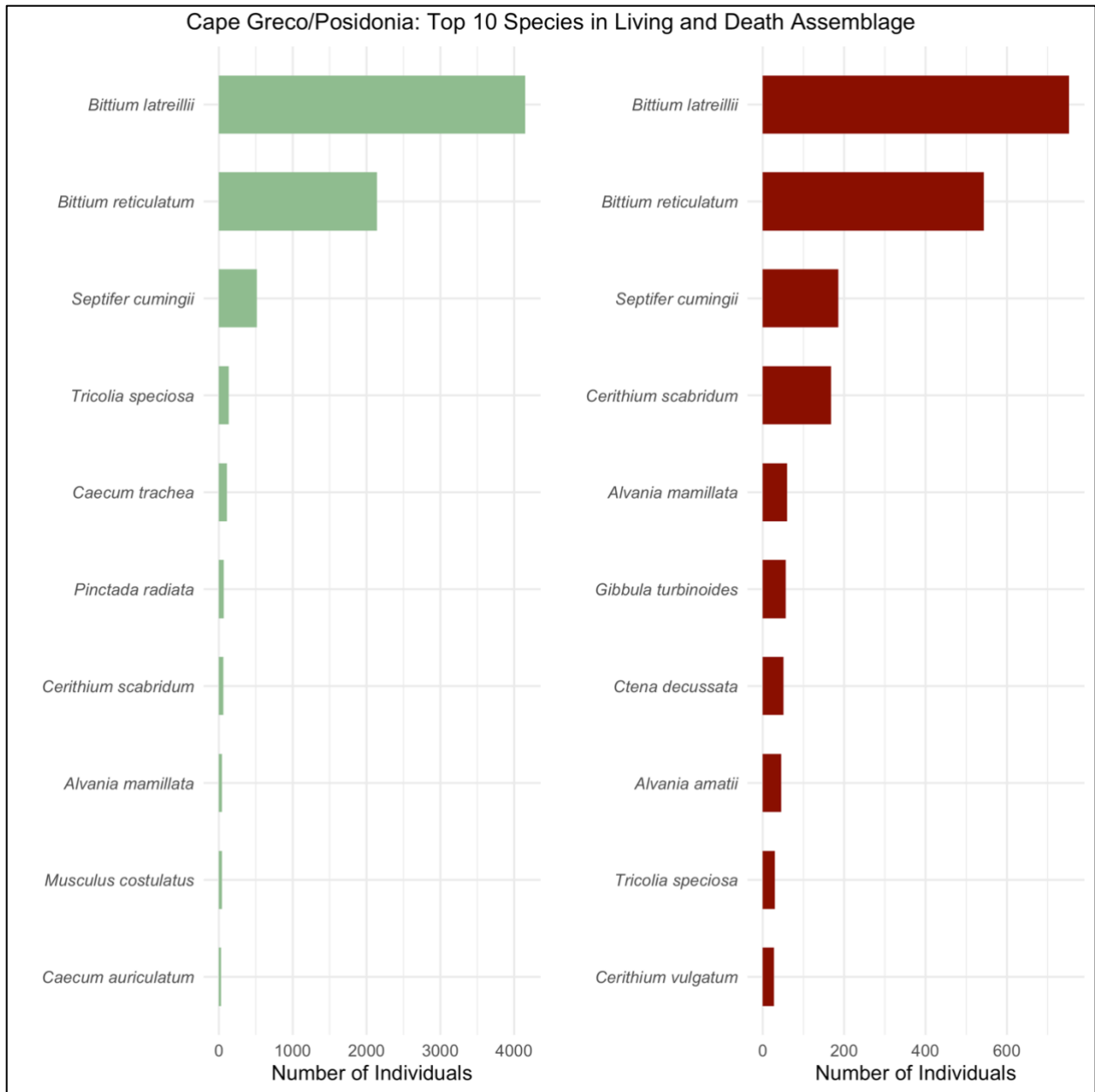


Appendix 11: (Spring) NMDS ordination representing the dissimilarity structure of the living and death assemblages. Each point represents a sample replicate. Stress value (=0.08) indicates a very good fit for the data. Green points represent the living assemblages and brown points represent the death assemblages.

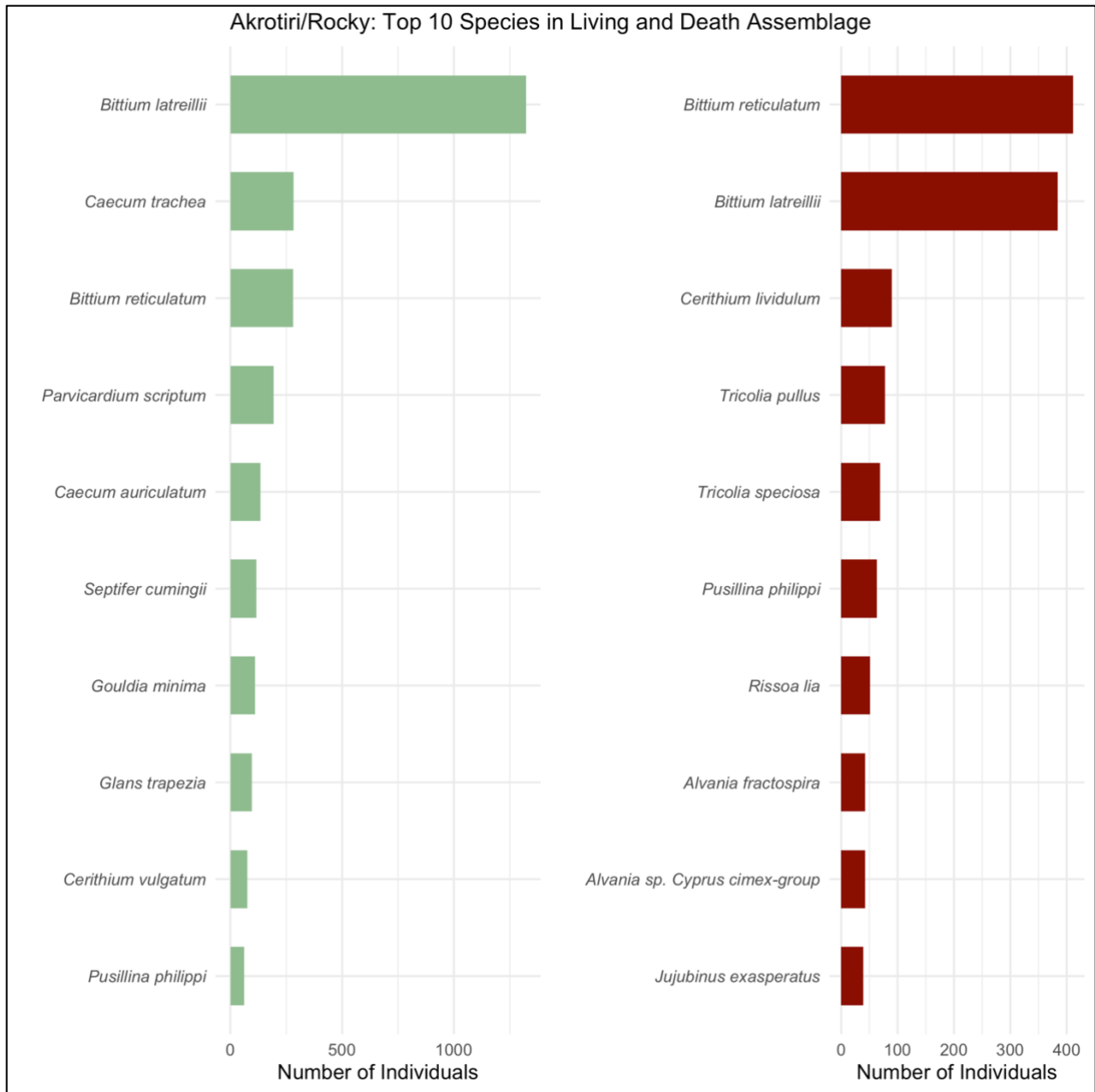


Appendix 12: (Autumn) NMDS ordination representing the dissimilarity structure of the living and death assemblages. Each point represents a sample replicate. Stress value (=0.07) indicates a very good fit for the data. Green points represent the living assemblages and brown points represent the death assemblages.

Top 10 species in DA/LA: Cape Greco/Posidonia meadow, Akrotiri/Rocky substrate



Appendix 13: Absolute abundance of top 10 species in Cape Greco/Posidonia meadow living and death assemblage. *Bittium latreillii*, *Bittium reticulatum*, and *Septifer cumingii* ranked 1st, 2nd, and 3rd in both the DA and the LA.



Appendix 14: Absolute abundance of top 10 species in Akrotiri/rocky substrate living and death assemblages. *Septifer cumingii*, an invasive species, was absent from the DA, but ranked 6th in abundance in the LA. *Bittium reticulatum*, an endemic micrograzer, saw a marked decrease in abundance from the DA to the LA.

Appendix 15: Number of native and non-indigenous species (S_{rar}) in each location/habitat's DA and LA.

	Native	Alien
APos_D	100	1
GPos_D	65	3
AR_D	84	0
GR_D	21	2
APos_L	48	2
GPos_L	16	3
AR_L	35	3
GR_L	10	2