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COMPARISON BETWEEN LIVING AND DEATH SHALLOW BENTHIC ASSEMBLAGES BETWEEN AREAS WITH DIFFERENT EXPOSURE TO SEAWATER TEMPERATURES IN THE EASTERN MEDITERRANEAN SEA

ANNA KARAMPET, 4526856

SUPERVISED BY KATJA PHILIPPART & PAOLO G. ALBANO

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Abstract

In the Mediterranean Sea's Levantine Basin, a region vulnerable to rising temperatures and biological invasions, the effects of climate change and the invasion of thermo-tolerant alien species threaten native organisms. This research project evaluates taxonomic diversity and composition variations between two thermally distinct regions within Cyprus, Cape Greco and Akrotiri. While in Cape Greco the sea temperature has risen, in Akrotiri the temperatures remain unaffected due to an upwelling phenomenon observed during the summer season in the area. The analysis was conducted by comparing living and death assemblages, encompassing not only different geographical locations but also distinct ecological habitats, rocky substrate and *Posidonia* meadow. The examination of death assemblages provides insights into the recent ecological history and helps quantify changes and losses; information highly valuable considering the lack of a speciation database in the region. The hypothesis is that the warmer Cape Greco exhibits reduced species richness and an increased number of non-native mollusks compared to Akrotiri, indicating an impacted area. Additionally, it is expected that *Posidonia* meadow faces higher rarified richness than rocky substrate, mainly due to photosynthesis that causes oxygen-super saturation which increases the thermo-tolerance of the species. In contrast with the expectations, the results show a pristine condition in both areas with alien species having a minor impact on the native benthic mollusks. However, Akrotiri maintains nearly triple the rarified richness compared to Cape Greco, suggesting an optimal refugia. *Posidonia* meadow has indeed greater rarified richness than the rocky substrate. This research aims to shed light on the biodiversity patterns and ecological relationships in the Mediterranean ecoregion, contributing to our understanding of the impacts of climate change and biological invasions on marine ecosystems.

1. Introduction

1.1. General Information

Climate change is a global phenomenon that transcends geographical boundaries, impacting ecosystems and biodiversity on a planetary scale. From the melting ice caps of the Arctic to the coral reefs of the tropics, its effects can be spotted across diverse landscapes and seascapes. However, certain regions emerge as points of ecological significance, where the convergence of geographical features and anthropogenic activities intensify the challenges posed by climate change. In the semi-enclosed Mediterranean Sea, the Levantine Basin stands out as a distinctive ecoregion of greater importance. With ocean warming being more intense than elsewhere in the Mediterranean, and the Suez Canal being so close facilitating biological invasions, this area faces significant ecological pressure (Albano et al., 2021). At the same time that alien species are more thermo-tolerant, the effects of climate change have pushed many native species in this region to the brink of their upper thermal tolerances, resulting in many cases in population collapse (Albano et al., 2021).

To quantify this loss in one of the warmest Mediterranean regions, the Israeli shelf, Albano et al. (2021) compared the current native mollusk richness with historical records derived from surface death assemblages (DA). The results indicate a significant decline, with only 12% and 5% of historically documented native species found on shallow soft and hard substrates, respectively, marking one of the most substantial climate-driven regional diversity losses documented in ocean ecosystems. In contrast, intertidal assemblages, which are more resilient to climate change, and the cooler mesophotic zone, exhibit approximately 50% of their historical native richness. Notably, about 60% of the native species recorded in the shallow subtidal areas do not reach reproductive maturity, making this zone a demographic sink. The forecast suggests that as the climate continues to warm, this native biodiversity decline will intensify and expand geographically, with potential migration from Indo-Pacific species entering through the Suez Canal.

Expanding upon Mediterranean research and seeking to explore its applicability in different locations, the next phase involves a detailed examination of mollusks in Cyprus (Figure 1). Cyprus, situated in the northeastern sector of the Levantine Basin, presents a unique opportunity to investigate local biodiversity patterns due to its distinctive oceanographic characteristics. While the northern and eastern coasts endure exceptionally warm summers, the southwestern coastline benefits from a notable cooling effect, attributed to coastal upwelling, maintaining temperatures 1 - 4 °C lower (Albano et al., 2021).

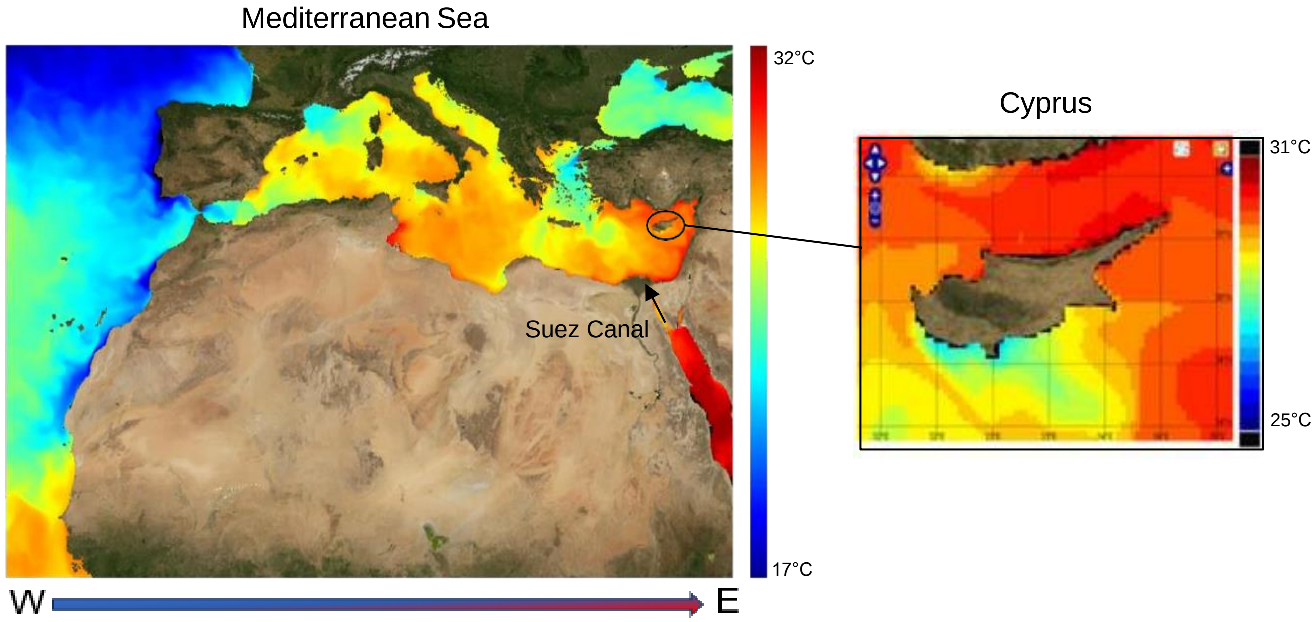


Figure 1 August 2018, mean sea surface temperature (SST) in the Mediterranean basin (left) and mean SST Cyprus (right). This figure shows a gradient of increasing sea surface temperature from northwest to southeast Mediterranean Sea, with Cyprus locating in the eastern Mediterranean (Figure by my Ocean Global Analysis PHY 001 024).

The primary objective of this thesis project is to examine the biological invasions and the climate change impact on shallow benthic mollusks between two thermally distinct regions of Cyprus, Cape Greco and Akrotiri. Variations in diversity richness and a comparative analysis between living and death assemblages were conducted for this purpose. The analysis took place not only between two different locations but also between two distinct ecological habitats, namely, *Posidonia* meadow (seagrass) and rocky substrate. The analysis of two different habitats is attributable to the findings of Albano and Sabelli (2011), which highlight notable disparities between habitat types. The methodology for comparing living and death assemblages was previously employed by Kidwell (2013). More specifically, Kidwell (2013) examined the progress made in two decades regarding the temporal precision and the capability of time-averaged skeletal collections to accurately capture ecological insights, with a primary focus on mollusks inhabiting soft-sediment seafloors. It subsequently shed light on two promising applications within the field of taphonomy. First, the live-dead comparison (LD) involves identifying differences in the species diversity, composition, and distribution of living assemblage (LA) compared to DA. Second, the time-averaged death assemblages. An approach that treats time-averaged collections of DAs as windows into regional biodiversity and long-term ecological baselines.

The benefits of live-dead comparison are numerous. LD helps stabilize natural variability by overcoming the inherent short-term fluctuations in ecosystems, allowing for a more stable and accurate assessment. These comparisons provide valuable site-specific insights into regional diversity composition and structure, historical baselines, range shifts, and species turnover. Highly valuable information, considering the scarcity of historical databases in the region (Kidwell & Tomasovych, 2013). LD can be applied across a broad spectrum of spatial scales, from specific sites to regional assessments, making them adaptable to diverse research contexts. Moreover, LD contributes to reducing the likelihood of false-positive results, thereby enhancing the reliability of ecological findings. Additionally, by focusing on a single group of organisms, live-dead comparisons help lessen the taxonomic expertise and identification workload required, simplifying data analysis and interpretation (Albano et al., 2023). However, composition differences between living and death assemblages can arise from several contributing factors. Firstly, differential preservation plays a role, potentially biasing the death assemblage against species or age classes with lower durability. Additionally, the transport processes, influenced by currents, tides, rafting, and predation, can result in the movement of specimens into or out of the area. Differential turnover rates within living populations can lead to an over-representation of species with shorter lifespans relative to longer-lived counterparts, an effect known as lifespan bias. Finally, environmental changes occurring within the time-averaging window can influence the death assemblage, potentially preserving past habitat or community states temporarily (Kidwell, 2013).

1.2. Study Area

The Mediterranean Sea is recognized as a biodiversity hotspot, hosting approximately 17,000 marine species, although this estimate is likely incomplete due to undiscovered species (Coll et al., 2010). In the Mediterranean Sea, there is a gradient of increasing sea surface temperature (SST) from northwest to southeast, with the eastern Mediterranean being the warmest sector of the region (Pisano et al. 2020; Figure 2).

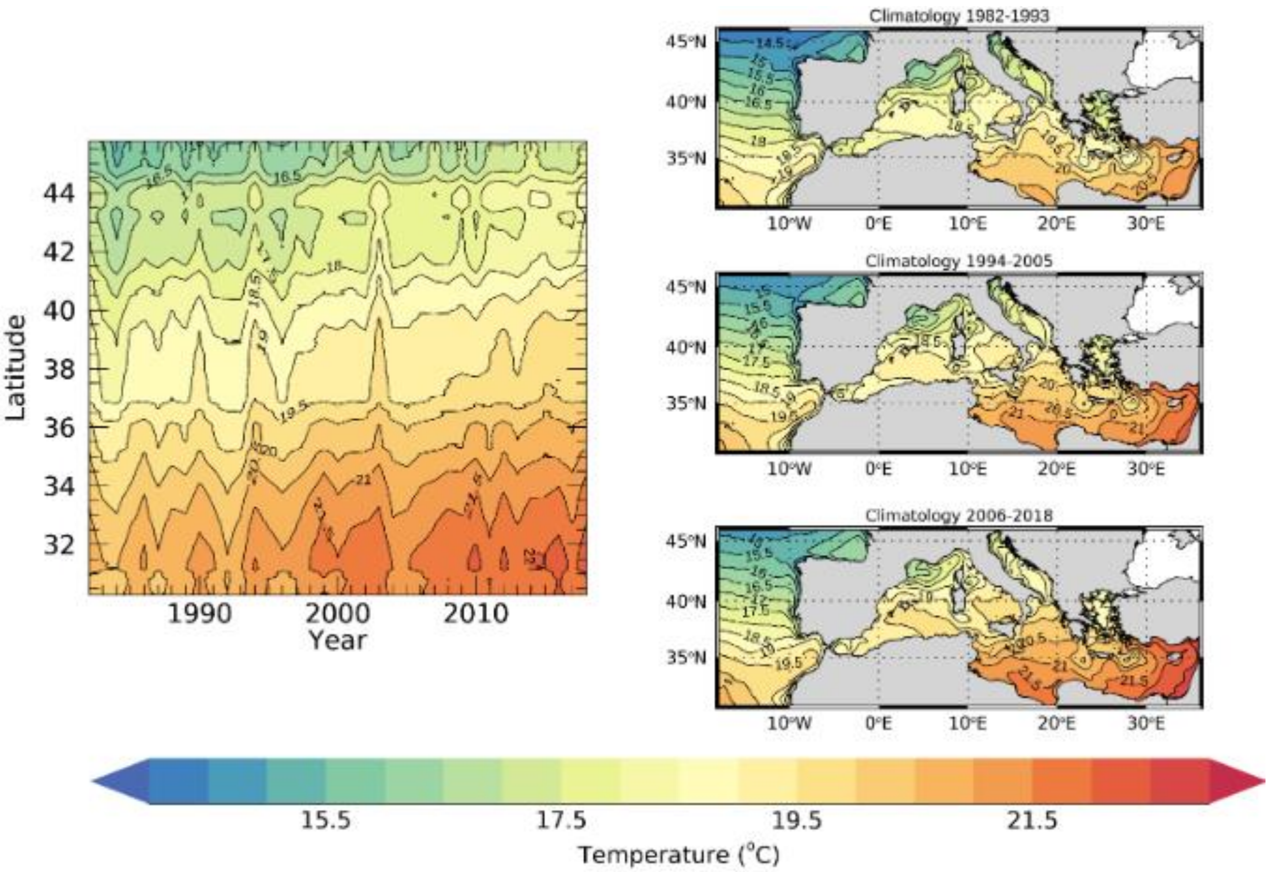


Figure 2 The left panel indicates a Hovmöller diagram of the SST from 1982 to 2018 in the Mediterranean Sea while the right panel shows the annual SSTs field from 1982 to 1993 (top), from 1994 to 2005 (middle) and from 2006 to 2018 (bottom). This figure indicates that the annual SST of the eastern Mediterranean Sea during the years has risen. While from 1982 to 1993 the annual SST was 20 – 21 °C, from 1994 till 2005 was risen to 20.5 – 22 °C, and from 2006 to 2018 picked to 21 – 22.5 °C (Figure by Pisano et al. 2020).

Cyprus, originally the Republic of Cyprus, is an island country located in the Eastern Mediterranean Sea. It is the third largest island in the Mediterranean, with an area of about 9,251 square kilometers and a population of 1.2 million in 2021 (WorldAtlas). The island is characterized by mountain ranges and Mediterranean climate, with hot, dry summers and mild, wet winters (WorldAtlas).

Within Cyprus, two areas were chosen for their differences in thermal characteristics, Cape Greco and Akrotiri (Figure 3). Cape Greco is in the southeastern part of Cyprus, and it is a part of Ayia Napa Municipality. Akrotiri is situated in the southern region of the island, near the city of Limassol, and it is one of the 14 British Overseas Territories (BOTs) (Secretary of State for Foreign and Commonwealth Affairs by Command of Her Majesty, 2012). Unlike Cape Greco, Akrotiri remains unaffected by the increasing sea temperatures. This is attributed to the upwelling effect during the summer season, which maintains temperatures up to 4 °C lower than elsewhere in Cyprus (Albano et al., 2021).

Figure 3 Cape Greco (left; by globaliayachting.com) and Akrotiri (right; by Birdlife Cyprus).

1.3. Stazione Zoologica Anton Dohrn

Although the sampling occurred in Cyprus, the project was conducted at the Stazione Zoologica Anton Dohrn (SZN) organization in Naples, Italy.

SZN was established in 1872 by Anton Dohrn and serves as a research center and as a museum. Until today, it remains dedicated to scientific and technological research (Stazione Zoologica Anton Dohrn Napoli - Italy, 2015). The experimental design, the laboratory work, and the analysis were all conducted in the laboratory of SZN in Naples.

1.4. Benthic Mollusks

Mollusks are a diverse group of invertebrate animals that belong to the phylum Mollusca. They encompass a wide range of species, with gastropods being the biggest class with over 100,000 living species (Marin et al., 2012). Mollusks are characterized by having soft bodies, often protected by a hard outer shell made of calcium carbonate. The shell is often used to determine the class and the species of a mollusk (Marin et al., 2012).

Benthic mollusks (Figure 4) are a category of mollusks that inhabit the benthic zone of aquatic environments, which refers to oceans, seas, lakes, and rivers. They include a wide variety of species, such as snails (Gastropoda), clams and mussels (Bivalvia). Benthic mollusks often feed on organic matter found in the sediment or filter nutrients from the water column, and they serve as food sources for various predators, contributing to the overall biodiversity and functioning of marine ecosystems.

Figure 4 Living mollusks from sample in Akrotiri. The mollusks were picked in the laboratory at SZN.

Native or indigenous species are those that naturally inhabit a specific area or habitat within its known natural range. Indigenous species have evolved and adapted to the local environmental conditions over time. In contrast, non-native species, also addressed as alien species, are those that have been introduced to an area outside of their natural range, often due to human activities. Non-native species can sometimes have detrimental effects on the local ecosystem, outcompeting native species or disrupting ecological balances, referred to as invasive species (Albano et al., 2021). The Mediterranean Sea is shielded by a solid thermic barrier, effectively isolating it from alien species (Sabelli & Taviani, 2014). However, throughout the warmest interglacial periods of the Pleistocene, this barrier ceased to exist and enabled the entrance of thermophilic biota, which primarily originated from West Africa, facilitating what is considered the most extensive marine biotic attrition to the Mediterranean Sea in history (Sabelli & Taviani, 2014). Although, in 1869, the construction of the Suez Canal breached the Indo-pacific province with the Mediterranean Sea, establishing a new record of species invasion. A direct link between the Levantine basin and the tropical species pool of the Red Sea facilitated the influx of hundreds of species, a phenomenon famously known as the Lessepsian invasion (Nunes et al., 2014).

1.5. Habitat

Rocky substrate and *Posidonia* meadow are crucial habitats for conservation due to their capacity to support greater biodiversity compared to soft substrates at similar depths, as noted by Guidetti (2000). The rocky subtidal habitat (Figure 5) includes all areas of the ocean bottom characterized by hard substrates. Rocky subtidal areas can be either extensions of shoreline features like headlands, cliffs, or rocky intertidal habitats, or they may exist as isolated patches of rock within areas dominated by soft bottom substrate. These rocky reefs exhibit diverse topography, with some barely protruding above the surrounding seafloor, while others rise several meters from the seabed or even extend above the surface to create islands within the Territorial Sea (Oregon Conservation Strategy). Rocky substrates are shaped by various factors including proximity to shore, water depth, local seafloor geology, erosional forces, and biological influences. Many rocky subtidal areas exhibit geology similar to adjacent landforms, often composed of erosion-resistant basalts or metamorphic rock typical of Oregon’s rocky headlands. Over time, these underwater rock formations have undergone uplift, bending, deformation, and exposure to both oceanic and terrestrial erosional forces due to successive ice ages and geological events leading to significant sea level changes. Consequently, a range of physical habitat features has emerged within reefs, such as flat rocky benches, stacks, jagged ridges, broken boulder fields, and numerous cracks and crevices providing shelter and substrate for diverse marine life. Moreover, oceanographic processes significantly influence the rocky subtidal environment. Subtidal reefs are subject to intense wave action, underwater currents, and the physical and chemical properties of the surrounding water (Oregon Conservation Strategy).

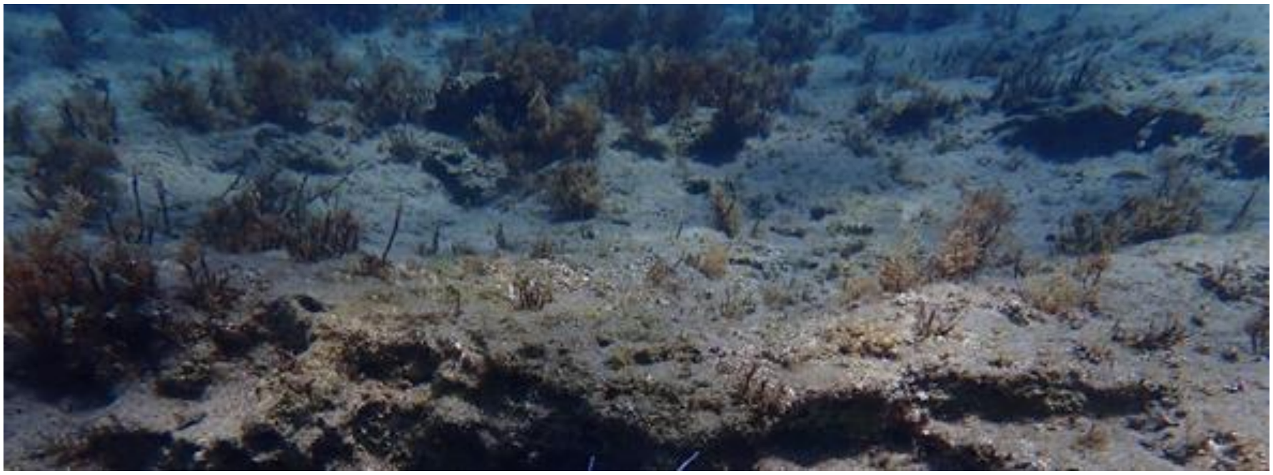


Figure 5 Rocky substrate (by Paolo Albano).

Posidonia oceanica (Linnaeus) Delile is a flowering seagrass species (Magnoliophyta), endemic to the Mediterranean Sea (Boudouresque et al. 2016). Grows in depths of up to 50 m (Vlachopoulou et al., 2012) and forms extensive meadows (Holzknecht & Albano, 2022; Figure 6). It is recognized as the most productive marine ecosystem in the Mediterranean (Duarte & Chiscano, 1999), offering numerous socio-economic benefits, and playing a vital role in preserving marine biodiversity (Hemminga and Duarte, 2000). *Posidonia oceanica* has rhizomes and a complex network of roots forming the matte. Creeping (plagiotropic) and erect (orthotropic) rhizomes can extend beyond 1 meter in length, taking nutrients and water from the sediment (Mediterranean Posidonia Network). These rhizomes have groups of 4 to 8 leaves (shoots; Mediterranean Posidonia Network). The leaves are narrow, typically green in color, and contain chlorophyll, the pigment responsible for photosynthesis, enabling the plant to produce oxygen and its food (Boudouresque et al. 2016). Net leaves are continuously produced throughout the year and typically have a lifespan ranging from 5 to 8 months (occasionally up to 13 months; Mediterranean Posidonia Network). Furthermore, every 5 to 10 years, during autumn, *Posidonia oceanica* produces small, inconspicuous flowers (Mediterranean Posidonia Network).

Figure 6 Posidonia oceanica meadow (by Graham Keeley).

Among the sixty-four species of seagrass, *Posidonia oceanica*, stands out as the most efficient in carbon fixation and storage (Pergent-Martini et al., 2021). Furthermore, *Posidonia* meadows increase the resilience of species to thermal stress and enhance their thermal tolerance by 2 °C to 4 °C (Giomi et al., 2019; Figure 7). Effects that are attributed to *Posidonia* oceanica's photosynthesis which increases the oxygen supersaturation levels and buffers the ocean warming effect. However, according to Pergent-Martini (2006), the placement of fish farming cages directly over *Posidonia oceanica* beds and the anchors of boats have yielded alarming consequences. The meadows have suffered severe degradation or complete disappearance, while the sediment has exhibited a marked rise in organic matter levels, potentially precipitating anoxia phenomena. To protect the *Posidonia* meadows, *Posidonia* has been included in European and international legislation. According to The Habitats Directive of 21 May 1992, *Posidonia oceanica* appears in Annex 1 (natural habitat types requiring Special Conservation Zones) and is classified as a priority habitat. Additionally, the Water Framework Directive (WFD, 2000) and the Marine Strategy Framework Directive (MSFD, 2008) contribute to *Posidonia* conservation by striving to achieve or maintain Good Environmental Status (GES) in all EU marine waters, with *Posidonia* often utilized as an environmental status indicator. Furthermore, the Common Fisheries Policy (CFP) includes protective measures for *Posidonia* meadows. Specifically, it prohibits the use of towed gears shallower than 50 meters deep and banned fishing activities with trawl nets, dredges, purse seines, boat seines, shore seines, or similar nets above seagrass beds, particularly those of *Posidonia oceanica* (Council of the European Union, 2006). Internationally it is protected under RAMSAR, Berne (Annex 1), and Barcelona (Annex 2) conventions (Mediterranean Posidonia Network).

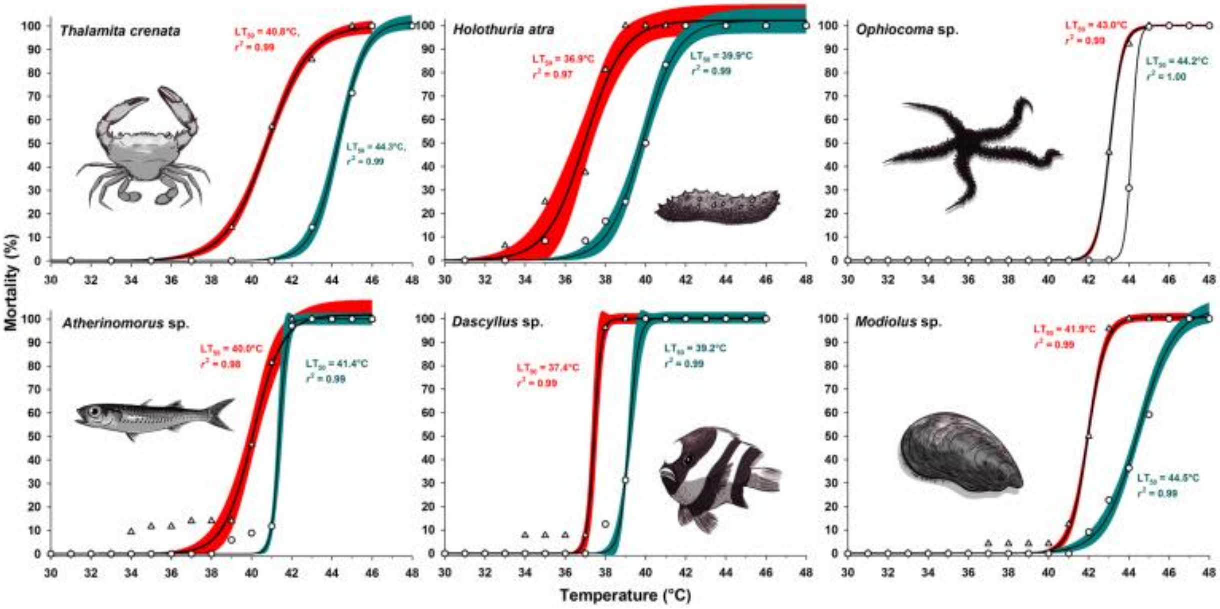


Figure 7 Normoxia (triangles) compared to hyperoxia (circles) in 6 different marine species. Red color stands for the mortality of marine species under normoxia while green for species under hyperoxia. In all cases the thermal tolerance increases when more oxygen is available (Figure by Allende Bodega Martinez).

1.6. Refugia

Refugia are environmental habitats characterized by both spatial and temporal dimensions that protect species from changing environmental conditions for millennia. They represent optimal locations for many species to withstand the impacts of climate change (Keppel, 2012). Two primary approaches exist for identifying and characterizing refugia. The first approach relies on biogeographic patterns, which serve as proxies for past processes, indicating the presence of refugia in a particular area at some point in history. This method involves gathering data on the paleobiology, ecology, and genetics of target organisms, enabling the quantification of the spatial and temporal dimensions of refugia. The second approach focuses on examining the processes likely to generate refugial habitats. This approach, which may be particularly valuable in identifying refugia in the context of anthropogenic climate change, entails identifying and quantifying the environmental conditions favorable for the formation and maintenance of refugia (Keppel, 2012).

1.7. Aim of Research

This study aims to shed light on the evolving biodiversity patterns and ecological relationships in the eastern Mediterranean ecoregion, contributing to our understanding of the impacts of climate change and biological invasions on marine ecosystems. The pivotal research questions are: 1) How does climate change impact the biodiversity patterns of benthic mollusks in the eastern Mediterranean Sea? 2) What are the differences between the two ecological habitats, rocky substrate and *Posidonia* meadow? 3) What is the impact of alien species? 4) Is there a potential refugium for native species?

The hypothesis is that Cape Greco will have fewer native species diversity and a high number of alien species when compared to Akrotiri. Akrotiri might be a potential refugium for native species and *Posidonia* meadow will exhibit higher species richness compared to rocky substrate, due to the benefits it offers to living organisms such as higher oxygen concentration.

This research project holds significant importance for several reasons. 1) It directly addresses pressing threats to native organisms, by focusing on the effects of climate change and the invasion of thermo-tolerant alien species in the vulnerable Levantine Basin. This is crucial for understanding and mitigating the impacts of these threats on marine ecosystems. 2) Evaluates biodiversity variations in taxonomic diversity and composition between two thermally distinct regions, provides valuable insights into how different environmental conditions affect biodiversity patterns, which is essential for effective conservation planning and management. 3) Analyzes living and death assemblages across different geographical locations and ecological habitats and delves into the ecological dynamics of these areas. This helps in understanding the historical context of ecological changes and quantifying losses, which is essential for informed decision-making. 4) The findings regarding the differences in species diversity and composition, as well as the higher rarified richness in Akrotiri, suggest that certain areas may serve as refugia for native organisms. This information is valuable for identifying priority areas for conservation efforts. 5) Contributes to understanding the evolving biodiversity patterns and ecological relationships in the eastern Mediterranean Sea. Shedding light on the impacts of climate change and biological invasions on marine ecosystems adds to the body of scientific knowledge in this field and informs future research and conservation initiatives.

2. Material and Methods

2.1. Material

To discuss the current literature and the implications of the methods of this study, the following definitions need to be mentioned. “Living assemblage” refers to a collection of organisms or species that are currently alive and actively inhabiting a specific ecological or environmental context, typically a particular site, habitat, or ecosystem. These assemblages represent the present-day composition and diversity of living organisms within a given area (Kidwell, 2013). “Death assemblage” comprises taxonomically identifiable organic remains that are lifeless or discarded and found within the uppermost mixed layer of a terrestrial landscape or the seafloor (Kidwell, 2013). “Time-averaging” is a natural phenomenon where the remnants of organisms from various periods accumulate together in a single assemblage and occurs as a result of sedimentation rates generally being slower than the rates at which shells degrade (Walker & Bambach, 1971; Fursich & Aberhan, 1990).

Within the upper mixed layer of the sea floor, both living assemblages (LA) and death assemblages (DA) coexist, as depicted in Figure 8. Death assemblages tend to be richer than living assemblages. While living mollusks have relatively short lifespans, dead mollusks persist for much longer periods, as illustrated in Figure 9, depending on the resilience of skeletal remains and the sedimentation rate.

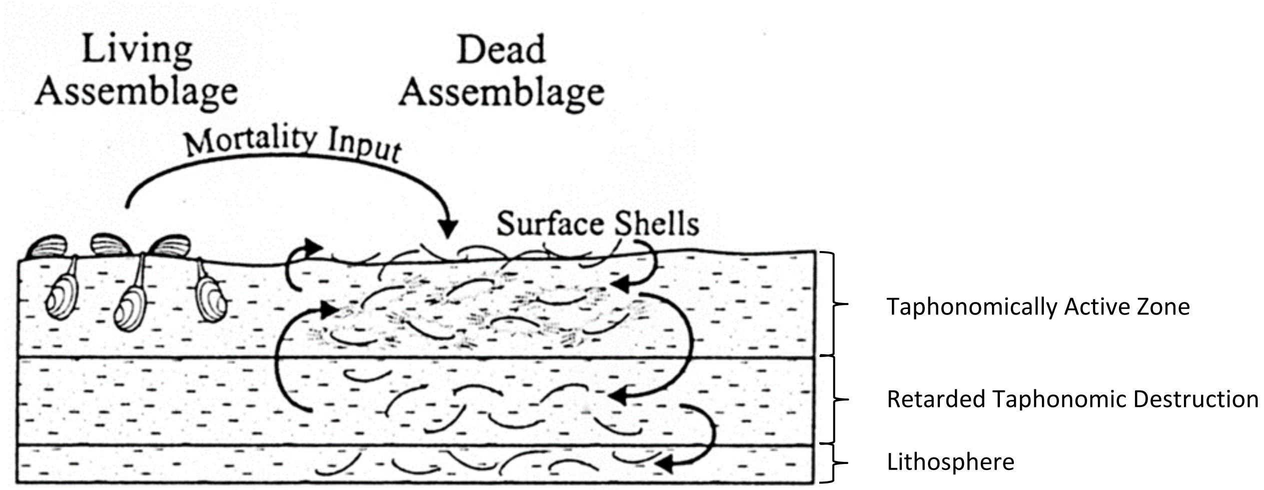


Figure 8 A representation of a simplified taphonomic process. Dead mollusks accumulate on the surface, adding to existing shells, and gradually become buried deeper within the Transition Altered Zone (TAZ), ultimately leading to the Retarded Taphonomic Destruction. Following Retarded Taphonomic Destruction, shells may either return to the TAZ or become permanently embedded in the lithosphere, unable to return to the surface. The shells resulting from recent mortality input and those that return after undergoing an extended journey in the Retarded Taphonomic Destruction process are the death assemblage (Figure by Olszewski, 1999).

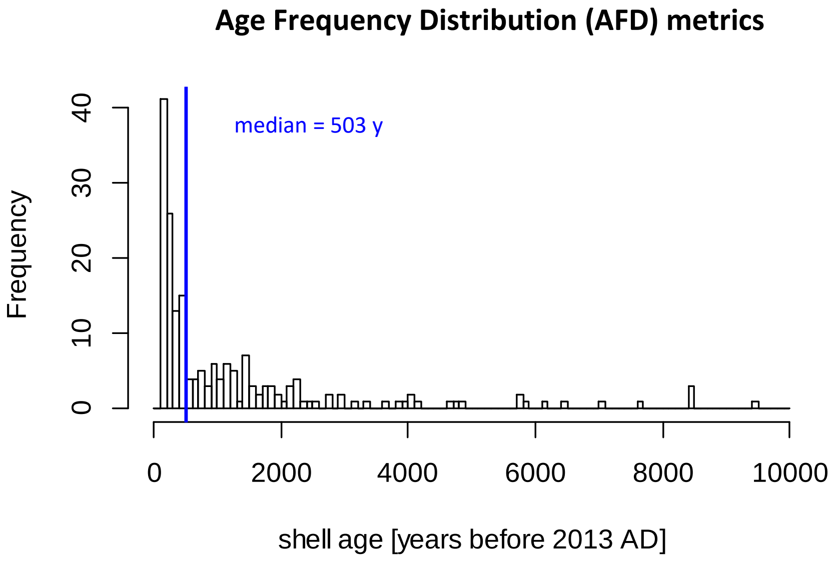


Figure 9 A representation of the frequency distribution of post-mortem ages of Laqueus erythraeus (Brachiopoda), a typical shell age distribution. The number of individuals used for this graph is 290. The minimum age is 105 years old while the maximum is 9431 years, with a total range of 9326 years and a median of 503 years (Figure by Tomasovych & Kidwell, 2017).

2.2. Methods

The sampling took place in Cape Greco (34.9851 °N, 34.0762 °E) and Akrotiri (34.5638 °N, 33.0124 °E) (Figure 10) in two different contrasting habitats, *Posidonia* meadow (both leaves and rhizomes) and rocky substrate in 2022.

Figure 10 The sample sites in Cyprus, Cape Greco and Akrotiri. Cape Greco is located in the eastern part, while Akrotiri is situated in the southern part of the island (Picture from Google Earth).

The samples were collected at 10 meters depth by scuba divers, using an air-lift suction sampler and a net, in an area of 1 m² per replicate (Figure 11).The research was conducted during spring and autumn to capture intra-annual variation. In summary, a total of 8 samples were collected, with 3 replicates for each sample, making for a comprehensive dataset. Two replicates from Akrotiri during the spring season were lost. The samples were rinsed in fresh water, dried, and sieved into three distinct fractions, fine (0.5 – 1 mm), medium (1 - 5 mm), and large (> 5 mm). Next, the fractions were stored in bags within boxes and transferred to the laboratory at SZN (see 1.3 Stazione Zoologica Anton Dohrn).

Figure 11 Sampling method in (a) Posidonia meadow and (b) Rocky substrate habitat. The samples were collected in both locations and habitats by scuba divers using an air-lift suction sampler. The sampling area for each replicate was 1 m^2.

Each fraction underwent microscopic analysis to detect and separate living and dead mollusks. To facilitate this process, several essential tools and materials were needed, including a microscope (model: LEICA EZ4), specialized tray, spoon for collecting portions of the sample and transferring them to the tray, fine brush, pointed tweezer to pick the living mollusks, and capsules to put the identified mollusks.

The primary guideline for picking and identifying dead mollusks is to maintain the integrity of the first shell layer. Additionally, the remains of the shell should be at least 50% without being broken or with big holes (predation marks did not count; Albano & Sabelli, 2011). For Gastropoda, when only the apex is available the identification is challenging while for Bivalvia specimens the hinge area is crucial for accurate identification (Albano and Sabelli, 2011). For the project, the data of the DA were given ready (the mollusks were picked and identified).

When picking the living mollusks from the sample and separating them from dead ones, an intact shell was necessary, acknowledging that partial shell damage may be present in certain circumstances. Additional criteria encompass the luminosity of the shell, the absence of extraneous sand or debris within the shell, the distinctive coloration of the shell, and, under specific circumstances, the presence of an operculum or desiccated remnants of the mollusk within the shell (Albano, 2014). Dead mollusks frequently exhibit fractures and predation marks, and they are commonly filled with sediment (Figure 12). Identifying them required heightened attention. Numerous species exhibited striking resemblances, making every detail crucial for accurate identification (Figure 13).

Figure 12 Differences between dead and living mollusks. Predation marks (a), shell field with sediment (b), an empty shell without the dry animal (c), and absence of operculum (d).

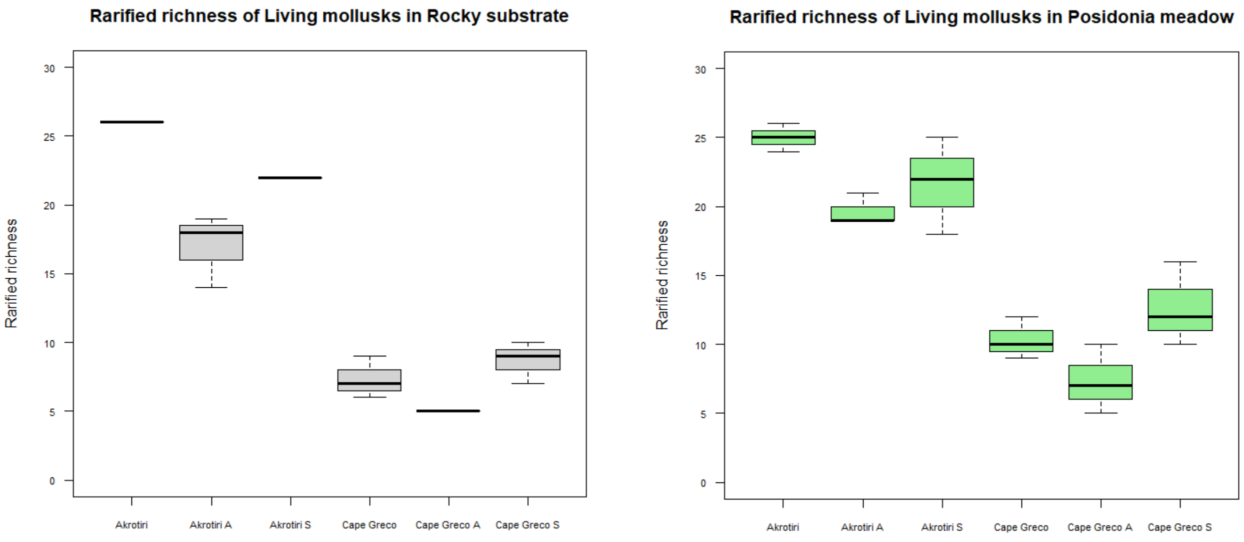
Figure 13 Living mollusks with similarities. In many cases, the species have similar characteristics. In order to identify them it is important to observe their differences. For instance, the shape, the size, the color, the tip, the structure, and the transparency of the shell.

Following the data collection, the next step was to meticulously analyze and interpret the information gathered. The statistical analysis was performed in PAST (https://past.en.lo4d.com/windows), in RStudio (www.r-project.org), and in Microsoft 365 Excel (https://www.microsoft.com/en-us/microsoft-365/excel). Rarified richness (Srar) and evenness (J') were conducted in PAST. The living and death assemblages' comparison was assessed by measuring the taxonomic similarity, the rank-order agreement in species relative abundance, the difference in species richness between living and death assemblages (deltaS), the difference in species evenness between living and death assemblages (deltaPIE), the non-metric multidimensional scaling (NMDS), and the permutational multivariate analysis of variance (PERMANOVA) in RStudio. Population comparison of native versus alien species was performed in Microsoft Excel. Srar represents the total number of the different species present between samples with the same number of individuals. J' is the species evenness in a sample, meaning how evenly the individuals are distributed among those species. This metric ranges from 0 (one or a few species dominating and others having very few individuals) to 1 (all species in the community have the same number of individuals). The taxonomic composition metric is based on Chao et al.'s (2005) abundance based Jaccard index to evaluate differences in living and death assemblages. The index ranges from 0 (no shared species) to 1 (all species occur in both alive and dead). To measure the rank-order agreement in species relative abundance, the correlation coefficient rho of the non-parametric Spearman rank-order test was used. This metric can show if the majority species in the LA are also the majority in the DA. The index ranges from -1 (perfect disagreement) to 1 (perfect agreement). deltaS were done by computing the difference between logarithmic values of species richness of death and living assemblages, $\Delta S = \log10\ SD - \log10\ SL$, with S = number of species, D = dead, and L = living mollusks (Olszewski and Kidwell, 2007). A higher abundance in the DA is expected and equals 1 while a higher abundance in the LA is unexpected and equals -1. The evenness based on the Probability of Interspecific Encounter ($PIE = [N / (N - 1)]\ (1 - \sum Si=1\ p2i)$, where N = number of individuals, S = number of species, pi =proportion of species, was measured at the difference between the samples of dead and living mollusks, $\Delta PIE = PIED - PIEL$, where D = dead and L = living mollusks (Olszewski & Kidwell, 2007) and represents the difference in probability of interspecific encounter between DA and LA. deltaPIE ranges from -1 till 1 with the extremes meaning a complete change in the probability of interspecific encounters between the samples while a value closer to zero would indicate that the probability remains the same. The multivariate analysis was conducted with NMDS to determine the relative similarity of the samples. Finally, the differences in composition between living and death assemblages were tested for significance using PERMANOVA (Anderson, 2001).

3. Results

3.1. Rarified Richness and Species Evenness

The rarified richness (Srar) in Akrotiri is approximately three times the rarified richness in Cape Greco in either habitat, rocky substrate or *Posidonia* meadow. This is displayed in Graph 1. In other words, Akrotiri has a greater diversity of species compared to Cape Greco. In Tables 1 and 2 one can drill down further on the results used for Graph 1 (column Srar).



Graph 1 Rarefied richness of living mollusks in rocky substrate (left) and Posidonia meadow (right). In both habitats, Akrotiri has a higher rarefied richness, with values ranging from 14 to 26 species while Cape Greco's values range from 5 to 16 species. A = autumn, S = spring.

When it comes to species evenness (J') between the two areas it is clear from Tables 1 and 2 columns (J') that Akrotiri exhibits higher evenness which means that the species are more evenly represented in the total population. On the other hand, it is found that in Cape Greco where evenness is lower, there are more dominant species.

Table 1 Srar and J' in Cape Greco. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, D = dead mollusks, L = living mollusks, and 1,2,3 = replicates.

ENTIRE YEAR	Srar	J'	**Spring**	Srar	J'	**Autumn**	Srar	J'
G_Pos_D	26	0.184	G_Pos_D	25	0.184	G_Pos_D	19	0.184
G_R_D	13	0.166	G_R_D	13	0.166	G_R_D	10	0.166
G_Pos_L1	9	0.116	G_Pos_L1	12	0.277	G_Pos_L1	5	0.135
G_Pos_L2	12	0.140	G_Pos_L2	16	0.318	G_Pos_L2	10	0.140
G_Pos_L3	10	0.124	G_Pos_L3	10	0.314	G_Pos_L3	7	0.124
G_R_L1	7	0.151	G_R_L1	9	0.122	G_R_L1	5	0.180
G_R_L2	6	0.227	G_R_L2	7	0.419	G_R_L2	5	0.227
G_R_L3	9	0.217	G_R_L3	10	0.311	G_R_L3	5	0.227

Table 2 Akrotiri during the entire year, spring, and autumn. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, D = dead mollusks, L = living mollusks, and 1,2,3 = replicates.

ENTIRE YEAR	Srar	J'	**Spring**	Srar	J'	**Autumn**	Srar	J'
A_Pos_D	47	0.221	A_Pos_D	45	0.2208	A_Pos_D	33	0.221
A_R_D	39	0.211	A_R_D	37	0.2106	A_R_D	27	0.211
A_Pos_L1	26	0.262	A_Pos_L1	25	0.3106	A_Pos_L1	19	0.339
A_Pos_L2	24	0.248	A_Pos_L2	18	0.3076	A_Pos_L2	19	0.248
A_Pos_L3	25	0.189	A_Pos_L3	22	0.1719	A_Pos_L3	21	0.345
A_R_L1	26	0.311	A_R_L1	22	0.2843	A_R_L1	19	0.603
A_R_L2	NA	NA	A_R_L2	NA	NA	A_R_L2	18	0.428
A_R_L3	NA	NA	A_R_L3	NA	NA	A_R_L3	14	0.250

To visualize biodiversity differences between Cape Greco and Akrotiri, below are two examples of living mollusks in *Posidonia* meadow at 10 m depth during autumn, in Cape Greco (Figure 14) and in Akrotiri (Figure 15) respectively.

Figure 14 A sample of living mollusks in Cape Greco, in Posidonia meadow, in 10 m depth. Green color stands for gastropods while brown is for bivalves. The figure displays 7 gastropods and 1 bivalve.

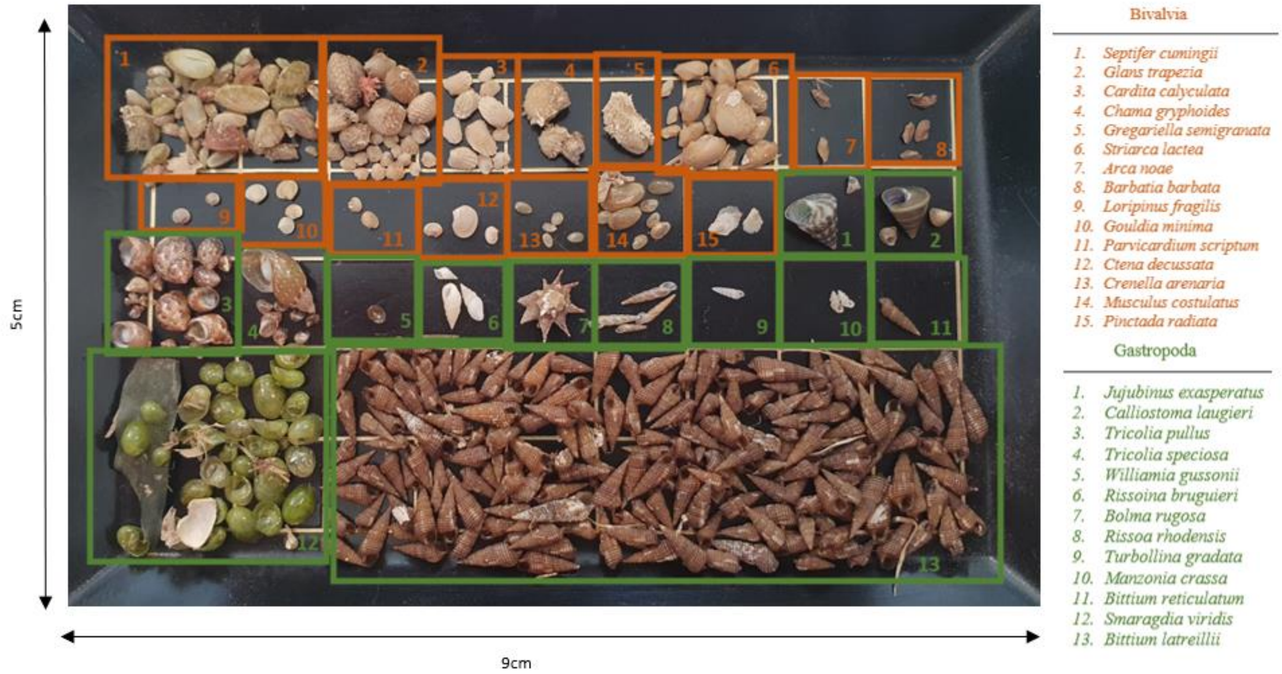
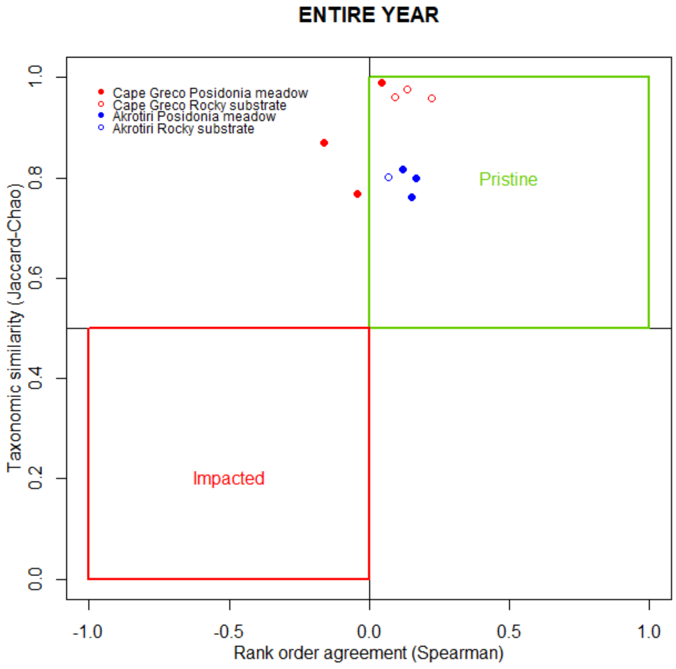


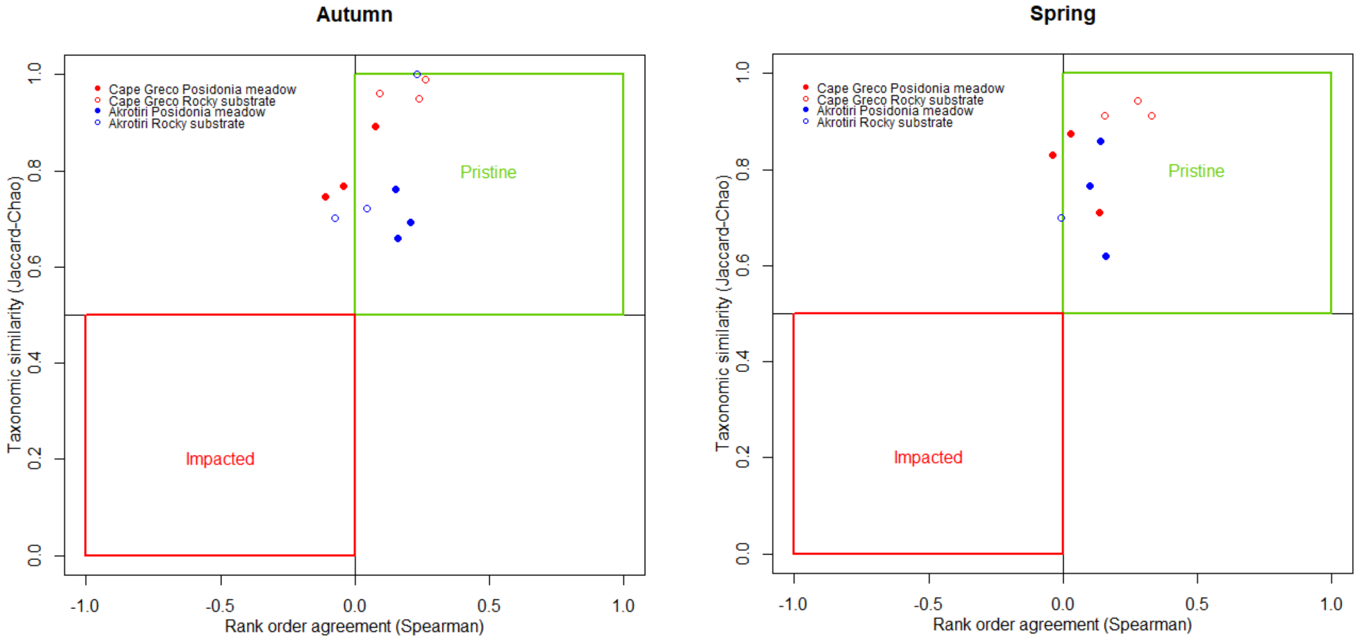
Figure 15 A sample of living mollusks in Akrotiri, in Posidonia meadow, in 10 m depth. Green color stands for gastropods while brown is for bivalves. The figure displays 13 gastropods and 15 bivalves.

3.2. Living and Death assemblages’ comparison

Taxonomic similarity and rank-order agreement in species relative abundance were examined in both areas and habitats, to investigate any potential impact. A combination of the two metrics helps to determine the status of the habitats. Samples scoring close to 1 (in both metrics) are considered with no impact. In Graph 2 this translates to the top right green quadrant. Cape Greco rocky substrate shows pristine conditions while *Posidonia* meadow appears to not be impacted. Akrotiri seems to be more consistent in both habitats as the metrics are almost identical and indicate pristine conditions. When considering seasonal impact (Graph 3) it is noticed that the same pattern appears.

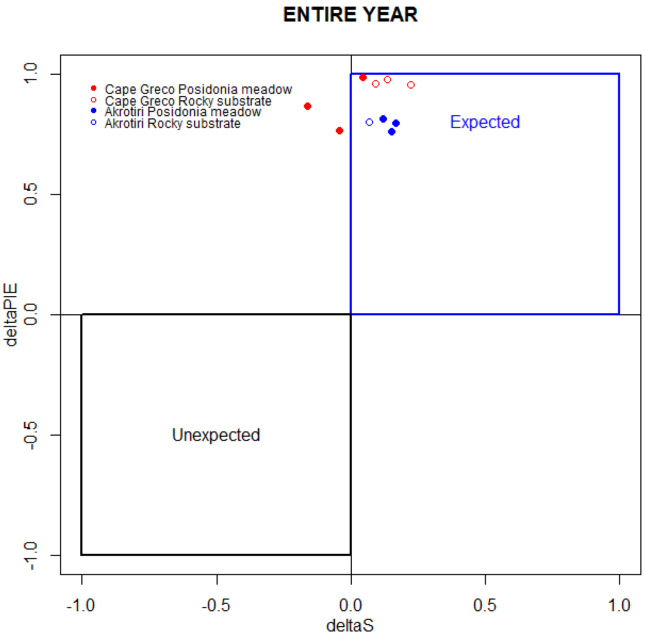


Graph 2 Cross-plot of LD in rank order agreement and taxonomic similarity during the entire year. The rank order agreement for species abundance (x-axis) in Cape Greco Posidonia meadow ranges from -0.2 to 0.1 and in Cape Greco rocky substrate from 0.1 to 0.3. The rank order agreement in Akrotiri hovers around 0.2 and 0.1 for Posidonia meadow and rocky substrate respectively. The taxonomic similarity (y-axis) for Cape Greco Posidonia meadow ranges from 0.7 to 1.0 and rocky substrate around 0.95 while for Akrotiri, it hovers around 0.8 for both habitats.

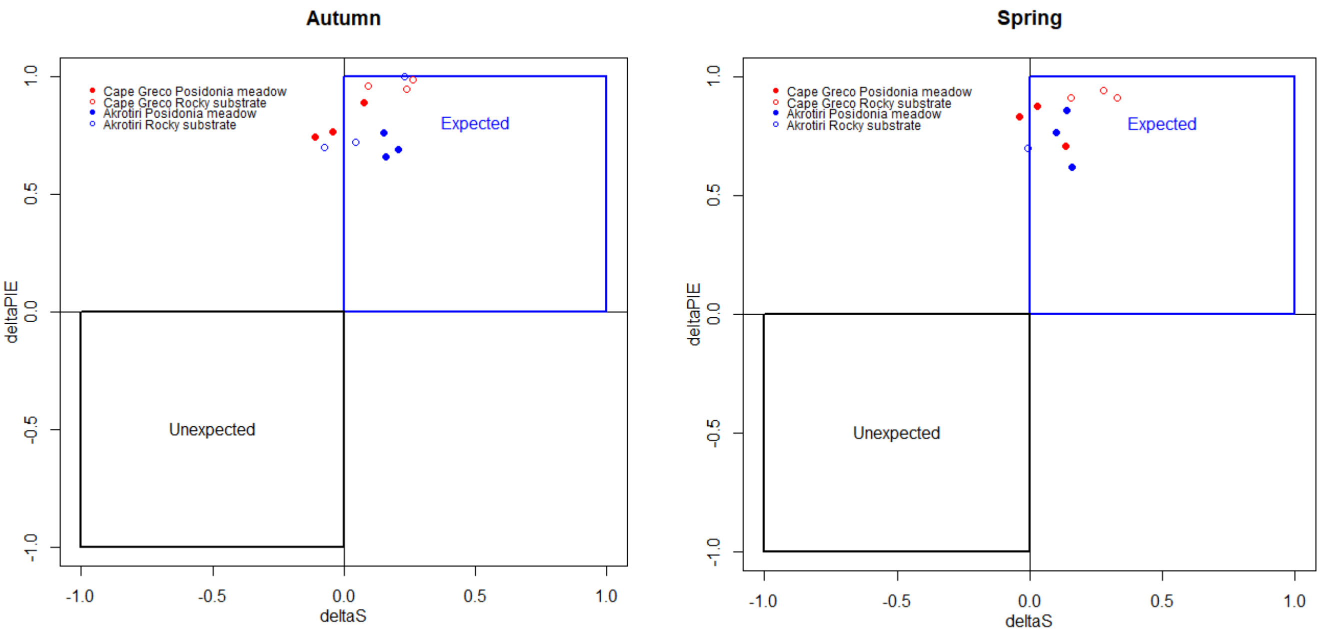


Graph 3 Cross-plots of LD in rank order agreement and taxonomic similarity for autumn (left) and spring (Right). The rank order agreement for species abundance (x-axis) in Cape Greco ranges from -0.1 to 0.1 and 0.1 to 0.3 in Posidonia meadow and rocky substrate respectively for both seasons. The rank order agreement in Akrotiri ranges from -0.1 to 0.2 from both habitats and seasons. The taxonomic similarity (y-axis) in Cape Greco Posidonia meadow ranges from 0.7 to 0.9 for both seasons, while for rocky substrate hovers around 1.0 for autumn and 0.9 in spring. The taxonomic similarity in Akrotiri Posidonia meadow ranges from 0.6 to 0.7 and from 0.6 to 0.8 in autumn and spring respectively while in rocky substrate from 0.7 to 1.0 in autumn and around 0.7 in spring.

To add to the previous findings and create a more complete picture and understanding of the potential changes in the examined areas, the differences in species richness (deltaS) and probability of interspecific encounters (deltaPIE) were also estimated. This was done for both areas and habitats between DA and LA. Findings that would range from 0 to 1 in both metrics can be considered as expected and vice versa for values lower than 0. As shown in Graph 4, for Akrotiri the findings in both habitats indicate that species richness is higher in the DA, as well as the probability of interspecific encounter. A finding that can be considered expected. As for Cape Greco, the findings can be considered also expected although the deltaPIE is significantly closer to 1 and for *Posidonia* meadow deltaS is slightly lower than 0 for most replicates, hinting higher species richness in the LA compared to the DA. Similarly to previous metrics, findings remain unaffected throughout different seasons in the year (Graph 5).

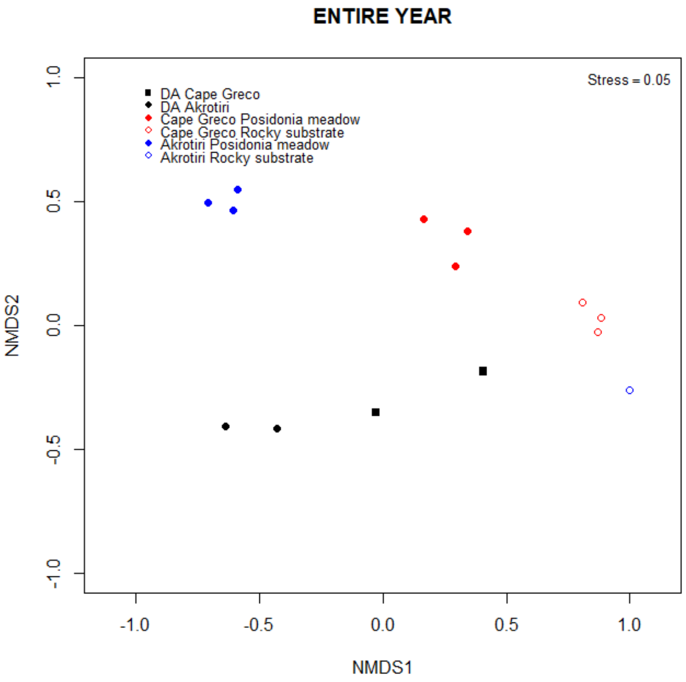


Graph 4 Cross-plot of LD in deltaS and deltaPIE during the entire year. deltaS in Cape Greco Posidonia meadow ranges from -0.2 to 0.1 while in rocky substrate from 0.1 to 0.3. deltaS in Akrotiri is approximately 0.1 for both habitats. deltaPIE in Cape Greco ranges from 0.7 to 1 in Posidonia meadow and is about 1.0 in rocky substrate while the values in Akrotiri hover around 0.7 for both habitats.

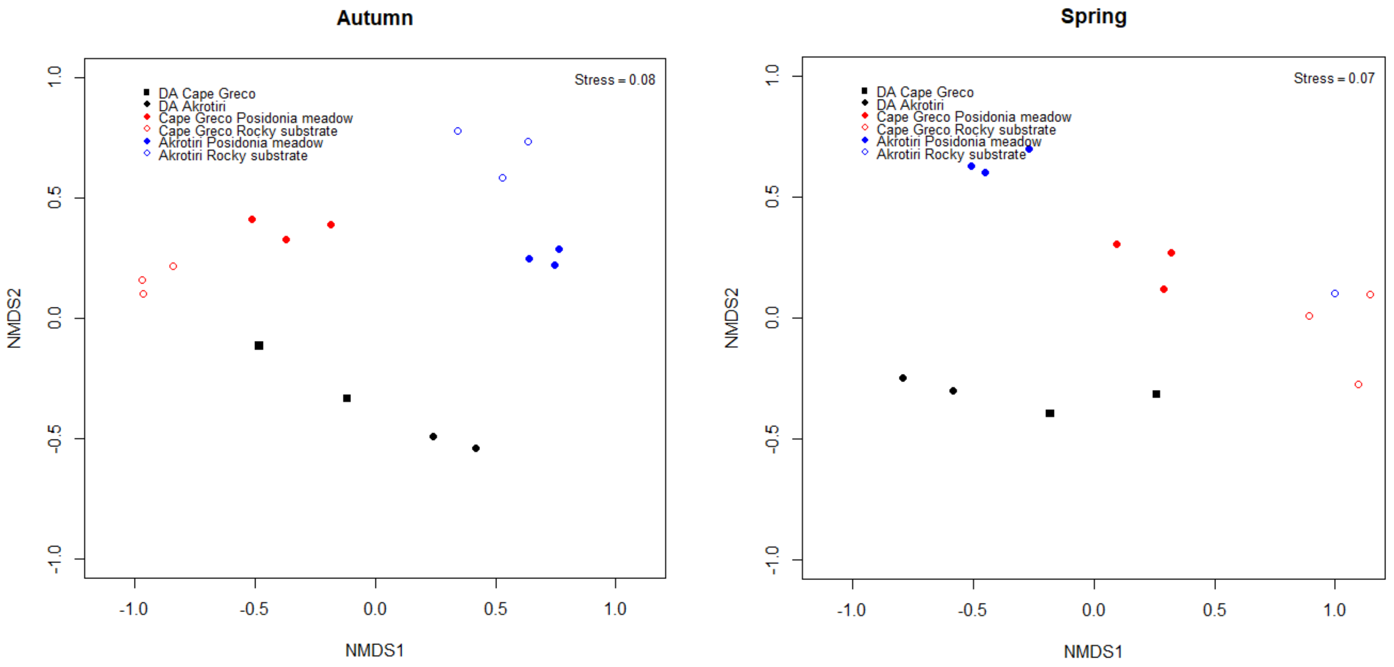


Graph 5 Cross-plots of LD in deltaS and deltaPIE during autumn and spring. deltaS in Cape Greco Posidonia meadow ranges from -0.1 to 0.2 and in rocky substrate from 0.1 to 0.3 in both seasons. deltaS in Akrotiri ranges from -0.1to 0.2 in both habitats and seasons. deltaPIE in Cape Greco ranges from 0.6 to 0.9 in Posidonia meadow and is about 1.0 in rocky substrate while in Akrotiri ranges from 0.5 to 1 for both habitats and seasons.

The differences among the samples and replicates were also considered. The data from both living and death assemblages were combined into a single abundance matrix for analysis using non-parametric multivariate methods. The NMDS plot (Graph 6) indicates differences in the samples between the locations as well as between the habitats. DA samples are also different to LA ones. On the other hand, replicates show resemblance among each other. The same pattern persists when considering this analysis on either spring or autumn (Graph 7). PERMANOVA shows statistically significant results, with p–value = 0.001.



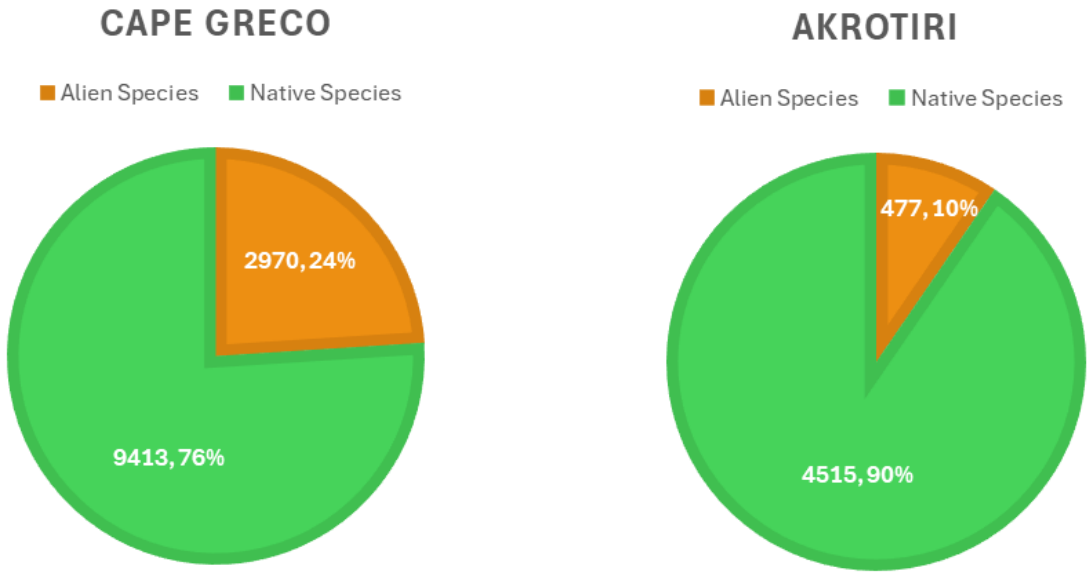
Graph 6 NMDS graph during the entire year. As the data points on the graph draw nearer, their similarity increases (each data point on the graph represents a sample). The graph indicates differences between the locations, Akrotiri and Cape Greco, the habitats, Posidonia meadow and rocky substrate, and the death and living assemblage.



Graph 7 NMDS in autumn (left) and in spring (right). As the samples draw nearer, their similarity increases. The graph indicates differences between the locations, Akrotiri and Cape Greco, the habitats, Posidonia meadow and rocky substrate, and the death and living assemblage, in both seasons.

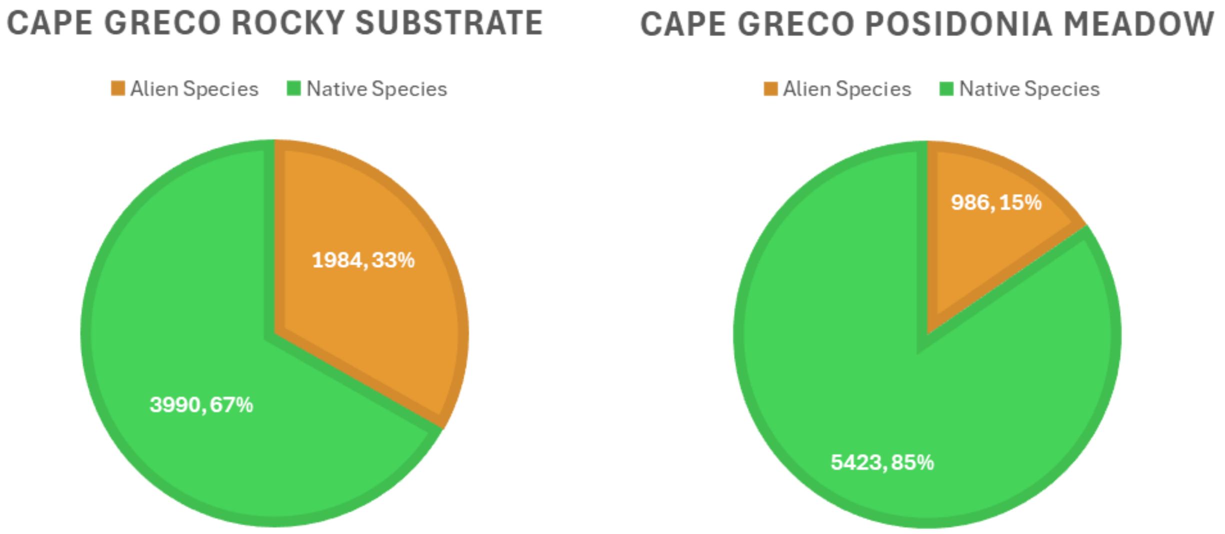
3.3. Alien Species Population

Alien species population in the areas of interest was examined. Graph 8 shows the total population of the alien species in Cape Greco and Akrotiri in LA compared to the native population in LA. Two alien species, *Septifer cumingii* and *Cerithium scabridum*, are those with sufficient sample size in both areas. If both were excluded from the analysis, only 1% of alien species would remain in both areas.

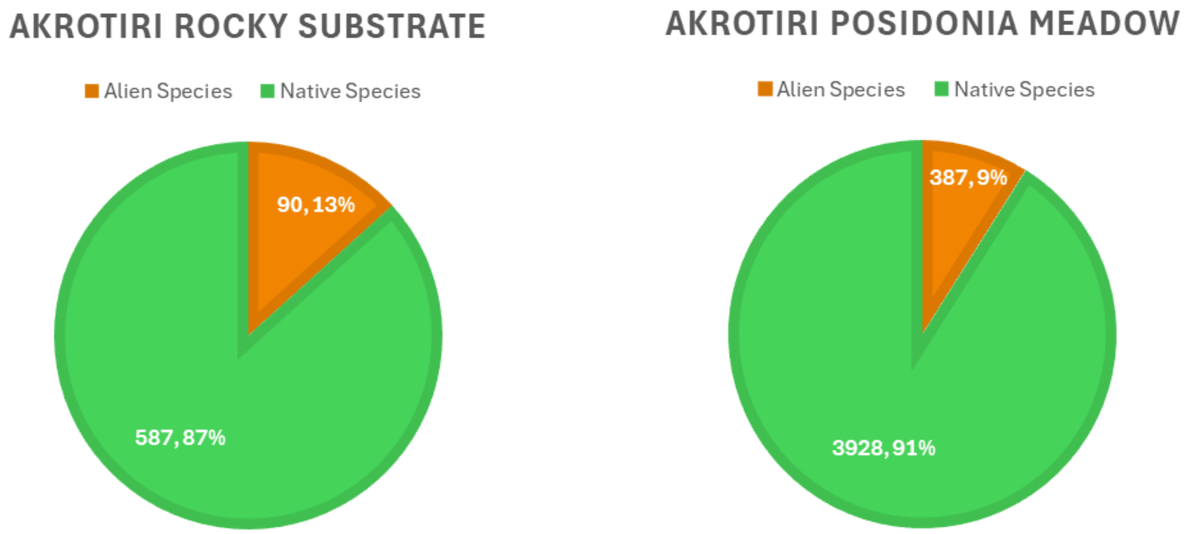


Graph 8 The number of individuals of alien species compared to the number of individuals of native species in Cape Greco (left) and Akrotiri (right). 74% of the population in Cape Greco is native species and the remaining 24% is alien species. 90% of the population in Akrotiri is native species while 10% is alien species.

Analyzing further the alien population results, in Cape Greco the rocky substrate has twice as many alien species as *Posidonia* meadow (Graph 9). In Akrotiri, there is no indication of differences between the two habitats (Graph 10).



Graph 10 The number of individuals of alien species compared to the number of individuals of native species in Cape Greco, in rocky substrate (left) and in Posidonia meadow (right). In rocky substrate 33% of the total population consists of alien species while in Posidonia meadow only 15% does.



Graph 9 The number of individuals of alien species compared to the number of individuals of native species in Akrotiri, in rocky substrate (left) and in Posidonia meadow (right). In rocky substrate and Posidonia meadow, alien species make up 13% and 9% respectively of the total population.

4. Discussion

In the Mediterranean Sea a temperature gradient from the Western to the Eastern Mediterranean can be observed, with the Eastern region being notably warmer (see 1.1. General Information, Figure 1). Typically, the annual SST in the Eastern Mediterranean Sea ranges from 20 to 22 °C (see 1.1. General Information, Figure 2), placing it between the cooler Atlantic Ocean (the donor of the Mediterranean species) and the warmer tropic Red Sea. As temperatures approach extremes (above 22 °C), only a select few species are capable of surviving.

As expected, Akrotiri proved to be more diverse, having approximately three times the species richness of Cape Greco and exhibits a higher evenness in its species population. With the most pronounced difference between the two areas being the upwelling phenomenon that keeps Akrotiri cooler than Cape Greco, it is an indication that the differences observed between the two areas occur due to the difference in water temperature, a result of climate change. According to Lawes et al. (2007), refugia exhibit higher species richness than the surrounding areas. As a result, the pristine condition in Akrotiri indicates a potential refugium for native species.

To draw better conclusions, examining how biodiversity patterns evolved in species richness and evenness through time between the two areas was necessary, the LD analysis was performed for this purpose. Having established Akrotiri as the more diverse of the two areas, the expectation was to come to the finding that Cape Greco is impacted due to climate change while Akrotiri shows resilience. Although Akrotiri seems to be in pristine condition when comparing LA and DA samples, that is also the case for Cape Greco. This is a finding that contrasts with the expectation for Cape Greco. It is important to consider the factors that could affect these findings. The DA in both areas is relatively young, with 95% of the shells being limited to a few decades (Albano et al., 2023). This suggests an already shifted baseline and an environmental impact that could have already taken place (Albano et al., 2023). The main reason behind the young DA in the rocky substrate is the transportation of the older death mollusks out of the habitat (Albano et al., 2023). In *Posidonia* meadow, it is mainly because of the dense structure of the root and rhizome system that buries the dead shells fast and limits the process of bioturbation (Albano et al., 2023).

When considering the different habitats within the two areas, *Posidonia* meadow appears to have greater rarefied richness compared to the rocky substrate, mainly in Cape Greco. One of the contributing factors could be the elevated oxygen concentration, which enhances thermal tolerance by up to 4 °C; a substantial characteristic distinguishing between Akrotiri and Cape Greco (see 1.5. Habitat, paragraph 4). These findings may imply that thriving seagrass meadows serve as refugia for native biodiversity, shielding them from the impacts of warming oceans and alien species (Pisano et al. 2020).

Another important consideration is the existence of alien species in the area (due to migration via the Suez Canal). The alien species population in Cape Greco is higher than in Akrotiri. When it comes to habitats, rocky substrate has a higher alien population than *Posidonia* meadow, mainly in Cape Greco. However, the total number of non-indigenous species could be considered low. Excluding the two most prominent alien species from the analysis, the alien population drops to 1% in all cases, exerting a minimal negative influence on local biodiversity. Additionally, there appears to be limited potential for active resource competition between native and non-indigenous species in the shallow benthic environment (Albano et al. 2021).

All in all, the area appears not to be heavily impacted especially when comparing it to nearby areas such as Israel. Despite the recorded trend of increasing SST in Cyprus, Cape Greco is currently positioned in the 22 °C annual isotherm (see 1.1. General Information, Figure 2). An increase of 1.5 °C compared to the 1982 - 1993 period, but still 1 °C lower than temperatures observed on the Israeli shelf, where the most significant impact has already occurred (Pisano et al., 2020).

5. Conclusion

The biodiversity of the Mediterranean Sea faces significant challenges, most likely due to increasing temperatures driven by climate change. This phenomenon has led to a decline in native mollusk diversity richness, highlighting the urgent need for conservation efforts. Akrotiri demonstrates remarkable diversity attributed to lower temperatures, contrasting with Cape Greco's comparatively lower species richness. *Posidonia* meadows emerge as vital refugia for native biodiversity, with their ability among others to buffer ocean warming effects and enhance thermal tolerance. In all cases the presence of alien species appears minimal, suggesting limited active resource competition in shallow benthic environments. Surprisingly, key metrics reveal pristine conditions in both Akrotiri and Cape Greco, challenging initial assumptions. The death assemblage in both areas is relatively young, limiting the ability to serve as useful baselines, while Cyprus is less impacted compared to neighboring Israel. Akrotiri's pristine condition and higher species richness hint at its potential as a refugium for native species. Overall, these findings underscore the importance of understanding and preserving the unique ecological dynamics of the Mediterranean region amidst ongoing environmental changes.

5.1. Outlook

Moving forward, further research efforts could focus on several key areas to enhance our understanding and conservation efforts in the Mediterranean region. Firstly, investigating the long-term impacts of climate change on native species and their habitats is crucial, particularly considering the potential for continued warming trends. Additionally, alternative research methods could be explored, as the death assemblage may not provide a reliable baseline for assessing biodiversity changes that occurred in the last decades. Moreover, class-specific research could offer valuable insights. Although not the focus of this project, an interesting finding is the difference in bivalves' population between the two areas and habitats (see Appendix B – Additional Graphs, Graph 13). Furthermore, in-depth studies on the interactions between native and non-indigenous species, as well as their responses to changing environmental conditions, would provide valuable insights into ecosystem dynamics and resilience. Additionally, exploring the role of potential refugia, such as Akrotiri, in supporting native biodiversity under changing climatic conditions warrants attention. Moreover, examining the effectiveness of conservation measures, including habitat restoration and invasive species management, could be essential for mitigating biodiversity loss and preserving ecosystem health. Overall, a multidisciplinary approach encompassing ecological, climatological, and conservation perspectives is needed to address the complex challenges facing the Mediterranean Sea in the coming years.

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Appendix A – Photographic Material

Photographic material captured from samples collected at SZN, Figures 16, 17, and 18.

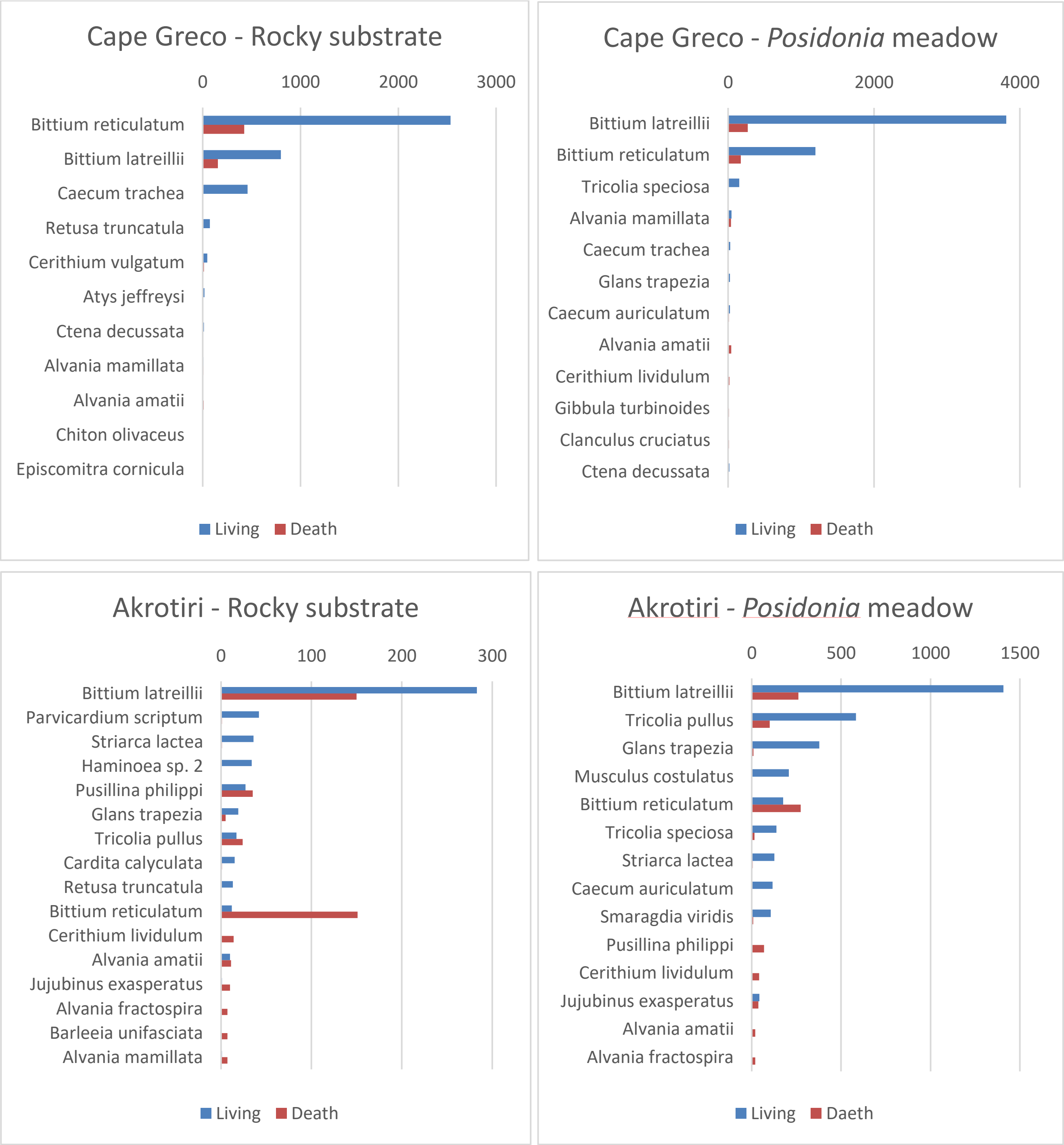
Figure 16 All living samples that underwent microscopic analysis at SZN.

Figure 17 All living mollusks that were picked and identified at SZN.

Figure 18 10 dead mollusks on the left and 10 living mollusks from the same species on the right. The photographic material was taken under the microscope at SZN laboratory. That picture was taken in order to show the differences between living and dead mollusks. Living mollusks have more colorful and shiny shells with the presence of the dry animal to many of them. Dead mollusks are usually filled with sediment, the shell is less colorful and shiny, with no indication of dry animal in it.

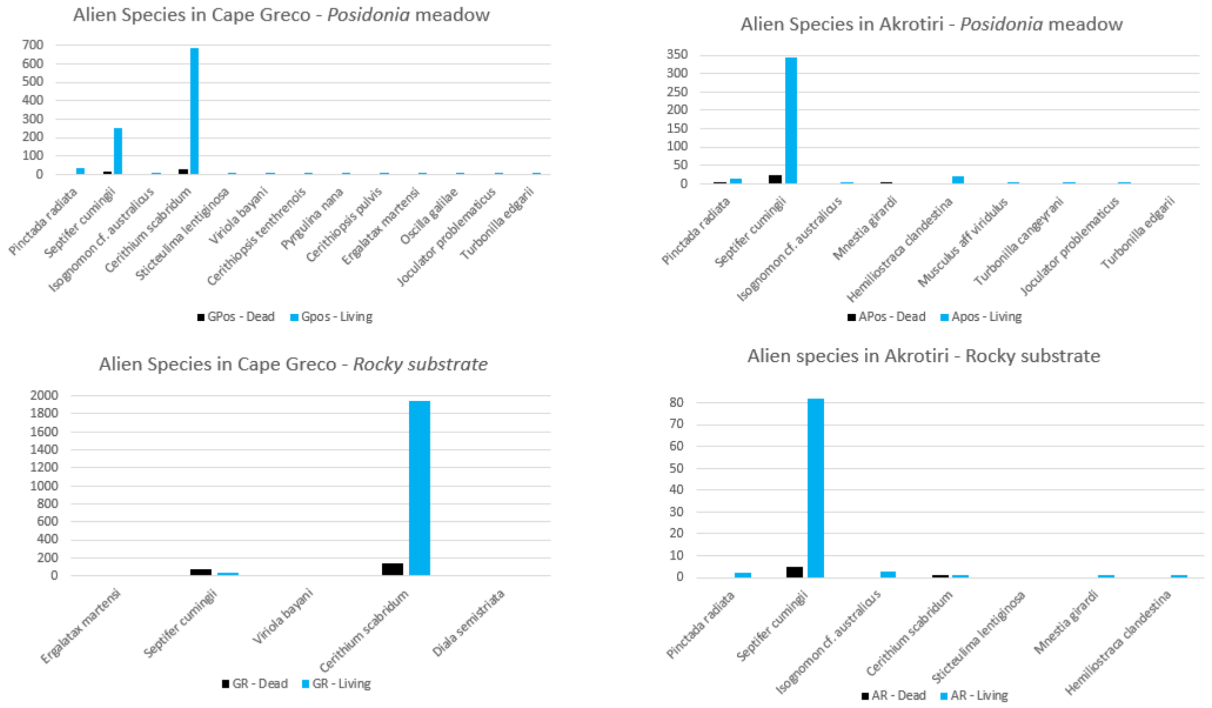
Appendix B – Additional Graphs

Combined representation of the ten most common native species among living mollusks and the ten most common among dead mollusks, Graph 11.



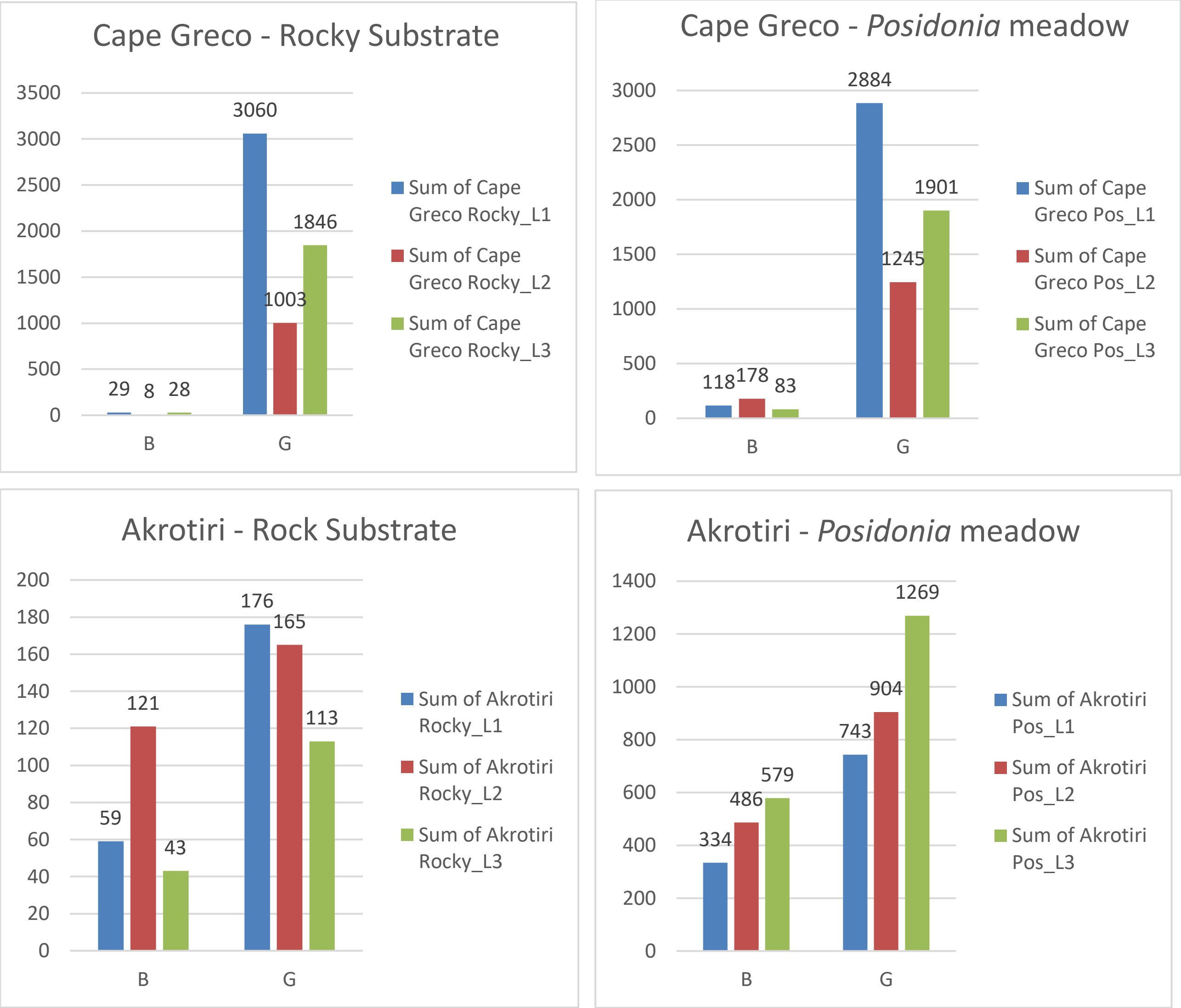
Graph 11 The 10 most common native species of living and dead mollusks. In all cases, 1) Cape Greco – Rocky substrate, 2) Cape Greco – Posidonia meadow, 3) Akrotiri – Rocky substrate, and 4) Akrotiri – Posidonia meadow, the most common species of DA and LA have similar patterns. In Cape Greco, two to three dominant species are consistently found in both habitats, appearing in both dead and living samples. The remaining species are also identical, with some exceptions. In Akrotiri – rocky substrate, one to two dominant species prevail, with one species in common, while numerous species, a few of which are shared between living and dead species exhibit substantial abundance. A similar pattern is observed in Akrotiri – Posidonia meadow, where dominance is not as pronounced.

Graph 12 indicates the alien species individuals in each habitat and locaiton for both death and living assamblages.



Graph 12 Alien species in Cape Geco in Posidonia meadow and rocky substrate (top and bottom left respectively) and Akrotiri in Posidonia meadow and rocky substrate (top and bottom right respectively).

The comparison between the total number of bivalves and the total number of gastropods in the samples indicates that Cape Greco's bivalves' population is limited. Graph 13 shows that in Cape Greco rocky substrate the number of gastropods is 100 times more than the number of bivalves and in Posidonia meadow approximately 20 time more. On the other hand, in Akrotiri, gastropods are only approximately 2 times more than bivalves for both habitats.



Graph 13 The total number of individuals of bivalves and gastropods. Cape Greco rocky substrate and Posidonia meadow are characterized by a significant presence of gastropods, while the number of bivalves is minimal. Akrotiiri rocky substrate and Posidonia meadow exhibits approximately double the number of gastropods compared to bivalves. Pos = Posidonia, L = living mollusks, and 1,2,3 = replicates.

Appendix C – Additional Tables

Table 3, 4, and 5 show the abundance (N), the observed species richness (Sobs), the rarified richness (Srar), and the species evenness (J') for the entire year, autumn, and spring respectively.

Table 3 N, Sobs, Srar, and J' for the entire year using PAST. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, D = dead mollusks, L = living mollusks, and 1,2,3 = replicates.

ENTIRE YEAR	N	Sobs	Srar	J'
G_Pos_D	717	56	26	0.184
G_R_D	882	32	13	0.166
A_Pos_D	1318	141	47	0.221
A_R_D	567	90	39	0.211
G_Pos_L1	3002	32	9	0.116
G_Pos_L2	1423	30	12	0.140
G_Pos_L3	1984	30	10	0.124
G_R_L1	3089	24	7	0.151
G_R_L2	1011	17	6	0.227
G_R_L3	1874	22	9	0.217
A_Pos_L1	1077	58	26	0.262
A_Pos_L2	1390	51	24	0.248
A_Pos_L3	1848	68	25	0.189
A_R_L1	235	33	26	0.311
A_R_L2	NA	NA	NA	NA
A_R_L3	NA	NA	NA	NA

Table 4 N, Sobs, Srar, and J' for autumn using PAST. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, D = dead mollusks, L = living mollusks, and 1,2,3 = replicates.

AUTUMN	N	Sobs	Srar	J'
G_Pos_D	717	56	19	0.184
G_R_D	882	32	10	0.166
A_Pos_D	1318	141	33	0.221
A_R_D	567	90	27	0.212
G_Pos_L1	2447	21	5	0.135
G_Pos_L2	1423	30	10	0.140
G_Pos_L3	1536	26	7	0.124
G_R_L1	2390	18	5	0.180
G_R_L2	1011	17	5	0.227
G_R_L3	1166	15	5	0.227
A_Pos_L1	554	41	19	0.339
A_Pos_L2	1390	51	19	0.248
A_Pos_L3	804	45	21	0.345
A_R_L1	92	20	19	0.603
A_R_L2	286	29	18	0.428
A_R_L3	156	20	14	0.250

Table 5 N, Sobs, Srar, and J' for spring using PAST. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, D = dead mollusks, L = living mollusks, and 1,2,3 = replicate.

SPRING	N	Sobs	Srar	J'
G_Pos_D	717	56	25	0.184
G_R_D	882	32	13	0.166
A_Pos_D	1318	141	45	0.221
A_R_D	567	90	37	0.211
G_Pos_L1	555	23	12	0.277
G_Pos_L2	361	23	16	0.318
G_Pos_L3	448	15	10	0.314
G_R_L1	700	18	9	0.122
G_R_L2	230	8	7	0.419
G_R_L3	708	16	10	0.311
A_Pos_L1	523	46	25	0.317
A_Pos_L2	424	28	18	0.308
A_Pos_L3	1044	57	22	0.172
A_R_L1	143	23	22	0.284
A_R_L2	NA	NA	NA	NA
A_R_L3	NA	NA	NA	NA

The analytical LD comparison results for the entire year are shown in Table 6, for autumn in Table 7, and for spring in Table 8. More specifically the results are the Spearman's Rank Correlation Coefficient (Spearman rho), the Spearman's p-value (Spearman p), the taxonomic similarity (Jaccard-Chao), the difference in species richness (deltaS), and the difference in species evenness (deltaPIE).

Table 6 Spearman rho, Spearman p. Jaccard-Chao, deltaS, and deltaPIE for the entire year using R Studio. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, L = living mollusks, and 1,2,3 = replicates.

ENTIRE YEAR	Spearman rho	Spearman p	Jaccard-Chao	deltaS	deltaPIE
G_Pos_L1	-0.163	0.175	0.869	0.520	0.194
G_Pos_L2	-0.044	0.719	0.768	0.369	0.209
G_Pos_L3	0.045	0.715	0.990	0.435	0.193
G_R_L1	0.223	0.145	0.959	0.347	0.046
G_R_L2	0.091	0.577	0.960	0.310	0.026
G_R_L3	0.136	0.390	0.977	0.290	-0.028
A_Pos_L1	0.122	0.128	0.817	0.354	0.022
A_Pos_L2	0.151	0.061	0.762	0.449	0.059
A_Pos_L3	0.167	0.034	0.798	0.371	0.082
A_R_L1	0.068	0.503	0.802	0.214	0.095
A_R_L2	NA	NA	NA	NA	NA
A_R_L3	NA	NA	NA	NA	NA

Table 7 Spearman rho, Spearman p. Jaccard-Chao, deltaS, and deltaPIE for autumn using R Studio. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, L = living mollusks, and 1,2,3 = replicates.

AUTUMN	Spearman rho	Spearman p	Jaccard-Chao	deltaS	deltaPIE
G_Pos_L1	-0.110	0.382	0.746	0.635	0.263
G_Pos_L2	-0.044	0.719	0.768	0.369	0.209
G_Pos_L3	0.076	0.542	0.891	0.464	0.241
G_R_L1	0.238	0.135	0.950	0.436	0.090
G_R_L2	0.091	0.577	0.960	0.310	0.026
G_R_L3	0.264	0.114	0.990	0.386	0.089
A_Pos_L1	0.161	0.050	0.660	0.385	0.025
A_Pos_L2	0.151	0.061	0.762	0.449	0.059
A_Pos_L3	0.208	0.010	0.693	0.415	0.028
A_R_L1	0.233	0.025	1.000	0.171	-0.015
A_R_L2	-0.076	0.450	0.702	0.321	-0.020
A_R_L3	0.045	0.663	0.721	0.321	0.257

Table 8 Table 7 Spearman rho, Spearman p. Jaccard-Chao, deltaS, and deltaPIE forspring using R Studio. G = Cape Greco, A = Akrotiri, Pos = Posidonia meadow, R = Rocky substrate, L = living mollusks, and 1,2,3 = replicates.

SPRING	Spearman rho	Spearman p	Jaccard-Chao	deltaS	deltaPIE
G_Pos_L1	-0.037	0.771	0.831	0.343	0.009
G_Pos_L2	0.029	0.819	0.875	0.259	0.004
G_Pos_L3	0.136	0.306	0.710	0.488	0.083
G_R_L1	0.280	0.084	0.942	0.204	0.419
G_R_L2	0.331	0.056	0.912	0.326	0.070
G_R_L3	0.155	0.347	0.911	0.257	0.010
A_Pos_L1	0.138	0.092	0.858	0.324	0.028
A_Pos_L2	0.161	0.051	0.619	0.496	0.129
A_Pos_L3	0.100	0.213	0.765	0.356	0.136
A_R_L1	-0.006	0.956	0.699	0.237	0.214
A_R_L2	NA	NA	NA	NA	NA
A_R_L3	NA	NA	NA	NA	NA

PERMANOVA results indicate statistically significant results for the entire year and the two seasons (Table 9).

Table 9 PERMANOVA results. F-statistic (Fd) = the ratio of variability between groups to variability within groups, R^2 = the proportion of variance in the data explained by the grouping variable(s), and p-value (p) = the probability of obtaining the observed Fd (or more extreme) if the null hypothesis were true.

	Fd	R^2	p
Entire Year	5.93	0.25	0.001
Autumn	7.02	0.24	0.001
Spring	7.11	0.28	0.001