



Universitat
de les Illes Balears

DOCTORAL THESIS

2023

**EPIPHYTIC FORAMINIFERA AS POTENTIAL
BIOINDICATORS IN *POSIDONIA OCEANICA* MEADOWS
IN THE BALEARIC ARCHIPELAGO (WESTERN
MEDITERRANEAN)**

Anna Khokhlova



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Doctor by the Universitat de les Illes Balears

*СЛАВА УКРАЇНІ
ГЕРОЯМ СЛАВА*

Борітеся — поборете!
Вам Бог помагає!
За вас правда, за вас слава.
І воля святая!

*Keep fighting — you are sure to win!
God helps you in your fight!
For fame and freedom march with you,
And right is on your side!*

Taras Shevchenko
"The Caucasus"
1845, Pereyaslav

(Translated by John Weir)

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FIGURE 17. Map with a general view of the sampling sites of the study areas associated with the three main objectives of the thesis:

A. Objective 1. General view of the sampling sites in the Western Mediterranean and Indian Ocean, where: 1. Locations of the Mallorca sampling stations: a) Sa Foradada (39°44'47.02"N, 2°36'58.47"E); b) Sant Elm (39°34'43.76"N, 2°20'55.59"E); 2. Detailed location of the Madagascar sampling site (Indian Ocean): c) Diego Suárez (12°12'52.54"S, 49°21'59.46"E).

B. Objective 2. Map of Mallorca and Cabrera Islands (Spain) in the Mediterranean Sea. 1. Mallorca Island with the detailed locations of the sampling stations in Port d'Andratx and Magaluf; 2. Cabrera Island with two sampling stations from Cala Santa Maria (Cabrera1 and Cabrera2).

C. Objective 3. General view of the locations from historical series of data of Mallorca (1): Cala Agulla (Ma-1-64), Cala Mendia (Ma-2-64), Cala Murada (Ma-3-64), Portocolom (Ma-4-64), Porto Petro (Ma-5-64), Cala Santanyí (Ma-6-64), Es Caragol (Ma-7-64), Colònia de Sant Jordi (Ma-8-64), Ses Covetes (Ma-9-64), Sa Ràpita (Ma-10-64), Cala Blava (Ma-11-65), S'Arenal (Ma-12-65), Port de Sóller (Ma-13-65), Camp de Mar (Ma-14-65), Santa Ponça (Ma-15-65) (Mateu, 1970); Ciutat Jardí (Ma-6-82), Portals Nous (Ma-7-82), Cap de Regana (Ma-8-84) (Gazá, 1988); Cala Fornells (Ma-17-89, Ma-1-90, Ma-7-91), Portocolom (Ma-22-89, Ma-6-90, Ma-12-91), Cala Murta (Ma-18-89, Ma-2-90, Ma-8-91), Es Barcarès (Ma-19-89, Ma-3-90, Ma-9-91), Es Carnatge (Ma-20-89, Ma-4-90, Ma-10-91), Port de Sóller (Ma-21-89, Ma-5-90, Ma-11-91) (Abril, 1993); Badia de Pollença (Ma-9-85, Ma-10-85) (Moreiro, 1993); Port de Sóller (Ma-1-00) (Mateu et al., 2001); Cala d'Or (Ma-2-08 and Ma-3-08), and Portals Vells (Ma-4-08 and Ma-5-08) (Mateu-Vicens et al., 2010); Cala Blava (Ma-5-12), Magaluf (Ma-8-12), Portals Nous (Ma-10-12), Son Verí (Ma-11-12), Ses Illetes (Ma-12-12), Santa Ponça (Ma-13-12), (Mateu-Vicens et al., 2014); Cala Blava (Ma-6.1-12 and Ma-6.2-12) (Racis, 2015); Port de Sóller (Ma-16-14) (Vaquer, 2016); Sant Elm (Ma-14-12), Sa Foradada (Ma-15-12) (Mateu-Vicens et al., 2016); Magaluf (Ma-7-12), Port d'Andratx (Ma-9-12); Alcanada (Ma-1-21 and Ma-2-22), Ca'n Picafort (Ma-3-21 and Ma-4-22), Cala Blava (Ma-5-21 and Ma-6-22), Cala Brafi (Ma-7-21 and Ma-8-22), Cala de Santa Ponça (Ma-9-21 and Ma-10-22), Capdepera (Ma-11-21 and Ma-12-22), Es Caragol (Ma-13-21 and Ma-14-22), Marina de Bonaire (Ma-15-21 and Ma-16-22), Port d'Andratx (Ma-17-21 and Ma-18-22), Port de Sóller (Ma-19-21 and Ma-20-22), Ses Illetes (Ma-21-21 and Ma-22-22) (Khokhlova and Mateu-Vicens, 2023). Menorca (2): Ses Fontanelles (Me-1-82), Cala Mitjana (Me-2-82), Cala En Blanes (Me-3-82), Arenal d'en Castell (Me-4-82) and Cala Galdana (Me-5-82) (Florit, 1983); Me-11-87, Ma-12-87, Me-13-87, Me-14-87, Me-15-87, Me-16-87 (Moreiro, 1993); and Cabrera (3): Cala Santa Maria (Ca-2-11), and Els Estels (Ca-4-12) (Mateu-Vicens et al., 2014); and Ca-3-12. Green area represents *Posidonia oceanica* coverage (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

FIGURE 18. Foraminiferal genera and their morphotype classification based on Mateu-Vicens et al. (2014), reported in the present study. AF*: 1 – *Planorbulina*, 2a, b – *Nubecularia*, 3 – *Cibicidella*, 4 – *Spirillina*; AR*: 5 – *Miniacina*; LM: 6 – *Peneroplis*, 7 – *Sorites*, 8 – *Marginopora*; LR: 9 – *Amphistegina*; B: 10a, b – *Rosalina*, 11a, b – *Lobatula*, 12 – *Cibicides*, C: 13 – *Elphidium*, 14 – *Astrononion*; D*: 15 – *Massilina*, 16 – *Bolivina*, 17 – *Sigmoilinita*.

FIGURE 19. Ecological status of coastal waters in 2017 using the POMI (*Posidonia oceanica* Multivariate Index), based on the combination of 11 metrics (modified from Santandreu et al. (2019)).

FIGURE 20. Orthophotos of Cala Santa Maria (Cabrera) from 1956 to 2019. A) 1956; B) 1984; C) 1989-90; D) 2002; E) 2006; F) 2008; G) 2010; H) 2012; I) 2015; J) 2018-19. Red arrows indicate the holes without *P. oceanica* coverage probably resulting from the military exercises. Green arrows indicate the same area before the military exercises without visible signs of damage. (<https://ideib.caib.es/visor/?locale=es>)

FIGURE 21. Map showing the sampling locations included in the historical data series of Mallorca: Cala Agulla (Ma-1-64), Cala Mendia (Ma-2-64), Cala Murada (Ma-3-64), Portocolom (Ma-4-64), Porto Petro (Ma-5-64), Cala Santanyí (Ma-6-64), Es Caragol (Ma-7-64), Colònia de Sant Jordi (Ma-8-64), Ses Covetes (Ma-9-64), Sa Ràpita (Ma-10-64), Cala Blava (Ma-11-65), S'Arenal (Ma-12-65), Port de Sóller (Ma-13-65), Camp de Mar (Ma-14-65), Santa Ponça (Ma-15-65) (Mateu, 1970).

- FIGURE 22.** Map showing the sampling locations of historical data series of Menorca: Ses Fontanelles (Me-1-82), Cala Mitjana (Me-2-82), Cala En Blanes (Me-3-82), Arenal d'en Castell (Me-4-82) and Cala Galdana (Me-5-82) (Florit, 1983).
- FIGURE 23.** Map showing the sampling locations of historical data series of Mallorca (Badia de Palma): Ciutat Jardí (Ma-6-82), Portals Nous (Ma-7-82), Cap de Regana (Ma-8-84) (Gazá, 1988).
- FIGURE 24.** Map showing the sampling locations of historical data series of Mallorca: Cala Fornells (Ma-17-89, Ma-1-90, Ma-7-91), Portocolom (Ma-22-89, Ma-6-90, Ma-12-91), Cala Murta (Ma-18-89, Ma-2-90, Ma-8-91), Es Barcarès (Ma-19-89, Ma-3-90, Ma-9-91), Es Carnatge (Ma-20-89, Ma-4-90, Ma-10-91), Port de Sóller (Ma-21-89, Ma-5-90, Ma-11-91) (Abril, 1993).
- FIGURE 25.** Map showing the sampling locations of historical data series of Mallorca: Badia de Pollença (Ma-9-85, Ma-10-85) and South Menorca (Me-11-87, Me-12-87, Me-13-87, Me-14-87, Me-15-87, Me-16-87) (Moreiro, 1993).
- FIGURE 26.** Map showing the sampling locations of historical data series of Mallorca: Cala d'Or (Ma-2-08 and Ma-3-08), and Portals Vells (Ma-4-08 and Ma-5-08) (Mateu-Vicens et al., 2010).
- FIGURE 27.** Map showing the sampling locations of historical data series of Mallorca: Cala Blava (Ma-5-12), Magaluf (Ma-8-12), Portals Nous (Ma-10-12), Son Verí (Ma-11-12), Ses Illetes (Ma-12-12), Santa Ponça (Ma-13-12); Cabrera: Cala Santa Maria (Ca-2-11), and Els Estels (Ca-4-12) (Mateu-Vicens et al., 2014).
- FIGURE 28.** Map showing the sampling locations of historical data series of Mallorca: Alcanada (Ma-1-21 and Ma-2-22), Ca'n Picafort (Ma-3-21 and Ma-4-22), Cala Blava (Ma-5-21 and Ma-6-22), Cala Brafi (Ma-7-21 and Ma-8-22), Cala de Santa Ponça (Ma-9-21 and Ma-10-22), Capdepera (Ma-11-21 and Ma-12-22), Es Caragol (Ma-13-21 and Ma-14-22), Marina de Bonaire (Ma-15-21 and Ma-16-22), Port d'Andratx (Ma-17-21 and Ma-18-22), Port de Sóller (Ma-19-21 and Ma-20-22), Ses Illetes (Ma-21-21 and Ma-22-22) (Khokhlova and Mateu-Vicens, 2023).
- FIGURE 29.** Percentage (%) of genera collected per morphotype and locality in this study.
- FIGURE 30.** Dual isotope scatter plot ($\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$) for each morphotype. A. Sa Foradada replicates; B. Sant Elm replicates; C. Madagascar replicates.
- FIGURE 31.** MDS analysis for morphotypes and localities of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ‰. F5 Sa Foradada (Mallorca, Western Mediterranean); SE5 Sant Elm (Mallorca, Western Mediterranean); M5 Diego Suárez (NE Madagascar, Indian Ocean).
- FIGURE 32.** Average squared distance of isotope values between localities and between morphotypes. Summary of SIMPER analyses.
- FIGURE 33.** In white squares ANOVA Model Box-plot of ecological indices values and FAI all sampling sites: A) FI', B) I_{LS}, C) FSI, D) H', and E) FAI; in grey squares ANOVA Model Box-plot of heavy metal concentrations from abnormal benthic foraminifera tests in every sampling site: F) Zn, G) Pb, H) Cu, I) Ni and J) As. Here "Andratx" means sampling stations from Port d'Andratx.
- FIGURE 34.** Principal Component Analysis (PCA) for heavy metals Pb, Zn, Cu, Ni and As in foraminifera tests (ppm); ecological indices FI', I_{LS}, FSI and H'; and FAI among sampling sites (Cabrera1, Cabrera2, Port d'Andratx and Magaluf). The table on the right side presents the PCA loadings of the different variables corresponding to PC1 and PC2 (the strongest values are marked in bold letters). The first two principal components explain up to 81.24% of the total variance.
- FIGURE 35.** Pearson correlation coefficient between heavy metal concentration (ppm) and percentage of different type of deformation of the foraminifera tests. Blue ellipses mean positive correlation; red ellipses mean negative correlation. Ellipses size shows the r (coefficient) value. Bold cells show the strong positive correlation ($r > 0.7$), and $p < 0.05$.
- FIGURE 36.** Map of the Balearic Islands with *P. oceanica* coverage (in green), and urban planning of all the municipalities (in red - urban land, in orange - developable land (actualized on 01/04/2022)), showing the location of the marine protected area (in blue) and the year of establishment: National park (Cabrera Archipelago), natural parks (Es Trenc-Salobrar de Campos, and s'Albufera des Grau), and marine reserves (Freu de Sa Dragonera, Badia de Palma, Illes del Toro i Malgrats, Migjorn de Mallorca, Llevant de Mallorca, Nord de Menorca, l'Illa de l'Aire) (from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_ReservesMarines/MapServer (actualized in 25/02/2023) with layers: GOIB_ReservesMarines, GOIB_MUIB, and GOIB_Posidonia_IB (Map Service

conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project) actualized in 09/03/2022)).

FIGURE 37. Maps of the Balearic Islands (Mallorca, Menorca, and Cabrera) with sampling locations showing the I_{LS} values during 1960's, 1980's, 1990's, 2000's, 2010's, and 2020's decades. The circle size is proportional to the I_{LS} . The green area represents the *Posidonia oceanica* cover (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

FIGURE 38. ANOVA Model Box-plot of ecological index values (I_{LS}) along a time series. Box-plot charts corresponding to a single sampling site are: 1) Cala Blava; 2) Portocolom; 3) Port de Sóller; 4) Port d'Andratx. Sampling localities are indicated by white dots within a grey ellipse. The green area represents *Posidonia oceanica* cover (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

FIGURE 39. ANOVA Model Box-plot of ecological index values (I_{LS}) along a time series. Box-plot charts corresponding to a different samples geographically close are grouped: 1) Santa Ponça and Cala de Santa Ponça; 2) Ses Illetes, Portals Nous, and Magaluf; 3) S'Arenal, Es Carnatge, and Ciutat Jardí; 4) Marina de Bonaire, Es Barcarès, and Badia de Pollença. The green area represents *Posidonia oceanica* cover (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

FIGURE 40. Dual isotope scatter plot ($\delta^{13}C$ vs. $\delta^{15}N$) of the mean values for each morphotype at each sampling locality and data obtained from literature corresponding to opportunistic foraminifera, filter-feeders, non-zooxanthellate and zooxanthellate corals, and cyanobacteria (Yamamuro, 1999; Muscatine et al., 2005; Carlier et al., 2007; Nomaki et al., 2008; Deudero et al., 2011; Lebreton et al., 2011; Wada et al., 2012; Jeffreys et al., 2015). Four shaded areas are defined corresponding to groups of organisms with different ecological significance. Notice also the different sizes of the symbols and letters. Larger symbols and names in bold correspond to our data and data from literature that are more relevant for the analysis of our results. **Opportunistic deposit-feeding foraminifera:** Allog: Allogromiidae, Astr: Astrorhizidae, Bagg: Bagginidae, Bol: Boliviniidae, Bul: Buliminidae, Chil: Chilostomellidae, Hapl: Haplophragmoididae, Non: Nonionidae, Plan: Planulinidae, Rhab: Rhabdaminiidae, Uvig: Uvigerinidae, Val: Valvulinidae. **Filter-feeding metazoans:** Biv: Bivalves, Cor: Non-zooxanthellate corals, Pol: Polychaetes, Spo: Sponges. **Zooxanthellate corals:** Z-cor. **Seagrass-related organisms.** Epiphytic foraminifera morphotypes: AF, AR, B*, C, D*, LM, LR; SG: Seagrass (including *P. oceanica* and genera Cymodocea, Halodule, Syringodium, Thalassia), Asc: Epiphytic ascidian, Bry: Epiphytic bryozoan. **Diatoms:** Diat. **Cyanobacteria:** Cyan. **Other.** Ser: Serpulid, Sed: Sediment from *P. oceanica* meadows from Mallorca, OM: Organic matter from *P. oceanica* meadows from Mallorca.

FIGURE 41. Fragments of non-dissolved shells of LM morphotype, A) Well-preserved *Peneroplis* (40x) and B) fragment of *Marginopora* (40x).

FIGURE 42. The transformation of shoreline of Magaluf from 1950s to 1960s.

SUMMARY

Marine ecosystems in the Mediterranean have experienced a significant increase in anthropogenic activities over the past century, leading to environmental pollution in the littoral zone. Coastal water quality has degraded due to human activity, and the ecological importance and fragility of *Posidonia oceanica* habitats have highlighted the need for effective bioindicators. Foraminifera assemblages have been found to be useful in monitoring coastal and shelf environments due to their well-studied ecologies and stress-tolerant species.

The aim of this thesis is to develop three specific objectives that exploit the stable association between *P. oceanica* seagrass and the epiphytic foraminiferal assemblage as an environmental monitoring tool. Firstly, to evaluate the ecological groups (morphotypes) that serve as the basis for bioindicator indices. Secondly, to conduct a comparative analysis of the environmental conditions of different *P. oceanica* meadows in the Balearic Islands that are subject to various types of anthropogenic pressure, using foraminiferal indices, abnormal-growth form frequencies, and trace-elemental concentrations within their tests to evaluate the impact of heavy-metal pollution exposure. Finally, to review published and grey literature on foraminiferal assemblages from *P. oceanica* meadows in the Balearic Islands and apply the new bioindicator indices to this historical dataset to detect the effects of human impact over the past 60 years (Chapter I).

The analysis was performed on 22 samples collected from *P. oceanica* seagrass meadows in the Balearic Islands, with the exception of multispecific seagrass meadows from tropical settings in Madagascar. The methodology employed included isotopic analysis to characterize the trophic structure of the foraminiferal assemblage using N and C stable isotopes, taxonomic and ecological description of foraminiferal assemblages for subsequent application of bioindicator indices (FI', I_{LS}, FSI, H'), detection of abnormal growth patterns, and geochemical analysis of foraminiferal tests to identify the presence of heavy metals (Cu, Zn, Cd, Pb, Co, Ni, As, Sn). In addition, a thorough review of theses, reports, and repositories from the 1960s to 2020s was conducted, based on foraminiferal assemblages described in seagrass meadows from the Balearic Islands (Mallorca, Cabrera, and Menorca) to gather a significant amount of information (Chapter II).

In regions with limited anthropogenic activity, such as the Cabrera Archipelago National Park, foraminiferal indices were found to be highest. This observation was supported by historical data analysis, which showed that the highest values were recorded in the 1960s, at the beginning of tourism development, and from areas with minimal human influence. Furthermore, it was evident that the FI' and I_{LS} indices have been increasing and stabilizing over the last two decades. However, there were contrasting results between high index values and anomalous foraminiferal tests with changes in geochemical test composition, which do not reflect the same environmental impacts (Chapter III). This apparent contradiction could be explained by differences in spatial scales. The FI' and I_{LS} reflect conditions over a large area and suggest an improvement in the marine environment and *P. oceanica* conditions, particularly after legislative protection was enforced. On the other hand, morphological and geochemical analyses of foraminiferal samples reveal localized but long-lasting impacts (Chapter IV).

The reliable classification of ecological groups was confirmed by the analysis of stable isotopes of carbon and nitrogen, which determined the trophic position of morphotypes in the food web. Thus, the FI' and I_{LS} indices, which are based on these morphotypes, along with other indicators such as FAI and heavy metal content in tests, were able to identify different stressors. The simplicity and ease of application of these indices make them ideal for use with a wide range of databases, allowing for powerful analysis of long-term trends in the health and environmental variability of *P. oceanica* meadows (Chapter V).

RESUMEN

Los ecosistemas marinos del Mediterráneo han experimentado un aumento significativo de actividades antropogénicas durante el último siglo, lo que ha llevado a la contaminación ambiental en la zona litoral. La calidad del agua costera se ha degradado debido a la actividad humana, y la importancia ecológica y fragilidad de los hábitats de *Posidonia oceanica* han resaltado la necesidad de bioindicadores efectivos, como foraminíferos epífitos debido a sus ecologías bien estudiadas.

El propósito de esta tesis consiste en desarrollar tres objetivos (Capítulo I) que aprovechen la relación estable entre la pradera de *P. oceanica* y la asociación de foraminíferos epífitos como una herramienta para el monitoreo ambiental: 1) evaluar los grupos ecológicos (morfotipos) que forman la base para los índices bioindicadores; 2) analizar las condiciones ambientales de diversas praderas de *P. oceanica* en las Islas Baleares sometidas a diferentes tipos de presión antropogénica. Este análisis se realizará mediante el uso de índices de foraminíferos epífitos, la proporción de formas aberrantes y la concentración de elementos traza en sus conchas para evaluar el impacto de la exposición a la contaminación por metales pesados; 3) revisar la literatura sobre las asociaciones de foraminíferos de las praderas de *P. oceanica* y aplicar los índices basados en foraminíferos epífitos al conjunto de datos históricos para detectar los efectos del impacto humano en los últimos 60 años.

Las 22 muestras analizadas fueron recolectadas de praderas de *P. oceanica* en las Islas Baleares, con la excepción de praderas de múltiples especies en entornos tropicales en Madagascar. La metodología empleada incluyó análisis isotópicos de N y C para caracterizar la estructura trófica de la asociación de foraminíferos, descripción taxonómica y la posterior aplicación de índices bioindicadores (FI', I_{LS}, FSI, H'), detección de patrones de crecimiento anormal y análisis geoquímico de las conchas para identificar la presencia de metales pesados (Cu, Zn, Cd, Pb, Co, Ni, As, Sn). Además, se realizó una revisión bibliográfica desde la década de 1960, basados en asociaciones de foraminíferos descritas en praderas de *P. oceanica* de las Islas Mallorca, Cabrera y Menorca (Capítulo II).

En áreas con actividad antropogénica limitada se encontraron los índices más altos. Esta observación fue respaldada por un análisis de datos históricos, que mostró los valores más altos en la década de 1960, al comienzo del desarrollo turístico, y en áreas con una influencia humana mínima. Además, FI' y I_{LS} han estado aumentando y estabilizándose durante las últimas dos décadas. Sin embargo, los resultados contrastantes entre valores de índices altos y formas de foraminíferos deformados con cambios en la composición, no reflejan los mismos impactos ambientales (Capítulo III). Esta aparente contradicción podría explicarse por diferencias en las escalas espaciales. Los índices FI' y I_{LS} reflejan condiciones sobre una gran área y sugieren una mejora en las condiciones de *P. oceanica*, especialmente después de que se aplicó la protección legislativa. Por otro lado, los análisis morfológicos y geoquímicos de las muestras de foraminíferos revelan impactos localizados, pero de larga duración (Capítulo IV).

La clasificación confiable de los grupos ecológicos fue confirmada por el análisis isotópico de carbono y nitrógeno, que determinaron la posición trófica de los morfotipos en la cadena alimentaria. Los índices FI' y I_{LS}, que se basan en estos morfotipos, junto con otros indicadores como FAI y el contenido de metales pesados en los foraminíferos, pudieron identificar diferentes factores de estrés. La simplicidad y facilidad de aplicación de estos índices los hacen ideales para su uso con una amplia gama de bases de datos, permitiendo un análisis poderoso de las tendencias a largo plazo en la salud y la variabilidad ambiental de las praderas de *P. oceanica* (Capítulo V).

RESUM

Els ecosistemes marins del Mediterrani han experimentat un augment significatiu d'activitats antropogèniques durant l'últim segle, el que ha portat a la contaminació ambiental a la zona litoral. La qualitat de l'aigua costanera s'ha degradat a causa de l'activitat humana, i la importància ecològica i fragilitat dels hàbitats de *Posidonia oceanica* han ressaltat la necessitat de bioindicadors efectius, com foraminífers epifits a causa de les seves ecologies ben estudiades.

El propòsit d'aquesta tesi és desenvolupar tres objectius que explotin l'associació estable entre la pradera de *P. oceanica* i l'associació de foraminífers epifítics com a eina de monitorització ambiental. En primer lloc, avaluar els grups ecològics (morfo-tipus) que serveixen com a base per als índexs bioindicadors. En segon lloc, dur a terme una anàlisi comparativa de les condicions ambientals de diferents praderes de *P. oceanica* a les Illes Balears que estan sotmeses a diversos tipus de pressió antropogènica, utilitzant índexs de foraminífers, la proporció de formes aberrants i concentracions d'elements traça en les seves closques per avaluar l'impacte de l'exposició a la contaminació per metalls pesants. Finalment, revisar la literatura sobre associacions de foraminífers de praderes de *P. oceanica* i aplicar els nous índexs a aquest conjunt de dades històriques per detectar els efectes de l'impacte humà en els últims 60 anys (Capítol I).

Les 22 mostres analitzades es varen recollir en praderes de *P. oceanica* a les Illes Balears, amb l'excepció de praderes de múltiples espècies en entorns tropicals a Madagascar. La metodologia emprada va incloure anàlisi isotòpics de N i C per caracteritzar l'estructura tròfica de l'associació de foraminífers, descripció taxonòmica i la posterior aplicació d'índexs bioindicadors (FI', I_{LS}, F_{SI}, H'), detecció de patrons de creixement anormal i anàlisi geoquímic de les closques per identificar la presència de metalls pesants (Cu, Zn, Cd, Pb, Co, Ni, As, Sn). A més, es va realitzar una revisió bibliogràfica des de la dècada de 1960, basats en associacions de foraminífers descrites en praderes de *P. oceanica* de les Illes Balears (Mallorca, Cabrera i Menorca) (Capítol II).

En àrees amb activitat antropogènica limitada es van trobar els índexs més alts. Aquesta observació va ser recolzada per un anàlisi de dades històriques, que va mostrar els valors més alts a la dècada dels 60, al començament del desenvolupament turístic, i en àrees amb una influència humana mínima. A més, els índexs FI' i I_{LS} han estat augmentant i estabilitzant-se durant les últimes dues dècades. No obstant això, els resultats contrastants entre valors d'índexs alts i formes de foraminífers deformats amb canvis en la composició, no reflecteixen els mateixos impactes ambientals (Capítol III). Aquesta aparent contradicció podria explicar-se per diferències en les escales espacials. Els índexs FI' i I_{LS} reflecteixen condicions sobre una àrea gran i suggereixen una millora en les condicions de *P. oceanica*, especialment després que es va aplicar la protecció legislativa. D'altra banda, els anàlisis morfològics i geoquímics de les mostres de foraminífers revelen impactes localitzats, però de llarga durada (Capítol IV).

La classificació fiable dels grups ecològics va ser confirmada per l'anàlisi isotòpic de carboni i nitrogen, que van determinar la posició tròfica dels morfotips en la cadena alimentària. Els índexs FI' i I_{LS}, que es basen en aquests morfotips, juntament amb altres indicadors com FAI i el contingut de metalls pesants en els foraminífers, van poder identificar diferents factors d'estrès. La simplicitat i facilitat d'aplicació d'aquests índexs els fan ideals per al seu ús amb una àmplia gamma de bases de dades, permetent una anàlisi potent de les tendències a llarg termini en la salut i la variabilitat ambiental de les praderes de *P. oceanica* (Capítol V).

PREFACE

The marine pollution increase and the interest to find the most effective, inexpensive and fast methods to detect the presence of contaminants and their source is currently of great interest in a Global Change context. The use of marine organisms as bioindicators is highly relevant and efficient, especially if the application provides rapid and cost-effective data, and does not require damaging any biota. In this sense, epiphytic foraminifera provide a good example of reliable bioindicators applicable in marine ecosystems such as seagrass meadows, to which this thesis is dedicated. During my master's degree, I created the bioindicators utilizing epiphytic foraminifera that were used in this thesis. The findings of my research were published in Mateu-Vicens et al., 2014.

The study of benthic foraminifera associated with seagrass meadows began in the Mediterranean in the first decades of the XXth century. The Balearic Islands, in particular, constitute one of the first scenarios of this discipline since the 1920s (e.g. Crespí, 1922). Colom (1942) later described the foraminiferal assemblages of the littoral zone, including *Posidonia oceanica* seagrass meadows, and defined the specific association as *Planorbulinitum mediterraneum*, whose occurrence became a very useful criterion for characterizing the *P. oceanica* ecosystems in the Mediterranean and, indirectly, assessing their environmental quality through foraminiferal analysis.

The present work represents the use of epiphytic foraminifera as bioindicators of *P. oceanica* ecosystems on the island of Mallorca (Balearic Islands, Spain), with characteristics comparable to other scenarios of the Western Mediterranean. The main objective proposed in this research are:

- To verify the robustness of bioindicator indices based on the revised classification of the ecological morphotypes and their role within the foodweb have been evaluated according to the stable isotopes (C and N) analysis.
- To apply the new bioindicator indices together with other indicators, such as the test morphology and the content of heavy metals in the foraminiferal tests, have been combined to provide a holistic perspective of the environmental conditions in selected *P. oceanica* meadows from Mallorca and Cabrera islands.
- To conduct a complete review of the published and grey literature on foraminiferal assemblages reported in *P. oceanica* meadows from Mallorca and Cabrera, from 1970s up to now, has been performed and, subsequently, the new indices have been applied to this historical dataset to detect the anthropogenic impact of the touristic development during the last 50 years.

This doctoral research has several publications linked to it. The first objective, which was to perform C and N stable isotope analysis in order to reinforce the epiphytic foraminiferal morphotypes, was developed in collaboration with Terean Sebastián-Pastor during their MSc research. The results of this research were published in the *Journal of Foraminiferal Research* (Mateu-Vicens et al, 2016). Additionally, the second objective led to another publication, which was published in 2022 under the name Khokhlova et al.

References:

Mateu-Vicens, G., Khokhlova, A., Sebastián-Pastor, T., 2014. Epiphytic foraminiferal indices as bioindicators in Mediterranean seagrass meadows. *Journal of Foraminiferal Research*, 44 (3): 325–339. <https://doi.org/10.2113/gsjfr.44.3.325>

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Khokhlova, A., Gudnitz, M. N., Ferriol, P., Tejada, S., Sureda, A., Pinya, S., Mateu-Vicens, G., 2022. Epiphytic foraminifera as indicators of heavy-metal pollution in *Posidonia oceanica* seagrass meadows. *Ecological Indicators*, 140, 109006. <https://doi.org/10.1016/j.ecolind.2022.109006>

CHAPTER 1

GENERAL INTRODUCTION

1.1. ORIGIN AND EVOLUTION OF SEAGRASS MEADOWS AND ASSOCIATED FORAMINIFERAL ASSEMBLAGES

Because of their physiological and morphological features, seagrasses are the only angiosperms able to live permanently underwater (Reich et al., 2015). This group of flowering plants with true leaves, stems, and roots occurs in tidal and shallow subtidal zones and form monospecific meadows, similar to terrestrial grasses (Duarte, 1991; Lee et al., 2018). Seagrass meadows vary in density and distribution, and they predominantly occur in the tidal and shallow subtidal zone, depending on the seagrass species and light penetration (Duarte, 1991). Moreover, the latitudinal distribution of species is mainly affected by light intensity and periodicity, but also by temperature, salinity, desiccation, turbidity, hydrodynamics, sediment thickness, grain size, and humic content (Brasier, 1975; Short et al., 2007). Seagrasses are distributed worldwide, from Australia and New Zealand to Iceland, Norway and Greenland, spanning the coasts of Africa, India, the Middle East, North and South America and Europe with the exception of Antarctica (Reich et al., 2015).

Seagrasses appeared during the early Late Cretaceous (Den Hartog, 1970; Brasier, 1975; Den Hartog and Polderman, 1975; Eva, 1980; Ivany et al., 1990; Reich et al., 2015) and their expansion started by the Early Eocene (late Ypresian), paralleling the global cooling trend and increased during the Late Eocene (Fig. 1) (Pomar et al., 2017). Through the middle-late Eocene and the Oligocene, seagrass ecosystems spread across the circum-tropical belt (Baceta and Mateu-Vicens, 2022 and references therein), and in the Eocene to Oligocene transition relative abundance and areal extent increased, associated with global cooling, and the resulting thermal gradients, both latitudinal and bathymetric, that characterize modern oceans (Pomar and Hallock, 2008; Pomar et al., 2017). Current distribution of seagrasses was achieved during the Miocene (Baceta and Mateu-Vicens, 2022), when the Tethys Sea split into three main bio-provinces (Indo-Pacific, Mediterranean and Caribbean), under a climate punctuated by brief glaciation pulses, a distinct warmer period [Mid-Miocene Climatic Optimum (MMCO)] and the final cooling trend across the Miocene–Pliocene transition (Zachos et al., 2001).

Foraminifera, along with other organisms such as red, green and brown algae, diatoms, cyanobacteria, bryozoans, and molluscs, represent the main biota inhabiting seagrass beds worldwide (Moncreiff and Sullivan, 2001; Deudero et al., 2011). Foraminifera are single-celled organisms, with Precambrian origin, forming a phylum included in the Chromista kingdom (WoRMS). According to morphologically-based taxonomy, there are 878 genera (Loeblich and Tappan, 1987) and about 10,000 current species, mostly benthic (only about 80 of them are planktonic), although their total diversity could reach around 15,000 taxa (Vickerman, 1992). Despite being exclusively unicellular organisms, they fulfill all the fundamental functions of life due to their high physiological complexity linked to their subcellular compartmentalization (Goldstein, 1999). These protists are characterized by the presence of the reticulopodium, constituted by a set of anastomosed filiform pseudopodia (they divide and rejoin) forming a structure similar to a network (Fig. 2). These

pseudopodia are essential, not only for locomotion and adherence to the substrate, but also for feeding, test construction and structuring, protection, and some aspects of respiration and reproduction (Travis et al, 1991; Murray, 2006).

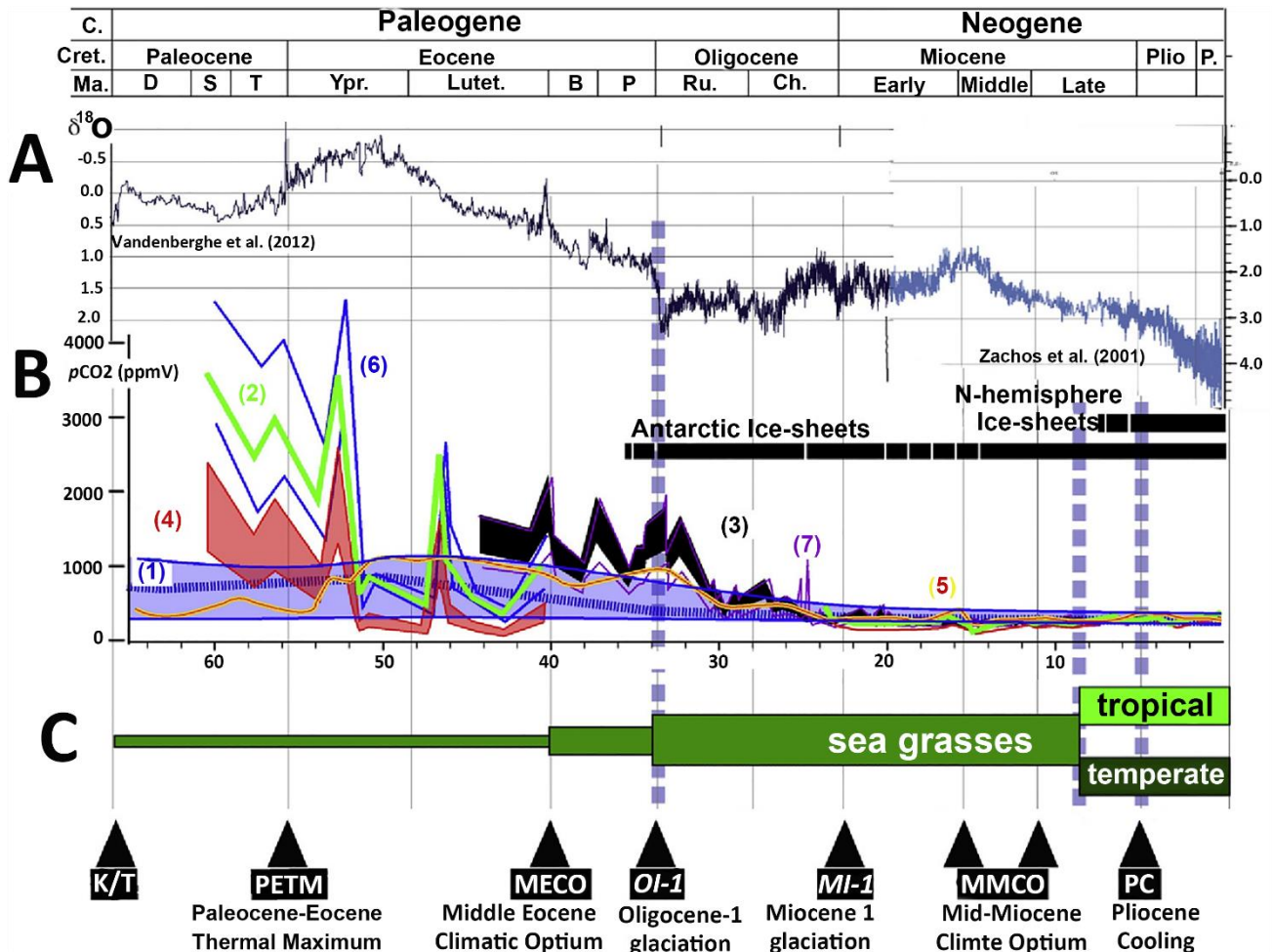


FIGURE 1. Relationships between: A) global temperature, B) CO₂ concentration in the atmosphere (1) Berner and Kothavala, 2001; (2) Pearson and Palmer, 2000; (3) Pagani et al., 2005; (4) Demicco et al., 2003; (5) Beerling and Royer, 2011; (6) Boron, Zachos et al., 2008; (7) Alkenones, Zachos et al., 2008, and C) expansion of seagrasses (modified from Pomar et al., 2017).

Another feature typical of foraminifera is the formation of a test that separates the cell from the external environment, whose chemistry, mineralogy and structure are crucial for classical taxonomical analyses (Sen Gupta, 2002). Sen Gupta (2002), based on Loeblich and Tappan (1992), separate four main groups according to the foraminifera wall composition (Fig. 3): Group 1, with the organic test, includes freshwater forms, with very poor fossil record; Group 2, with agglutinated test made of particles selected from the surrounding environment; Group 3, with calcium carbonate test, includes benthic (green boxes) and planktonic (blue boxes, with one extinct taxa Favusellacea (in red)) forms, and extinct order Fusulinida; Group 4, with silica test, imperforate, includes only Family Silicoloculinidae.

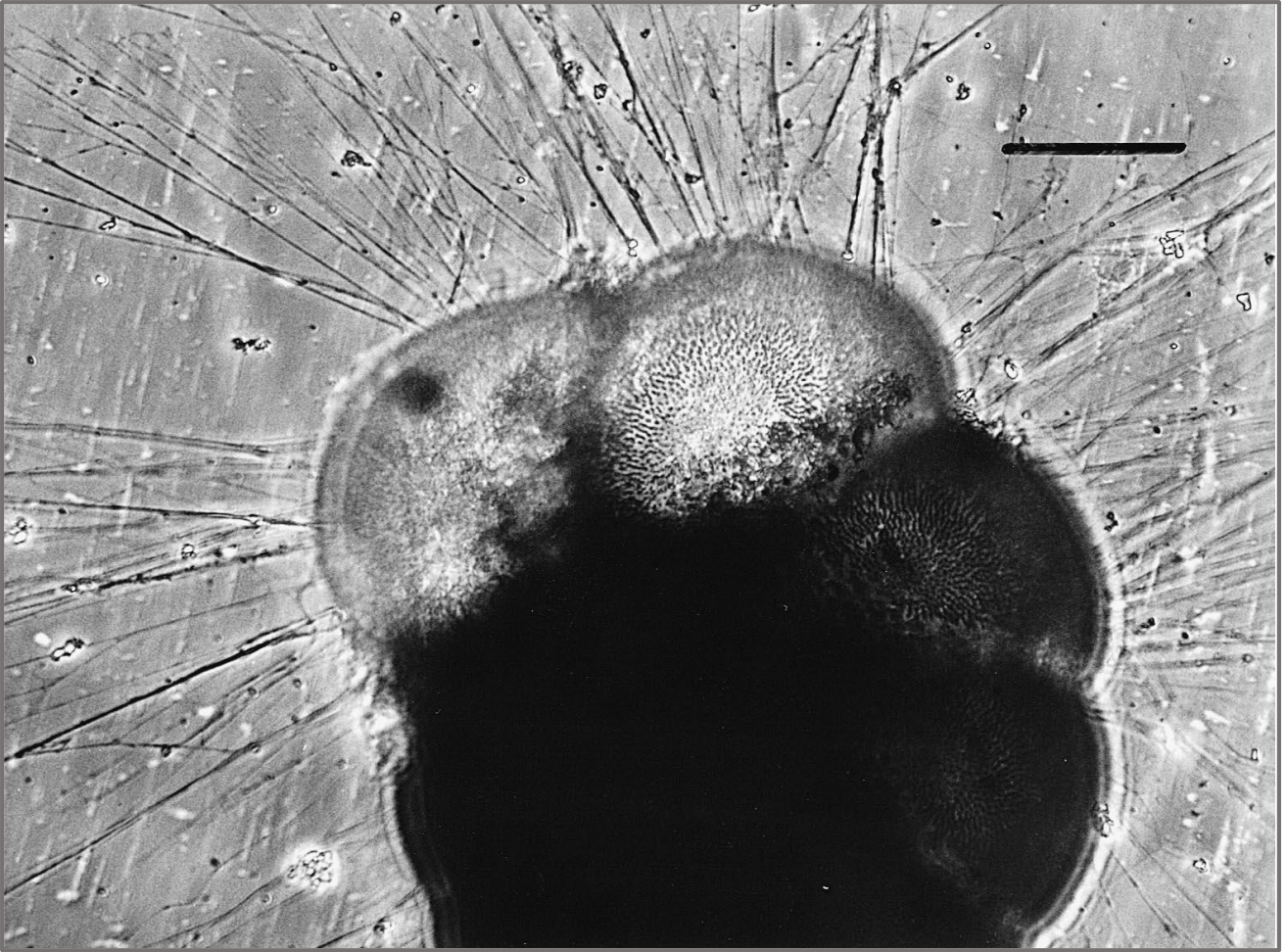


FIGURE 2. *Ammonia tepida*, umbilical view, showing the pseudopodia regularly distributed around the test (scale bar = 0.100 mm) from Debenay et al. (1998).

The most primitive taxa possess an organic test (not mineralized), which, forward in the evolution within this group, give way to those species with an agglutinated test, constructed from particles adhered by the foraminifera itself (Arenillas et al., 2002). The highest complexity is achieved in the calcareous tests secreted under very precisely-controlled processes that result in porcelaneous and hyaline tests (Fig. 4 and 5) (de Nooijer et al., 2009).

Hyaline tests (Fig. 5 A) are produced by transferring CO_3^{2-} and Ca^{2+} to the calcification site where low-Mg calcite is precipitated forming the translucent test wall. Porcelaneous tests in Miliolida (Fig. 5 B) are opaque and result from the accumulation of microscopic high-Mg calcite needles, previously precipitated in vesicles that are subsequently transported to the cell membrane, where they are released and form the test wall. As a result of their mineral composition, foraminifera can be preserved in sediments, facilitating their study in the fossil record as well as reconstructing time series for ecological analyses of present-day environments.

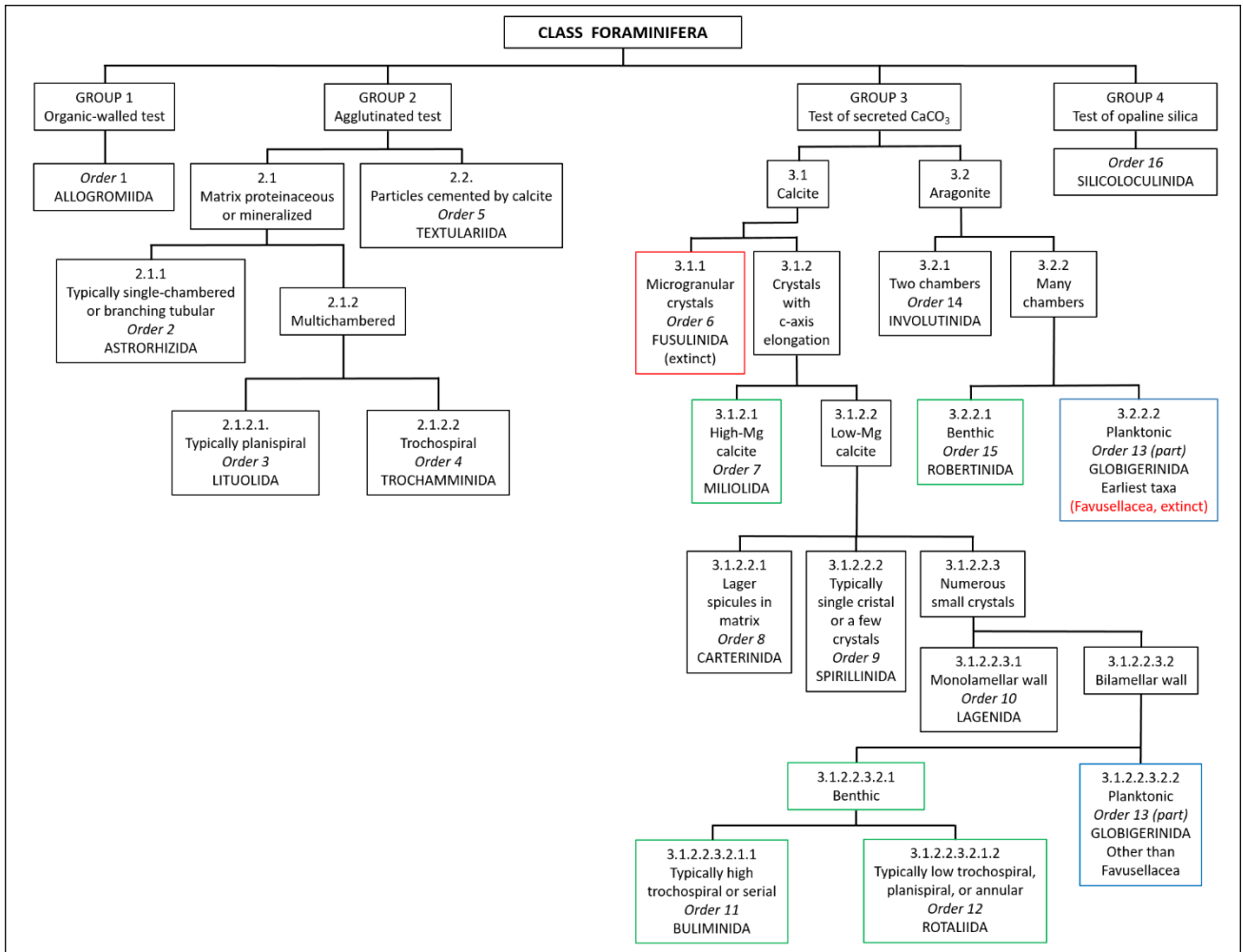


FIGURE 3. Simplified morphological key to foraminiferal orders based on the Loeblich and Tappan (1992) system of foraminifera classification (from Sen Gupta (2002)) with four main groups according to the foraminifera wall composition. Group 3 corresponds to taxa secreting calcareous tests, which include benthic (green boxes) and planktonic (blue boxes) forms, with one extinct taxa Favusellacea (in red)), and one extinct order Fusulinida (red box).

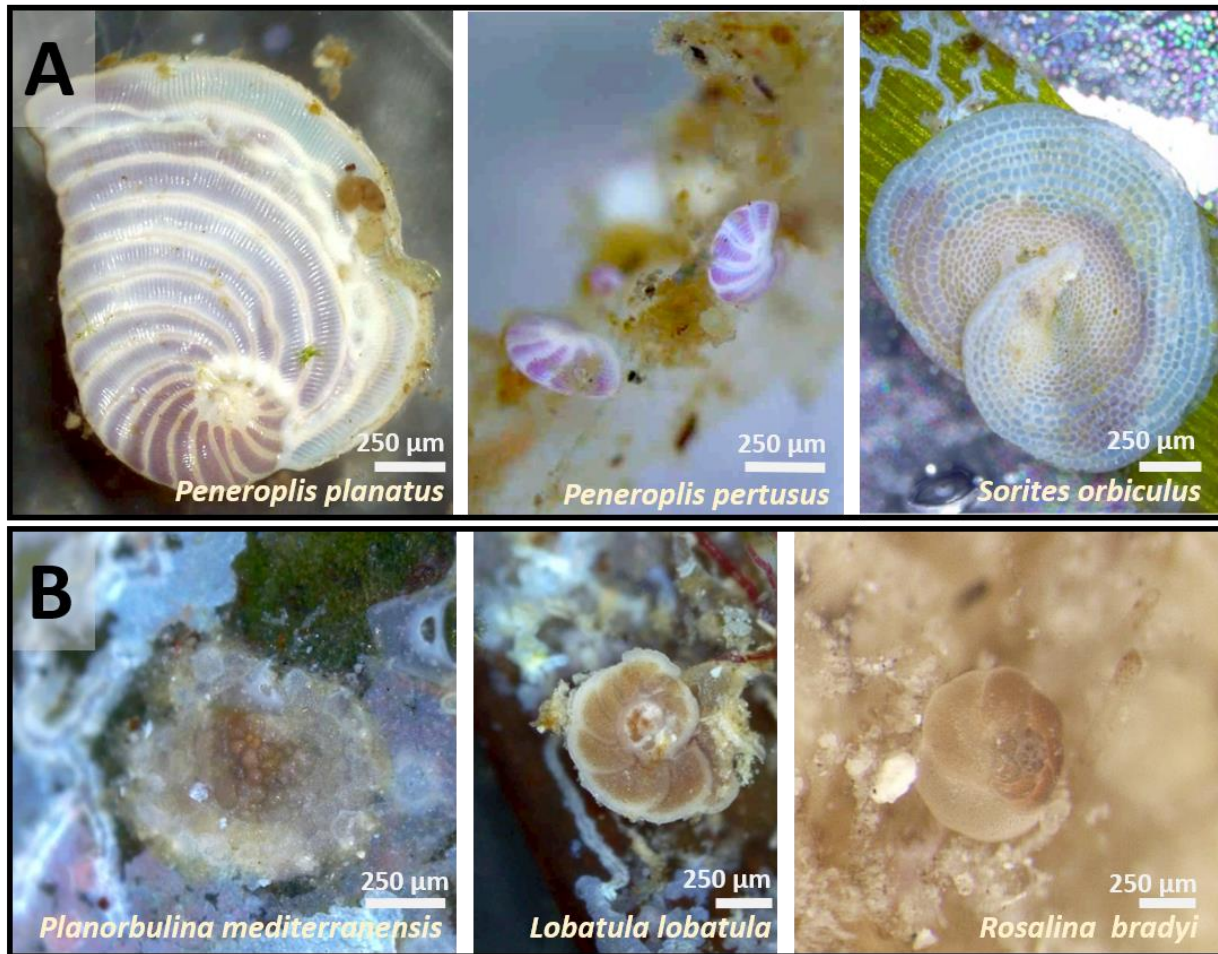


FIGURE 4: Some examples of benthic foraminifera with different types of calcareous test: (A) Porcelaneous tests in *Peneroplis planatus*, *P. pertusus*, and *Sorites orbiculus*; (B) Hyaline tests in *Planorbulina mediterranensis*, *Lobatula lobatula* and *Rosalina bradyi* (Photos: Guillem Mateu-Vicens).

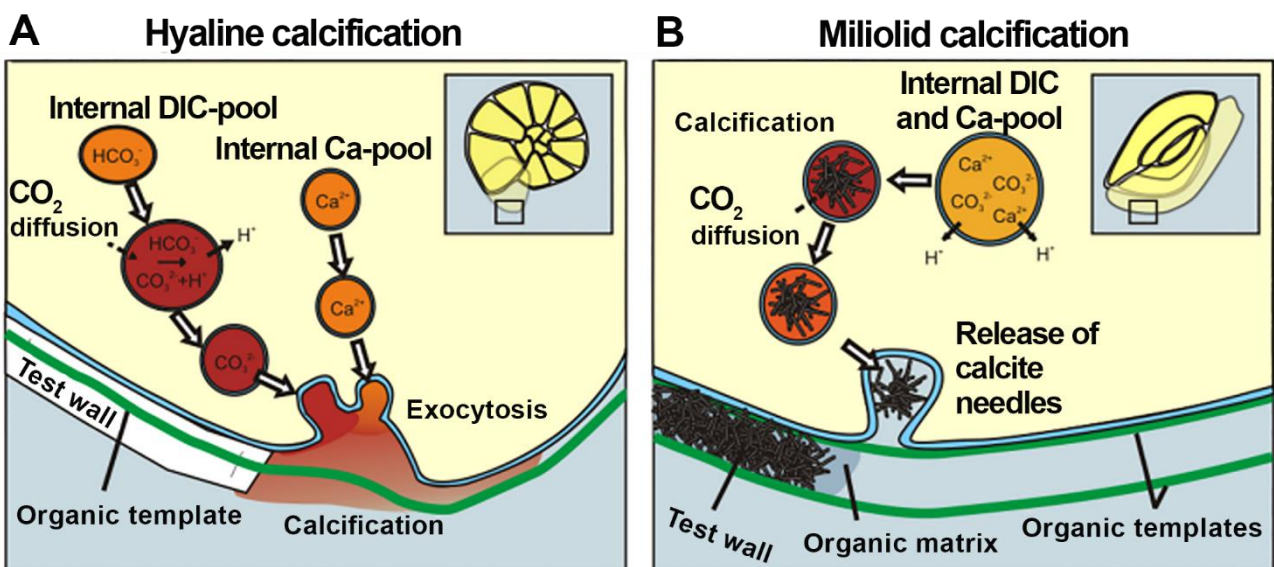


FIGURE 5. Hyaline and porcelainous test wall formation: (A) The hyaline test calcification produces lamellar layers of well-arranged calcite crystals, where CO_3^{2-} and Ca^{2+} precipitate at the calcification sites forming translucent test walls; (B) The porcelainous test calcification (miliolid calcification) is formed by tiny, randomly oriented calcite crystals, previously precipitated in intracellular vesicles and then transported to the cell membrane (scheme from de Nooijer et al. (2009)).

1.1.1. Coevolution of Foraminifera and Seagrasses

Since their expansion in the Cenozoic, seagrasses have occupied a relatively wide bathymetric range within the euphotic zone and provided a plethora of ecological niches for epiphytic organisms, including foraminifera (Fig. 6) (Pomar et al., 2017).

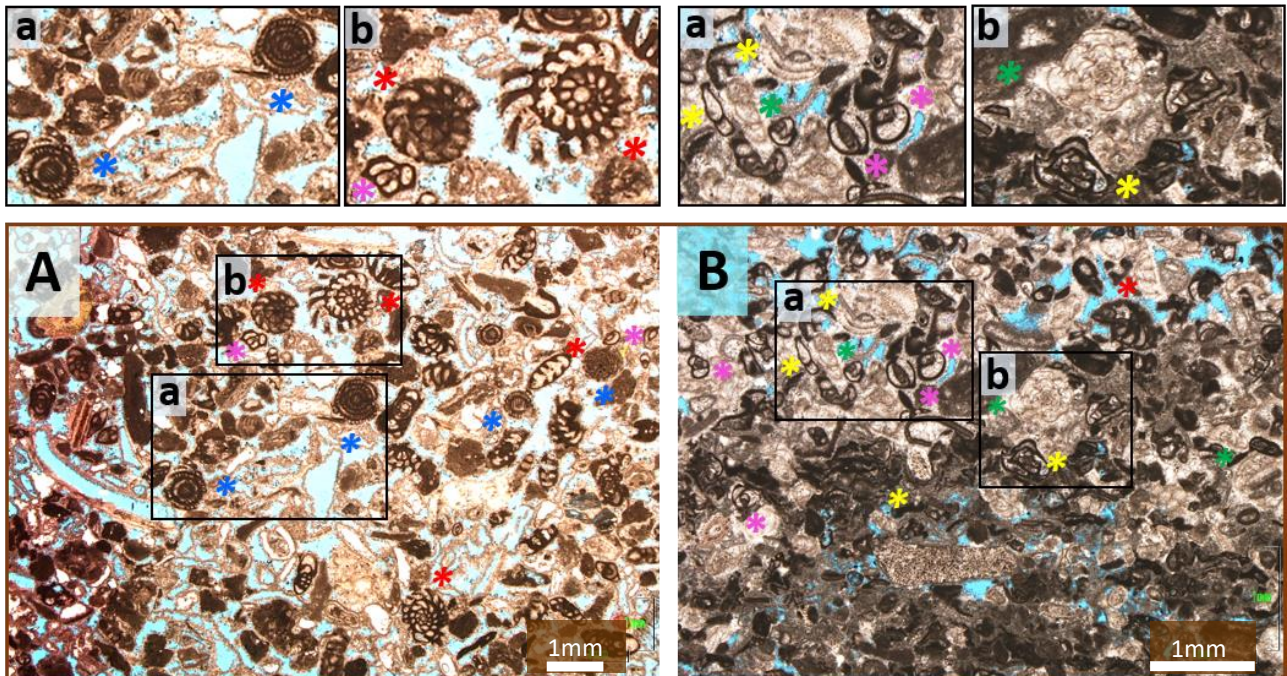


FIGURE 6. Thin sections with fossil content of epiphytic foraminifera from Upper Tortonian-Messinian of Mallorca, with *Peneroplis**, *Planorbulina**, *Borelis**, *Nubecularia** and small miliolids*. (A) The foraminiferal assemblage is dominated by morphotype SB, which can be associated with the shallowest settings of a seagrass meadow; (B) The foraminiferal assemblage includes encrusting morphotype A* and morphotype D* is dominant, which indicates slightly deeper conditions than those inferred from (A) (Mateu-Vicens and Baceta, 2022).

Among the foraminifera, encrusting genera (e.g., *Planorbulina*, *Miniacina*, *Nubecularia*) and those with reduced motility (e.g., *Lobatula*, *Cibicides*, *Rosalina*) have life spans synchronized with leaf-turnover rates. In contrast, more opportunistic, less specialized taxa (e.g., miliolids and textularids) that are also common in other environments have shorter life cycles. Most of these small epiphytic foraminifera appeared during the Late Cretaceous and the Paleogene, coincidentally with seagrass emergence and expansion periods (Fig. 7).

On the other hand, symbiont-bearing benthic foraminifera also found novel niches for colonization and evolved accordingly. Thus, large miliolids with discoid tests (extinct meandropsinids, peneroplids, soritids), recurrently developed since the Late Cretaceous and along the Cenozoic, when attached to a leaf surface, maximize nutrient absorption and optimize exposure surfaces to light (Hallock et al., 1991), indicating evolutionary adaptation (Fig. 8).

In contrast to large miliolids, only a few symbiont-bearing rotalids have structurally evolved to develop epiphytic behaviors. This is the case of *Amphistegina*, some miogypsinids, and the stellate calcarinids. In the middle Miocene, irregularly shaped *Miogypsina* probably thrived attached to the seagrass, adopting the shape of the substrate (Boudagher-Fadel, 2008), offering less exposed surface and hindering test entrainment.

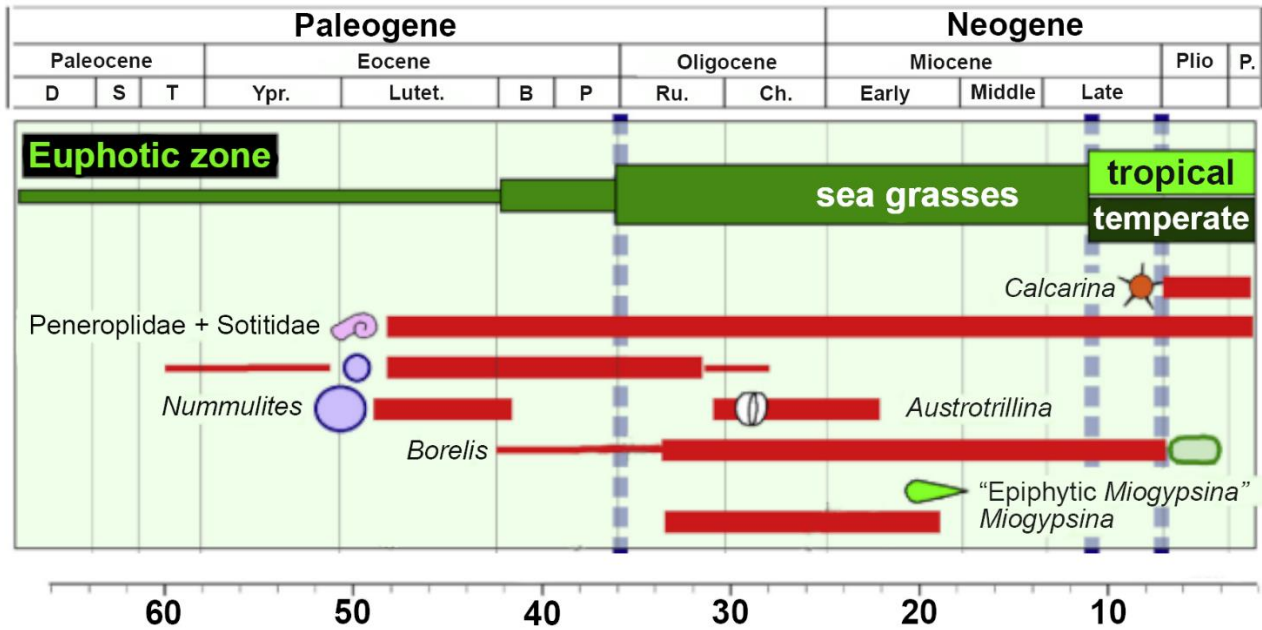


FIGURE 7. Expansion of seagrasses, and appearance, disappearance and changes in habitat of the main groups of associated symbiont-bearing benthic foraminifera through the Cenozoic. (Modified from Pomar et al. (2017)).

Other, non-disc-shaped, symbiont-bearing miliolids have occurred in seagrass meadows since the end of the Oligocene. These taxa include *Austrotrillina* and some alveolinids that evolved from elongate (e.g., *Alveolina*) to subspherical forms of the genus *Borelis* (Boudagher-Fadel, 2008) and colonized the seagrass vegetated bottoms during the Eocene-Oligocene transition. In contrast, not all symbiont-bearing rotalids reported in seagrass meadows are exclusive of these environments, although taxa such as flat nummulitids (e.g. small *Nummulites*, *Operculina*, *Heterostegina*, *Planostegina*) and amphisteginids (*Amphistegina*) have adapted their ecological requirements to vegetated niches since the Paleogene, under calm and oligophotic conditions at shallow depths, below the leaf canopy (Racey, 1995; Mateu-Vicens et al., 2012).

Symbiont-bearing rotalid and miliolid taxa living in seagrass meadows have distinct adaptive trends, related to the algal symbionts hosted by each group (Fig. 9). Miliolids host a variety of unicellular algae (red algae, green algae, dinoflagellates, diatoms) that have established univocal symbiotic relationships mostly at family and subfamily levels. Such an array of algal symbionts assures that photosynthesis can be performed over a fairly broad wavelength range, regardless of the inherent opacity of the miliolid test (Hallock, 1988a, b). Thus, soritids hosting green algae or dinoflagellates (Hallock, 2002) are better adapted to well-illuminated settings such as the foliar stratum of seagrasses. In contrast, peneroplids, with red-algal symbionts, are adapted to lower light conditions such as those occurring close to the rhizome. Alveolinids and symbiont-bearing rotalids host diatoms, which use a wide light spectrum and are less specialized to particular photic conditions. Thus, diatom bearing taxa (i.e., *Amphistegina*) are often found beneath the leaf canopy, but also in deeper environments with equivalent low light intensity.

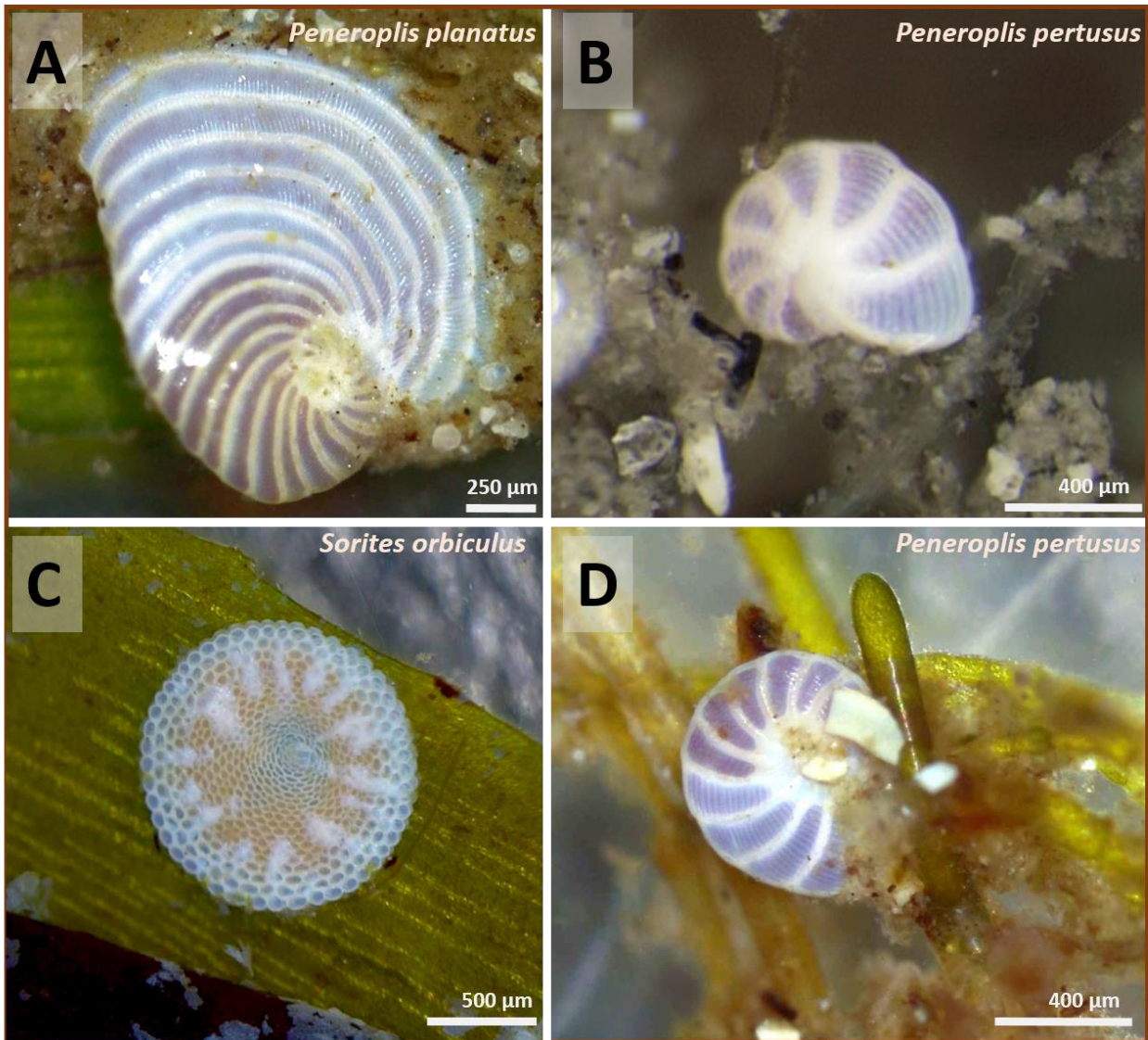


FIGURE 8. Evolutive adaptation to light in some symbiont-bearing benthic foraminifera with flat to discoid tests. (A) flat flabelliform *Peneroplis planatus* and (C) discoid *Sorites orbiculus* attached to the leaf surface of *Posidonia oceanica*; (B) and (D) planispiral *Peneroplis pertusus* in sediment and attached to filamentous algae (Photos: Guillem Mateu-Vicens).

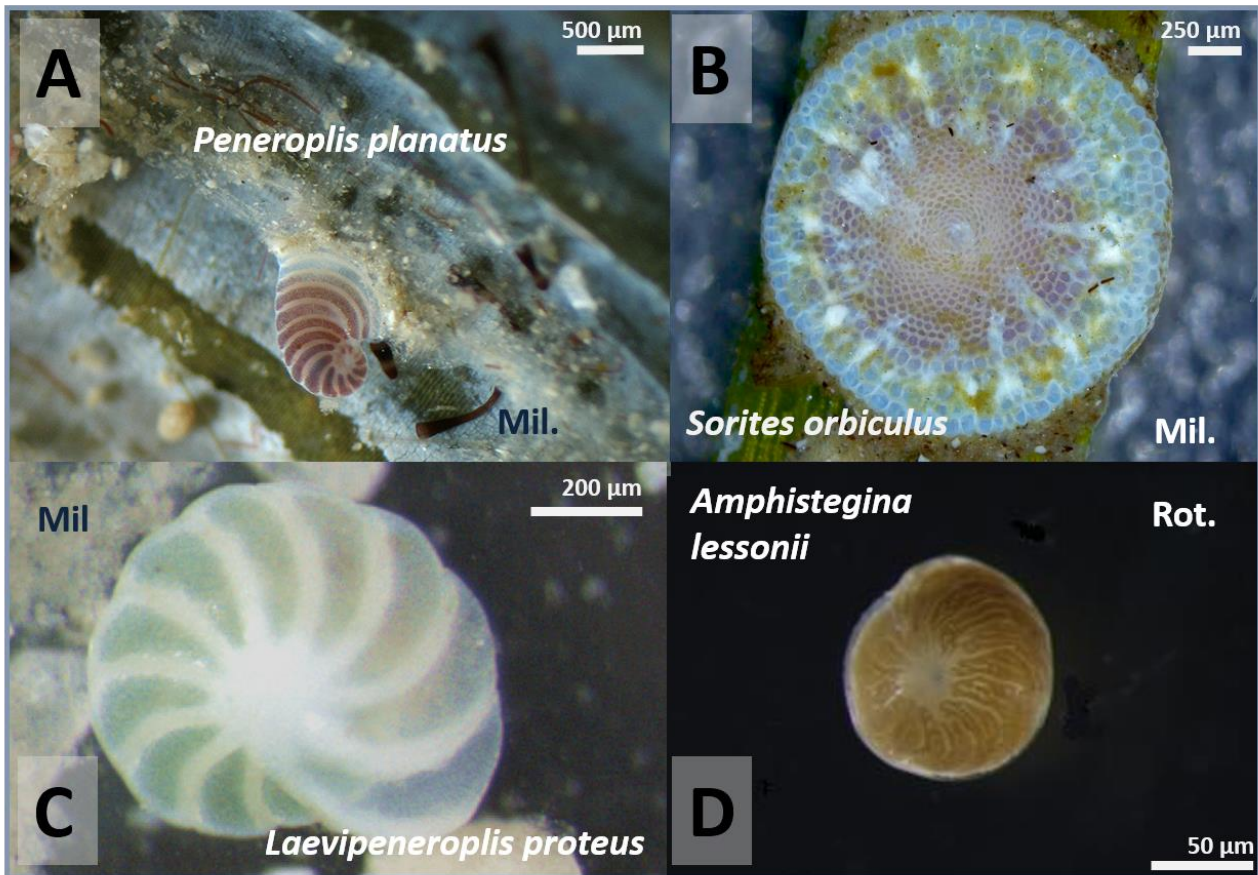


FIGURE 9. Adaptations of symbiont-bearing foraminifera to different light conditions hosting different algal taxa: (A) *Peneroplis planatus* with red-algal symbionts, (B) *Sorites orbiculus* with dinoflagellate symbionts, (C) *Laevipeneroplis proteus* with green algae symbionts; or modifying their shape: (D) *Amphistegina lessonii* with diatom symbionts, adapted to wide light spectrum. *Amphistegina* spp. may change their thickness-to-surface ratio with depth to optimize their light interception. Mil. – miliolids, Rot. – rotalids (Photos: Guillem Mateu-Vicens).

1.2. THE *POSIDONIA OCEANICA* MEADOW: AN ECOSYSTEM ENDEMIC TO THE MEDITERRANEAN.

Mediterranean infralittoral soft-bottoms are dominated by extensive seagrass meadows of the endemic phanerogam *Posidonia oceanica* (L.) Delile (Duarte and Chiscano, 1999; Short *et al.*, 2007), which cover about 50,000 km² of coastal sandy, and, occasionally, rocky areas (Bethoux and Cópín-Motegut, 1986). These meadows constitute the climax ecosystem, occupying a depth range between 0.5 mwd and 45 mwd (Duarte, 1991; Langer, 1993; Larkum *et al.*, 2006; Marbà *et al.*, 2002). *Posidonia oceanica* densities reported from western Mediterranean localities range between 150 shoots m⁻² and 800 shoots m⁻², exceeding 1,500 shoots m⁻² under exceptionally favourable conditions (Marbà *et al.*, 2005). The fully grown leaves reach a length of 60-80 cm and they are 0.5-1 cm wide. Moreover, they show a maximum life-span of 12 months, whereas seagrass rhizomes serve as a substrate for about two years (Langer, 1993).

Posidonia oceanica meadows are considered very important ecosystems in the Mediterranean because of their high number of services (Boudouresque, 2004), which include their ability to host exceptionally biodiversity communities (Macpherson *et al.*, 1997; Guidetti, 2000). Indeed, *P. oceanica* leaves, rhizomes and roots host almost 20% of all species living in the

Mediterranean Sea and offer substrata suitable for settlement and growth of a number of different taxonomic groups of sessile organisms (Canals and Ballesteros, 1997; Vasarri et al., 2021). Seagrasses also provide shelter and food for assemblages of fish, including species of commercial importance (Jimenez et al., 1996), and for many endangered species, such as the biggest Mediterranean bivalve *Pinna nobilis* (Duarte, 2000; Boudouresque et al., 2012). Seagrass meadows are recognised as one of the most productive ecosystems on Earth due to the accumulation of plant biomass and the amount of autotrophic epiphytes strongly associated with this plant (Canals and Ballesteros, 1997; Vasarri et al., 2021).

The large number of organisms associated with seagrasses could be explained by its structural complexity that permits the juvenile forms of different species to acquire food and protection from predators (Heck and Orth, 2006), which in turn, facilitates lower mortality rates (Bell and Pollard, 1989; Larkum et al., 2006). The leaves of *P. oceanica* play an essential role in the marine food chain as a rich source of biochemical components and essential trace elements (Vasarri et al., 2021). After the leaves fall, large amounts of their detritus accumulate at the sea bottom and support high rates of secondary production (Cebrián and Duarte, 2001). Furthermore, in the benthic food web, detritus is more relevant for benthic organisms than seagrass living tissues (Vizzini et al., 2002).

Moreover, because *P. oceanica* leaves are able to photosynthesize, they play an important role in the oxygenation of coastal waters (Gazeau et al., 2005). Despite the oxygen absorbed during tissue degradation, the amount of oxygen produced exceeds its consumption and *P. oceanica* still constitutes a major source of this gas (Mateo and Romero, 1996). Moreover, photosynthetic activity and community metabolism of *Posidonia oceanica* meadows can modify pH in the water column, which may alleviate the expected impacts of ocean acidification (Invers et al., 1997; Orr et al., 2005; Gazeau et al., 2007; Hendriks et al., 2014; Ramajo et al., 2019).

Posidonia oceanica also plays an important role in coastal hydrodynamic processes (Fig. 10) because of its ability to reduce the impact of waves on the sea bottom at shallow depths, absorbing between 30% and 40% of the total energy (Gacia et al., 1999; Terrados and Duarte, 2000; Balata and Tola, 2018; Vasarri et al., 2021). By acting as hydrodynamic sinks, these plants contribute to sediment deposition (Gacia et al., 1999; Terrados and Duarte, 2000).

The growth mode of *P. oceanica* results in a type of bioconstruction known as *matte* (Fig. 10), which prevents the displacement of sediment towards deeper zones, favouring its accumulation *in situ* and providing a particle source for the maintenance of the sandy coastline (Gacia et al., 1999). A *matte* structure is characterized by live and dead rhizomes and roots within the sediment, where rhizomes and leaf shoots can grow horizontally (plagiotropic rhizomes) or vertically (orthotropic rhizomes) to colonize the substrate (Boudouresque and Meinesz, 1982), thus trapping autochthonous and allochthonous sediment (Boudouresque et al., 2016). Moreover, turbulence dissipation and sediment trapping increase water transparency of coastal environments (Duarte, 2000; Boudouresque et al., 2012). After death, leaves and rhizomes accumulate onshore, forming *banquettes* (Boudouresque and Meinesz, 1982) that cover and protect beaches from erosion, especially during the autumn and winter months.

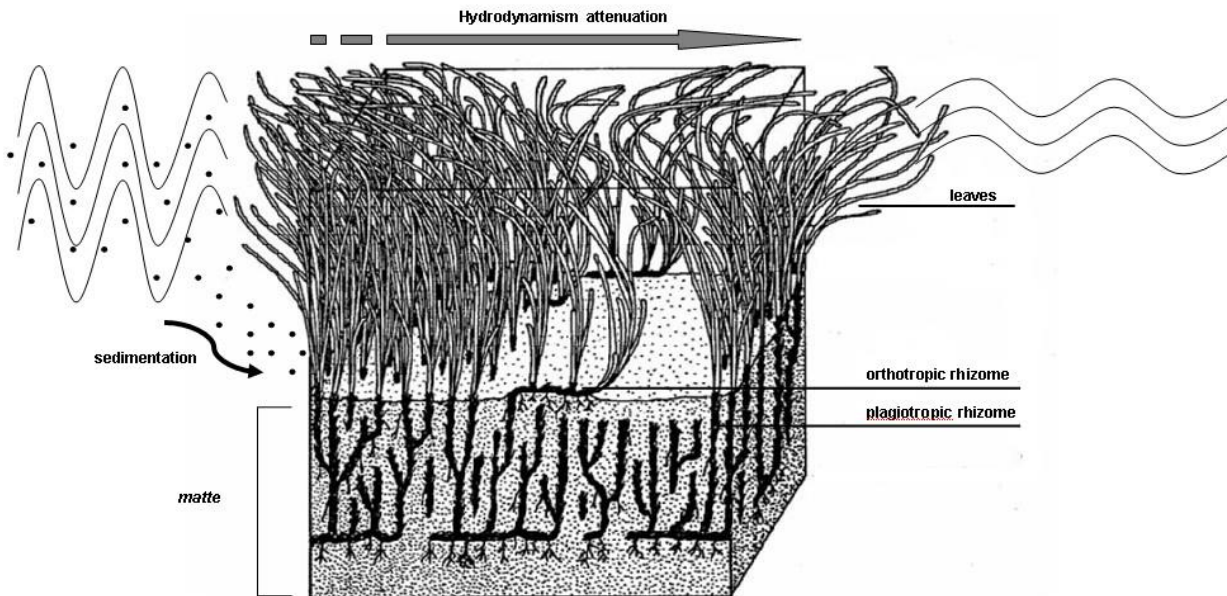


FIGURE 10. Effect of *Posidonia oceanica* meadows (*matte*, rhizomes and leaves) on reduction of hydrodynamics and its effects on sediment trapping and stabilization from Boudouresque and Meinesz (1982), modified by Migliore et al. (2007).

1.3. CHARACTERIZATION OF FORAMINIFERAL ASSEMBLAGES OF *P. OCEANICA* AND THEIR ECOLOGICAL SIGNIFICANCE

The systematic studies of the foraminiferal assemblages on *Posidonia oceanica* bottoms started by middle XXth century (Colom, 1942) and have continued up to the present (Colom, 1964; Blanc-Vernet, 1969; Mateu, 1970; Langer, 1993; Ribes et al., 2000; Mateu-Vicens et al., 2010; Frezza et al., 2011; Mateu-Vicens et al., 2012; Gaglianone et al., 2015; El Kateb et al., 2020; Di Bella et al., 2022). The foraminiferal assemblage typical of *P. oceanica* was formally described in Mallorca (Colom, 1942) and named as *Planorbulinatum mediterraneasae*, due to the abundance of the epiphytic, encrusting, species *Planorbulina mediterraneensis*. However, some years earlier, in the same study area, Crespí (1922) and Colom (1935) had already described the assemblages of peneroplids and soritids, which are typically found in seagrass meadows as epiphytic forms.

Since the end of the 1960s, Mateu (1966; 1970) and Mateu et al. (1984), who was the first in Spain to draw attention to the importance of using foraminifera as bioindicators, developed his research on *P. oceanica* meadows of the Balearic Islands and Catalonia. His results and those from subsequent works on seagrass meadows from the Balearic Islands by Florit (1983), Gazá (1988), Abril (1993), Moreira (1993), Mateu et al. (2001), Mateu-Vicens et al. (2010), Mateu-Vicens et al. (2014), Racis (2015), Vaquer (2016), Mateu-Vicens et al. (2016), Khokhlova et al. (2022), Khokhlova and Mateu-Vicens (2023), provide a data series of about 60 years, which allows the comparative analysis of the foraminiferal assemblages and the associated environmental changes of the *P. oceanica* meadows along a time series.

Posidonia oceanica-associated assemblages represent a climax community (Blanc-Vernet, 1969), dominated by foraminiferal species well adapted to the temporal availability of phytal substrates and the structural features of plant microhabitats (Langer, 1993). Among the most common species for such association, there are *Planorbulina mediterraneensis* d'Orbigny, 1826,

Lobatula lobatula (Walker and Jacob, 1798) and *Nubecularia lucifuga* DeFrance, 1825 and, at a lesser extent, *Astrononion stelligerum* (d'Orbigny, 1839), *Rosalina* spp., *Planogypsina acervalis* (Brady, 1884), *Cibicidoides variabilis* (d'Orbigny, 1826), *Peneroplis pertusus* (Forsskål in Niebuhr, 1775), *Sorites orbiculus* (Forsskål in Niebuhr, 1775), and genera *Quinqueloculina* and *Siphonaperta* (Colom, 1942; Blanc-Vernet, 1969; Mateu, 1970; Mateu et al., 2003; Langer, 1993; Mateu-Vicens et al., 2010). Based on morphological and ecological features, Langer (1993) grouped the epiphytic foraminiferal taxa into four morphotypes (A–D, Fig. 11). Sessile species permanently attached to the substrate, with glycosaminoglycans, were assigned to morphotype A. These foraminifera are preferably found on larger plants (i.e., phanerogams) that have life spans > 10 months. Species belonging to morphotype B occur on different phytal substrates and often constitute a major portion of epiphytic foraminiferal assemblages. These species are generally motile, but temporarily attach their test to the substrate. Their flat umbilical surface is well-adapted to smooth substrates. However, their small size and short life span, 3–5 months, facilitate the colonization of a variety of structurally different plant microhabitats. Motile, suspension-feeding foraminifera with short life-spans (3–4 months) are assigned to morphotype C and correspond to keeled elphidiids. Taxa of this morphotype extend their pseudopods through the opening of a complex canal system, forming a dense meshwork that facilitates the capture of food particles. Finally, permanently motile, grazing foraminifera with short life spans are assigned to morphotype D, which includes many opportunistic species and a few symbiont-bearing taxa that dominate in the rhizomes and surrounding sediment.





Langer (1993) morphotypes	Motility	Attachment to the substrate	Shape	Life span	
	A	Sessile	Glycosaminoglycans	Flat concave attachment surface	1 year
	B	Temporary motile	Pseudopodial network	Wide apertural faces, often with papillae	2-3 months
	C	Motile	Extrusion of pseudopodes	Multiple apertural openings for pseudopodes	3-4 months
	D	Permanently motile	Striding on the apertural face in position	Bottle-neck apertures	short

FIGURE 11. Langer (1993) morphotypes and their ecological features, including motility and life span.

Langer (1993) argued that diversity of epiphytic foraminifera (Fig. 12) mainly depends on nutritional gradients, and temporal and spatial components. Thus, low-diversity epiphytic assemblages occur on small algae with short life spans, while high-diversity assemblages are typical of phytal substrates that have annual or perennial life spans. Nevertheless, the results of comparative analysis of epiphytic foraminifera in sediments colonized by the seagrass *P. oceanica* and macroalgae of the genus *Caulerpa* showed that assemblages in areas dominated by *P. oceanica* and *Caulerpa racemosa*, are characterized by lower numbers of species than those of *C. taxifolia* and *C. prolifera* (Mateu-Vicens et al., 2010). According to these authors, the low foraminiferal diversity in *P. oceanica* assemblages is related to an advanced stage in the ecological succession with few, dominant epiphytic foraminiferal taxa strongly adapted to life cycle of the phanerogam. On the contrary, lower

foraminiferal diversities reported in the *Caulerpa* spp. assemblages are associated with a type of substrate that provides only limited surface for epiphytic colonization.

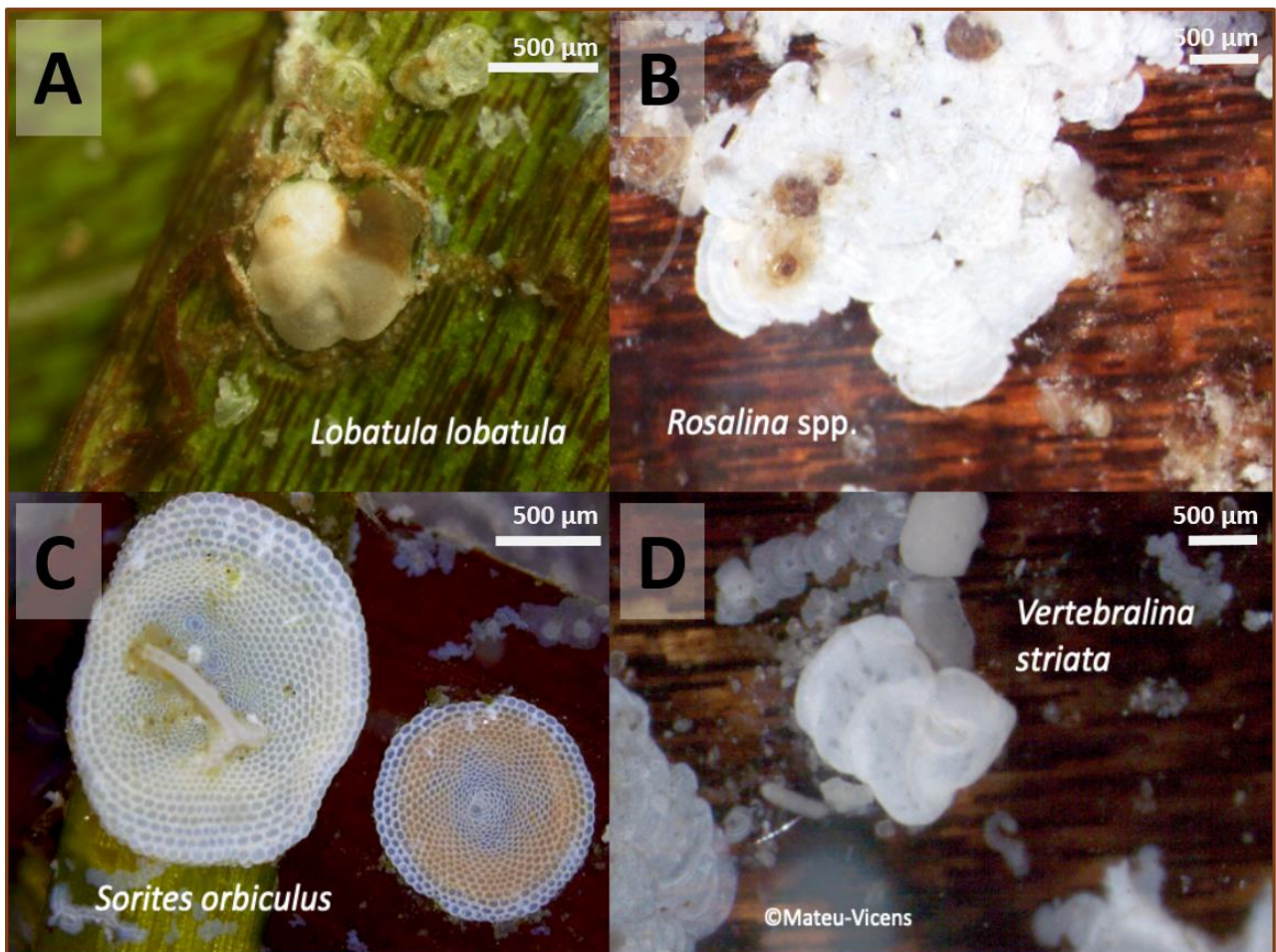


FIGURE 12. Some examples of the morphotypes of living epiphytic foraminifera according to Langer's (1993) groups: (A) *Lobatula lobatula* and (B) *Rosalina* spp. - morphotype B, (C) *Sorites orbiculus* - morphotype A, (D) *Vertebralina striata* - morphotype D (Photos: Guillem Mateu-Vicens).

1.4. ANTHROPOGENIC THREATS AND REGULATORY FRAMEWORK TO PROTECT *P. OCEANICA* MEADOWS

During centuries, and in particular, since the middle XIXth Century, human activity around the Mediterranean has deeply impacted the littoral zone, affecting considerably the *P. oceanica* meadows. Since the last decades, the impacts have become more intense associated with the population increase and the unsustainable use of resources (Marbà et al., 2005). Currently, the main reasons for this problem come from illegal otter trawling in shallow depth (González-Correa et al., 2005); uncontrollable moorings (Lloret et al., 2008); contamination by hydrocarbons, detergents, paints or wastewaters, heavy metals and other toxic elements (Balestri et al., 2004; Burkholder et al., 2007; Martínez-Colón et al., 2009); the presence of invasive species (Sureda et al., 2017), fish farming (Ruiz et al., 2001), hypersaline water from desalination plants (Telesca et al., 2015), as well as warming and ocean acidification (Marbà et al., 2014), among others.

1.4.1. Anthropogenic Threats in the Balearic Islands

In the Balearic Islands, trawling and other types of fishing were unregulated before the 1960s and caused irreparable damage to the *P. oceanica* meadows (Álvarez et al., 2015). Due to the increased size and effectiveness of the boats after 1988, and to avoid this type of impact, it was decided to make trawling illegal at less than 50 meters of depth (Royal Decree 679/1988), and the placement of anti-trawling artificial reefs was carried out (Guillén et al., 1994; Ramos et al., 2000). Unfortunately, in parallel, the construction of dikes, docks, and other port facilities that started at the end of the XIXth century, drastically increased between the 1950s and 1980s, affecting wide extensions of *P. oceanica* meadows (Álvarez et al., 2015). In addition, during the beginning of the 1970s, water purification and the subsequent use of purified water for agriculture began, which allowed sanitation and regeneration of some of the abandoned aquifers (Santarrufina, 2007). Notwithstanding, treatment plants discharged their contents directly into the sea (Fig. 13a), mostly after storms. The drainage of these waters still negatively impacts *P. oceanica* ecosystems (Álvarez et al., 2015). As a consequence, the eutrophication of the sediment became one of the biggest problems for the seagrass (Díaz-Almela et al., 2008). In addition, desalination has become another stress since the 1990s, when desalination plants started to discharge brine at shallow depths (less than 20 m) near *P. oceanica* meadows, causing serious damage, reducing leaf growth, and reducing shoot survival, even at small increases in salinity (Ruíz et al., 2009; Marín-Guirao et al., 2011, Capó et al., 2020).

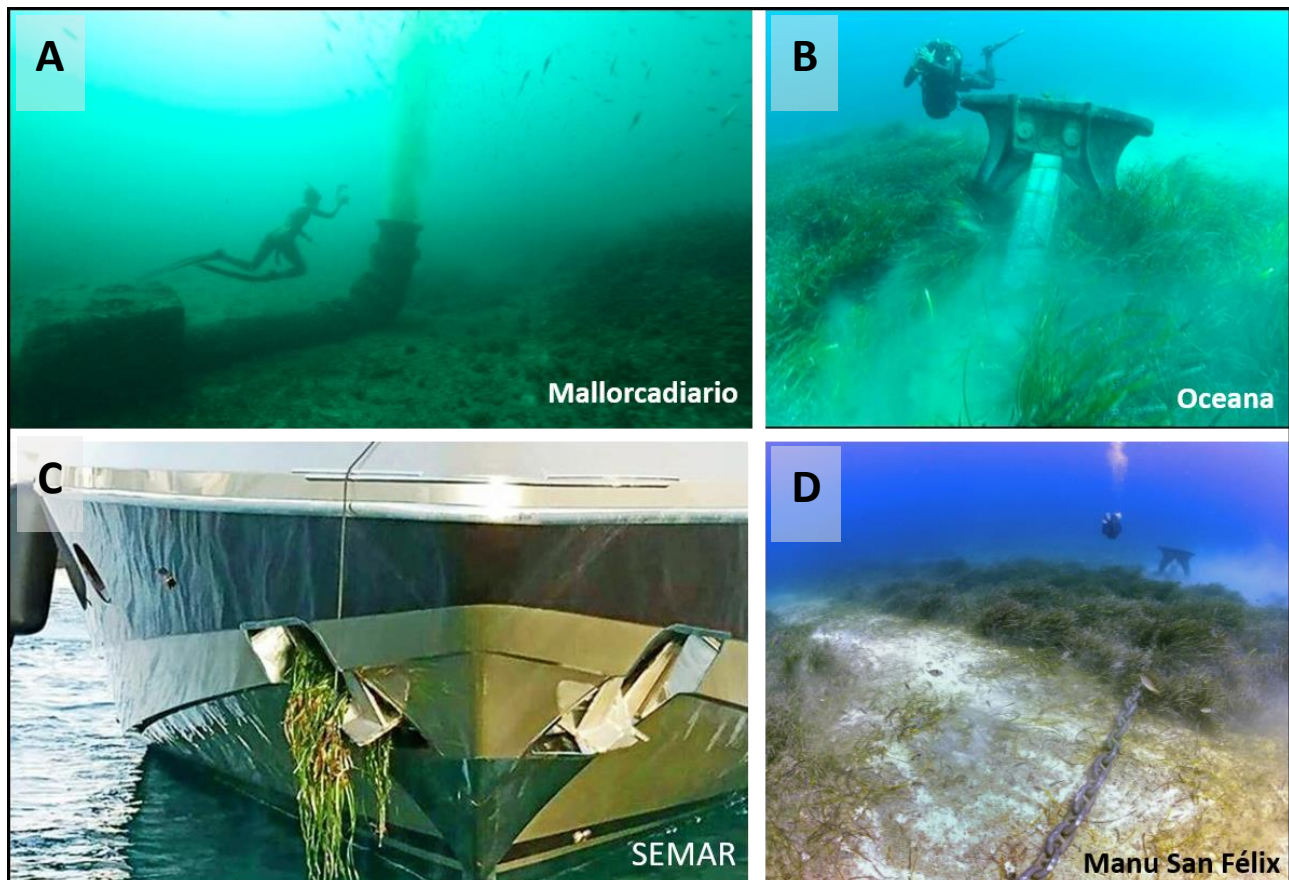


FIGURE 13. Some examples of negative anthropogenic impact on *P. oceanica*: (A) - degradation of the meadows, caused by the discharge of the outfall; (B), (C), and (D) - the direct physical damage to seagrass from unregulated anchoring of boats by anchors and chains.

P. oceanica meadows are also negatively affected by unregulated anchoring of boats (Fig. 13b, c, d), especially during the summer period (Balaguer et al., 2011). It has caused an adverse effect on the seagrass meadows, decreasing their cover and shoot density (Vázquez-Luis et al., 2015). To diminish the direct physical damage to the fields, in 2006, as part of the LIFE-POSIDONIA project, regulated anchorages (Fig. 14) were installed within specific areas of Community Interest (SCI (LIC in Spanish)) in *Posidonia* fields (Fig. 15) (Álvarez et al., 2015). In the Balearic Islands on the whole, 45 SCI areas have been declared (16 in Mallorca, 16 in Menorca, and 13 in Ibiza and Formentera) (Xarxa Natura a les Illes Balears, 2022).



FIGURE 14. Regulated anchorages installed in the Balearic Islands in specific areas of Community Interest (SCI) in *Posidonia* fields, to prevent boats from anchoring and destroying seagrass meadows.

In addition to mechanical damage, recreational boats also contribute to the spread of invasive species (Williams, 2007). The presence of exotic algae may cause irreversible changes and the decline of the *P. oceanica* ecosystem. In particular, the rapid distribution of alien species within the genus *Caulerpa* (*C. taxifolia* (M. Vahl) C. Agardh and *C. racemosa* (Forsskål) J. Agardh) led to competitive displacement of *P. oceanica* in the Mediterranean (Ballesteros et al., 2007; Holmer et al., 2009; Deudero et al., 2010). The presence of *C. racemosa* has caused massive changes in benthic assemblages (Argyrou et al., 1999) and significantly elevated sulphate reduction rates, favouring the increase of hydrogen sulphide concentration in the sediment, which is highly toxic to plants (Holmer et al., 2004).

Red alga *Lophocladia lallemandii* (Montagne) F. Schmitz, was observed in the Mediterranean for the first time in the 1990s (Álvarez et al., 2015). It grows epiphytically on the leaves and rhizomes of *P. oceanica*, forming very voluminous masses that cover the meadow and cause a decrease in its size, growth and survival of the bundles (Marbà et al., 2014). The most recently reported invasive alga in the Balearic Islands is *Halimeda incrassata* (J. Ellis) J.V. Lamoroux, which shows high rates of colonization of shallow sedimentary habitats although it is not a competitor for *P. oceanica* (Sureda

et al., 2015). However, Alós et al. (2016) cited that the presence of this algae could pose a threat to the Mediterranean Sea's sediment dynamics, food web, and interactions with native seaweeds and seagrasses.

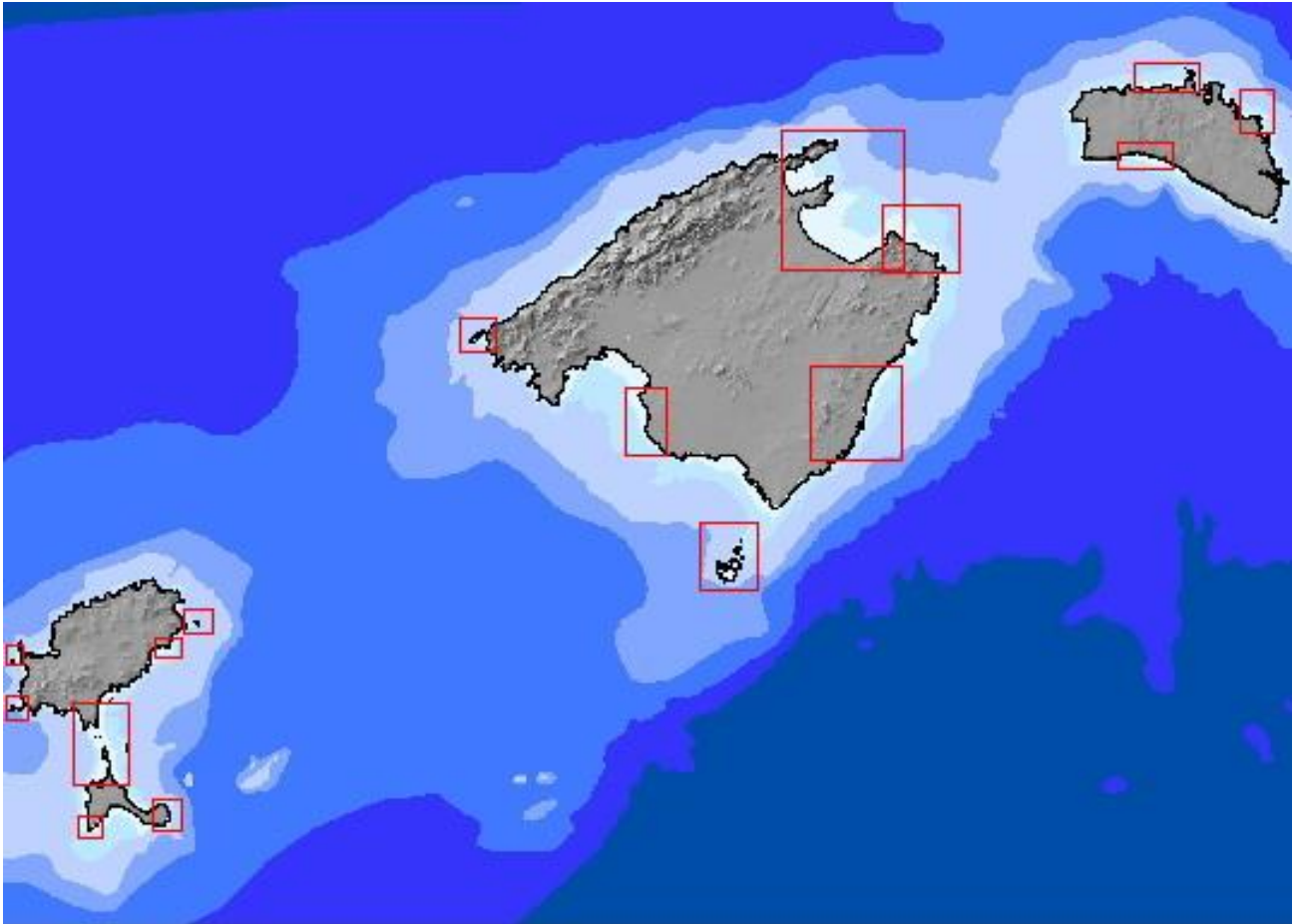


FIGURE 15. Map of of *Posidonia* fields situated within the special areas of Community Interest (SCI) of the Balearic Islands (https://lifeposidonia.caib.es/user/carto/index_cs.htm).

Pollutants such as fuels, detergents, pesticides, antibiotics, metals, hydrocarbons, etc. can cause alterations in the metabolism and growth of the plant or even its death (Chesworth et al. 2004; Bucalossi et al. 2006). There are different sources of these substances, including recreational boating that can be associated with the input of heavy metals from anti-fouling paints and fuels (Copat et al., 2012). These trace elements accumulate in the food chain, producing negative impacts on the health of the biota and humans (Leon and Warnken, 2008, Lloret et al., 2008). Other sources, less common, but locally relevant, may include ammunition fragments derived from military activity (see Objective 2 (Methodology)).

Lastly, the rise in temperature within the current context of climate change imposes a highly negative impact on seagrass globally, and particularly in the Mediterranean, where a warming trend has been observed since 1900 (Vargas-Yáñez et al., 2010). Temperature is one of the main ecological factors defining seagrass distribution, and both its decrease and increase beyond certain thresholds negatively impact the development and survival success of *P. oceanica* (Marbà and Duarte, 2010;

Guerrero-Meseguer et al., 2017; Ontoria et al., 2019). Accordingly, a seawater temperature increase up to 27.5°C–28.0°C tripled the mortality rate of *P. oceanica* shoots in the Balearic Islands (Marbà and Duarte, 2010). The cover reduction of *P. oceanica* worsens not only the conditions of the seagrass-associated assemblages, but negatively changes the marine ecosystem of the Mediterranean Sea in general (Boudouresque et al., 2012). Therefore, it is imperative to study and develop a comprehensive approach to the problem of negative anthropogenic influence and take measures to reduce the consequences.

1.4.2. Regulatory Framework

Rapid degradation in water quality resulting from anthropogenic influence, and the ecological importance and fragility of *P. oceanica* habitats have promoted their inclusion in European protective regulations such as the Water Framework Directive (WFD 2000/60/EC). The main objective of this trans-national strategy is to establish the basis of policies for the monitoring, protection and enhancement of the status of aquatic systems for all water bodies across Europe (Romero et al., 2007; Bennett et al., 2011). The water masses are classified according to their ecological status through the use of specific biological quality elements (BQE), which are determined by monitoring and assessing biological indicators (Mascaró et al., 2012). In particular, for coastal waters, phytoplankton, macroalgae and angiosperms, and benthic invertebrate fauna have been considered as BQEs (Romero et al., 2007).

Several directives and laws, as well as national and regional regulations, have been developed based on the WFD 2000/60/EC. Thus, the Marine Strategy Framework Directive (MSFD 2008/56/EC) of the European Parliament and of the Council, of June 17th, 2008, establishes a framework for community action on marine environment policy (Marine Strategy Framework Directive). At a national scale, the Law 62/2003, of December 30th, on fiscal, administrative and social order measures, includes the modification (article 129) of the text of the Water Law, approved by Royal Legislative Decree 1/2001, of 20th July, which, in turn, incorporates the directive WFD 2000/60/EC into Spanish law, providing a community framework for action in the field of water policy. The Royal Decree 907/2007, of July 6th, approved the Hydrological Planning Regulation and the Decree Law 1/2015, of April 10th, establishes the Hydrological Planning Instruction for the intra-community hydrographic demarcation of the Balearic Islands (BOIB no. 52 of April 11th, 2015) (Vaquer-Sunyer et al., 2021). Following the guidelines of WFD 2000/60/EC, the regulations provide means to protect coastal ecosystems. They also include the development of indicators that can be used to assess the current ecological status of Mediterranean coastal waters, and the Balearic Islands in particular. These indicators are based on the seagrass species *P. oceanica*. At the level of the Spanish State, each autonomous community is responsible for applying the WFD. Therefore, in the Balearic Islands, the Govern de les Illes Balears is in charge of implementing the Directive as it had that competence transferred (Barón et al., 2011).

In addition to the regulation of aquatic systems, a number of precautionary measures were adopted for the regulation and protection of *P. oceanica* meadows in particular. This ecosystem is protected at European level by the Habitats Directive of the European Union (Council Directive 92/43/EEC on the conservation of natural habitats and wild fauna and flora), which was modified various times and adapted to technical and scientific progress. Council directive 97/62/EC of 27 October 1997 (having regard to Council Directive 92/43/EEC) included the updated, replaced, Natura 2000-based codes, which identified each natural habitat type and species. Subsequently, Directive

2006/105/CE, of November 20, 2006, adapted Directives 73/239/CEE, 74/557/CEE and 2002/83/CE on the environment, on the occasion of accession by Bulgaria and Romania. These directives included *P. oceanica* meadows in Annex 1 (habitat 1120), as a priority habitat to be conserved within the territory of the European Union (Telesca et al., 2015). In the Mediterranean, the European Union Fisheries Regulation (Council Regulation (EC) No. 1626/94) expressly prohibits trawling in seagrass meadows, and Council Regulation (EC) No. 1967/2006, regarding management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, allows trawling on bottoms deeper than 50 meters, beyond the deepest limit of *P. oceanica* distribution (Álvarez et al., 2015).

In Spain, *P. oceanica* meadows, as a habitat, have been protected since 1995 (Royal Decree 310/1995) with the adaptation of the European Habitats Directive, and, as a species, since 2011 with its inclusion in the List of Wild Species under the Special Protection Regime (Royal Decree 139/2011). Nevertheless, after the Spanish Administration restricted coastal and littoral trawling in the Mediterranean back in 1962, *P. oceanica* received some informal protection in Spain, and as of 1988 (Royal Decree 679/1988; Royal Decree 1440/1999), bottom trawling at depths less than 50 meters and trawling over meadows of *P. oceanica* are prohibited (Álvarez et al., 2015).

In the Balearic Islands, after the modification of its Statute of Autonomy (Law 1/2007, of February 28th), the power of decision in matters of environmental management, ecology and protected natural spaces is included. Based on Law 1/2007, Law 42/2007, of December 13th, on natural heritage and biodiversity was developed. The third chapter of Title II of Law 42/2007 focused on the Natura 2000 European Ecological Network in the Marine Environment, which includes protection figures such as a Special Conservation Zone (SCZ), a Site of Community Importance (SCI), and a Special Protection Zone for Birds or Special Protection Area (SPA) (BOE No. 299, of December 14th, 2007). The Natura 2000 Network presents the most valuable and threatened species and habitats in Europe, listed in the Habitats Directive (Council Directive 92/43/EEC, of May 21st, 1992) and Birds (Directive 2009/147/EC, of 30th November 2009) (Ministry for the Ecological Transition and the Demographic Challenge, 2022). When applying this regulation, each Autonomous Community defines these spaces and communicates them to the Ministry of the Environment to communicate them to the European Commission, as well as to establish the necessary conservation measures (Website of the European Commission, 2022).

Later, the Royal Decree 139/2011 of February 4th, added *P. oceanica* to the List of Wild Species under Special Protection as an endangered species, taking into consideration the marine-environment protection Law 41/2010 of December 29th, where the standards of protection were specified (Morelle, 2019). Most recently, the Balearic Islands Decree 25/2018, of July 27th, aimed to guarantee the protection and conservation of *P. oceanica* and its habitat. This Decree regulates activities that may impact the seagrass-meadow ecosystem, as well as promoting specific measures to help maintain and preserve seagrass meadows.

1.5. FORAMINIFERA AS (BIO) INDICATORS

In recent years, the Water Framework Directive (WFD 2000/60/EC), as well as the Marine Strategy Framework Directive (MSFD 2008/56/EC), have given impetus to the quest for the most effective bioindicators of coastal water quality. Due to its high sensitivity to disturbances (such as reduction of water transparency, eutrophication, pollution, erosion, etc.), wide distribution along the Mediterranean coast, and dominance among marine plants in the Balearic Islands, in

particular, *P. oceanica* was recognized as a reliable bioindicator to be used (Romero et al., 2007; Barón et al., 2011; Vaquer-Sunyer et al., 2021). Following the WFD 2000/60/EC requirements, Romero et al. (2007) proposed the *Posidonia oceanica* Multivariate Index (POMI), based on previous knowledge about the *P. oceanica* ecosystem, and its structural and functional attributes. There was designed a list with 14 metrics and their expected response to some impacts or changes in environmental quality (Romero et al., 2007). Accordingly, the EQR (Ecological Quality Ratio) stabling boundaries of five ecological statuses from adverse to high were calculated, as required by the WFD 2000/60/EC.

The strength of POMI relies on combining an extensive list of data (metrics) to discard possible experimental errors or regular, natural inter-annual changes with no relation to stress. In addition, this method also takes into account the slow rate of *P. oceanica* meadow recovery (Meinesz and Lefèvre, 1984; González-Correa et al., 2005) by combining various indicators and parameters related to different levels of biological organisation (Romero et al., 2007). Thus, when meadow cover or shoot density are poor, plant physiological descriptors may indicate a gradual improvement in water quality. The implementation of POMI is restricted to areas where seagrass meadows occur. As a result, slow recolonization following major disturbances (Meinesz and Lefèvre, 1984; Boudouresque et al., 2012) can make it difficult to fully assess the ecological status of a water body and implement improvement measures. Also, this index cannot be used in places such as the mouths of coastal rivers, with low salinity, increased turbidity, and abundant sediment input, where *P. oceanica* cannot physically grow (Pasqualini et al., 1998).

Thus, POMI facilitates the interpretation of multiple-data-based information into a simple index, which is relatively easy to apply and is very useful in coastal management in the Mediterranean Sea. However, the number of parameters to be analyzed and the physical elimination of protected species by cutting their leaves and rhizomes for laboratory analysis, lead to the search for alternatives based on other indicators, such as foraminifera.

1.5.1. Bioindicators Based on Foraminifera Taxonomy

Many organisms that inhabit *P. oceanica* meadows are also effective bioindicators of coastal water quality, providing significant additional information about the state of the ecosystem because they have adapted well to the plant throughout its evolutionary history (Pomar et al., 2017; Baceta and Mateu-Vicens, 2022). The benthic macrofauna is the group most used to assess the ecological quality status (EcoQS) in marine environments and to calculate the indices based on the requirements of the WFD 2000/60/EC and the MFSO 2008/56/EC (Dauvin et al., 2012; Alve et al., 2016; El Kateb et al., 2020). One of the most successful in its application to different geographical areas, as well as to different environments, is the AZTI Marine Biotic Index (AMBI) developed by Borja et al. (2000). This index was proposed to be applied to the European coastal zone. It is based on the five ecological groups of species in the macrobenthic communities of soft bottoms, according to their sensitivity to an increase in organic matter. More than 900 species were identified to be used in a single formula to calculate the index (Borja et al., 2000).

Subsequently, the AMBI index has been successfully adapted to other geographical regions outside of Europe such as South America (Muniz et al., 2005), West Greenland (Josefson et al., 2008), Algeria (Bakalem et al., 2009), China (Cai et al., 2003; 2015), Tunisian coast and lagoons (Afli et al., 2008), Southwest Indian Ocean (Bigot et al., 2008), Eastern Canada (Callier et al., 2008), Florida (Borja

and Tunberg, 2011), Southern California (Teixeira et al., 2012), Morocco (Daief et al., 2014), etc., and new species have been added (Borja et al., 2019). However, the use of only macroinvertebrate species may be rather ineffective in naturally-stressed and low-diversity communities, which may confer certain limitations on AMBI use (Muxika et al., 2005; O'Malley et al., 2021). Therefore, the use of meiofauna, which occurs in high density in small areas, including benthic foraminifera, appears particularly suitable for biomonitoring (Alve et al., 2016).

The analysis of foraminiferal assemblages has proven to be useful in the assessment and monitoring of coastal and shelf environments because their ecologies are well-studied, and several species have known stress tolerances (Alve, 1999; Yanko et al, 1999; Coccioni, 2000; Scott et al., 2001; Samir and El-Din, 2001; Hallock et al, 2003; Frontalini and Coccioni, 2008; Frontalini et al., 2009; Khokhlova, 2013; Mateu-Vicens et al., 2014). Moreover, foraminifera are useful for long-term monitoring, as they create either a carbonate or an agglutinated test that is readily preserved in the sediment and constitutes records of environmental conditions through time (Murray, 1971, 2006; Yanko et al, 1999; Martínez-Colón et al., 2009; Gupta and Singh, 2011). Due to their special qualities and a low impact on the environment during their sampling (Hallock et al., 2003) the AMBI criteria were adapted to these protists by the international FOraminiferal BloMONitoring (FOBIMO) Group (Schönfeld et al., 2012). The main objective was to develop specific standardized foraminiferal biomonitoring tools (method for sampling, storing, processing, and analyzing sediment samples for living benthic foraminiferal assemblages), and then calculate the biotic index based on foraminifera response to an environmental stress gradient (Foram-AMBI, Alve et al., 2016), easy to implement in a wider community (Jorissen et al., 2018). The main complication was that the same species of foraminifera respond differently to the same stress in different environmental conditions (Bradshaw, 1961; Nikulina et al., 2008; Jorissen et al., 2018). Consequently, it was decided to study NE Atlantic and Arctic ecosystems (Alve et al., 2016), transitional environments, tropical environments, and Mediterranean ecosystems separately (Jorissen et al., 2018; El Kateb et al., 2020).

The goal of the Foram-AMBI index was to assess the health of the benthic environments by assigning benthic foraminiferal species to one of five predefined ecological groups according to their tolerance/sensitivity to organic matter enrichment (based on AMBI classification). In the Mediterranean 199 common benthic foraminifera species (with a clear response to organic enrichment) were included and were classified into five ecological categories: sensitive (I group with 79 species), indifferent (II group with 60 species), 3rd order opportunists (III group with 46 species), 2nd order opportunists (IV group with 12 species), and the last 1st order opportunists (V group with 2 species) (Jorissen et al., 2018). However, that list appeared insufficiently effective in *P. oceanica* meadows, where frequently identified species were not included in the master list (El Kateb et al., 2020).

Alternatively, there are other indices such as the Foram Stress Index (FSI) (Dimiza et al., 2016) for ecological and environmental assessments of the status of soft-benthic meiofaunal communities from the Eastern Mediterranean (Saronicos Gulf and Elefsis Bay), but may be extrapolated to the whole Mediterranean region. The main advantage of this index is its simplicity in comparison with the Foram-AMBI, as it uses only two ecological groups of species: sensitive (Sen) and stress-tolerant (Str) to organic material enrichment. The percentage contribution of Str between 10% and 50% represents sites of high ECoQ, and the highest quality status is characterized by Str less than 10%, while the poor-quality status corresponds to areas with relative abundances of Str over 90% (Dimiza et al., 2016). However, neither of the indices can distinguish whether a given organic enrichment is natural or anthropogenic (Dimiza et al., 2016).

Foram-AMBI and FSI based on taxonomic analysis turned out to be useful in areas with a high level of pollution. However, their application presents some limitations, especially for samples with low foraminiferal densities of living specimens (300 living individuals were recommended to be picked according to the protocol of FOBIMO) (Schönfeld et al., 2012; Alve et al., 2016; Dimiza et al., 2016; Jorissen et al., 2018). Moreover, the sample must be collected from surface soft sediment using an interface corer or box corer, not grabbing, for keeping the sediment intact (Schönfeld et al., 2012), which is technically difficult on the *P. oceanica* matte. Additionally, both Foram-AMBI and FSI are species-based and require high taxonomical skills, which prevents their general use for routine monitoring. This issue can be solved with the development and application of non-taxonomically based indicators, which are not so time-consuming, and do not require highly-qualified staff to use them (Hallock et al, 2003).

1.5.2. Non-taxonomical Bioindicator Indices

Considering the complexity of taxonomy-based protocols, foraminifera have been used to create, non-taxonomical, bioindicator indices for the environmental assessment of particularly sensitive ecosystems such as coral reefs and seagrass meadows. Thus, the “FORAM” (Foraminifera in Reef Assessment and Monitoring) Index (FI) was developed as an ecology-based index for the assessment of habitat quality, to evaluate whether water quality is sufficient to support reef recovery based on the foraminiferal community as a sensitive indicator (Hallock et al., 2003, Oliver et al., 2014). In general, the FORAM Index is calculated on three components: the proportion of stress-tolerant taxa (Po); the proportion of large, algal symbiont-bearing taxa (Ps); and other small, heterotrophic foraminiferal taxa (Ph). If symbiont-bearing taxa occur, FI values increase. On the contrary, FI decreases if stress-tolerant taxa become abundant (Hallock, 2012).

The advantages of this protocol are the minimal impact on the study area, the low economic cost of sample collection and analysis, and the simplicity of the classification that allows the sampling team (e.g., public employees, students, volunteers) to be easily instructed.

Although this method does not provide detailed information about the taxonomic composition of the assemblages, its utility and reliability have been successfully proven in reef ecosystems worldwide (Florida, Brazil, Pacific islands, Australia; Hallock et al., 2003; Hallock, 2012) and, more recently, in areas of the eastern Mediterranean (Koukousioura et al., 2011). However, the scarcity and low diversity of symbiont-bearing taxa in the western Mediterranean hinder the application of FI in this region. Over time it was subsequently modified and adapted to different global physical environmental conditions such as water temperature, salinity, and nutrients, among others (Hallock, 2012; Dimiza et al., 2016; Prazeres and Renema, 2019).

However, applying a similar methodology, based on different ecological assemblages of foraminifera, specific bioindicator indices for *P. oceanica* meadows were produced in my MSc. Theses (Khokhlova, 2013; Mateu-Vicens et al., 2014) for environmental assessments carried out in the western Mediterranean. The ecological groups on which these indices are based consist of a modification of the epiphytic foraminifera morphotypes described by Langer (1993) and reflect behavioral characteristics, feeding patterns, and lifespans. This modification aims to solve the problems of the original classification. In Langer's (1993) morphotypes, symbiont-bearing taxa are divided into categories A and D, representing opposite life forms. A morphotype represents long-lived, often encrusting taxa. By contrast, morphotype D refers mainly to opportunistic species. To resolve these inconsistencies, Khokhlova (2013) grouped symbiont-bearing taxa into a single

morphotype (SB), and morphotypes A and D, renamed A* and D*, correspond exclusively to long-lived, immobile, and opportunistic taxa, respectively (Fig. 16). However, the validity of the modification must be tested with tests independent of observations about life span and behaviour. The analysis of the trophic structure of the epiphytic foraminiferal assemblage, with the help of stable N and C isotopes, enables us to determine whether the different morphotypes use trophic resources in a particular way and, as a consequence, reflect an adaptation to nutrient acquisition.






Khokhlova, 2013 Mateu-Vicens et al., 2014	Motility	Description	Life span
	A*	Encrusting Mostly flat forms, permanently attached to the substrate by glycosaminoglycans. Ex: <i>Planorbulina</i> , <i>Nubecularia</i> , <i>Miniacina</i>	1 year
	SB	Sessile Symbiont-bearing taxa. Ex: <i>Peneroplis</i> , <i>Sorites</i> , <i>Amphistegina</i> (Eastern Mediterranean)	1 year
	B	Temporary motile Same as in Langer's (1993) classification	2-3 months
	C	Motile Same as in Langer's (1993) classification	3-4 months
	D*	Permanently motile Single aperture in upright position. Opportunistic forms. Ex: <i>Quinqueloculina</i> , <i>Triloculina</i> , <i>Adelosina</i> , <i>Textularia</i>	short

FIGURE 16. Modified morphotype categories and their ecological features based on Langer (1993), proposed in Khokhlova (2013), and Mateu-Vicens et al. (2014), where symbiont-bearing taxa are grouped into a new single morphotype SB (symbiont-bearing); renamed morphotype A* corresponds to long-lived, immobile taxa; and renamed morphotype D* corresponds to opportunistic taxa species; the morphotypes B and C correspond to the morphotypes of Langer (1993) without changes.

During the analysis of the samples to create the bioindicator indices, in some localities unusually high frequencies of epiphytic foraminifera with aberrant growth were detected following a patchy distribution. Foraminifera with abnormal tests are often associated with some type of stress, including heavy metal contamination. These samples coincide with places where the bivalve *Pinna nobilis* had high levels of heavy metals in its tissues (Morató et al., 2013). The detection of these abnormal foraminifera raised the question about the types of impacts and the response of the foraminiferal assemblage to their intensity and exposure time. In this thesis, this problem is addressed by analyzing the frequency of aberrant growth patterns and the composition of the foraminiferal tests. This is done together with information about the environmental state obtained from the bioindicator indices.

1.6. OBJECTIVES OF THE Ph.D. THESIS

The implementation and use of these indices based on epiphytic foraminifera were carried out according to the particularities of each study area. This included substrate type, temperature gradient, salinity concentration, light penetration, presence of organic matter, level, and type of pollution. Taking into account all these features and looking for a way to take advantage of the stable association between *Posidonia oceanica* seagrass and the epiphytic foraminiferal assemblage as a tool for environmental monitoring, the present thesis aims to develop the following specific objectives:

- Evaluation of the ecological groups (epiphytic foraminiferal morphotypes) upon which the bioindicator indices are based, and use of these bioindicators, which are highly cost-effective and easy to use, to assess the state of *P. oceanica* meadows, and contribute to their conservation. These inexpensive and easy-to-use indexes are targeted as management tools for short and mid-term scale evaluations.
- Performing a comparative analysis of the environmental conditions of different *P. oceanica* meadows located in the Balearic Islands (Spain) that have been subjected to different types of anthropogenic pressures using 1) bioindicator indices based on epiphytic foraminifera, 2) the frequency of abnormal-growth forms and 3) the trace-elemental concentrations within their tests to assess the impact of exposure to heavy-metal pollution.
- A review of published and grey literature on foraminiferal assemblages from *P. oceanica* meadows from Mallorca, Cabrera, and Menorca from the 1960s to now, and to apply the new bioindicator indices to this historical dataset to detect the effects of tourism since the 1970s.

CHAPTER 2

MATERIALS AND METHODS

To achieve the three main goals proposed in this thesis, the samples analyzed have been obtained mostly from *P. oceanica* meadows from the Balearic Islands (Spain), except for those representing multispecific seagrass meadows from tropical settings (Madagascar).

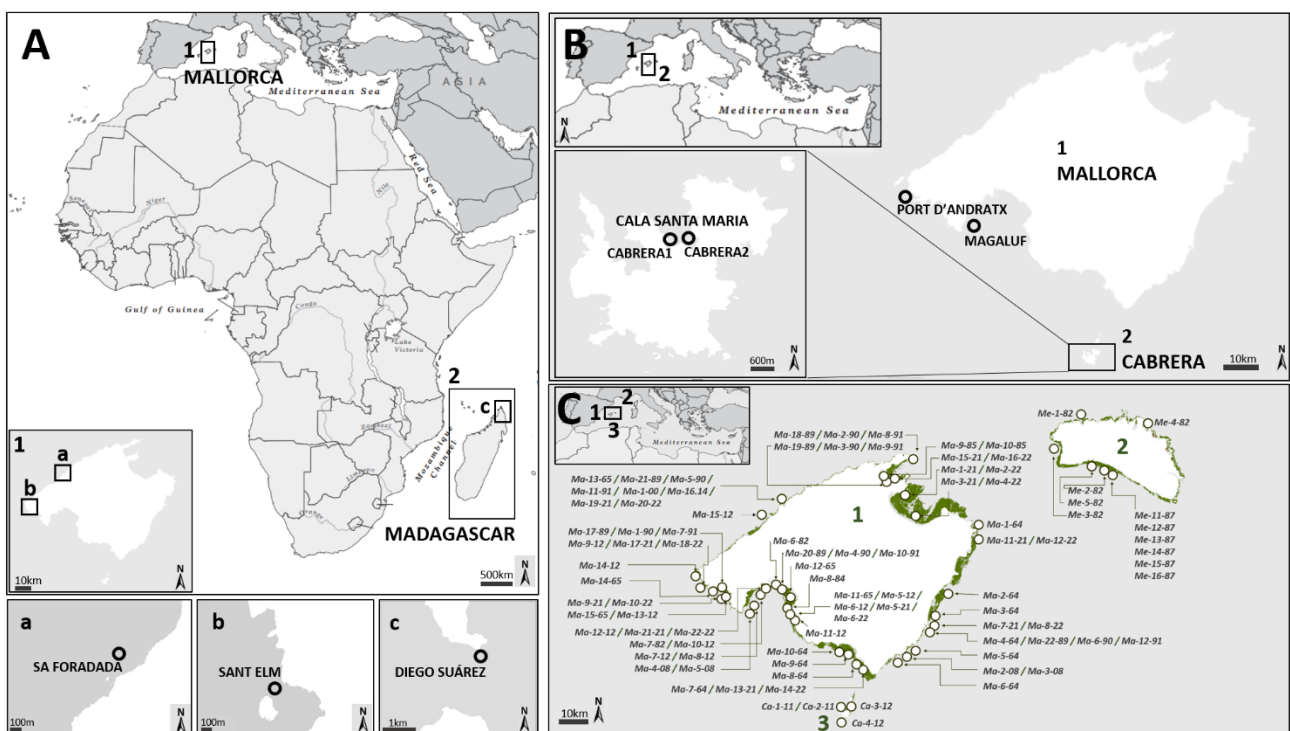


FIGURE 17. Map with a general view of the sampling sites of the study areas associated with the three main objectives of the thesis:

A. Objective 1. General view of the sampling sites in the Western Mediterranean and Indian Ocean, where: 1. Locations of the Mallorca sampling stations: a) Sa Foradada ($39^{\circ}44'47.02''N$, $2^{\circ}36'58.47''E$); b) Sant Elm ($39^{\circ}34'43.76''N$, $2^{\circ}20'55.59''E$); 2. Detailed location of the Madagascar sampling site (Indian Ocean): c) Diego Suárez ($12^{\circ}12'52.54''S$, $49^{\circ}21'59.46''E$).

B. Objective 2. Map of Mallorca and Cabrera Islands (Spain) in the Mediterranean Sea. 1. Mallorca Island with the detailed locations of the sampling stations in Port d'Andratx and Magaluf; 2. Cabrera Island with two sampling stations from Cala Santa Maria (Cabrera1 and Cabrera2).

C. Objective 3. General view of the locations from historical series of data of Mallorca (1): Cala Agulla (Ma-1-64), Cala Mèndia (Ma-2-64), Cala Murada (Ma-3-64), Portocolom (Ma-4-64), Porto Petro (Ma-5-64), Cala Santanyí (Ma-6-64), Es Caragol (Ma-7-64), Colònia de Sant Jordi (Ma-8-64), Ses Covetes (Ma-9-64), Sa Ràpita (Ma-10-64), Cala Blava (Ma-11-65), S'Arenal (Ma-12-65), Port de Sóller (Ma-13-65), Camp de Mar (Ma-14-65), Santa Ponça (Ma-15-65) (Mateu, 1970); Ciutat Jardí (Ma-6-82), Portals Nous (Ma-7-82), Cap de Regana (Ma-8-84) (Gazà, 1988); Cala Fornells (Ma-17-89, Ma-1-90, Ma-7-91), Portocolom (Ma-22-89, Ma-6-90, Ma-12-91), Cala Murta (Ma-18-89, Ma-2-90, Ma-8-91), Es Barcarès (Ma-19-89, Ma-3-90, Ma-9-91), Es Carnatge (Ma-20-89, Ma-4-90, Ma-10-91), Port de Sóller (Ma-21-89, Ma-5-90, Ma-11-91) (Abril, 1993); Badia de Pollença (Ma-9-85, Ma-10-85) (Moreiro, 1993); Port de Sóller (Ma-1-00) (Mateu et al., 2001); Cala d'Or (Ma-2-08 and Ma-3-08), and Portals Vells (Ma-4-08 and Ma-5-08) (Mateu-Vicens et al., 2010); Cala Blava (Ma-5-12),

Magaluf (Ma-8-12), Portals Nous (Ma-10-12), Son Verí (Ma-11-12), Ses Illetes (Ma-12-12), Santa Ponça (Ma-13-12), (Mateu-Vicens et al., 2014); Cala Blava (Ma-6.1-12 and Ma-6.2-12) (Racis, 2015); Port de Sóller (Ma-16-14) (Vaquer, 2016); Sant Elm (Ma-14-12), Sa Foradada (Ma-15-12) (Mateu-Vicens et al., 2016); Magaluf (Ma-7-12), Port d'Andratx (Ma-9-12); Alcandada (Ma-1-21 and Ma-2-22), Ca'n Picafort (Ma-3-21 and Ma-4-22), Cala Blava (Ma-5-21 and Ma-6-22), Cala Brafí (Ma-7-21 and Ma-8-22), Cala de Santa Ponça (Ma-9-21 and Ma-10-22), Capdepera (Ma-11-21 and Ma-12-22), Es Caragol (Ma-13-21 and Ma-14-22), Marina de Bonaire (Ma-15-21 and Ma-16-22), Port d'Andratx (Ma-17-21 and Ma-18-22), Port de Sóller (Ma-19-21 and Ma-20-22), Ses Illetes (Ma-21-21 and Ma-22-22) (Khokhlova and Mateu-Vicens, 2023). Menorca (2): Ses Fontanelles (Me-1-82), Cala Mitjana (Me-2-82), Cala En Blanes (Me-3-82), Arenal d'en Castell (Me-4-82) and Cala Galdana (Me-5-82) (Florit, 1983); Me-11-87, Ma-12-87, Me-13-87, Me-14-87, Me-15-87, Me-16-87 (Moreiro, 1993); and Cabrera (3): Cala Santa Maria (Ca-2-11), and Els Estels (Ca-4-12) (Mateu-Vicens et al., 2014); and Ca-3-12. Green area represents *Posidonia oceanica* coverage (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

Moreover, a considerable amount of information has been obtained from a detailed and exhaustive review of the published literature. This review has been based on the foraminiferal assemblages described in seagrass meadows from the Balearic Islands in theses, reports, and repositories. The methodology used comprises a taxonomic and ecological description of the foraminiferal assemblages for the subsequent application of bioindicator indices, detection of abnormal growth patterns; and molecular analysis including the characterization of the trophic structure of the foraminiferal assemblage using N and C stable isotopes and the geochemical composition of the foraminiferal tests to detect the presence of pollutants such as heavy metals. The following section will be organized by the three main objectives to be achieved.

2.1. OBJECTIVE 1: N AND C ISOTOPIC ANALYSIS TO VALIDATE KHOKHLOVA'S (2013) ECOLOGICAL GROUPS ON WHICH THE BIOINDICATOR INDICES ARE BASED.

2.1.1. Study Site.

The study of N and C stable isotopes to characterize the trophic structure of the epiphytic foraminiferal assemblage and to confirm the validity, regardless of the type of seagrass, of the proposed morphotypes in Khokhlova (2013), has been carried out by comparing samples from different locations in Mallorca (Sant Elm and Sa Foradada) and Madagascar (Diego Suárez) (Fig. 17.A).

However, before isotopic analysis, some issues must be considered. Test size and abundance differ widely among epiphytic taxa. Thus, the number of specimens collected (Table 1) to obtain a detectable signal is highly variable, depending on the dominant species within each morphotype. Previous studies (Nomaki et al., 2008) point out that, to obtain a useful isotopic signal for small-sized taxa, the required number of specimens is much higher (between 50 and 300) than for bigger-sized species. Despite Nomaki et al. (2008) do not refer to an explicit size threshold to separate small from large taxa, the only species studied from 50 individuals per analysis was *Cyclammia cancellata* Brady, 1879. This species is typically 3–4 mm in diameter. In this study, the size threshold between large and small specimens was 2 mm in maximum diameter.

The analyzed specimens came from opportunity samples, two from Mallorca (Balearic Islands, NW Mediterranean) and one from Madagascar. The two sites from Mallorca were located along the

Serra de Tramuntana, a mountain range that extends NW-SE on the northern side of the island (Fig. 17. A1): more precisely, Sa Foradada (39°44'47.02"N, 2°36'58.47"E) and Sant Elm (39°34'43.76"N, 2°20'55.59"E). In Madagascar (Fig. 17. A2), the samples were collected in the NE at Diego Suarez (12°12'52.54"S, 49°21'59.46"E). Samples from the Mallorca sites consist of three replicates (1, 2, and 3) in Sa Foradada and two (1 and 2) in Sant Elm, which were all obtained at similar depths (ca. 15 m). Sample collection was performed manually in November 2012. Based on foraminiferal densities on leaves and rhizomes of *Posidonia oceanica* reported by Langer (1993), six to seven shoots per replicate were collected and placed in a plastic bag to transport them to the laboratory for freezing and storage at -20°C until use. In Madagascar, two random replicates (1 and 2) were sampled in January 2013 at less than 5 m water depth. In contrast to Mallorca sampling sites, in Madagascar, seagrass meadows were formed by more than a single species and included *Syringodium* sp., *Halodule* sp., and *Cymodocea nodosa*. Once collected, samples were placed in a plastic bag, frozen, and subsequently transported to the laboratory, where they were kept at -20°C until their use.

TABLE 1. Total number of foraminiferal individuals collected by localities and morphotypes (M). Non-acidified samples (No Acid) and acidified samples (Acid). Genera forming the different morphotypes at each locality are specified in Fig. 18.

M	Genera	Sa Foradada						Sant Elm				Madagascar				
		1		2		3		1		2		1		2		
		NA	A	NA	A	NA	A	NA	A	NA	A	NA	A	NA	A	
AF*	<i>Planorbulina</i>	105	70	143	207	53	52	36	39	47	47	21	21	17	16	
	<i>Nubecularia</i>	70	135	227	268	193	221	86	68	37	36	/	/	/	/	
	<i>Cibicidella</i>	56	62	84	92	21	25	145	130	131	130	/	/	/	/	
	<i>Cyclocibicides</i>	/	/	/	/	3	/	/	/	/	/	/	/	/	/	
	<i>Spirulina</i>	6	6	21	22	43	89	3	2	1	/	/	/	/	/	
		237	273	475	589	313	387	270	239	216	213	21	21	17	16	
AR*	<i>Miniacina</i>	31	31	40	40	27	27	15	15	9	8	/	/	/	/	
LM	<i>Peneroplis</i>	24	/	74	/	33	/	4	/	2	0	2	1	10	12	
	<i>Sorites</i>	3	/	11	/	6	/	3	/	/	/	/	/	/	/	
	<i>Marginopora</i>	/	/	/	/	/	/	/	/	/	/	15	15	5	5	
		27	0	85	0	39	0	7	0	2	0	17	16	15	17	
LR	<i>Amphistegina</i>	/	/	/	/	/	/	/	/	/	/	36	35	6	6	
B	<i>Lobatula</i>	65	108	207	246	68	22	37	37	50	50	9	9	/	/	
	<i>Rosalina</i>	190	164	393	463	283	402	219	218	207	208	56	56	9	8	
	<i>Cibicides</i>	/	/	/	/	/	/	/	/	/	/	49	49	21	21	
		255	272	600	709	351	424	256	255	257	258	114	114	30	29	
C	<i>Elphidium</i>	16	/	54	/	36	/	6	/	5	/	51	/	27	/	
	<i>Astrononion</i>	31	/	32	/	22	/	8	/	9	/	/	/	/	/	
		47	0	86	0	58	0	14	0	14	0	26	25	14	13	
D*	<i>Quinqueloculina</i>	107	113	355	243	309	230	33	33	47	47	121	121	95	94	
	<i>Bolivina</i>	1	/	2	3	8	2	1	1	/	/	/	/	/	/	
		108	113	357	246	317	232	34	34	47	47	121	121	95	94	
Total		705	689	1,643	1,584	1,105	1,070	596	543	545	526	335	332	177	175	10,025

A major control, for the analysis, in addition to the specimen size, is the abundance of each morphotype. There is insufficient organic matter for isotopic analysis without a minimum number of specimens per morphotype. Therefore, for large, encrusting forms such as *Miniacina miniaceae* (Pallas, 1766), the number of specimens required to acquire sufficient organic matter was much

fewer than in the case of the smaller taxa forming the B morphotype. This has been particularly limiting for morphotype C (mostly keeled *Elphidium* spp.), which is generally very scarce. Indeed, only in Sa Foradada were specimens corresponding to morphotype C sufficiently abundant to obtain the minimum number established for small taxa (50 individuals).

In the laboratory, the leaves and rhizomes were analyzed to identify the different morphotypes and ecological groups of foraminifera considered (Fig. 18). Only colored foraminifera, indicating the presence of cytoplasm and/or symbiont algae (i.e., symbiont-bearing taxa), were separated by hand picking using a binocular stereomicroscope Leica MZ16. Individuals corresponding to each foraminiferal morphotype of all replicates were stored in separate Eppendorf tubes with distilled water. Morphotype classification was based on Langer (1993) and its subsequent modification (Khokhlova, 2013; Mateu-Vicens et al., 2014). Moreover, some taxa were considered apart from their corresponding morphotype to determine whether the location of the permanently attached individuals and the type of algae determine the utilization of trophic resources (Table 1). Thus, the A* morphotype was split into AF* and AR*, corresponding to the taxa attached to the leaves and those living in the rhizome, respectively. Symbiont-bearing foraminifera (SB morphotype) were grouped into LR (Large rotalids), only occurring in Madagascar and including species hosting diatoms exclusively; and LM (Large miliolids), with taxa harboring, in this study, red algae and dinoflagellates.

2.1.2. N and C Isotopic Analysis

The ecological groups on which the bioindicator indices for *P. oceanica* meadows are based correspond to a review of the morphotype classification of epiphytic foraminifera described by Langer (1993). The updated classification aims to correct the inconsistencies pointed out by Langer in his original work, but it needs to be validated by independent criteria.

To proceed with the validation, a total of seven samples (3 from Sa Foradada, 2 from Sant Elm, and 2 from Madagascar) for stable-isotopic analyses were dried (60°C, 24 h) and approximately one-half of the specimens available were used with intact shells ($\delta^{15}\text{N}$) whereas the other half was acidified with 1 M HCl during 3 h, and only the cytoplasm was considered for $\delta^{13}\text{C}$ analysis (Ng et al., 2007). The samples were then rinsed with distilled water and dried again at 60°C for at least 24 hours. Each dry sample, containing several individuals from the same morphotype, was ground to a homogeneous powder and 1.9–2.1 mg, weighed, and stored in ultra-clean tin capsules, which were combusted for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic composition by continuous-flow isotope-ratios spectrometry (CF-IRMS THERMO delta X PLUS mass spectrometry). One sample of reference material was analyzed after every eight samples to calibrate the system. The reference material used for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic analyses was Bovine Liver Standard BSL (1577b) from the U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD 20899. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ deviations of 107 analyses of a regularly calibrated reference standard were, for BSL, 0.012‰ and 0.09‰, respectively.

Stable-isotopic data were calculated by comparing the ratio of the two most abundant isotopes ($^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$) in the sample to international isotopic standards. Results are expressed in terms of parts per thousand (‰) deviations from the standards according to the following equation:

$$\delta X = [(R_{\text{sample}} / R_{\text{reference}}) - 1] \times 10^3$$

where X is either ^{13}C or ^{15}N and R is the corresponding $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratio (Peterson and Fry, 1987; Deudero et al., 2011).

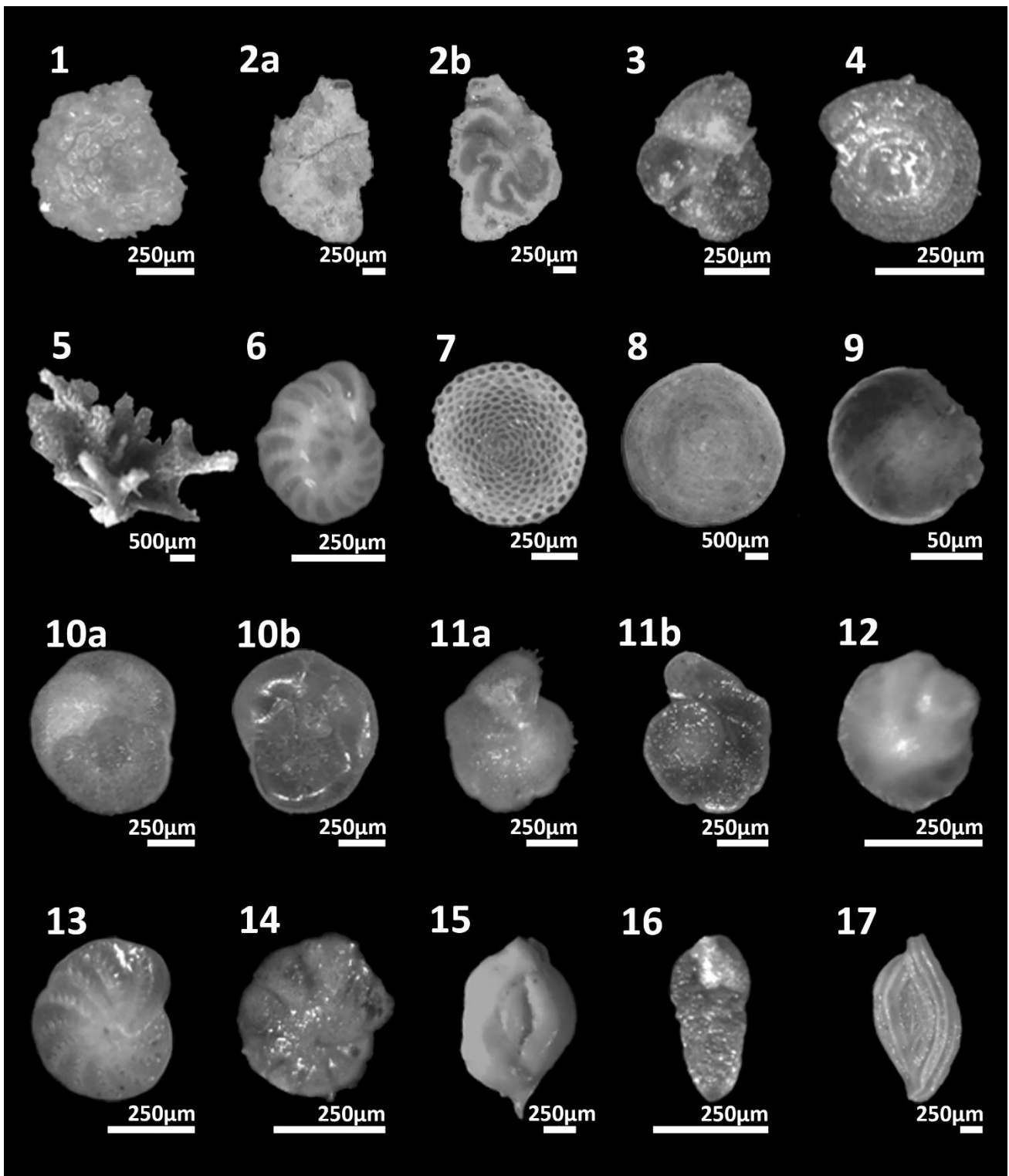


FIGURE 18. Foraminiferal genera and their morphotype classification based on Mateu-Vicens et al. (2014), reported in the present study. AF*: 1 – *Planorbulina*, 2a, b – *Nubecularia*, 3 – *Cibicidella*, 4 – *Spirillina*; AR*: 5 – *Miniacina*; LM: 6 – *Peneroplis*, 7 – *Sorites*, 8 – *Marginopora*; LR: 9 – *Amphistegina*; B: 10a, b – *Rosalina*, 11a, b – *Lobatula*, 12 – *Cibicides*, C: 13 – *Elphidium*, 14 – *Astrononion*; D*: 15 – *Massilina*, 16 – *Bolivina*, 17 – *Sigmoilinita*.

2.1.3. Statistical Analysis

Multivariate techniques were used to compare $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ variations by morphotype and locality. The analysis was based on the modified Euclidean distance dissimilarity on the isotopic signatures of carbon and nitrogen, as it is widely used in other studies using stable isotopes as tracers of trophic ecology (e.g., Kürten et al., 2014). Non-metric Multi-Dimensional Scaling (MDS) was applied to correlate morphotype abundance and locality variability with isotopic values. Two-way Crossed Analysis of Similarities (ANOSIM) was applied to test whether there were differences among $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ datasets, and the ratios between both isotopic values ($\delta^{13}\text{C}/\delta^{15}\text{N}$) associated with the sampling sites or epiphytic morphotypes. ANOSIM values lie between -1 and +1; zero represents the null hypothesis of no differences among a set of samples (Clarke, 1993). Therefore, small negative or positive values close to zero confirm the null hypothesis. The significance criterion considered in this analysis is $p < 0.05$.

Similarity Percentages analysis (SIMPER) was performed to calculate the dissimilarity percentages between the different localities and morphotypes and to identify the corresponding contribution of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. These tests were followed by non-parametric procedures to achieve more robust statistics. Kruskal-Wallis analysis was applied to compare the effect of locality and morphotype on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ variability patterns. Multivariate analyses were performed using PRIMER 6 and IBM-SPSS v.19.

2.2. OBJECTIVE 2: COMPARATIVE ANALYSIS OF THE ENVIRONMENTAL CONDITIONS OF *P. OCEANICA* MEADOWS FROM THE BALEARIC ISLANDS (SPAIN) USING BIOINDICATOR INDICES, ABNORMAL-GROWTH FORMS, AND TRACE-ELEMENTAL CONCENTRATIONS.

2.2.1. Study Site.

The application of the proposed bioindicator indices combined with the analysis of heavy metals in the foraminiferal tests has been conducted in different locations of the islands of Mallorca (Port d'Andratx and Magaluf (Fig. 17. B1)) and Cabrera (Cala Santa Maria) (Fig. 17. B2). The study sites were selected because they include a wide spectrum of conservation statuses and are associated with different anthropogenic activities.

2.2.1.1. Mallorca Island

Mallorca is the largest island in the Balearic Archipelago in the western Mediterranean (Fig. 17. B1). The mean surface water temperature ranges between 16°C in winter and 31°C in summer. Salinities are higher in winter (>38 psu) than in summer and autumn (37 psu: Fernández de Puellas, 2007). Nutrient concentrations are nearly undetectable in the surface waters during the summer but are slightly more abundant during spring and autumn (Fernández de Puellas et al., 2003). Light intensity is high throughout the year (Canals and Ballesteros, 1997). A vast area of the coastal zone

is occupied by the seagrass *Posidonia oceanica*, with shoot densities ranging between ca. 60 shoots m^{-2} and 600 shoots m^{-2} , and a bottom cover from 30% to 90% (Santandreu et al., 2019).

The study was performed on sediment samples from Magaluf, a tourist area under anthropogenic influence. The materials have been used for both applying the bioindicator indices and analyzing the heavy-metal content in the foraminifera shells. In Magaluf, *P. oceanica* meadows cover an average of 85% of the bottom, with a mean shoot density of 164 shoots m^{-2} . Another study site was Port d'Andratx, which hosts a small professional fishing fleet and a considerable amount of recreational boats (Vázquez-Luis et al., 2016). In the Port d'Andratx area, the shoot density of *P. oceanica* ranged from 128 to 360 shoots m^{-2} , which corresponds to a total coverage of ~65–70% of the bay's surface. *P. oceanica* meadows in these sampling sites are in good condition according to the WFD 2000/60/EC criteria, applied to the assessment of coastal water quality in the Balearic Islands (Fig. 19) (Santandreu et al., 2019).

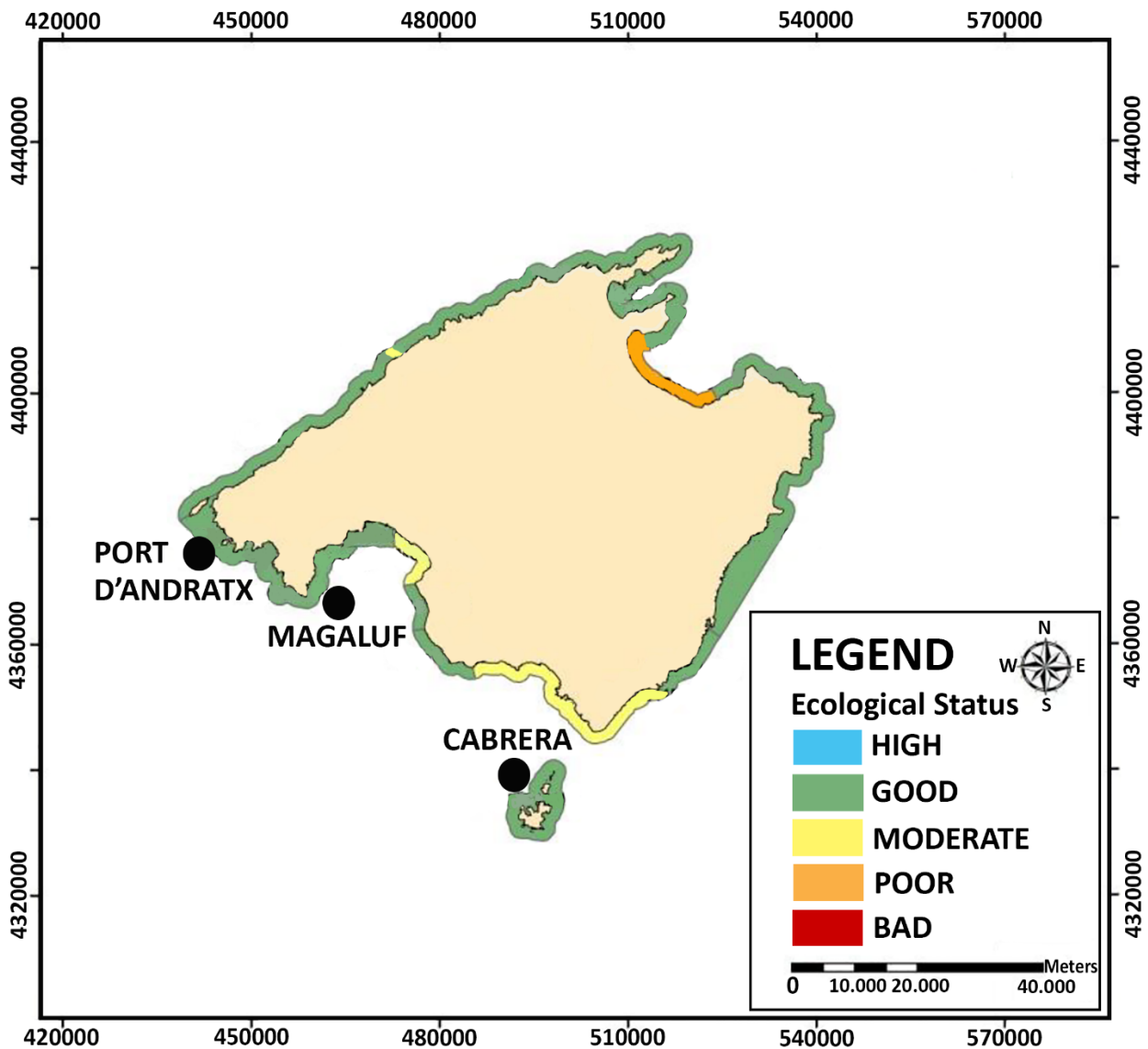


FIGURE 19. Ecological status of coastal waters in 2017 using the POMI, based on the combination of 11 metrics (modified from Santandreu et al. (2019)).

2.2.1.2. Cabrera Island

Study areas from Cabrera Island were selected as better-preserved counterparts to compare with those from Mallorca. The island of Cabrera is subjected, *a priori*, to lower anthropogenic impacts since it was declared a Marine Protected Area more than 20 years before the present sampling occurred. However, before this declaration and National Park status, the Cabrera Archipelago was a site for war games from 1916 to 1991, impacting the terrestrial and aquatic ecosystems. Between 1973 and 1986, particularly during the months of May and June, the Spanish Armed Forces used the area to train with real ammunition. The effect of the bombing may be inferred from the series of orthophotos of Cala Santa Maria taken between 1956 and 2019 (Fig. 20) that shows some uncovered areas within the seagrass meadow, clearly visible since 1984, when the military exercises were extremely intensive, evident in all the images up to date. These areas might represent impact points rather than being associated with other activities such as boat anchoring, since it is a restricted-access zone. Even so, previous assessments of the site (Sancho et al., 1987) performed to support the National Park declaration concluded that the military presence did not cause any negative effect on the benthic community.

To preserve its natural assets, the area has been under the highest level of protection since 1991, when the Maritime-Terrestrial National Park of the Cabrera Archipelago was created (Law 14/1991). Initially, the protected area comprised 19 small islands and covered an area of 100.21 km², of which 87.03 km² were maritime. However, in 2019, the National Park was extended to cover up to 908 km². Under this protection, direct exploitation of natural resources is prohibited, scuba diving requires special permission, and navigation around the island is limited; all of which has helped to conserve the ecosystem, including the extensive areas of high-density seagrass meadows.

On the northern side of Cabrera island two study sites (Cabrera1 and Cabrera2 (Fig. 17. B2)) are located in Cala Santa Maria, where *P. oceanica* meadows cover up to 84% of the bottom, with an average shoot density of 408 shoots m⁻², and according to the WFD 2000/60/EC, the ecological status of the coastal waters for this area has been determined as high (Fig. 19) (Santandreu et al., 2019).

2.2.2. Collection Method and Sample Treatment

This part of the thesis was performed on dead foraminiferal assemblages (thanatocoenoses) of *P. oceanica* meadow sediments. The sediment thanatocoenosis records a time span that covers the seasonal cyclic behaviour of *P. oceanica* and, therefore, the sampling frequency required to obtain representative information about the study area is considerably reduced (Samir and El-Din, 2001; Murray, 2006), which allows comparison between different samples while retaining the seasonal bias. The studied sediments correspond to opportunity samples from Port d'Andratx and Magaluf (Mallorca) from May 2012, and Cala Santa Maria (Cabrera) from November 2011 in Cabrera1 and from May 2012 in Cabrera2, collected by members of the Balearic Oceanographic Center of the Spanish Institute of Oceanography (IEO), while monitoring populations of the mollusk *Pinna nobilis* (Vázquez-Luis et al., 2016). The sediments were manually collected (~100 g) and placed in plastic bags while scuba diving at shallow depths between 8–12 m from seagrass meadows. Replicate sediment samples were obtained in Cabrera, at Cabrera1 (SMRA-1, SMRA-2, SMRA-3), and at Cabrera2 (SMRA-4, SMRA-5, SMRA-6 and SMRA-7). Four replicates for both Port d'Andratx and Magaluf were surveyed (ADTX-1, ADTX -2, ADTX -3, ADTX-4 and MGLF-1, MGLF-2, MGLF-3, MGLF-4).

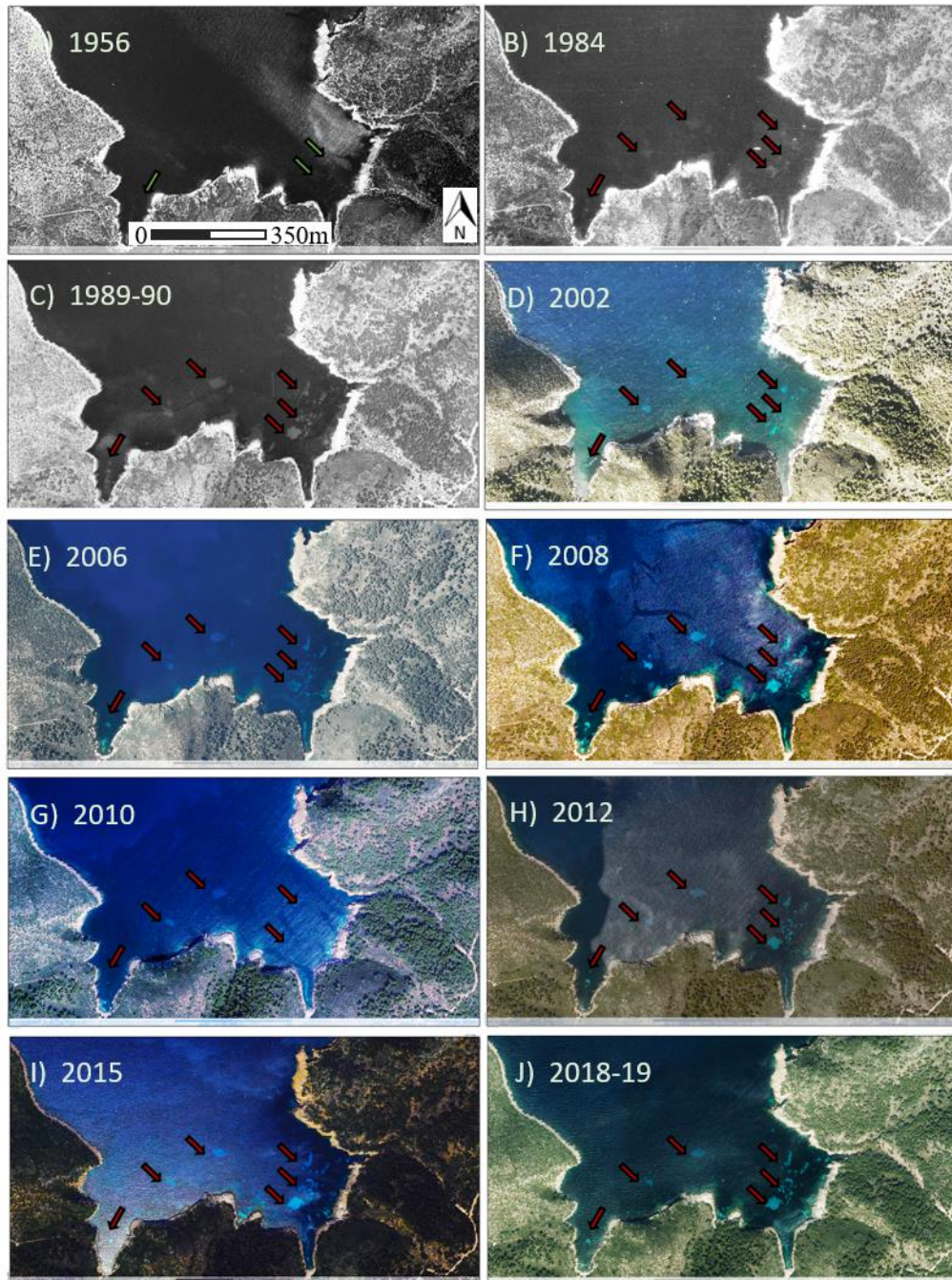


FIGURE 20. Orthophotos of Cala Santa Maria (Cabrera) from 1956 to 2019. A) 1956; B) 1984; C) 1989-90; D) 2002; E) 2006; F) 2008; G) 2010; H) 2012; I) 2015; J) 2018-19. Red arrows indicate the holes without *P. oceanica* coverage probably resulting from the military exercises. Green arrows indicate the same area before the military exercises without visible signs of damage. (<https://ideib.caib.es/visor/?locale=es>)

The samples were initially treated with hydrogen peroxide solution for 24 hours to eliminate organic matter attached to the foraminiferal tests. The sediments were thoroughly washed with tap water over a 0.063 mm wire mesh sieve and left to dry. Foraminifera were separated using a camera-equipped Leica MZ16 binocular stereomicroscope (camera Leica DFC295). One hundred and fifty foraminifera were identified per replicate sample.

To avoid any taphonomic bias, only well-preserved specimens, without evidence of transport and/or reworking, were collected regardless of the presence or absence of deformations. After the specimens were identified, their relative abundance and the number of deformed individuals per sample were calculated. Some authors (Langer, 1993; Geslin et al., 2000; Martínez-Colón et al., 2009) have advised against using epiphytic foraminiferal taxa as stress indicators since many of them develop irregular tests that conform to their substrate of attachment; however, not all growth patterns and morphological features are the consequence of substrate attachment. In this thesis, the aberrant morphological categories are those described by Coccioni et al. (1997) and correspond to substrate-independent growth patterns such as abnormal coiling, aberrant chamber shape and size, poor development of the last whorl, twisted chamber arrangement, supernumerary chambers, protuberances, multiple apertures, irregular keel, twinning, lateral asymmetry, and lack of ornamentation.

2.2.3. Foraminiferal Assemblage Analysis

2.2.3.4. Taxonomical analysis and biodiversity

Taxonomic identifications were based on Le Calvez and Le Calvez (1958), Barker (1960), Colom (1974), Loeblich and Tappan (1987), Cimerman and Langer (1991), Sgarrella and Moncharmont Zei (1993), Langer and Schmidt-Sinns (2006). Nomenclature was updated according to the latest version of WoRMS (World Register of Marine Species, 2022).

Abundances were recorded for each replicate, and then average values per sample were calculated. For analyzing the community structure, biodiversity was calculated using the Shannon-Weaver index (H').

$$H' = - \sum_{i=0}^R p_i \ln (p_i)$$

where p_i is the relative abundance of each species, i is the number of individuals of a given species, and R represents the species richness (actual number of species).

2.2.3.5. Epiphytic morphotypes and ecological analysis

Epiphytic species were classified according to the modified Langer's (1993) morphotype classification (Khokhlova, 2013; Mateu-Vicens et al., 2014). While retaining Langer's original B and C morphotypes, the revised classification splits his A and D morphotypes into three groups, including one for symbiont-bearing taxa (SB). As a result, the proposed new categories with ecological significance are: A* that corresponds to encrusting and sessile forms (Langer's A morphotype without the symbiont-bearing species); D* includes small, short-living, opportunistic forms (Langer's D morphotype without the symbiont-bearing foraminifera); and SB that clusters all symbiont-bearing taxa (e.g., *Sorites orbiculus* (Forsskål in Niebuhr, 1775), *Peneroplis* spp., *Amphistegina lessonii* d'Orbigny in Deshayes, 1830), whose occurrences are limited by the 15°C winter isotherm (Murray, 1976; 2006; Langer and Hottinger, 2000).

2.2.4. Epiphytic Foraminifera-Based Bioindicator Indices: A Summary

This section is included to explain in detail the scientific foundation of the indices applied in this thesis. The following is a summary of the research conducted as part of my MSc (2013), published in Mateu-Vicens et al. (2014), where two epiphytic foraminifera-based bioindicator indices were specifically developed to assess the environmental state of *P. oceanica* meadows. Since then, these indices have been applied to meadows in Tunisia (el Kateb et al., 2020), in Australia, for *Posidonia* spp. (Buosi et al., 2020), and for paleoecological analysis in the Quaternary (Mariani et al., 2022 a; 2022b). The indices that I have developed have been inspired by other existing ones, based on ecological groups, rather than a detailed systematic analysis of the association of foraminifera. This enables the creation of easy-to-use, inexpensive, and widely applicable tools, such as the FORAM Index (FI) (Hallock et al., 2003) that, over time, has been subsequently adapted to different global physical environmental conditions such as water temperature, salinity, and nutrients, among others (Hallock, 2012; Dimiza et al., 2016; Prazeres and Renema, 2019). Originally, the FI was intended to provide environmental managers with a tool to assess whether water quality supports reef growth and/or recovery after major stress episodes (Prazeres et al., 2020).

This index was developed as a foraminiferal-based indicator of water and sediment quality in warm subtropical/tropical coastal and shelf environments with limited flux of inorganic or organic nutrients (Hallock et al., 2003; Hallock, 2012). The formula was empirically based, considering the total assemblages, which are mostly dead specimens in reef environments:

$$FI = (10 \times P_s) + (P_o) + (2 \times P_h);$$

where P_s , P_o , and P_h represent the proportions of symbiont-bearing, opportunistic and small heterotrophic taxa in the foraminiferal assemblage.

The rationale that supports this index is that environments where symbiont-bearing organisms proliferate have sediments with at least 25–30% of the foraminiferal tests corresponding to species with algal symbionts. Thus, a sample containing 25% larger foraminiferal tests and 75% tests of other small taxa has a FI = 4. Environments with sediments devoid of symbiont-bearing foraminifera have a FI \leq 2. In sediments from areas with coral reefs with marginal growth that does not allow recovery after mortality events, FI values are between 2 and 4. More recently, the FI has been used to analyze water quality in shallow-water environments of the Greek coasts (Koukousioura et al., 2011) since symbiont-bearing taxa are abundant in the eastern Mediterranean. In contrast, symbiont-bearing taxa in the western half of the Mediterranean show a restricted distribution due to thermal constraints, suggesting that the straightforward application of the FI might induce some interpretative mistakes.

The FI separates foraminifera into three groups with very different ecological requirements: sensitive, long lived taxa (i.e. symbiont-bearing foraminifera); stress-tolerant opportunistic species; and small heterotrophic forms that require well-oxygenated environments. The same distinction can be made with epiphytic foraminifera, in which the sensitive, long-lived species are represented by the SB and A* groups, the opportunists correspond to D*, and the small heterotrophic forms are represented by B and C morphotypes. Consequently, we modified the FI as follows:

$$FI' = 10 \times (P_{A^*} + P_{SB}) + P_{D^*} + 2 \times (P_B + P_C)$$

where P_{A^*} , P_{SB} , P_{D^*} , P_B and P_C represent the proportion of A^* , SB, D^* , B and C forms, respectively.

As in FI, values of $FI' > 4$ represent excellent environmental conditions. Sites with $FI' > 4$ correspond to good to high ecological status of *P. oceanica* meadows, such as those of the Balearic Islands (Barón et al., 2011), while $FI' < 2$ point to stressed conditions, such as meadows influenced by strong terrigenous inputs.

Notwithstanding, *Posidonia oceanica* foraminiferal assemblages contain very abundant B and C forms that remain more or less consistent regardless of environmental conditions. This is because they do not have as strict requirements for light and substrate as the A^* and SB groups. Moreover, B and C taxa are not as well-adapted to stressful conditions as D^* taxa, with the exception of a few taxa, such as *Ammonia* spp. (Bernhard and Sen Gupta, 2002; Murray, 2006). Therefore, to magnify the differences between well-preserved and stressed *P. oceanica* meadows, a novel index was calculated, called the "long vs. short life-span" index (I_{LS}), which is expressed as:

$$I_{LS} = (3.5 \times (P_{A^*} + P_{SB}) + 0.1) / (P_{D^*} + 0.1),$$

where P_{A^*} , P_{SB} , and P_{D^*} represent the proportion of A^* , SB, and D^* forms, respectively.

Since species included in D^* (mostly small miliolids and textulariids) have short life-spans (3–4 months; Langer, 1993) compared to those of symbiont-bearing and encrusting taxa (1 year; Langer, 1993), the number of D^* specimens produced in a given time span corresponds to an average of 3.5× the amount of longer-living A^* and SB forms.

To solve the infinity issue, 0.1 was added to both the numerator and denominator and the values range between 0 and 36. Thus, in the case of A^* and SB forms being totally absent and D^* being dominant, the index would be very close to 0. If D^* were totally absent and the dominant epiphytic forms corresponded to A^* and SB, the value would be very close to 36. However, the latter scenario never occurs in natural conditions as there is a significant amount of low-oxygen tolerant, opportunistic forms associated with decaying seagrass in the sediment.

In contrast, the FSI, obtained by Dimiza et al. (2016) uses the proportion of only two ecological groups of benthic foraminiferal species: sensitive (Sen) and stress-tolerant (Str), according to their tolerance/sensitivity to organic material enrichment:

$$FSI = (10 \text{ Sen}) + (\text{Str})$$

2.2.5. Heavy-Metal Concentration in the Foraminiferal Tests

Up to 20 abnormal individuals of *Sorites orbiculus* and *Peneroplis planatus* were picked from each sample (Cabrera1 (6), Cabrera2 (5), Magaluf (3), and Port d'Andratx (3)). For the control analysis, one non-deformed individual specimen was selected from Cabrera1, Cabrera2, and Magaluf samples. The deformed and control foraminifera larger than 1 mm were fixed on double-sided sticky tape

under an SEM aluminium stub. Subsequently, the aluminium stub was embedded in epoxy resin inside of mold cups (~1 cm in diameter) and vacuum was applied to extract the air and thus avoid high-porosity domains for analysis accuracy and precision (Kozdon, 2013). Once dried, the sample was polished with 2000 grit sandpaper under tap water to expose the margin of the shell walls.

The polished sample was sent to the Geochronology and Isotopic Geochemistry (Ibercron) Service of the University of the Basque Country UPV/EHU (Leioa) to perform heavy-metal detection and quantification on the foraminiferal tests. The analysis was carried out by a quadrupole mass spectrometer with a plasma source (Q-ICP-MS), model iCAP-Qc (Thermo). To improve the sensitivity of the equipment a second vacuum pump was used at the interface of the system.

A 193 nm wavelength excimer laser ablation system, model RESOLUTION SE (Applied Spectra), was applied for sample introduction. Specific sampling points in the analyzed tests were carried out with a nominal diameter of 100 μm , under a fluence of 2 J/cm^2 and a frequency of 5 Hz. The Spell out what NIST stands for (NIST) 612 glass provided by NIST (Danyushevsky et al., 2011) was used for equipment tuning and results in calibration. To control the results, the NIST 610 and NIST 614 glasses provided by NIST (Jochum et al., 2011) were repeatedly analyzed under the same conditions as those found in the test samples. To obtain the concentrations of Cu, Zn, Cd, Pb, Co, Ni, As, and Sn, data processing was conducted with the Lolite 3.32 software (Paton et al., 2011; Paul et al., 2012), using Ca^{2+} as an internal standard, with the ideal concentrations for calcite.

2.2.6. Statistical Analysis

Statistical analyses were completed using the Paleontological Statistics (PAST) software package v. 4.05 (Hammer et al., 2001). Box plots were used to display the differences between the ecological indices used for the study (FS' , I_{LS} , FSI , and H') and the percentage of deformed foraminifera among sites. Each plot represents the data value of the determined index for each sampling site (two in Cabrera, Port d'Andratx, and Magaluf). Subsequently, the same method was utilized to illustrate the concentrations of each heavy metal (Cu, Zn, Pb, Ni, and As) present in the foraminiferal tests. One-factor ANOVA test was applied to detect significant differences ($p < 0.05$) of the heavy-metal concentrations among sites. If the ANOVA test indicated a significant difference (in the case of Zn, Pb, and As), then a post-hoc Tukey test was applied to examine if the distributions are significantly different for Cabrera1 and Cabrera2. Principal Components Analysis (PCA) was calculated to determine the correlation between the spatial variation of heavy metals concentrations in foraminiferal shells, the values of the foraminiferal indices explained above (F' , I_{LS} , FSI , H'), but also the percentage of deformed foraminiferal shells among sites (FAI , sensu Coccioni et al., 2003; 2005). Finally, to determine if there is a correlation between each type of test deformation and the concentration of heavy metals measured in the shells, a Pearson analysis was performed. Only abnormal features appearing at more than two sampling sites were included in the correlation analysis. Thus, the categories “deformed side view” and “multiple apertures” were excluded.

2.3. OBJECTIVE 3: APPLICATION OF THE BIOINDICATOR INDICES TO HISTORICAL DATA SERIES BASED ON INFORMATION FROM DIFFERENT LOCALITIES OF THE BALEARIC ISLANDS (MALLORCA, MENORCA, AND CABRERA).

2.3.1. Localities from Previous Literature

Information about the foraminiferal assemblages reported in *P. oceanica* meadows from various sites of the Balearic Archipelago (Fig. 17. C) has been collected from published and unpublished literature to provide a complete spectrum of environmental situations.

For comparing the information from the different historical sources, the samples have been renamed in a homogeneous way using a new nomenclature (Table 2). "Ma" was used for Mallorca, "Me" for Menorca, and "Ca" for Cabrera; the second number refers to the sequential number of samples for each research source, and the last number corresponds to the year of sampling.

TABLE 2. The original names of the samples for each location, and their counterparts, unified in the present work, where Ma – Mallorca, Me – Menorca, Ca – Cabrera, the first number indicates the sequential number of the sample for each research source, and the last number indicates the year of sampling.

REFERENCE	LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE
Mateu, 1970	Cala Agulla	ACM	Ma-1-64
	Cala Mendia	CPM	Ma-2-64
	Cala Murada	FM	Ma-3-64
	Portocolom	PCM	Ma-4-64
	Porto Petro	SPM	Ma-5-64
	Cala Santanyí	CSM	Ma-6-64
	Es Caragol	PCS	Ma-7-64
	Colònia de Sant Jordi	CSJ	Ma-8-64
	Ses Covetes	SC	Ma-9-64
	Sa Ràpita	LRC	Ma-10-64
	Cala Blava	CB	Ma-11-65
	S'Arenal	ARE	Ma-12-65
	Port de Sóller	PS	Ma-13-65
	Camp de Mar	CdM	Ma-14-65
	Santa Ponça	SP	Ma-15-65
Florit, 1983	Ses Fontanelles	SF	Me-1-82
	Cala Mitjana	CM	Me-2-82
	Cala En Blanes	CB	Me-3-82
	Arenal d'en Castell	AC	Me-4-82
	Cala Galdana	CG	Me-5-82
Gazá, 1988	Ciutat Jardí	T-2	Ma-6-82
	Portals Nous	T-10	Ma-7-82
	Cap de Regana	ST-14	Ma-8-84
Moreiro, 1993	Badia de Pollença	4017	Ma-9-85
	Badia de Pollença	4027	Ma-10-85
	South Menorca	4174	Me-11-87

	South Menorca	4175	Me-12-87
	South Menorca	4177	Me-13-87
	South Menorca	4182	Me-14-87
	South Menorca	4184	Me-15-87
	South Menorca	4189	Me-16-87
Abril, 1993	Cala Fornells	CF	Ma-17-89
	Cala Murta	CM	Ma-18-89
	Es Barcarès	BA	Ma-19-89
	Es Carnatge	EC	Ma-20-89
	Port de Sóller	PS	Ma-21-89
	Portocolom	PC	Ma-22-89
	Cala Fornells	CF	Ma-1-90
	Cala Murta	CM	Ma-2-90
	Es Barcarès	BA	Ma-3-90
	Es Carnatge	EC	Ma-4-90
	Port de Sóller	PS	Ma-5-90
	Portocolom	PC	Ma-6-90
	Cala Fornells	CF	Ma-7-91
	Cala Murta	CM	Ma-8-91
	Es Barcarès	BA	Ma-9-91
	Es Carnatge	EC	Ma-10-91
	Port de Sóller	PS	Ma-11-91
	Portocolom	PC	Ma-12-91
Mateu et al., 2001	Port de Sóller	PS-2001	Ma-1-00
Mateu-Vicens et al., 2010	Cala d'Or	PO1	Ma-2-08
	Cala d'Or	PO2	Ma-3-08
	Portals Vells	PO3	Ma-4-08
	Portals Vells	PO4	Ma-5-08
Khokhlova, 2013 -	Magaluf	MGL	Ma-8-12
Mateu-Vicens et al., 2014	Portals Nous	PRT	Ma-10-12
	Son Verí	SVR	Ma-11-12
	Ses Illetes	ILL	Ma-12-12
	Santa Ponça	SP	Ma-13-12
	Cala Santa Maria	CAB-2	Ca-2-11
	Els Estels	CAB-1	Ca-4-12
	Cala Blava	CBL	Ma-5-12
Racis, 2015	Cala Blava	CB2	Ma-6.1-12
	Cala Blava	CB3	Ma-6.2-12
Vaquer, 2016	Port de Sóller	PS2014	Ma-16-14
Mateu-Vicens et al., 2016	Sant Elm	SE	Ma-14-12
	Sa Foradada	SF	Ma-15-12
Khokhlova et al., 2022	Cala Santa Maria	CABRERA1	Ca-1-11
	Cala Santa Maria	CABRERA2	Ca-3-12
	Magaluf	MGLF	Ma-7-12
	Port d'Andratx	ADTX	Ma-9-12
Khokhlova and	Alcanada	ALKN1	Ma-1-21
Mateu-Vicens, 2023	Alcanada	ALKN2	Ma-2-22
	Ca'n Picafort	IBAP1	Ma-3-21
	Ca'n Picafort	IBAP2	Ma-4-22

Cala Blava	CLBL1	Ma-5-21
Cala Blava	CLBL2	Ma-6-22
Cala Brafi	CBRF1	Ma-7-21
Cala Brafi	CBRF2	Ma-8-22
Cala de Santa Ponça	IBSO1	Ma-9-21
Cala de Santa Ponça	IBSO2	Ma-10-22
Capdepera	PIPK1	Ma-11-21
Capdepera	PIPK2	Ma-12-22
Es Caragol	CRGL1	Ma-13-21
Es Caragol	CRGL2	Ma-14-22
Marina de Bonaire	CDRL1	Ma-15-21
Marina de Bonaire	CDRL2	Ma-16-22
Port d'Andratx	ADTX1	Ma-17-21
Port d'Andratx	ADTX2	Ma-18-22
Port de Sóller	SLLR1	Ma-19-21
Port de Sóller	SLLR2	Ma-20-22
Ses Illetes	ILLT1	Ma-21-21
Ses Illetes	ILLT2	Ma-22-22

The information analyzed in this historical time series corresponds to data on foraminiferal dead assemblages reported in sediments (thanathocoenosis) and living assemblages from plants (biocoenosis), representing a time lapse between the mid-late 1960s and the early 2020s. For each station, the noted information (if available) was: 1) location, with the names of the localities modified according to the current Catalan grammar, 2) sampling date, 3) depth, 4) type of sample (sediment, leaves or rhizomes), and 6) bibliographic reference. These parameters were used for statistical analysis. Furthermore, the systematic classification of all taxa reported was updated according to WoRMS (World Register of Marine Species) (2022). Below is the list of literature used in this section, including a short description of the study area and the sampling methodology used in each case:

2.3.1.1. *Mateu, 1970* (Figure 21; Table 3)

The sediment samples were obtained between 11/10/1964 and 03/01/1965 in Cala Agulla (Ma-1-64), Cala Mendia (Ma-2-64), Cala Murada (Ma-3-64), Portocolom (Ma-4-64), Porto Petro (Ma-5-64), Cala Santanyí (Ma-6-64), Es Caragol (Ma-7-64), Colònia de Sant Jordi (Ma-8-64), Ses Covetes (Ma-9-64), Sa Ràpita (Ma-10-64), Cala Blava (Ma-11-65), S'Arenal (Ma-12-65), Port de Sóller (Ma-13-65), Camp de Mar (Ma-14-65), and Santa Ponça (Ma-15-65).

The other seven locations have not been included in this dataset due to a lack of detailed information. The next sediment samples the author described as very poor for foraminifera, without reporting the total number of foraminifera found: only few miliolids, rare peneroplids, and isolated nubecularids in Peguera, 26 species in Port d'Alcúdia, ten species in Cala Sant Vicenç, four species in Es Barcarès, rare peneroplids and remnants of miliolids were reported by Mateu (1970). In Canyamel no foraminifera were found.

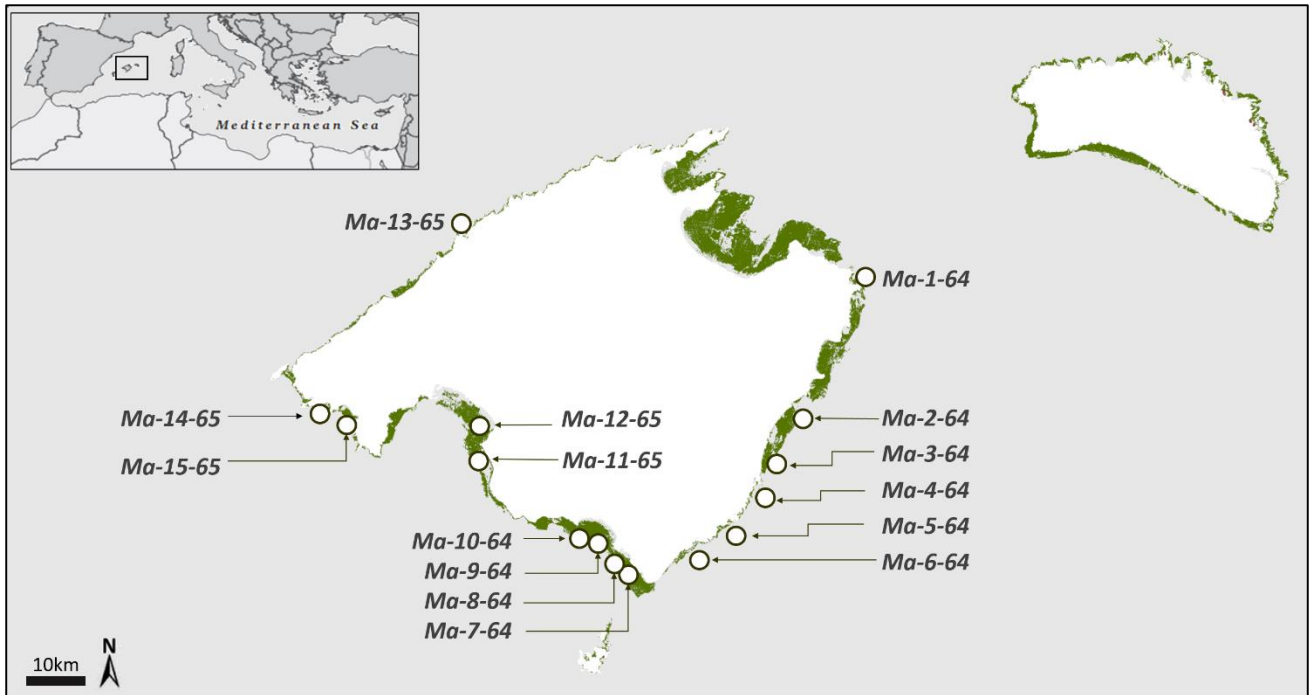


FIGURE 21. Map showing the sampling locations included in the historical data series of Mallorca: Cala Agulla (Ma-1-64), Cala Mendia (Ma-2-64), Cala Murada (Ma-3-64), Portocolom (Ma-4-64), Porto Petro (Ma-5-64), Cala Santanyí (Ma-6-64), Es Caragol (Ma-7-64), Colònia de Sant Jordi (Ma-8-64), Ses Covetes (Ma-9-64), Sa Ràpita (Ma-10-64), Cala Blava (Ma-11-65), S'Arenal (Ma-12-65), Port de Sóller (Ma-13-65), Camp de Mar (Ma-14-65), Santa Ponça (Ma-15-65) (Mateu, 1970).

Samples consist of fine sand sediment, collected in the swash zone by surface dragging with a small trowel. Once in the laboratory, samples were washed with fresh water and left overnight in the stove at about 50°C. For the quantitative analysis, the abundances of the classified taxa of the described biocoenoses and thanatocoenoses were expressed either proportionally or according to Margalef's (1953) progressive scale.

TABLE 3. Location, date, and depth of sampling with a short description of samples from Mateu (1970). Only epiphytic foraminifera were considered. Information about sampling stations was literally translated from the original work.

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES (from the original work)	DEPTH (m)	DATE
Cala Agulla	ACM	Ma-1-64	sand, fragments of spicules, and tests	0	11/10/1964
Cala Mendia	CPM	Ma-2-64	clean and calm water	0	12/10/1964
Cala Murada	FM	Ma-3-64	clean and calm water	0	12/10/1964
Portocolom	PCM	Ma-4-64	gravel and fragments of <i>Posidonia</i>	0	11/10/1964
Porto Petro	SPM	Ma-5-64	gravel and fragments of <i>Posidonia</i>	0	11/10/1964
Cala Santanyí	CSM	Ma-6-64	fine sand, calm water	0	20/12/1964
Es Caragol	PCS	Ma-7-64	fine sand, remains of <i>Posidonia</i>	0	20/12/1964
Colònia de Sant Jordi	CSJ	Ma-8-64	strong waves, sand with <i>Posidonia</i>	0	20/12/1964
Ses Covetes	SC	Ma-9-64	lots of tests	0	20/12/1964
Sa Ràpita	LRC	Ma-10-64	coarse, reworked sand with rounded grains	0	20/12/1964
Cala Blava	CB	Ma-11-65	coarse, reworked sand with rounded grains	0	03/01/1965

S'Arenal	ARE	Ma-12-65	coarse, reworked sand with rounded grains	0	03/01/1965
Port de Sóller	PS	Ma-13-65	very reworked sands	0	03/01/1965
Camp de Mar	CdM	Ma-14-65	very reworked sands	0	03/01/1965
Santa Ponça	SP	Ma-15-65	sands with remains of <i>Posidonia</i>	0	03/01/1965

2.3.1.2. Florit, 1983 (Figure 22; Table 4)

In this study, samples consist of *P. oceanica* leaves and rhizomes, collected manually, between 3 and 5 mwd, from five beaches of the littoral of Menorca: Ses Fontanelles, Cala Mitjana, Cala En Blanes, Arenal d'en Castell, and Cala Galdana. In the original study, the same quantity of leaves and rhizomes were collected at each station (without specifying how many samples were picked). Ses Fontanelles, located in the North of Menorca, is a small and narrow cove with a rocky/sandy marine bottom tightly covered by *P. oceanica*. During the early 1980s, the cove was not urbanized, but during summer fishing boats were very frequent. Cala Mitjana was not urbanized during the sampling period. It is a cove with a sandy marine bottom, covered with *P. oceanica* from 7-8 mwd. In contrast, Cala En Blanes, located on the western side of Menorca, was urbanized. It has a sandy bottom covered by *P. oceanica* at the sampling depth, between 8 and 10 mwd. Arenal d'en Castell, located in the Northern East of the island, was already quite developed when sampling took place. In this locality, *P. oceanica* meadows cover the bottom at shallow depths. Finally, the last sampling point from the south of Menorca corresponds to Cala Galdana. This is one of the most visited areas on the island, urbanized, with sandy bottoms inhabited by *P. oceanica* from 5 mwd.

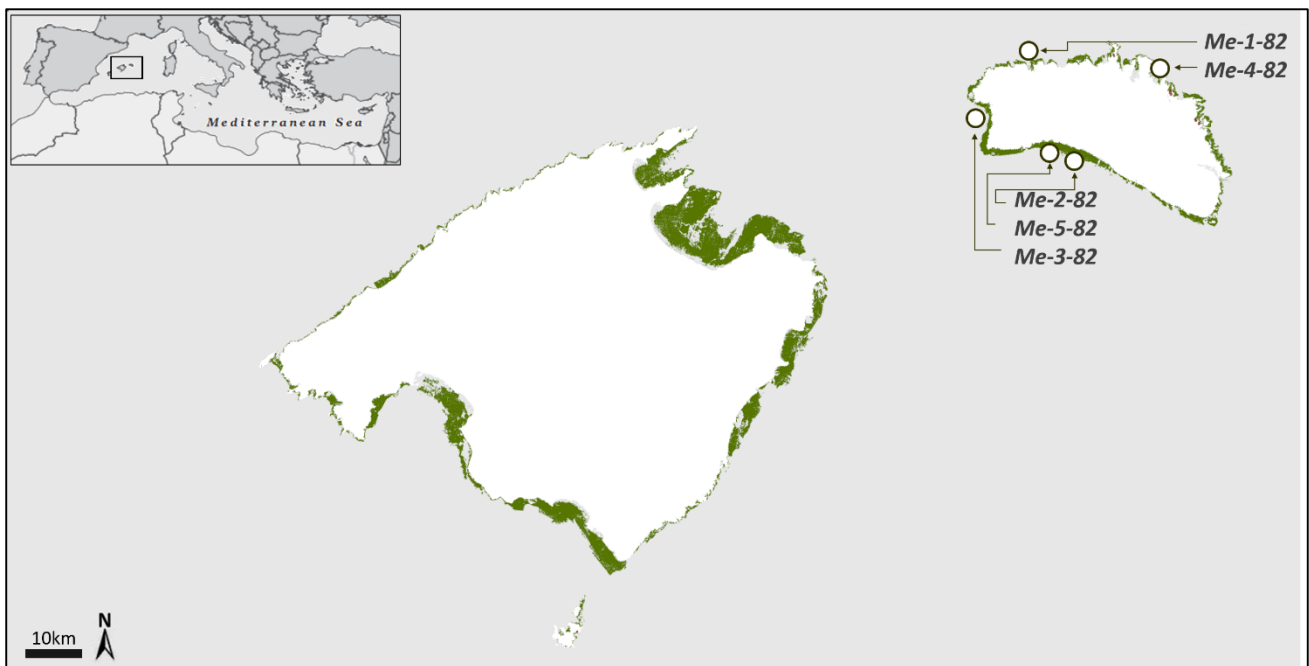


FIGURE 22. Map showing the sampling locations of historical data series of Menorca: Ses Fontanelles (Me-1-82), Cala Mitjana (Me-2-82), Cala En Blanes (Me-3-82), Arenal d'en Castell (Me-4-82) and Cala Galdana (Me-5-82) (Florit, 1983).

After sampling, up to 1000 individuals were picked from leaves and rhizomes (in case of insufficient quantity, the total amount of foraminifera was considered) and classified up to species level.

TABLE 4. Location, date and depth of sampling with a short description of samples from Florit (1983).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES (from the original work)	DEPTH (m)	DATE
Ses Fontanelles	1	Me-1-82	Rocky/sandy bottom with a large amount of <i>Posidonia</i>	3–5	19/09/1982
Cala Mitjana	2	Me-2-82	Sandy bottom, <i>Posidonia</i> at 7-8 mwd	3–5	05/08/1982
Cala En Blanes	3	Me-3-82	Sandy bottom, <i>Posidonia</i> at 8-10 mwd	3–5	27/09/1982
Arenal d'en Castell	4	Me-4-82	Sandy bottom with <i>Posidonia</i> forming shallow meadows	3–5	13/09/1982
Cala Galdana	5	Me-5-82	Sandy bottom, <i>Posidonia</i> at 5 mwd	3–5	16/08/1982

2.3.1.3. Gazá, 1988 (Figure 23; Table 5)

These data correspond to the Ph.D. thesis of Gazá (1988). The sediment samples were obtained using a piston corer during scientific cruises performed in 1982 and 1984 by the Spanish Institute of Oceanography. The sediment was sampled from the Badia de Palma in Ciutat Jardí (Ma-6-82), Portals Nous (Ma-7-82), Cap de Regana (Ma-8-84), which represent moderate levels of anthropogenic impacts. Despite being located in Palma, a highly populated area, during the late 1980s, these localities were moderately urbanized.

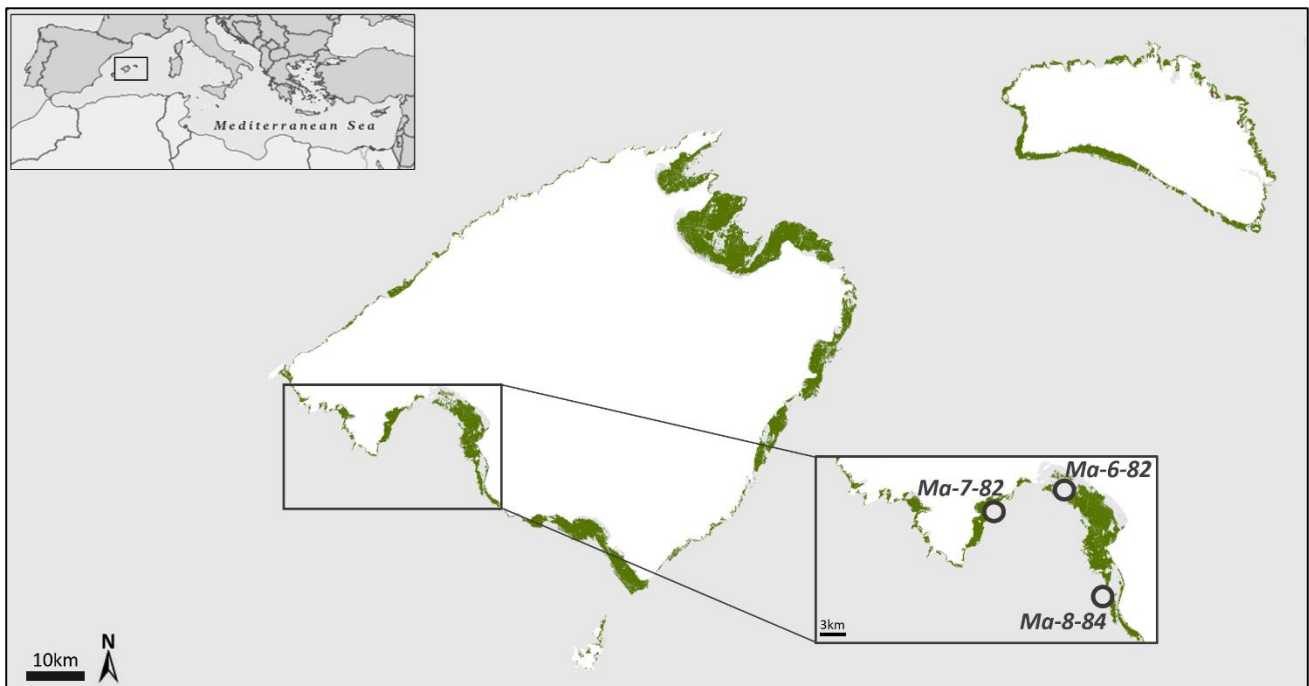


FIGURE 23. Map showing the sampling locations of historical data series of Mallorca (Badia de Palma): Ciutat Jardí (Ma-6-82), Portals Nous (Ma-7-82), Cap de Regana (Ma-8-84) (Gazá, 1988).

Sampled sediment cores were frozen to preserve the internal structure, and later the first 2 cm for Ma-6-82 and Ma-7-82, and 4–5 cm approximately for Ma-8-82 were treated by wet sieving to separate the samples by size. Then, sediments were sieved through 0.800 mm, 0.800–0.500 mm, 0.500–0.200 mm meshes, and dried for 24 hours at 60°C. 2 g for each fraction, and therefore 6 g for

each sample were analyzed and the total amount of foraminifera was picked. Species identifications (73067 individuals in total) were based on d'Orbigny (1839), Cushman (1949, 1959), Loeblich and Tappan (In Moore, 1964), Sigal (1966), and Colom (1974).

TABLE 5. Location, date and depth of sampling with a short description of samples from Gazá (1988).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES (from the original work)	DEPTH (m)	DATE
Ciutat Jardí	T-2	Ma-6-82	Coarse sand with <i>Posidonia</i>	20	11/1982
Portals Nous	T-10	Ma-7-82	Coarse sand with <i>Posidonia</i>	22	11/1982
Cap de Regana	ST-14	Ma-8-84	Coarse sand with <i>Posidonia</i>	20	04/1984

2.3.1.4. Abril, 1993 (Figure 24; Table 6)

For her Ph.D. thesis, Abril (1993) collected manually the first centimeter of sediment between 3 and 6 mwd from the soft bottoms colonized by *P. oceanica*, but also the leaves and rhizomes at each site. In the original work information about the exact amount of samples was not indicated. Samplings were carried out every spring, summer, autumn, and winter from 1989 to 1991. For the present work, data corresponding to sediment samples, leaves and rhizomes were considered separately to discriminate between biocoenosis and thanatocoenosis. The study localities are Cala Fornells (Ma-17-89, Ma-1-90, Ma-7-91), Portocolom (Ma-22-89, Ma-6-90, Ma-12-91), Cala Murta (Ma-18-89, Ma-2-90, Ma-8-91), Es Barcarès (Ma-19-89, Ma-3-90, Ma-9-91), Es Carnatge (Ma-20-89, Ma-4-90, Ma-10-91), Port de Sóller (Ma-21-89, Ma-5-90, Ma-11-91).

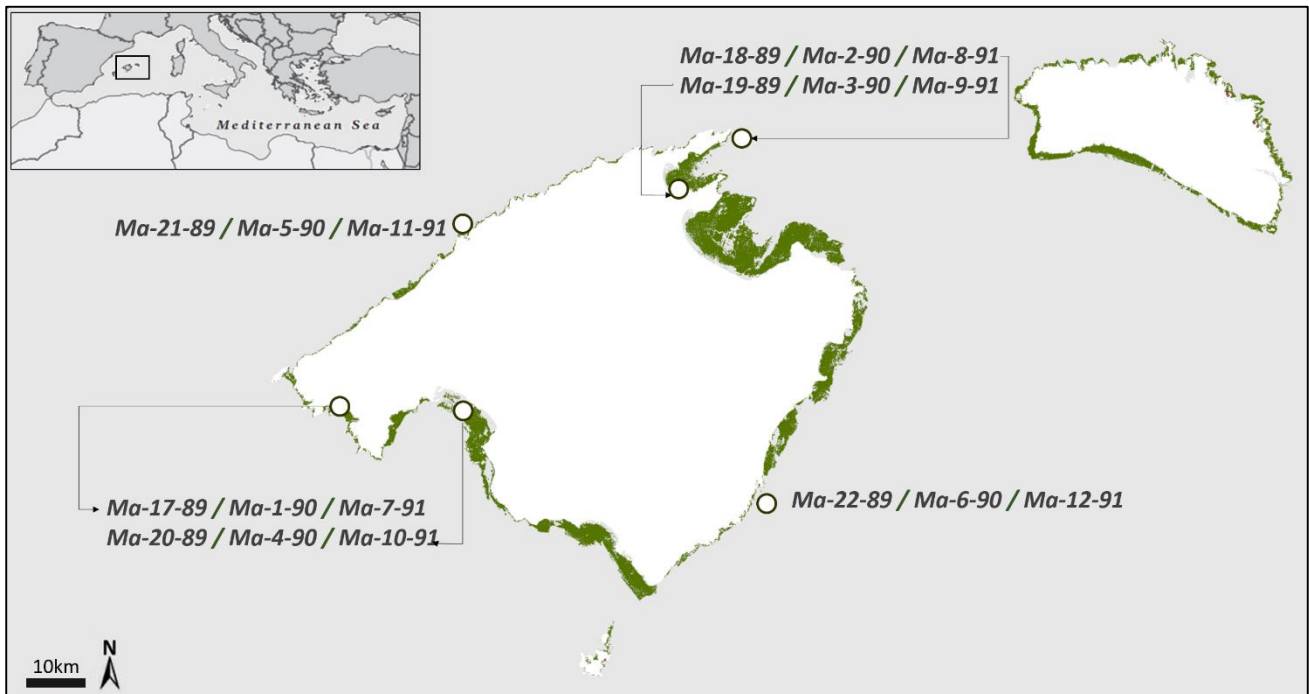


FIGURE 24. Map showing the sampling locations of historical data series of Mallorca: Cala Fornells (Ma-17-89, Ma-1-90, Ma-7-91), Portocolom (Ma-22-89, Ma-6-90, Ma-12-91), Cala Murta (Ma-18-89, Ma-2-90, Ma-8-91), Es Barcarès (Ma-19-89, Ma-3-90, Ma-9-91), Es Carnatge (Ma-20-89, Ma-4-90, Ma-10-91), Port de Sóller (Ma-21-89, Ma-5-90, Ma-11-91) (Abril, 1993).

Sediments were thoroughly washed with tap water over 0.250–1.00 mm wire mesh sieves, dried, and weighed 2 g for each sample. Leaves and rhizomes of *P. oceanica* were left for 24 hours in fresh water to separate foraminifera from its substrate by an osmotic shock. Then, the detached particles were sieved through 0.250–1.00 mm meshes. Foraminiferal taxonomic analysis was performed by randomly selecting all the individuals from 2 g of sediments and 500 individuals from leaves and rhizomes for each sample. Species identifications (130119 foraminifera in total, 25956 individuals from leaves and rhizomes, and 130119 from sediment samples) were based on d’Orbigny (1839), Cushman (1949, 1959), Loeblich and Tappan (in Moore, 1964), Sigal (1966) and Colom (1974).

TABLE 6. Location, date, and depth of sampling with a short description of samples from Abril (1993).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES	DEPTH (m)	DATE
Cala Fornells	CF	Ma-17-89	No data in literature	3–6	1989
		Ma-1-90			1990
		Ma-7-91			1991
Portocolom	PC	Ma-22-89	No data in literature	3–6	1989
		Ma-6-90			1990
		Ma-12-91			1991
Cala Murta	CM	Ma-18-89	No data in literature	3–6	1989
		Ma-2-90			1990
		Ma-8-91			1991
Es Barcarès	BA	Ma-19-89	No data in literature	3–6	1989
		Ma-3-90			1990
		Ma-9-91			1991
Es Carnatge	EC	Ma-20-89	No data in literature	3–6	1989
		Ma-4-90			1990
		Ma-10-91			1991
Port de Sóller	PS	Ma-21-89	No data in literature	3–6	1989
		Ma-5-90			1990
		Ma-11-91			1991

2.3.1.5. Moreiro, 1993 (Figure 25; Table 7)

Moreiro (1993), in her Ph.D. thesis, sample sediments corresponding to *P. oceanica* meadows were obtained from two stations in Mallorca and six in Menorca. Samples were collected manually by scuba diving, on the 22nd-28th of June 1985, in Badia de Pollença (Mallorca), and in the Menorca area, the sediment samples were taken on the 20th-26th of June 1987, manually, by scuba diving and by grab dredger. Sampling was performed by members of the Department of Ecology (University of Barcelona), the section of Marine Geology of the University of Barcelona, and by members of the Balearic Islands Oceanographic Center (Spanish Institute of Oceanography-IEO).

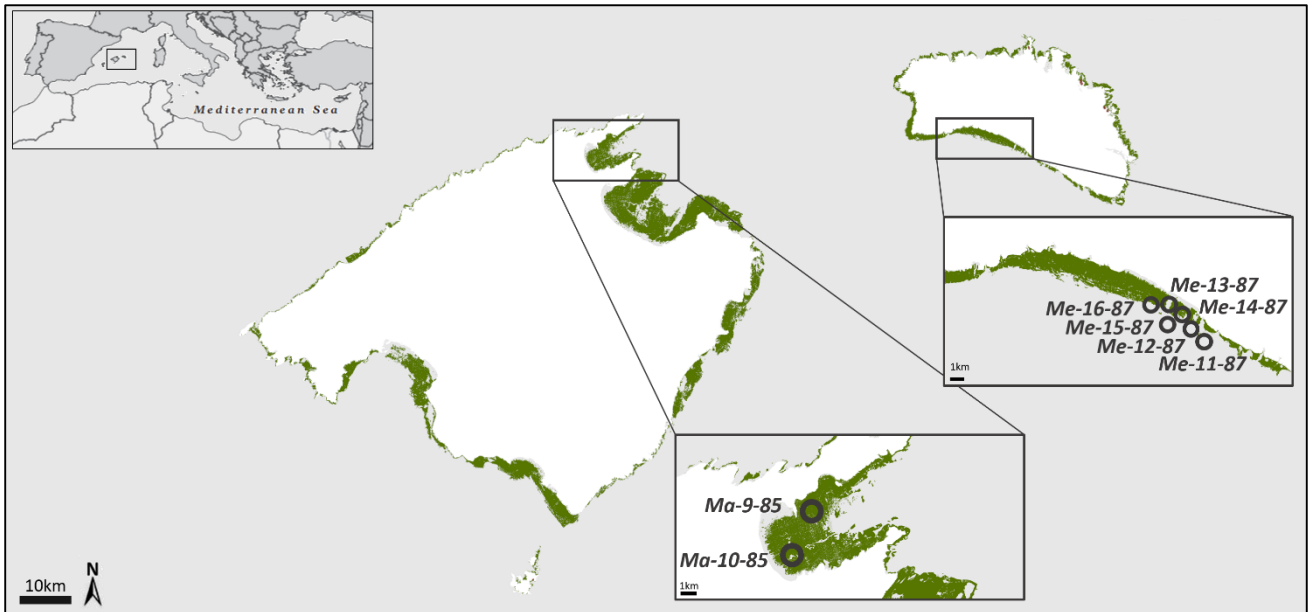


FIGURE 25. Map showing the sampling locations of historical data series of Mallorca: Badia de Pollença (Ma-9-85, Ma-10-85) and South Menorca (Me-11-87, Me-12-87, Me-13-87, Me-14-87, Me-15-87, Me-16-87) (Moreiro, 1993).

To eliminate organic matter attached to the foraminiferal tests all samples were treated with hydrogen peroxide solution from 48 hours to two weeks. Then, sediments were thoroughly washed with tap water over a 0.063 mm wire mesh sieve, dried, and weighed 2 g for each sample. The qualitative and quantitative analyses were performed on dead (thanatocoenosis) assemblage. The foraminiferal species were assigned to 5491 individuals from Mallorca samples and 8087 from Menorca samples, based on Agip Mineraria (1957), Le Calvez and Le Calvez (1958), Cushman (1959), Barker (1960), Moore (1964), Ellis and Messina (1965), Colom (1974), Van Morkhoven et al. (1986), and Be (1967) were used for present research.

TABLE 7. Location, date and depth of sampling with a short description of samples from Moreiro (1993).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES (from the original work)	DEPTH (m)	DATE
Badia de Pollença	4017	Ma-9-85	Bioclastic sandy bottom, with extensive coverage of <i>Posidonia</i> extending down to 35 m.	20	06/1985
Badia de Pollença	4027	Ma-10-85		7	06/1985
South Menorca	4174	Me-11-87	Bioclastic sandy bottom	29	06/1987
South Menorca	4175	Me-12-87	Muddy sands with <i>Posidonia</i> forming shallow meadows	19	06/1987
South Menorca	4177	Me-13-87	Bioclastic sandy bottom	19	06/1987
South Menorca	4182	Me-14-87	Muddy sands with <i>Posidonia</i> forming shallow meadows	7	06/1987
South Menorca	4184	Me-15-87	Bioclastic sandy bottom	29	06/1987
South Menorca	4189	Me-16-87	Bioclastic sandy bottom. <i>Posidonia</i> extending down to 30 m of depth.	30	06/1987

2.3.1.6. Mateu et al., 2001

In this study, samples consist of *Posidonia oceanica* leaves from eight replicates in Port de Sóller (Ma-1-00) (Fig. 17. C), which were obtained at ca. 12 mwd, in 2000. Environmental conditions for the 10 years before sampling had not deteriorated, especially since domestic water and faecal discharges were diverted beyond the port, towards the open sea. Once extracted, leaves were kept for 24 hours in freshwater to detach epiphytic foraminifera. Then the dregs were sieved through 0.500 mm, 1 mm, and 2 mm meshes and all foraminifera from 25 cm³ were handpicked. Up to 693 individuals were used for further taxonomic analysis, based on Colom (1974), Cimerman and Langer (1991), Sgarrella and Moncharmont Zei (1993), Loeblich and Tappan (1987).

2.3.1.7. Mateu-Vicens et al., 2010 (Figure 26; Table 8)

In this work, sediment samples were collected by scuba divers in Cala d'Or and Portals Vells at 6–8 mwd, in soft bottoms colonized by invasive *Caulerpa taxifolia* and *C. racemosa*, as well as by established *C. prolifera* and the seagrass *P. oceanica*. For the present study, only samples collected from *P. oceanica*-dominated areas are considered: Ma-2-08 and Ma-3-08 samples from Cala d'Or, and Ma-4-08 and Ma-5-08 samples from Portals Vells. Samples at both study sites were collected at least 100 m apart to avoid pseudoreplication.

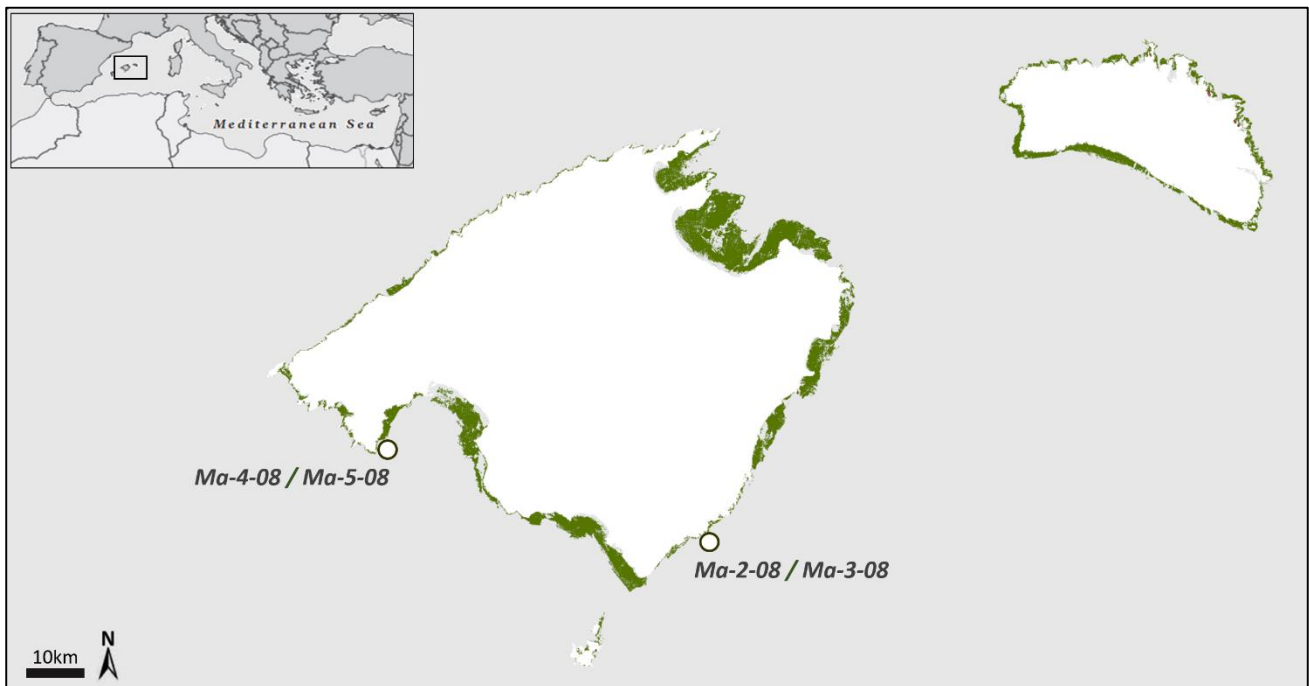


FIGURE 26. Map showing the sampling locations of historical data series of Mallorca: Cala d'Or (Ma-2-08 and Ma-3-08), and Portals Vells (Ma-4-08 and Ma-5-08) (Mateu-Vicens et al., 2010).

For each sample, granulometric analysis was carried out by sieving 100 g of sediment. However, the 0.500–0.125 mm fractions were combined and foraminiferal taxonomic analysis was performed on the dead assemblage (thanatocoenosis) by picking up to 300 specimens per sample. Taxonomic analysis was based on Colom (1974), Cimerman and Langer (1991), Sgarrella and Moncharmont Zei (1993), and Loeblich and Tappan (1987).

TABLE 8. Location, date, and depth of sampling with a short description of samples from Mateu-Vicens et al. (2010).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES (from the original work)	DEPTH (m)	DATE
Cala d'Or	PO1/PO2	Ma-2-08/Ma-3-08	Both are enclosed bays of similar bathymetry (6–8 m). Samples were	6–8	2008-2009
Portals Vells	PO3/PO4	Ma-4-08/Ma-5-08	collected over soft bottoms colonized by the seagrass <i>P. oceanica</i>	6–8	2008-2009

2.3.1.8. Khokhlova, 2013 - Mateu-Vicens et al., 2014 (Figure 27; Table 9)

This case study corresponds to my M.Sc. thesis that was adapted and published. Sediment samples were collected from the surface of soft bottoms colonized by *P. oceanica* at depths of 4–20 mwd from May–November 2012. All samples were obtained by scuba diving by members of the Balearic Islands Oceanographic Center (Spanish Institute of Oceanography-IEO) and the Mediterranean Institute for Advanced Studies (IMEDEA).

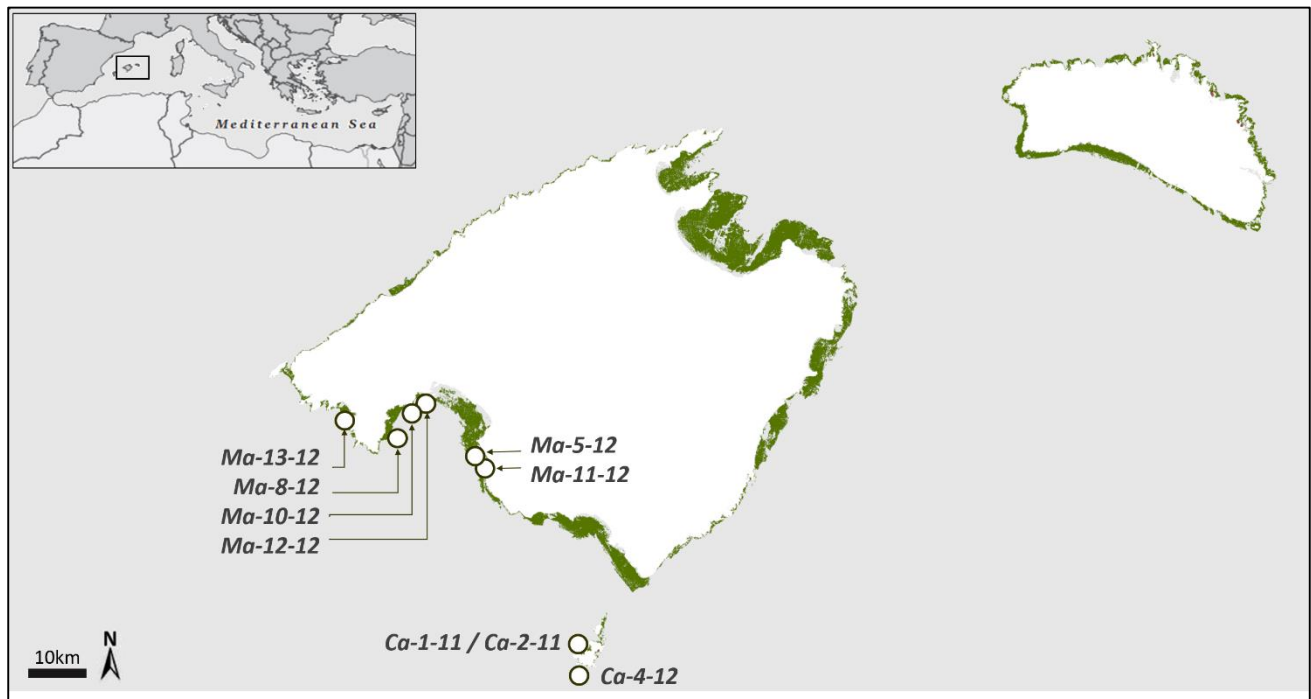


FIGURE 27. Map showing the sampling locations of historical data series of Mallorca: Cala Blava (Ma-5-12), Magaluf (Ma-8-12), Portals Nous (Ma-10-12), Son Verí (Ma-11-12), Ses Illetes (Ma-12-12), Santa Ponça (Ma-13-12); Cabrera: Cala Santa Maria (Ca-2-11), and Els Estels (Ca-4-12) (Mateu-Vicens et al., 2014).

Sample replicates were obtained at least several tens of meters from each other to avoid pseudoreplication. Two Cabrera samples were collected from 20 mwd and 10 mwd water depths at Els Estels (Ca-4-12) and three from 8–10 mwd at Cala Santa Maria (Ca-2-11). At Santa Ponça (Ma-13-12) and Ses Illetes (Ma-12-12), three replicates for each location were obtained from 4–5 mwd. Sediment samples were also collected from 5–6 mwd at Cala Blava (Ma-5-12), 6–8 mwd at Portals Nous (Ma-10-12), 6–7 mwd at Son Verí (Ma-11-12), and 6–7 mwd at Magaluf (Ma-8-12).

TABLE 9. Location, date, and depth of sampling with a short description of samples from Mateu-Vicens et al. (2014) with data about *P. oceanica* from Santandreu et al. (2019).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES	DEPTH (m)	DATE
Els Estels	CAB-1	Ca-4-12	Maritime-Terrestrial National Park with extensive and high-density seagrass meadows. <i>P. oceanica</i> meadows cover an average of 84.33% of the bottom, with a mean shoot density of 408 shoots/m ²	10–20	2012
Cala Santa Maria	CAB-2	Ca-2-11		10	2012
Cala Blava	CBL	Ma-5-12	Marine protected area with consequently low anthropogenic influence and high covering of seagrass meadows. <i>P. oceanica</i> meadows cover an average of 89% of the bottom, with a mean shoot density of 408 shoots/m ²	5–6	2012
Son Verí	SVR	Ma-11-12		6–7	2012
Magaluf	MGL	Ma-8-12	Impacted area by different levels of human activity. <i>P. oceanica</i> meadows cover an average of 85% of the bottom, with a mean shoot density of 164 shoots/m ²	6–7	2012
Ses Illetes	ILL	Ma-12-12		4–5	2012
Portals Nous	PRT	Ma-10-12		6–8	2012
Santa Ponça	SP	Ma-13-12	An intensely urbanized tourist area with. <i>P. oceanica</i> meadows cover an average of 79.17% of the bottom, with a mean shoot density of 291.20 shoots/m ²	4–5	2012

Cala Blava and Son Verí sites (Fig. 27) are included within a marine protected area and consequently have low anthropogenic influence, resulting in high *P. oceanica* shoot-density values (see objective 2 (Methodology)). Magaluf, Portals Nous, and Ses Illetes are situated on the west side of Badia de Palma and are impacted by different levels of human activity. Another sampling site, Santa Ponça, located in the municipality of Calvià, an intensely urbanized tourist area where the runoff into the sea is from urban and agro-gardening waste (Basterretxea et al., 2007; Garcés et al., 2011). *P. oceanica* meadows in sampling sites are in moderate condition according to the WFD 2000/60/EC criteria, applied to the assessment of coastal water quality in the Balearic Islands (Santandreu et al., 2019).

Two hundred, well-preserved, foraminifera per sample were handpicked (3000 in total) using a camera-equipped (Leica MZ16) binocular stereomicroscope (Leica DFC295). Species identifications were based on Le Calvez and Le Calvez (1958), Barker (1960), Colom (1974), Cimerman and Langer (1991), Sgarrella and Moncharmont Zei (1993), Langer and Schmidt-Sinns (2006). Taxonomic nomenclature followed Loeblich and Tappan (1987).

2.3.1.9. Racis, 2015

Racis (2015), in his degree thesis, analyzed sediment samples from Cala Blava (Ma-6-12) (39°29'9.21"N, 2°44'1.90"E). Two replicates (Ma-6.1-12 and Ma-6.2-12) were collected by scuba divers of the "Spanish Institute of Oceanography (IEO)" and the "Mediterranean Institute for Advanced Studies (CSIC-UIB)" of the Balearic Islands between May and November 2012.

Well-preserved foraminifera were handpicked using a camera-equipped Leica MZ16 binocular stereomicroscope (Leica DFC295). Samples were identified up to the species level (201 individuals for Ma-6.1-12, and 237 for Ma-6.2-12), according to Colom (1974), Loeblich and Tappan (1994), Cimerman and Langer (1991) and updated following the WoRMS database.

2.3.1.10. Vaquer, 2016

For his degree thesis, Vaquer (2016) collected in October 2014, 3 sediment samples (~60 g), in the *P. oceanica* meadow in front of Can Repic beach (39°47'43.4"N, 2°41' 53.1"E), Port de Sóller (Ma-16-14), at 4-7m of depth. The *Posidonia oceanica* meadow extended from 3 to 12 m on a soft bottom of fine sediment, being more abundant in areas between 2.7 and 3.5 m and less abundant between 10 and 12 m of depth. Samples were taken close to the mouth of the Torrent Major, a torrent that collects water from Sóller and other nearby municipalities (Fornalutx, Escorca, Bunyola, and Deià). The sampling area is very urbanized and includes a traditional small fishing fleet and a considerable number of recreational boats.

Sediment samples were wet sieved to eliminate silt and clay (Rosa-del Río et al. 2011), obtaining a grain fraction of 0.125–0.500 mm for the foraminiferal-semblage analysis. The sediment was dried for 24 hours at 24°C. Then foraminifera (894 individuals in total) were picked and classified up to genus level based on Le Calvez and Le Calvez (1958), Colom (1974), Cimerman and Langer (1991), Sgarrella and Moncharmont Zei (1993). Nomenclature was reviewed according to the WoRMS database.

2.3.1.11. Khokhlova et al., 2022 (Table 10)

The detailed description is presented in OBJECTIVE 2.

TABLE 10. Location, date, and depth of sampling with a short description of samples from Khokhlova et al. (2022).

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES (from the original work)	DEPTH (m)	DATE
Cala Santa Maria	CABRERA1	Ca-1-11	Bioclastic sandy bottom with a good ecological status of the coastal waters.	8	25/11/11
Cala Santa Maria	CABRERA2	Ca-3-12	<i>P. oceanica</i> meadow presents high shoot density, 626 shoots/m ² an average (Marbà et al., 2002).	8–12	23/05/12
Port d'Andratx	ADTX	Ma-9-12	Rocky/sandy bottom, with a small professional fishing fleet and a considerable amount of recreational boats. The shoot density of <i>P. oceanica</i> ranged from 350 to 460 shoots/m ² (Barón et al., 2011).	8–12	15/05/12
Magaluf	MGLF	Ma-7-12	Tourist areas under anthropogenic influence. The shoot density showed an average value of 632 shoots/m ² (Barón et al., 2011)	6–7	16/05/12

2.3.1.12. Khokhlova and Mateu-Vicens, 2023 (Figure 28; Table 11)

Three sediment samples were obtained for each site from 11 stations, twice a year (spring-summer and autumn-winter) to cover seasonal changes. Samples were collected manually (~100 g) by snorkeling at shallow depths between 2 and 4 m, from soft bottoms colonized by *P. oceanica*. The selected sites covered a wide spectrum of environmental conditions, including different degrees of anthropogenic influence on the *P. oceanica* meadows.

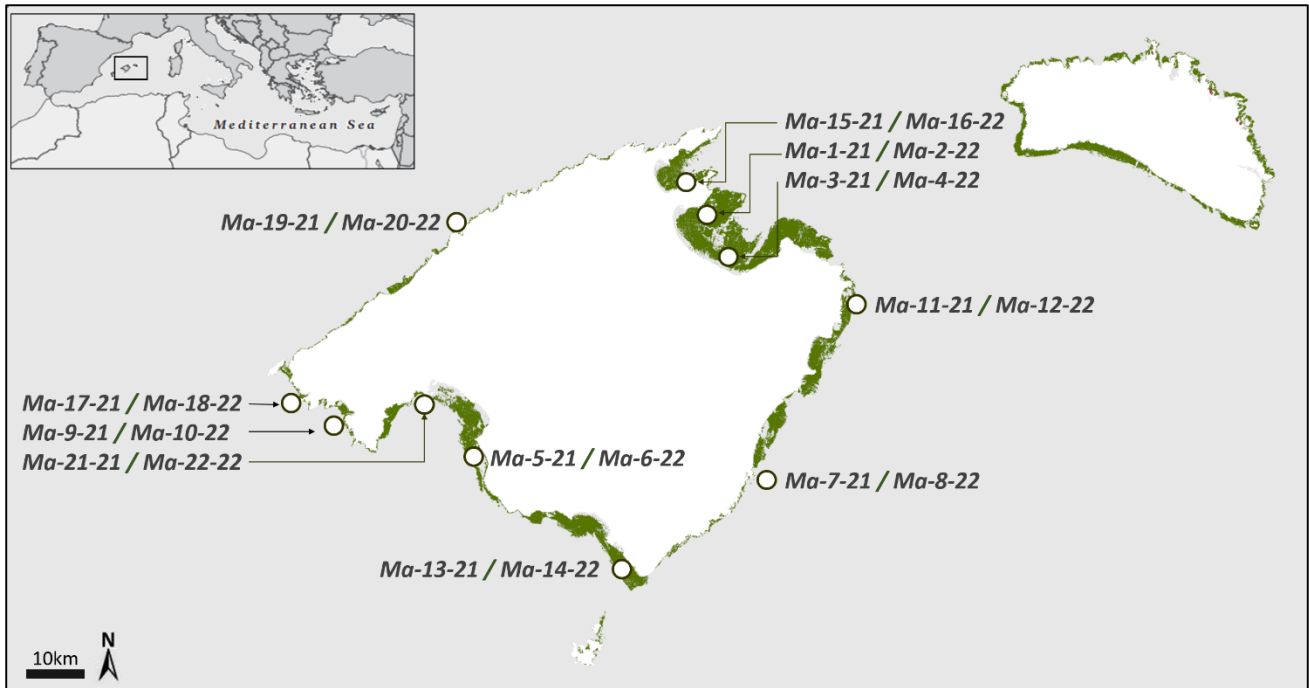


FIGURE 28. Map showing the sampling locations of historical data series of Mallorca: Alcanada (Ma-1-21 and Ma-2-22), Ca'n Picafort (Ma-3-21 and Ma-4-22), Cala Blava (Ma-5-21 and Ma-6-22), Cala Brafi (Ma-7-21 and Ma-8-22), Cala de Santa Ponça (Ma-9-21 and Ma-10-22), Capdepera (Ma-11-21 and Ma-12-22), Es Caragol (Ma-13-21 and Ma-14-22), Marina de Bonaire (Ma-15-21 and Ma-16-22), Port d'Andratx (Ma-17-21 and Ma-18-22), Port de Sóller (Ma-19-21 and Ma-20-22), Ses Illetes (Ma-21-21 and Ma-22-22) (Khokhlova and Mateu-Vicens, 2023).

Samples were treated with hydrogen peroxide solution for 24 hours to eliminate organic matter attached to the foraminiferal tests. The sediments were thoroughly washed with tap water over a 0.063 mm wire mesh sieve and left to dry. Foraminifera were separated by hand picking using a camera-equipped Leica MZ16 binocular stereomicroscope (Leica DFC295). One hundred and fifty foraminifera were identified per replicate sample. 1650 individuals were identified to morphotype level based on the literature (Frezza et al., 2011; Mateu-Vicens et al., 2014; Khokhlova et al., 2022).

TABLE 11. Location, date, and depth of sampling with a short description of samples from Khokhlova and Mateu-Vicens (2023). The sampling station's descriptions were based on personal observations.

LOCATION	ORIGINAL NAME OF SAMPLE	UNIFIED NAME OF SAMPLE	SAMPLING NOTES	DEPTH (m)	DATE
Alcanada	ALKN1	Ma-1-21	Muddy sands with <i>Posidonia</i> from shallow depth,	2–4	2021
	ALKN2	Ma-2-22	urbanized area with high anthropogenic pressure	2–4	2022
Ca'n Picafort	IBAP1	Ma-3-21	Rocky/sandy bottom with a large amount of <i>Posidonia</i> ,	2–4	2021
	IBAP2	Ma-4-22	touristic zone with high anthropogenic pressure	2–4	2022
Cala Blava	CLBL1	Ma-5-21	Sandy bottom with a large amount of <i>Posidonia</i> from shallow depth, protected area, close to an urbanized area	2–4	2021
	CLBL2	Ma-6-22		2–4	2022
Cala Brafi	CBRF1	Ma-7-21	Rocky/sandy bottom with a large amount of <i>Posidonia</i> ,	2–4	2021
	CBRF2	Ma-8-22	not urbanized area	2–4	2022
Cala de Santa Ponça	IBSO1	Ma-9-21	Gravels with a large amount of <i>Posidonia</i> , the hotel's cost	2–4	2021
	IBSO2	Ma-10-22		2–4	2022
Capdepera	PIPK1	Ma-11-21	Sandy bottom, with <i>Posidonia</i> from 4m of depth,	2–4	2021
	PIPK2	Ma-12-22	hotel's cost, with high anthropogenic pressure	2–4	2022

Es Caragol	CRGL1	Ma-13-21	Fine sand, <i>Posidonia</i> from shallow depth, not urbanized	2–4	2021
	CRGL2	Ma-14-22	area	2–4	2022
Marina de Bonaire	CDRL1	Ma-15-21	Rocky/sandy bottom close to the port with high	2–4	2021
	CDRL2	Ma-16-22	anthropogenic pressure	2–4	2022
Port d’Andratx	ADTX1	Ma-17-21	Rocky/sandy bottom, with a small professional fishing	2–4	2021
	ADTX2	Ma-18-22	fleet and a considerable amount of recreational boats	2–4	2022
Port de Sóller	SLLR1	Ma-19-21	Rocky/sandy bottom with muddy sands, port with high	2–4	2021
	SLLR2	Ma-20-22	anthropogenic pressure	2–4	2022
Ses Illetes	ILLT1	Ma-21-21	Fine sand, with <i>Posidonia</i> from shallow depth, hotel’s	2–4	2021
	ILLT2	Ma-22-22	cost, with high anthropogenic pressure	2–4	2022

2.3.2. Epiphytic Foraminifera-Based Bioindicator Indices (FI', I_{LS}, FSI)

Application

The indices adopted for this study are the modified FORAM (Foraminifera in Reef Assessment and Monitoring) Index (FI'), the “Long vs Short life span” index (I_{LS}) calculated by Khokhlova, 2013 and Mateu-Vicens et al. (2014) built to illustrate the differences between well preserved and stressed *P. oceanica* meadows, and the Foram Stress Index (FSI) obtained by Dimiza et al. (2016).

2.3.3. Statistical Analysis

For statistical analysis Paleontological Statistics (PAST) software package v. 4.05 (Hammer et al., 2001) was applied. A box-plot analysis was carried out to visualize the variation of the ecological index I_{LS} among the years for different sample stations in Mallorca. Each plot represents a sample station (Cala Blava, Port de Sóller, Portocolom, and Port d’Andratx), in which was possible to calculate the index I_{LS} for at least three years. In other cases, in which the data from nearby sampling stations correspond to a single year, they have been grouped into zones to obtain a temporal trend of the indices. These plots represent zones with two or three sample stations geographically close and with similar physical characteristics (Santa Ponça and Cala de Santa Ponça; Ses Illetes, Portals Nous and Magaluf; S’Arenal, Es Carnatge, and Ciutat Jardí; Marina de Bonaire, Es Barcarès, and Badia de Pollença). Box-plot analysis could not be applied to Menorca and Cabrera due to the lack of data to compare. OpenStreetMaps geographical database (in PAST) was used to display data distribution of ecological index I_{LS} for each sample location (defined by a latitude and longitude) in the Balearic Islands for decades (grouped by: 1960s, 1980s, 1990s, 2000s, 2010s, 2020s) if data were available. Taken from the PAST spreadsheet, the bubble radius represents the I_{LS} value for each location in each decade. As I_{LS} and FI' are highly correlated and the former index magnifies the differences between good and bad ecological conditions, the FI' was not represented on OpenStreetMaps.

CHAPTER 3

RESULTS

The results obtained are presented below, following the sequence of objectives addressed in this thesis. Objective 1 “N and C isotopic analysis to validate Khokhlova’s (2013) ecological groups on which the bioindicator indices are based” has been tackled through the analysis of samples collected in *P. oceanica* meadows from Mallorca and multispecific seagrass meadows from Madagascar. On the other hand, objective 2 “Comparative analysis on the environmental conditions of different *P. oceanica* meadows from the Balearic Islands (Spain) using bioindicator indices, abnormal-growth forms, and trace-elemental concentrations” has been approached by studying samples picked in *P. oceanica* meadows from Mallorca and Cabrera islands, representing a variety of anthropogenic impacts affecting this kind of ecosystem. Finally, the third main objective of the thesis, “Application of the bioindicator indices to historical data series based on information from localities of the Balearic Islands (Mallorca, Menorca, and Cabrera)” is based on the information gathered from published literature, reports, and theses studying the foraminiferal assemblages of the *P. oceanica* meadows from the Balearic Islands since the 1960s.

3.1. OBJECTIVE 1: N AND C ISOTOPIC ANALYSIS TO VALIDATE KHOKHLOVA’S (2013) ECOLOGICAL GROUPS ON WHICH THE BIOINDICATOR INDICES ARE BASED

To achieve this objective, a total of 10,025 living individuals were collected, classified into genera (Table 1), and assigned to one of the morphotypes established in Khokhlova (2013) although the A* (long life span taxa) and SB (symbiont bearing) groups were split into AF* (epifoliar A* taxa) and AR* (epirhizomatic A* taxa), and LM (large miliolids) and LR (large rotalids), respectively. Relative abundances of the different morphotypes varied among the localities. Thus, AF* and B morphotypes were the most abundant (Table 12) in Sa Foradada ($33.9\% \pm 4.6$ and $38.1\% \pm 4.3$, respectively) and Sant Elm ($40.6\% \pm 1$ and $44.8\% \pm 4.2$, respectively). In contrast, the D* morphotype was the most abundant in Madagascar ($45\% \pm 10$). The LM and C morphotypes were generally the least abundant of the morphotypes found in all three localities. The AR* morphotype was only found in Sa Foradada and Sant Elm samples, with similar abundances in both sites (ca. 3%). Finally, the LR morphotype was only observed in Madagascar ($7\% \pm 4.2$).

The most abundant genera of the AF* morphotype (Fig. 29) were *Nubecularia* ($16.4\% \pm 4$) in Sa Foradada and *Cibicidella* ($23.3\% \pm 1.3$) in Sant Elm. In Madagascar, the AF* morphotype was represented exclusively by the genus *Planorbulina* ($7.8\% \pm 1.8$), and the LR morphotype was represented exclusively by the genus *Amphistegina* ($7.0\% \pm 4.2$). For the LM morphotype, *Peneroplis* occurred at all three localities, with an abundance range between $3.6\% \pm 0.8$ (Sa Foradada) and $0.5\% \pm 0.2$ (Sant Elm); *Sorites* was found only in Sant Elm and Sa Foradada, with similar abundances (ca. 0.5%); and *Marginopora*, was observed only in Madagascar, with a $3.7\% \pm 1$ abundance. The most abundant genus in the B morphotype was *Rosalina* in Sa Foradada ($27.9\% \pm 5.2$) and Sant Elm (37.1%

± 2.3); in Madagascar, the most abundant genus was *Cibicides* ($13.3\% \pm 1.6$). The genus *Lobatula* was included within the B morphotype; showing similar abundances in Sa Foradada and Sant Elm and being much scarcer in Madagascar (Fig. 29). The C morphotype included *Elphidium* and *Astrononion* in both Sa Foradada and Sant Elm, and only *Elphidium* in Madagascar, where it was most abundant ($7.7\% \pm 0.2$). *Quinqueloculina* was the main genus of the D* morphotype in all three localities (19.9% in Sa Foradada, 9.8% in Sant Elm, and 45% in Madagascar). *Bolivina* was rarely found (Fig. 29) in either Sa Foradada or Sant Elm and was not recorded in the samples from Madagascar.

TABLE 12. Mean of the individual abundance (number of specimens) and mean percentage (%) collected for each locality and by morphotype (M).

M	Sa Foradada	Sant Elm	Madagascar	Sa Foradada (%)	Sant Elm (%)	Madagascar (%)
AF*	379 \pm 133.5	234.58 \pm 26.4	18.8 \pm 2.6	33.9 \pm 4.6	40.6 \pm 1.0	7.8 \pm 1.8
AR*	32.7 \pm 6.0	19.3 \pm 12.4		3.1 \pm 1.0	3.2 \pm 1.9	
LM	25.2 \pm 33.7	2.3	16.3 \pm 1.0	4.2 \pm 0.9	0.4	7.0 \pm 2.4
LR			20.8 \pm 17.0			7.0 \pm 4.2
B	435.2 \pm 183.5	265.5 \pm 1.3	71.8 \pm 48.8	38.1 \pm 4.3	44.8 \pm 4.2	25.5 \pm 10.1
C	31.8 \pm 37.1	7	19.5	5.7 \pm 0.8	2.37	7.7 \pm 0.2
D*	228.8 \pm 102.5	40.5 \pm 7.5	107.8 \pm 15.3	19.9 \pm 5.2	9.8 \pm 1.3	45.0 \pm 10.0

3.1.1. Stable Isotopic Ratios

The sample sets (3 from Sa Foradada and 2 each from Sant Elm and Madagascar) were split into two subsets, one for non-acidification treatment ($\delta^{15}\text{N}$) and the other for acidification treatment ($\delta^{13}\text{C}$). The LM and C morphotypes from Sa Foradada and Sant Elm localities were only assessed for $\delta^{15}\text{N}$, as the number of individuals was insufficient for both treatments.

The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios for each morphotype and each location (Fig. 30) revealed a relatively narrow range of $\delta^{15}\text{N}$ values (0.5–3‰), and substantial variability in $\delta^{13}\text{C}$ values, (-19– 0.1) at all three locations. Some samples of the LM, C and D* morphotypes yielded insufficient organic matter to provide isotopic signals for the different localities (Table 13).

The most enriched $\delta^{15}\text{N}$ values (5.13‰) were from one sample of specimens of the AF* morphotype from Madagascar (Fig. 30C), whereas in Sa Foradada the most enriched $\delta^{15}\text{N}$ was found in B (2.90‰) and C (2.80‰) morphotypes; and in Sant Elm in D* morphotype (2.25‰). The most depleted $\delta^{15}\text{N}$ values were -0.88‰ and 0.32‰, corresponding to LM morphotype in Madagascar and AR* morphotype in Sa Foradada, respectively (Figs. 30A, C). In Sant Elm, the lowest $\delta^{15}\text{N}$ value is 0.39‰ (Fig. 30B) measured from AR* morphotype. For the LR morphotype the only reported $\delta^{15}\text{N}$ value is 0.96‰, from Madagascar.

The most-enriched values for $\delta^{13}\text{C}$ (-0.90‰ to -1.85‰) were found in the LM and LR morphotypes in Madagascar (Fig. 30C). The Madagascar samples showed the only strong negative linear trend of increasingly depleted $\delta^{13}\text{C}$ with increasingly enriched $\delta^{15}\text{N}$. In Sa Foradada, the $\delta^{13}\text{C}$ ranged between -2‰ and -11‰, except for -18.25‰ in the B morphotype of one sample), with the most enriched values corresponding to AR morphotype (Fig. 30A). In Sant Elm, $\delta^{13}\text{C}$ values were consistently more depleted for B and D* morphotypes, down to -15.87‰ and -17.02‰, respectively (Fig. 30B). The AF* (-4.27‰) and AR* (-2.78‰) recorded the most-enriched values for this locality.

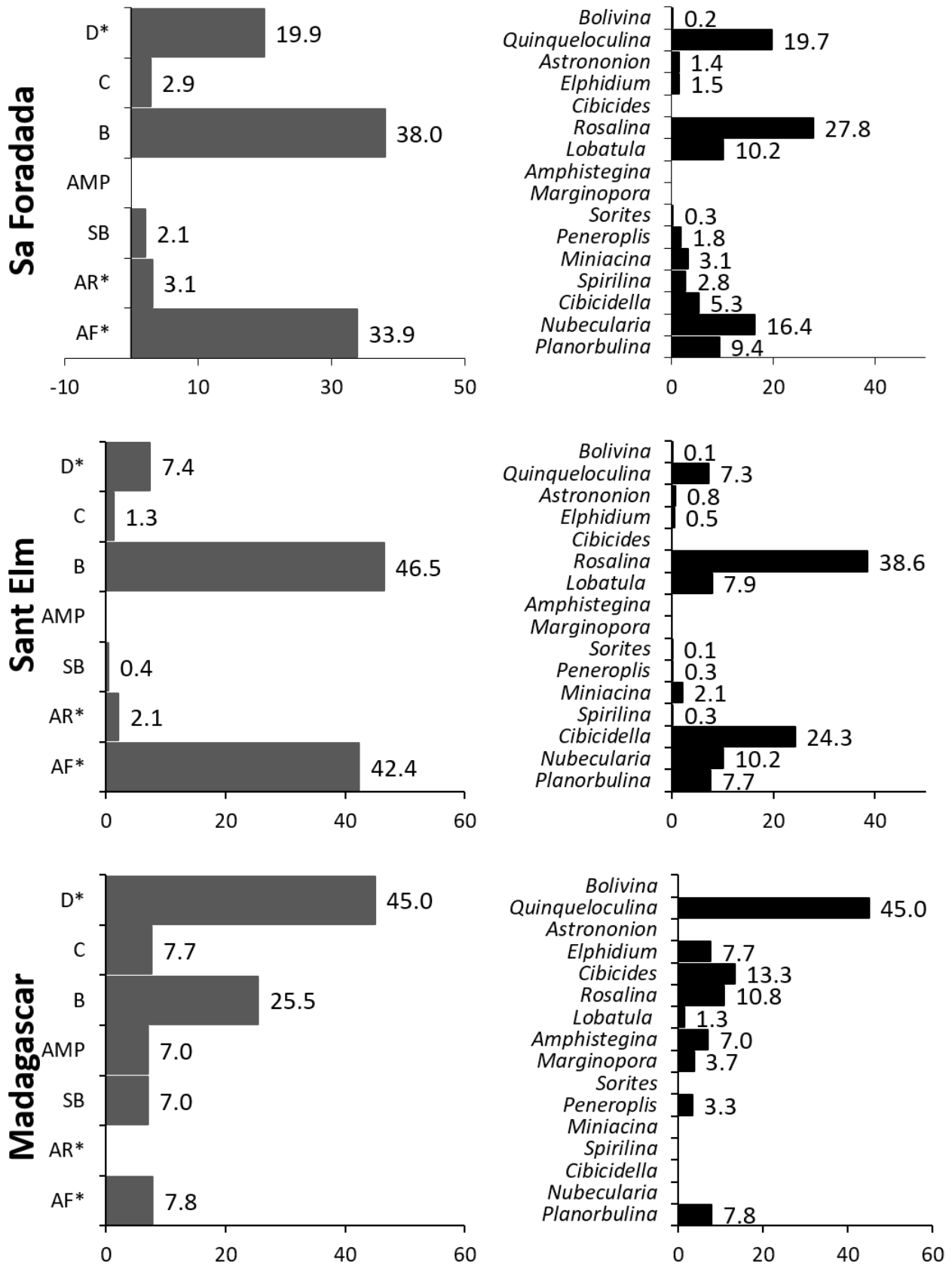


FIGURE 29. Percentage (%) of genera collected per morphotype and locality in this study.

Overall (Fig. 30), the AF*, B and C morphotypes were highly variable, though most samples revealed depleted $\delta^{13}\text{C}$, and some had higher $\delta^{15}\text{N}$ ranges. The LM and LR morphotypes, which were only sufficiently abundant to analyse in the Madagascar samples, presented highly depleted $\delta^{15}\text{N}$ values (-0.88‰) and very enriched $\delta^{13}\text{C}$ values (-0.90‰). The AR* morphotypes also were enriched in $\delta^{13}\text{C}$.

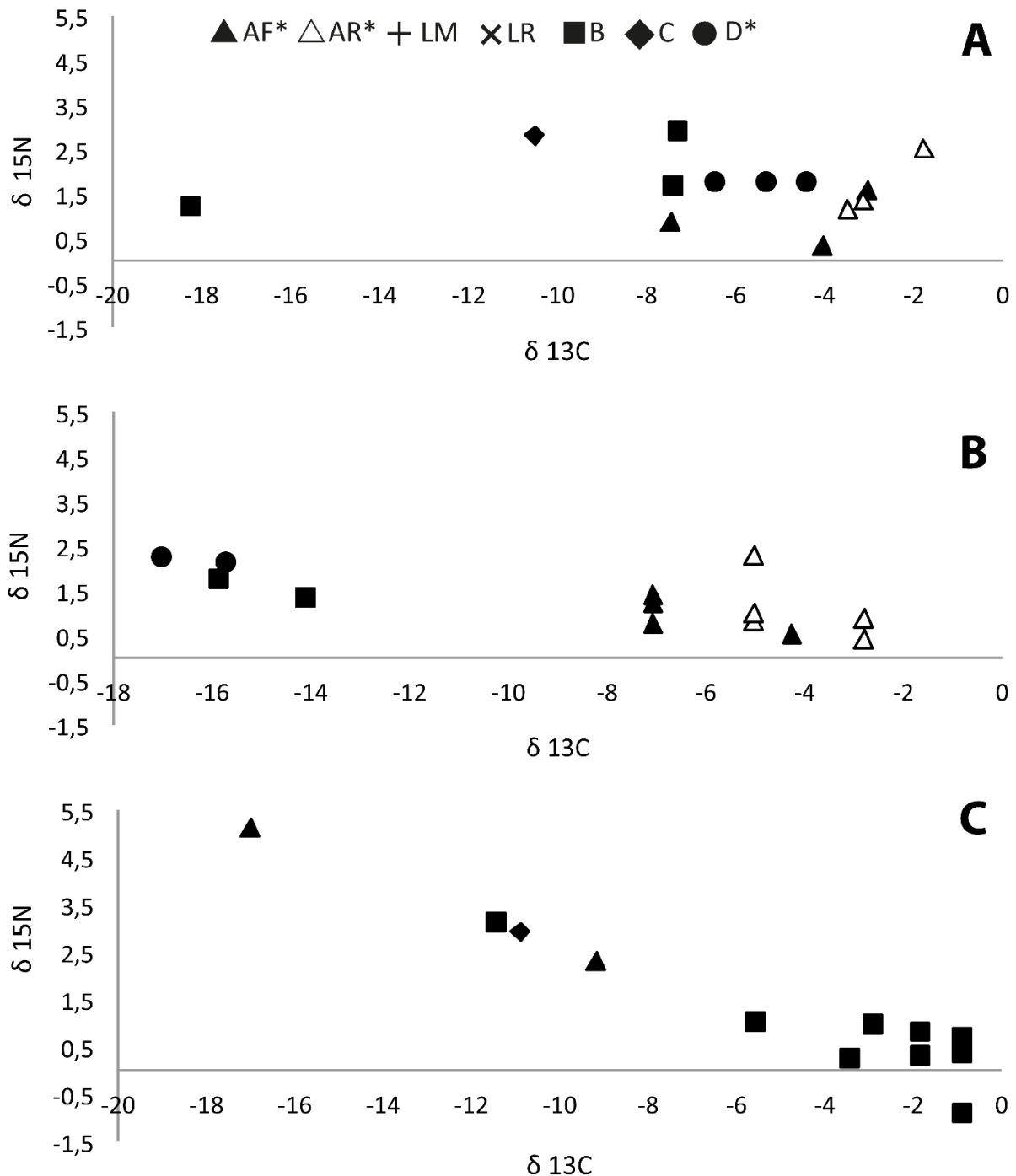


FIGURE 30. Dual isotope scatter plot ($\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$) for each morphotype. A. Sa Foradada replicates; B. Sant Elm replicates; C. Madagascar replicates.

TABLE 13. Values of $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ measured for each morphotype and locality. Empty spaces correspond to samples with insufficient material to produce a detectable signal.

Morphotype	Sa Foradada		Sant Elm		Madagascar	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
AF*	-7.45	0.88	-4.27	0.53	-17.01	5.14
	-4.06	0.32	-7.08	1.40	-9.17	2.32
	-3.04	1.59	-7.08	0.75		
			-7.08	1.22		
AR*	-3.48	1.14	-2.79	0.40		
	-3.14	1.36	-2.79	0.88		
	-1.77	2.53	-5.01	2.29		
			-5.01	0.82		
		-5.01	1.01			
LM					-0.90	0.36
					-0.90	-0.89
					-0.90	0.72
					-1.86	0.31
LR					-1.86	0.83
					-2.92	0.97
B	-18.26	1.23	-15.88	1.77	-11.42	3.13
	-7.42	1.68	-14.11	1.34	-3.44	0.26
	-7.32	2.91			-5.56	1.02
C	-10.52	2.80			-10.89	2.94
D*	-6.46	1.78	-17.03	2.26		
	-5.32	1.78	-15.73	2.14		
	-4.42	1.78				

The obtained results were statistically analyzed. The MDS analysis (Fig. 31) confirmed the relationships shown in Figure 30, including the relatively wide range of values for $\delta^{13}\text{C}$ and the limited range for $\delta^{15}\text{N}$. The LM and LR morphotypes were grouped to the far left, with the AR* samples just to their right. The AF* values from Mallorca were somewhat to the right, though the AF* samples from Madagascar were widely dispersed, as were those from the B and D morphotypes. Interestingly, the C morphotype had similar, intermediate $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values at both Sa Foradada and Madagascar (Figs. 30, 31). Kruskal-Wallis analyses (Table 14) revealed no significant differences by locality but significant differences among morphotypes, with especially strong differences in $\delta^{13}\text{C}$.

The results of the SIMPER analysis (Fig. 32) performed for both localities and morphotypes indicate stronger similarities between the Mallorca localities. For the morphotypes, the highest similarity occurs between symbiont-bearing taxa (LM and LR), and between permanently-attached species (AF* and AR*). In contrast, maximum dissimilarity is observed when comparing symbiont-bearing and permanently-attached morphotypes.

The results (Table 14) obtained from the analysis combining $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values revealed no significant differences among samples by locality ($R=0.26$; Sig. $L = 2.4\%$), and small, but significant, differences by morphotype ($R=0.59$; Sig. $L = 0.01\%$). The ANOSIM analysis performed on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ separately further revealed no significant differences for $\delta^{15}\text{N}$ related to locality ($R = 0.18$; Sig. $L = 7.5\%$) or to morphotype ($R = 0.23$; Sig. $L = 2.2\%$). In contrast, results for the $\delta^{13}\text{C}$ do indicate significant differences associated with morphotype ($R = 0.60$; Sig. $L = 0.01\%$) but not with locality ($R = 0.30$; Sig. $L = 1.7\%$).

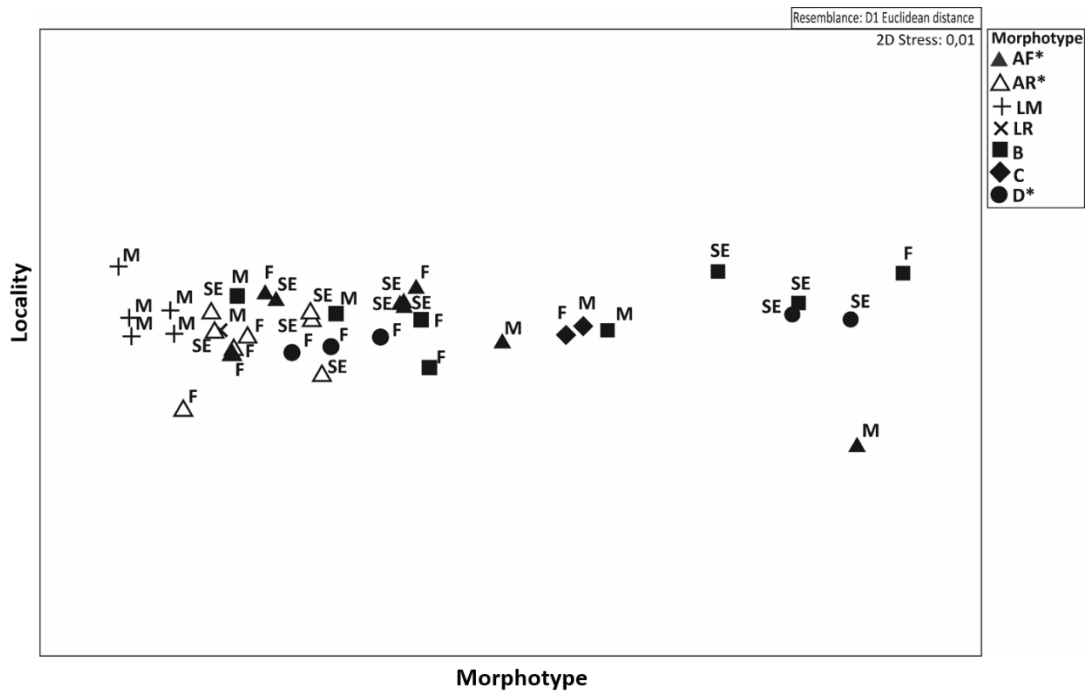


FIGURE 31. MDS analysis for morphotypes and localities of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ‰. F5 Sa Foradada (Mallorca, Western Mediterranean); SE5 Sant Elm (Mallorca, Western Mediterranean); M5 Diego Suárez (NE Madagascar, Indian Ocean).

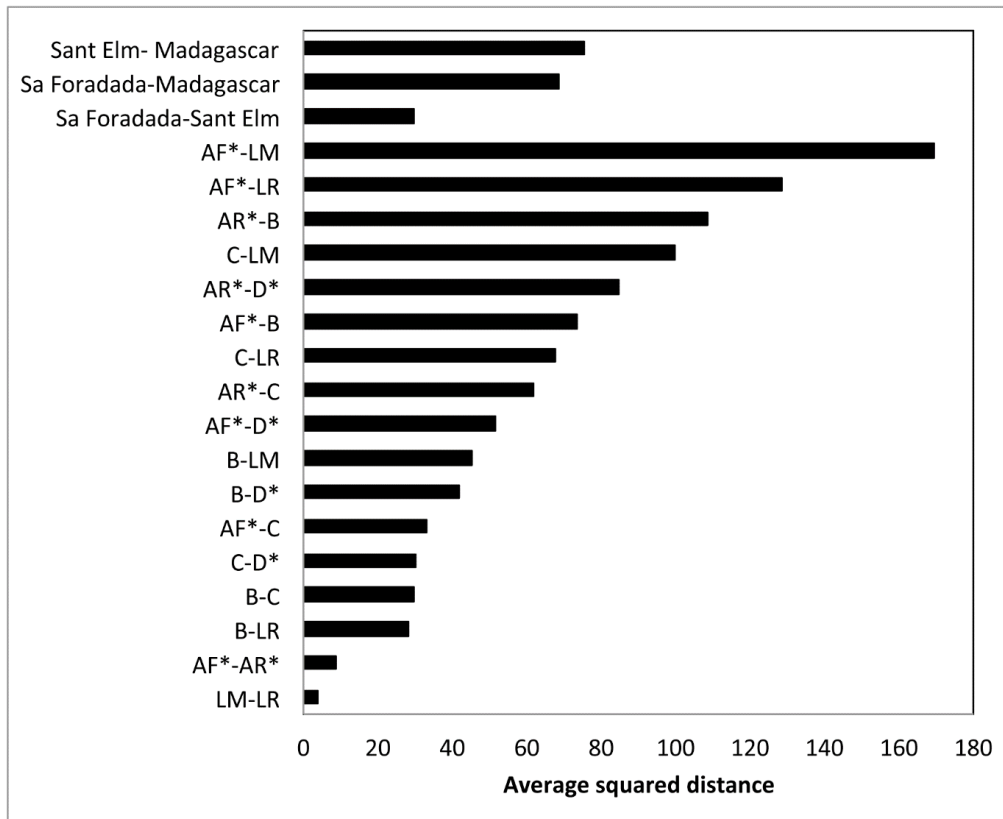


FIGURE 32. Average squared distance of isotope values between localities and between morphotypes. Summary of SIMPER analyses.

TABLE 14. Differences among $\delta^{13}\text{C}\text{‰}$, $\delta^{15}\text{N}\text{‰}$, datasets and the ratio $\delta^{13}\text{C}\text{‰}$, $\delta^{15}\text{N}\text{‰}$, associated with localities or morphotypes. Summary of ANOSIM analyses and Kruskal-Wallis tests. $P < 0.05$; R=the sample statistic (Global R); Sig. L= significance level of sample statistic.

	ANOSIM						Kruskal-Wallis	
	$\delta^{13}\text{C}\text{‰}$		$\delta^{15}\text{N}\text{‰}$		$\delta^{13}\text{C}$ and $\delta^{15}\text{N}\text{‰}$		$\delta^{13}\text{C}\text{‰}$	$\delta^{15}\text{N}\text{‰}$
	R	Sig. L (%)	R	Sig. L (%)	R	Sig. L (%)	p-value	p-value
Locality	0.298	1.7	0.176	7.5	0.262	2.4	0.274	0.303
Morphotype	0.597	0.01	0.226	2.2	0.588	0.01	0.001	0.016

3.2. OBJECTIVE 2: COMPARATIVE ANALYSIS OF THE ENVIRONMENTAL CONDITIONS OF *P. OCEANICA* MEADOWS USING 1) BIOINDICATOR INDICES BASED ON EPIPHYTIC FORAMINIFERA, 2) THE FREQUENCY OF ABNORMAL-GROWTH FORMS AND 3) THE TRACE-ELEMENTAL CONCENTRATIONS WITHIN THEIR TESTS.

The achievement of this objective is based on the analysis of sediment samples of localities from Mallorca and Cabrera islands. A total of 106 foraminiferal species were identified from 2,250 individuals (150 for every sampling site) in this study (Table 15). The FI' , I_{LS} , FSI, H' , FAI and ratio P/H (porcelaneous and hyaline tests) were calculated for each sample (Table 15, Fig. 33 (A-E)). The highest FI' , I_{LS} and H' values were found in Cabrera stations, whereas FSI values were high for all samples. The highest FAI (23%) (Table 15) occurred in Cabrera1. In contrast, in Cabrera2 there much lower FAI (4%) similar to those reported in the two stations of Mallorca, Port d'Andratx (6%) and Magaluf (4%).

Regarding the percentage of each type of deformation (Table 16), aberrant chamber shape and size was dominant in the samples from Cabrera island (42.2 % in Cabrera1 and 51.2% in Cabrera2) and Port d'Andratx (48.4%), when in Magaluf, abnormal coiling (45.4%) was the predominant deformity followed by aberrant chamber shape and size (35.7%).

TABLE 15. The value of FI' , I_{LS} , FSI, H' , FAI (total and on average) and ratio of P/H (porcelaneous and hyaline tests) for the sampling stations of Cabrera1, Cabrera2, Port d'Andratx and Magaluf with the date of sampling.

Locality	Sampling site	FI'	FI' Av.	I_{LS}	I_{LS} Av.	FSI	FSI Av.	H'	H' Av.	FAI	FAI Av.	Ratio P/H	
CABRERA1	SMRA-1	5.94	5.48	5.50	5.14	9.70	9.68	3.42	3.62	26	23	2.00	
	25/11/11	SMRA-2	5.24		5.72		9.64		3.79		22		1.25
	SMRA-3	5.27		4.20		9.70		3.64		22		1.67	
CABRERA2	SMRA-4	4.93	4.59	4.02	3.98	9.82	9.78	3.45	3.50	4	4	1.25	
	23/05/12	SMRA-5	4.47		5.41		9.76		3.56		1		1.43
	SMRA-6	4.71		3.77		9.82		3.47		4		1.11	
	SMRA-7	4.24		2.74		9.70		3.51		6		1.11	
PORT D'ANDRATX	ADTX-1	5.04	4.64	4.22	3.67	9.94	9.91	3.32	3.39	10	6	1.11	
	15/05/12	ADTX-2	4.43		3.59		9.82		3.44		5		0.91
	ADTX-3	4.03		2.68		9.94		3.45		2		0.91	
	ADTX-4	5.07		4.18		9.94		3.37		6		1.11	
MAGALUF	MGLF-1	3.72	3.79	2.11	2.29	9.46	9.60	3.59	3.49	7	4	1.00	
	16/05/12	MGLF-2	4.03		2.68		9.76		3.30		4		0.77
	MGLF-3	3.45		1.84		9.76		3.58		3		1.00	
	MGLF-4	3.96		2.55		9.40		3.48		3		1.11	

TABLE 16. Percentage (%) of the different type of test abnormality observed in foraminiferal species of the Cabrera1, Cabrera2, Port d'Andratx and Magaluf samples.

	CABRERA1	CABRERA2	PORT D'ANDRATX	MAGALUF
Aberrant chamber shape and size	42.2	51.2	48.4	35.7
Abnormal coiling	21.2	33.0	23.7	45.4
Protuberances	10.7	8.3	19.9	12.7
Twinning	11.2	2.5	2.5	0.0
Deformed side view	8.6	0.0	0.0	0.0
Supernumerary chambers	3.0	2.5	5.4	6.3
Multiple apertures	3.0	2.5	0.0	0.0

The highest heavy-metal concentrations in foraminiferal tests were reported in samples from Magaluf and Port d'Andratx (Table 17, Fig. 33 (F-J)). In Magaluf, the Pb concentrations ranged from 28.13 to 92.75 ppm, mean 57.54 ppm; compared to Port d'Andratx, Pb (11.21—40.83 ppm, mean, 22.71 ppm). In contrast, in Cabrera there are remarkable differences between the values reported in the two sampling site. In Cabrera1 the mean Pb concentration was 15.59 ppm, (6.15—34.70 ppm). While in Cabrera2, the concentration of Pb in foraminiferal tests is almost 3 times lower (4.16 ppm) than in Cabrera1, ranging from 2.97 to 5.84 ppm. Lead concentrations in the control samples ranged from 8.70 (Cabrera2) to 20.62 (Magaluf) ppm, with a mean of 12.78 ppm.

TABLE 17. The mean value, minimum and maximum of deformed foraminifera for the concentrations of heavy metals such as Pb, Zn, Cd, Cu, Co, Ni, As and Sn (ppm).

		Pb (ppm)	Zn (ppm)	Cd (ppm)	Cu (ppm)	Co (ppm)	Ni (ppm)	As (ppm)	Sn (ppm)
CABRERA1	mean	15.59	18.82	0.13	2.33	0.30	1.44	1.28	0.10
	min. - max.	6.15 - 34.70	11.35 - 30.42	0.09 - 0.23	1.08 - 3.45	0.16 - 0.49	0.52 - 2.35	0.79 - 1.87	0.04 - 0.18
CABRERA2	mean	4.16	8.46	0.53	2.82	0.06	1.06	1.42	0.06
	min. - max.	2.97 - 5.84	5.23 - 13.82	0.08 - 1.60	1.47 - 4.77	0.03 - 0.09	0.77 - 1.42	1.16 - 2.16	0.03 - 0.07
MAGALUF	mean	57.54	36.46	0.36	6.47	0.38	2.33	3.36	1.16
	min. - max.	28.13 - 92.75	34.00 - 40.90	0.35 - 0.37	4.70 - 9.05	0.18 - 0.50	2.08 - 2.57	1.22 - 5.12	0.12 - 2.8
PORT D'ANDRATX	mean	22.71	36.13	2.24	10.23	0.65	3.30	3.18	0.33
	min. - max.	11.21 - 40.83	12.08 - 59.35	1.22 - 4.13	3.64 - 21.74	0.21 - 1.22	1.52 - 5.77	2.16 - 3.89	0.21 - 0.42
CONTROL	mean	12.78	12.16	0.17	2.49	0.14	2.09	1.51	0.14
	min. - max.	8.70 - 20.62	8.95 - 17.10	0.11 - 0.25	1.67 - 3.65	0.06 - 0.28	0.83 - 4.14	1.09 - 1.85	0.07 - 0.27

The average Zn concentrations in foraminiferal tests from Magaluf and Port d'Andratx were very similar; however, their ranges were considerably different (34.00—40.90 ppm, Magaluf vs. 12.08—59.35 ppm, Port d' Andratx). The mean Zn concentration in Cabrera1 (18.82 ppm) more than doubles that of Cabrera2 (8.46 ppm), with ranges between 11.35 and 30.42 ppm, and between 5.23 and 13.82 ppm in Cabrera2. In the control samples, the Zn concentrations range from 8.95 (Cabrera2) to 17.10 (Magaluf) ppm, with a mean of 12.16 ppm. All these differences were statistically significant ($p < 0.05$) among the study sites.

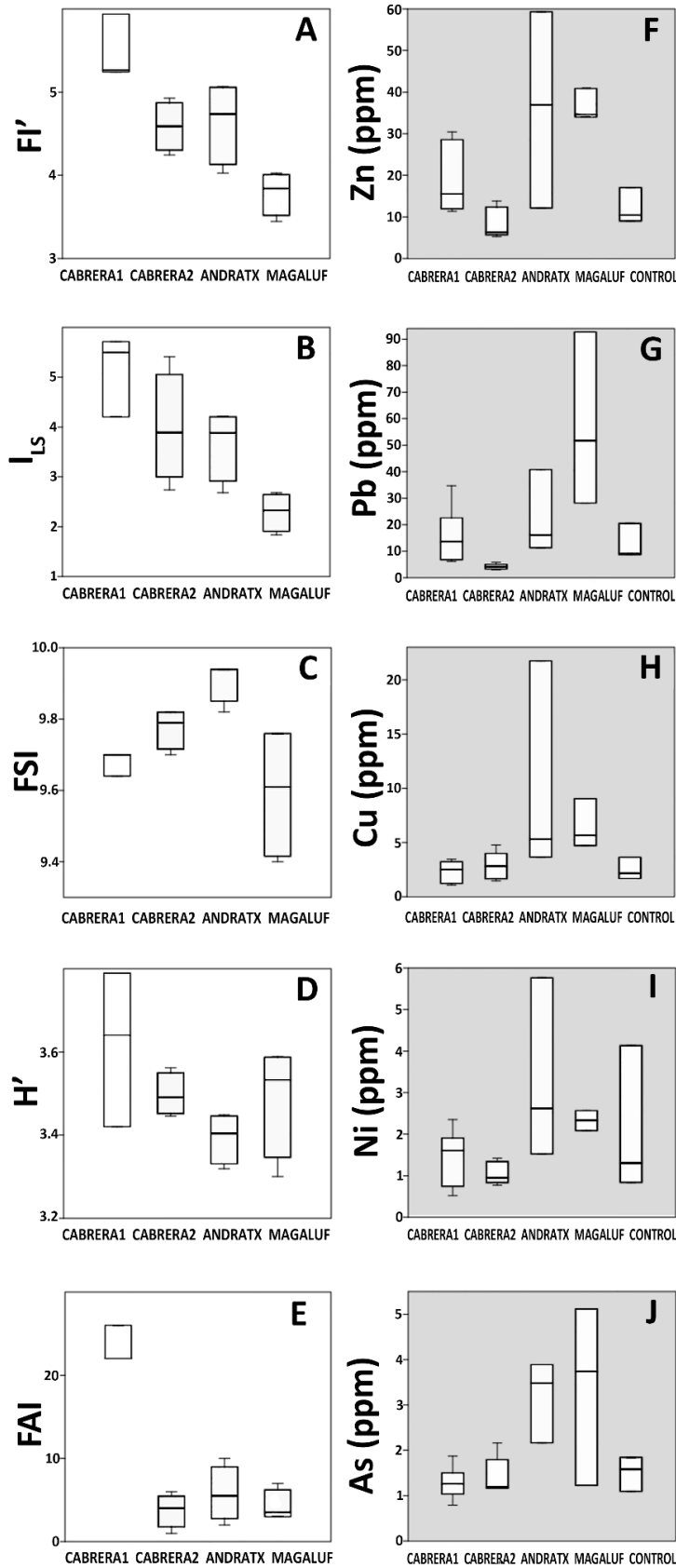


FIGURE 33. In white squares ANOVA Model Box-plot of ecological indices values and FAI all sampling sites: A) FI' , B) I_{LS} , C) FSI , D) H' , and E) FAI ; in grey squares ANOVA Model Box-plot of heavy metal concentrations from abnormal benthic foraminifera tests in every sampling site: F) Zn, G) Pb, H) Cu, I) Ni and J) As. Here “Andratx” means sampling stations from Port d’Andratx.

Differences in Cu concentration were not statistically significant among the study sites ($p < 0.05$). The highest mean value of Cu was observed in Port d'Andratx (10.23 ppm) and considerably lower (6.47 ppm) in Magaluf. Lower Cu levels were observed in Cabrera1 (2.33 ppm) and Cabrera2 (2.82 ppm). In the control samples, Cu concentrations ranged from 1.67 (Cabrera2) to 3.65 (Cabrera1) ppm, with a mean of 2.49 ppm.

As for Cu, the Ni content in the foraminiferal tests was not significantly different among sample sites ($p < 0.05$). The maximum mean concentration was detected in Port d'Andratx (3.30 ppm) and was lower (2.33 ppm) in Magaluf. There was little difference in the mean concentrations of Ni in Cabrera1 (1.44 ppm) and Cabrera2 (1.06). Ni concentrations in the control samples ranged from 0.83 (Cabrera2) to 4.14 (Cabrera1) ppm, with a mean concentration of 2.09 ppm.

The As concentration in the foraminiferal tests were significantly different ($p < 0.05$) in all the study sites except at the Cabrera stations. In Cabrera1 and Cabrera2 the mean values were 1.28 and 1.42 ppm, and ranged from 0.79 to 1.87 ppm, and from 1.16 to 2.16 ppm, respectively. Nearly the same values were observed in the control samples, which ranged from 1.09 (Cabrera2) to 1.85 (Cabrera1) ppm, with a mean concentration of 1.51 ppm. The highest concentrations were detected in Magaluf (mean = 3.36 ppm) and Port d'Andratx (mean = 3.18 ppm).

Cd, Co and Sn concentrations are included in Table 17, but they are not considered in the statistical analyses as the internal error shown in the heavy-metal detection is $> 20\%$. This high percentage of error is likely attributed to low concentrations of analyte in the foraminiferal test, or to measurements performed on very thinly walled tests. However, the capture of Cd by symbiotic algae should not be disregarded (Mashiotta et al., 1997; Lea, 2003).

In the PCA analysis (Fig. 34), up to 81% of the variance is explained by PC1 (57%) and PC2 (24%). Stronger loadings in PC1 (Fig. 34) correspond to the different heavy-metal contents present in the foraminiferal tests, mostly Pb, followed by Zn and Cu, and to a lesser extent, Ni and As. In contrast, PC2 loadings mostly correspond to the percentage of deformed foraminifera. The different bioindicator indices do not play a major role in PC1 and PC2 loadings. The analysis shows a strong correlation between Cabrera1, the percentage of deformed foraminifera (PC2), and the concentrations of Pb and Zn in foraminifera tests (PC1). In contrast, there was no evident influence of heavy-metal concentrations and the percentage of deformed shells in Cabrera2 samples. The PCA also showed that high concentrations of Pb, Zn, Ni, As and Cu (PC1) explain the distribution of samples from the Magaluf and Port d'Andratx sites.

The Pearson correlation coefficient resulted in a significant ($p < 0.05$) correlation between certain heavy-metal concentrations (Fig. 35) such as Cu and Zn, Cu and Ni, Cu and As, Zn and Pb, Zn and As. Other significant correlations appeared between the presence of protuberances in the foraminiferal tests and Ni concentrations; and between the occurrence of supernumerary chambers and Zn and As concentrations. However, although not significant (p -values between 0.088 and 0.180), a relatively strong correlation was observed between some types of abnormalities and the occurrence of certain heavy metals (i.e., "supernumerary chambers" and As, Zn, Pb, and Ni; "protuberances" and Cu, Zn, and Ni; "aberrant chamber shape and size" and Pb) (Fig. 35).

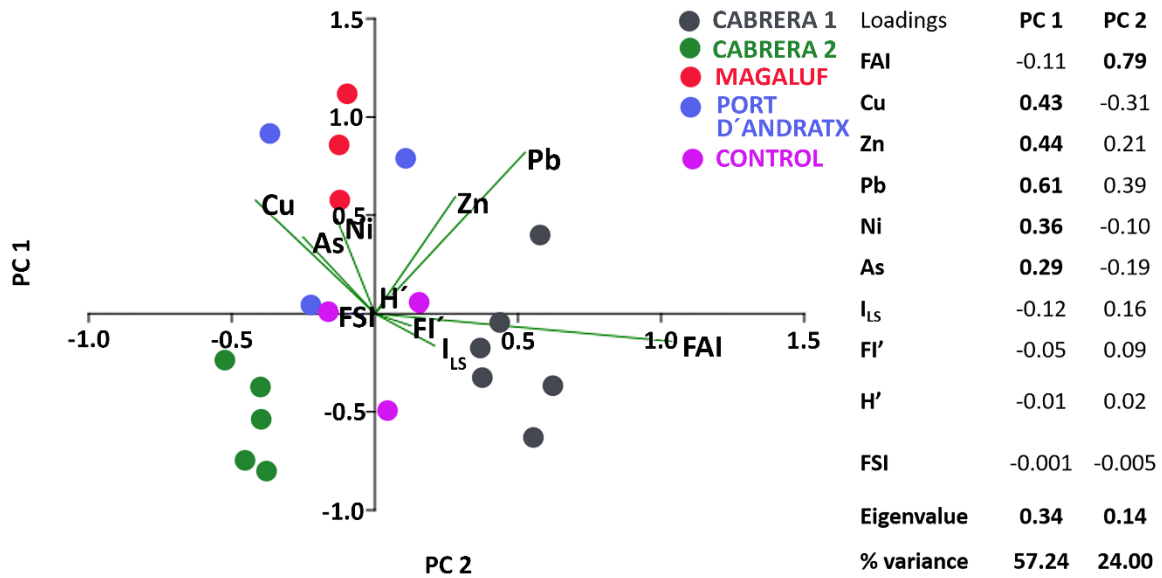


FIGURE 34. Principal Component Analysis (PCA) for heavy metals Pb, Zn, Cu, Ni and As in foraminifera tests (ppm); ecological indices FI', ILS, FSI and H'; and FAI among sampling sites (Cabrera1, Cabrera2, Port d'Andratx and Magaluf). The table on the right side presents the PCA loadings of the different variables corresponding to PC1 and PC2 (the strongest values are marked in bold letters). The first two principal components explain up to 81% of the total variance.

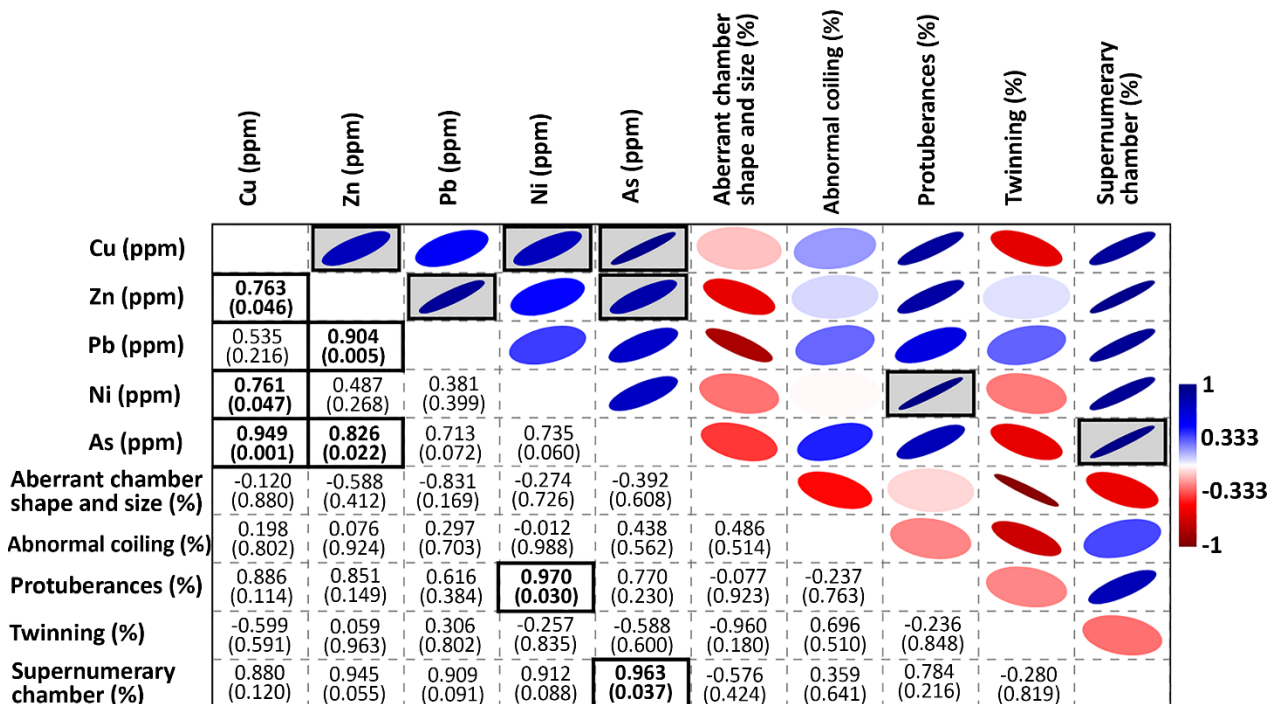


FIGURE 35. Pearson correlation coefficient between heavy metal concentration (ppm) and percentage of different type of deformation of the foraminifera tests. Blue ellipses mean positive correlation; red ellipses mean negative correlation. Ellipses size shows the r (coefficient) value. Bold cells show the strong positive correlation (r > 0.7), and p < 0.05.

3.3. OBJECTIVE 3. APPLICATION OF THE BIOINDICATOR INDICES TO HISTORICAL DATA SERIES BASED ON INFORMATION FROM LOCALITIES OF THE BALEARIC ISLANDS (MALLORCA, MENORCA, AND CABRERA).

Thirteen distinct research sources were consulted to fulfill the final goal, which was to ascertain the species (or genera) composition of epiphytic foraminifera in *P. oceanica* meadows. Data was collected from 45 locations across Mallorca, Menorca, and Cabrera islands to gather information about the leaves and rhizomes of *P. oceanica*, as well as the sediments below. This information spans from 1964, when the ecological investigation of benthic foraminifera associated with seagrass meadows in the Balearic Islands was initiated, until 2022.

The selected data cover full seasonality and a bathymetric spectrum from 0 (sediment collected in the swash zone of the beach by surface dragging with a small trowel) to 30 m (the deepest point where *P. oceanica* was collected) (Table 18, SM 1). Also, different levels of anthropogenic influence were considered (Fig. 36, and SM. 2-4), including zones close to port areas (Badia de Pollença, Ca'n Picafort, Cala d'Or, Es Barcarès, Marina de Bonaire, Port d'Andratx, Port de Sóller, Portals Nous, Portals Vells, Porto Petro, Portocolom, and S'Arenal (see SM. 2), urban areas (Alcanada, Cap de Regana, Ciutat Jardí, Santa Ponça, Cala En Blanes, Cala Fornells, Cala Galdana, Cala Murta, Es Carnatge, Ses Illetes); touristic areas (Arenal d'en Castell, Cala Brafí, Cala Mendia, Cala Murada, Camp de Mar, Capdepera, Magaluf); zones far from urban area (Sa Foradada, Ses Fontanelles, and Cala Mitjana); marine reserves with limited human activity (Cala Agulla, Cala Blava, Son Verí, Sa Ràpita, Colònia de Sant Jordi, Es Caragol, Sant Elm); an area corresponding to a natural park (Ses Covetes); and areas with the maximum protection status at the Balearic Islands (Cabrera Archipelago Maritime-Terrestrial National Park (see SM. 4).

The I_{LS} and FI' were calculated for each sample (Table 18, SM 1), and the highest values of these two indices were found in Mallorca, at Es Caragol (Ma-7-64) station in 1964, with I_{LS} - 14.65, and FI' - 8.94. These high values are associated with the predominance of foraminifera of morphotypes A* (0.53) and SB (0.35). Moreover, the relative abundance of morphotype D* was low (0.12), and there were not reported (Mateu, 1970) morphotypes C and B. The second highest values of the ecological indices were reported in Cala Blava (Ma-11-65), and Cala d'Or (Ma-2/3-08), with I_{LS} - 11.52, FI' - 5.71, and I_{LS} - 10.66, FI' - 6.75 respectively. In Cala Blava site, morphotypes A* (0.32) and SB (0.21) accounted for more than half of the foraminifera assemblage. Morphotype C was low (0.14), and morphotype D* had the lowest proportion (0.07). The morphotype B was totally absent.

In Menorca, the highest values of these indices were measured in the South of the island (Me-12-87) with I_{LS} of 12.99, and FI' of 8.30, corresponding to a very high presence of morphotypes A* (0.67) and SB (0.13). In addition, morphotypes D*, B, and C were scarce (0.12, 0.07, and 0.01 respectively). In Cala En Blanes (Me-3-82) I_{LS} (9.09) and FI' (6.10) were also high, and although morphotype SB was absent, the proportion of A* was very high (0.48). The morphotype C had the lowest proportion (0.01), morphotypes D* and B were scarce, with relative abundances of 0.13 and 0.31 respectively.

In Cabrera, the highest values of I_{LS} (4.68), and FI' (5.48) were obtained at Cala Santa Maria (Ca-1-11), where morphotypes A* (0.22) and SB (0.26) represented almost half of the foraminiferal assemblage. However, the proportion of D* foraminifera was high (0.28), in contrast to morphotypes B and C, which only corresponded to relative abundances of 0.16 and 0.07 respectively.

TABLE 18. Average value of morphotype (A*, B, C, D*, SB) relative abundances and average values of I_{LS}, FI', FSI for all sampling stations of Mallorca, Menorca, and Cabrera Islands grouped according to each publication, with data of depth for each sample and the decade when each sample was taken. ND (for FSI) – no data. The highest values are highlighted in bold cells, and the lowest - in cursive. 1 - Mateu, 1970, 2 - Gazá, 1988, 3 - Moreiro, 1993, 4 - Abril, 1993, 5 - Mateu et al., 2001, 6 - Mateu-Vicens et al., 2010, 7 - Khokhlova, 2013 and Mateu-Vicens et al., 2014, 8 - Racis, 2015, 9 - Mateu-Vicens et al., 2016, 10 - Vaquer, 2016, 11 - Khokhlova et al., 2022, 12 - Khokhlova and Mateu-Vicens, 2023, 13 - Florit, 1983.

MALLORCA AND CABRERA												
	LOCALITY	SAMPLING NAME	DECADES	DEPTH (m)	A* Av.	B Av.	C Av.	D* Av.	SB Av.	I _{LS} Av.	FI' Av.	FSI Av.
1	Cala Agulla	Ma-1-64	1960	0	0.19	0.29	0.16	0.19	0.08	3.69	3.81	8.21
	Cala Mendia	Ma-2-64	1960	0	0.39	0.17	0.06	0.22	0.14	6.17	6.05	9.65
	Cala Murada	Ma-3-64	1960	0	0.47	0.16	0.04	0.26	0.06	5.39	6.00	10.00
	Portocolom	Ma-4-64	1960	0	0.10	0.00	0.20	0.50	0.01	<i>0.81</i>	<i>1.98</i>	8.22
	Porto Petro	Ma-5-64	1960	0	0.14	0.05	0.14	0.19	0.10	3.21	2.95	6.57
	Cala Santanyí	Ma-6-64	1960	0	0.31	0.06	0.08	0.39	0.14	3.39	5.11	9.75
	Es Caragol	Ma-7-64	1960	0	0.53	0.00	0.00	0.12	0.35	14.65	8.94	10.00
	Colònia de Sant Jordi	Ma-8-64	1960	0	0.19	0.11	0.06	0.32	0.28	4.21	5.42	9.65
	Ses Covetes	Ma-9-64	1960	0	0.23	0.09	0.06	0.29	0.24	4.53	5.35	9.22
	Sa Ràpita	Ma-10-64	1960	0	0.22	0.15	0.10	0.30	0.21	4.03	5.12	9.60
	Cala Blava	Ma-11-65	1960	0	0.32	0.00	0.14	0.07	0.21	11.52	5.71	7.75
	S'Arenal	Ma-12-65	1960	0	0.24	0.04	0.04	0.29	0.37	5.81	6.57	9.82
	Port de Sóller	Ma-13-65	1960	0	0.34	0.10	0.09	0.30	0.17	4.76	5.74	9.91
	Camp de Mar	Ma-14-65	1960	0	0.32	0.09	0.15	0.19	0.16	6.15	5.42	9.16
	Santa Ponça	Ma-15-65	1960	0	0.19	0.14	0.04	0.27	0.31	5.03	5.70	9.64
2	Ciutat Jardí	Ma-6-82	1980	20	0.11	0.45	0.14	0.26	0.00	<i>1.28</i>	<i>2.47</i>	9.38
	Portals Nous	Ma-7-82	1980	22	0.25	0.18	0.10	0.45	0.00	1.95	3.49	9.56
	Cap de Regana	Ma-8-84	1980	20	0.02	0.18	0.10	0.31	0.33	3.26	4.38	9.17
3	Badia de Pollença	Ma-9-85	1980	20	0.21	0.19	0.03	0.33	0.22	3.74	5.06	9.54
	Badia de Pollença	Ma-10-85	1980	7	0.51	0.04	0.01	0.43	0.00	3.58	5.62	9.83
4	Cala Fornells	Ma-17-89	1980	3-6	0.12	0.33	0.10	0.29	0.14	2.61	3.72	9.43
	Port de Sóller	Ma-18-89	1980	3-6	0.39	0.39	0.03	0.11	0.04	7.97	5.16	9.52
	Es Barcarès	Ma-19-89	1980	3-6	0.18	0.36	0.03	0.17	0.24	6.85	5.17	9.81
	Portocolom	Ma-20-89	1980	3-6	0.14	0.54	0.06	0.13	0.10	4.20	3.65	9.51
	Es Carnatge	Ma-21-89	1980	3-6	0.20	0.42	0.02	0.22	0.01	3.04	3.20	8.56

	Cala Murta	Ma-22-89	1980	3-6	0.12	0.51	0.05	0.17	0.12	3.50	3.68	9.76
	Cala Fornells	Ma-1-90	1990	3-6	0.09	0.38	0.11	0.24	0.15	3.00	3.58	9.63
	Cala Murta	Ma-2-90	1990	3-6	0.39	0.38	0.03	0.12	0.03	7.20	5.22	9.59
	Es Barcarès	Ma-3-90	1990	3-6	0.23	0.33	0.02	0.21	0.17	5.01	4.90	9.68
	Es Carnatge	Ma-4-90	1990	3-6	0.18	0.38	0.08	0.25	0.06	2.78	3.58	9.13
	Port de Sóller	Ma-5-90	1990	3-6	0.20	0.43	0.03	0.22	0.01	3.58	3.23	8.72
	Portocolom	Ma-6-90	1990	3-6	0.17	0.49	0.04	0.18	0.11	3.85	3.99	9.82
	Cala Fornells	Ma-7-91	1990	3-6	0.07	0.26	0.28	0.19	0.17	5.31	3.59	9.48
	Cala Murta	Ma-8-91	1990	3-6	0.34	0.39	0.03	0.17	0.04	5.31	4.73	9.62
	Es Barcarès	Ma-9-91	1990	3-6	0.10	0.34	0.03	0.30	0.20	2.92	4.03	9.78
	Es Carnatge	Ma-10-91	1990	3-6	0.08	0.44	0.09	0.23	0.10	2.25	3.09	9.38
	Port de Sóller	Ma-11-91	1990	3-6	0.14	0.34	0.03	0.31	0.01	1.77	2.63	8.46
	Portocolom	Ma-12-91	1990	3-6	0.07	0.56	0.05	0.17	0.13	3.13	3.42	9.78
5	Port de Sóller	Ma-1-00	2000	12	0.04	0.78	0.03	0.08	0.03	1.97	2.42	9.69
6	Cala d'Or	Ma-2/3-08	2000	6-8	0.49	0.24	0.05	0.11	0.12	10.66	6.75	10.00
	Portals Vells	Ma-4/5-08	2000	6-8	0.47	0.28	0.05	0.11	0.09	9.83	6.36	9.97
7	Els Estels	Ca-4-12	2010	10-20	0.28	0.26	0.07	0.28	0.14	4.04	4.93	9.84
	Cala Santa Maria	Ca-2-11	2010	10	0.19	0.20	0.08	0.34	0.19	3.26	4.63	9.64
	Ses Illetes	Ma-12-12	2010	4-5	0.13	0.24	0.13	0.28	0.17	2.97	3.90	9.24
	Santa Ponça	Ma-13-12	2010	4-5	0.19	0.17	0.09	0.41	0.05	1.85	3.30	9.04
	Cala Blava	Ma-5-12	2010	5-6	0.12	0.19	0.10	0.44	0.14	1.82	3.50	9.69
	Portals Nous	Ma-10-12	2010	6-8	0.27	0.27	0.14	0.30	0.03	2.87	4.06	9.78
	Son Verí	Ma-11-12	2010	6-7	0.20	0.20	0.11	0.37	0.12	2.56	4.13	9.78
	Magaluf	Ma-8-12	2010	6-7	0.21	0.22	0.11	0.36	0.09	2.49	3.95	9.64
8	Cala Blava	Ma-6-12	2010	5-6	0.27	0.13	0.05	0.33	0.21	4.23	5.39	9.86
9	Sa Foradada	Ma-14-12	2010	15	0.20	0.71	0.01	0.08	0.01	4.36	3.58	9.98
	Sant Elm	Ma-15-12	2010	15	0.32	0.43	0.03	0.20	0.02	4.36	4.51	9.98
10	Port de Sóller	Ma-16-14	2010	4-7	0.36	0.03	0.15	0.06	0.40	3.15	2.42	ND
11	Cala Santa Maria	Ca-1-11	2010	8	0.22	0.16	0.07	0.28	0.26	4.68	5.48	9.70
	Cala Santa Maria	Ca-3-12	2010	8-12	0.21	0.20	0.12	0.31	0.16	3.52	4.59	9.78
	Port d'Andratx	Ma-9-12	2010	8-12	0.25	0.26	0.09	0.28	0.12	3.65	4.65	9.94
	Magaluf	Ma-7-12	2010	6-7	0.21	0.26	0.11	0.36	0.06	2.36	3.83	9.63

12	Alcanada	Ma-1-21	2020	2-4	0.22	0.14	0.05	0.28	0.32	5.17	6.01	ND
	Ca'n Picafort	Ma-3-21	2020	2-4	0.21	0.28	0.15	0.17	0.20	5.67	5.08	ND
	Cala Blava	Ma-5-21	2020	2-4	0.12	0.25	0.09	0.23	0.31	4.98	5.26	ND
	Cala Brafi	Ma-7-21	2020	2-4	0.16	0.28	0.12	0.26	0.20	3.73	4.52	ND
	Cala de Santa Ponça	Ma-9-21	2020	2-4	0.30	0.29	0.08	0.32	0.02	2.95	4.24	ND
	Capdepera	Ma-11-21	2020	2-4	0.23	0.27	0.11	0.18	0.23	5.99	5.40	ND
	Es Caragol	Ma-13-21	2020	2-4	0.26	0.16	0.10	0.24	0.25	5.52	5.80	ND
	Marina de Bonaire	Ma-15-21	2020	2-4	0.22	0.25	0.12	0.18	0.25	6.20	5.54	ND
	Port d'Andratx	Ma-17-21	2020	2-4	0.30	0.33	0.09	0.26	0.04	3.55	4.39	ND
	Port de Sóller	Ma-19-21	2020	2-4	0.24	0.23	0.09	0.31	0.14	3.44	4.64	ND
	Ses Illetes	Ma-21-21	2020	2-4	0.16	0.31	0.10	0.21	0.23	4.84	4.92	ND

MENORCA

	LOCALITY	SAMPLING NAME	DECADES	DEPTH (m)	A* Av.	B Av.	C Av.	D* Av.	SB Av.	I _{LS} Av.	FI' Av.	FSI Av.
3	South Menorca	Me-11-87	1980	29	0.01	0.49	0.20	0.14	0.03	1.10	2.01	9.02
	South Menorca	Me-12-87	1980	19	0.67	0.07	0.01	0.12	0.13	12.99	8.30	10.00
	South Menorca	Me-13-87	1980	19	0.31	0.15	0.03	0.21	0.14	5.47	5.09	9.78
	South Menorca	Me-14-87	1980	7	0.24	0.39	0.05	0.16	0.16	5.78	5.00	9.94
	South Menorca	Me-15-87	1980	29	0.30	0.23	0.03	0.42	0.01	2.30	4.05	9.72
	South Menorca	Me-16-87	1980	30	0.04	0.11	0.11	0.59	0.00	0.38	1.47	8.55
13	Ses Fontanelles	Me-1-82	1980	3-5	0.25	0.40	0.02	0.23	0.10	4.11	4.51	ND
	Cala Mitjana	Me-2-82	1980	3-5	0.28	0.57	0.03	0.07	0.03	6.86	4.24	ND
	Cala En Blanes	Me-3-82	1980	3-5	0.48	0.31	0.01	0.13	0.06	9.09	6.10	ND
	Arenal d'en Castell	Me-4-82	1980	3-5	0.22	0.39	0.01	0.26	0.07	3.12	3.96	ND
	Cala Galdana	Me-5-82	1980	3-5	0.35	0.39	0.00	0.16	0.08	6.28	5.22	ND

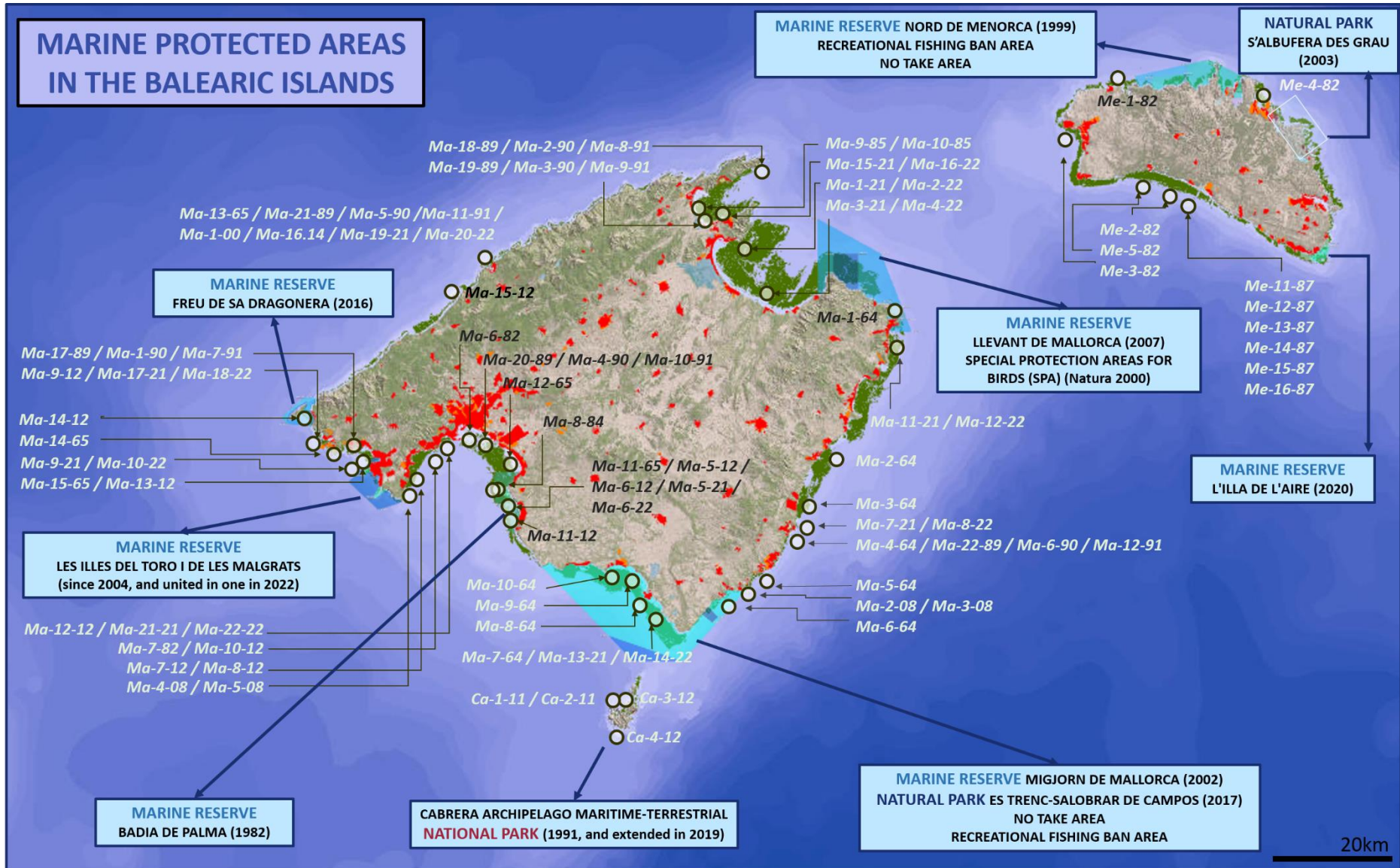


FIGURE 36. Map of the Balearic Islands with *P. oceanica* coverage (in green), and urban planning of all the municipalities (in red - urban land, in orange - developable land (actualized on 01/04/2022)), showing the location of the marine protected area (in blue) and the year of establishment: National park (Cabrera Archipelago), natural parks (Es Trenc-Salobrar de Campos, and s'Albufera des Grau), and marine reserves (Freu de Sa Dragonera, Badia de Palma, Illes del Toro i Malgrats, Migjorn de Mallorca, Llevant de Mallorca, Nord de Menorca, l'Illa de l'Aire) (from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_ReservesMarines/MapServer (actualized in 25/02/2023) with layers: GOIB_ReservesMarines, GOIB_MUIB, and GOIB_Posidonia_IB (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project) actualized in 09/03/2022)).

In contrast, in Mallorca, the lowest values of I_{LS} and FI' were found in Portocolom (Ma-4-64), with I_{LS} - 0.81, and FI' - 1.98, as morphotypes A* and SB accounted for relative abundances of only 0.10 and 0.01 respectively; while morphotype D* accounted for half of the epiphytic foraminiferal assemblage (0.50), and C corresponded to 0.20. No B foraminifera were identified. In Ciutat Jardí (Ma-6-82) I_{LS} (1.28), and FI' (2.47) were also among the lowest values reported since SB morphotype was completely absent and the A* was low (0.11), whereas morphotype B (0.45) was the most abundant, and D* and C showed were 0.26 and 0.14, respectively.

The lowest values reported in Menorca of I_{LS} (0.38), and FI' (1.47) were calculated in South Menorca (Me-16-87), with no morphotype SB and a minimal presence of A* (0.01). The morphotype D* was the most abundant (0.59), and both C and B presented relative abundances of 0.11. In the Me-11-87 sampling site the I_{LS} (1.10) and FI' (2.01) were also very low, as in that sample the morphotype A* relative abundance was 0.01, and SB was 0.03. The morphotype D* (0.49) was the most abundant, followed by morphotypes B (0.20) and C (0.14).

Despite the fact that in Cabrera I_{LS} and FI' were high for all samples due to the abundances of morphotypes A* and SB (with the lowest value of I_{LS} (3.26) at Ca-2-11, and FI' (4.59) at Ca-3-11), foraminifera of morphotype D* were highly represented, and their proportion varied from 0.28 in Cala Santa Maria (Ca-1-11), as well as in Els Estels (Ca-4-12), to 0.34 in Ca-2-11. In the same time the values reported for B were higher (0.26 in Els Estels and 0.20 in Cala Santa Maria), then for C (0.07 in Els Estels and till 0.12 in Cala Santa Maria).

FSI values were high for all samples with a maximum value of 10 for Es Caragol (Ma-7-64), Cala Murada (Ma-3-64), South Menorca (Me-12-87), and Cala d'Or (Ma-2/3-08). The minimum values of FSI were fixed in Porto Petro (Ma-5-64) (6.57), in Cala Blava (Ma-11-65) (7.75), and in Cala Agulla (Ma-1-64) (8.21). FSI could not be calculated for Ses Fontanelles (Me-1-82), Cala Mitjana (Me-2-82), Cala En Blanes (Me-3-82), Arenal d'en Castell (Me-4-82), Cala Galdana (Me-5-82), Alcanada (Ma-1-21/Ma-2-22), Ca'n Picafort (Ma-3-21/Ma-4-22), Cala Blava Ma-5-21/Ma-6-22), Cala Brafí (Ma-7-21/Ma-8-22), Cala de Santa Ponça (Ma-9-21/Ma-10-22), Capdepera (Ma-11-21/Ma-12-22), Es Caragol (Ma-13-21/Ma-14-22), Marina de Bonaire (Ma-15-21/Ma-16-22), Port d'Andratx (Ma-17-21/Ma-18-22), Port de Sóller (Ma-19-21/Ma-20-22), and Ses Illetes (Ma-21-21/Ma-22-22) because of absence of data about numerical species composition.

The I_{LS} values for each sample were displayed by decades, from 1960s to 2020s, on maps generated using the OpenStreetMap (OSM) software to visualize the variation of the index along time. The Figure 37 shows a decreasing general trend in the circle surface representing I_{LS} values, becoming more homogeneous with time, especially for the 2010s and 2020s decades.

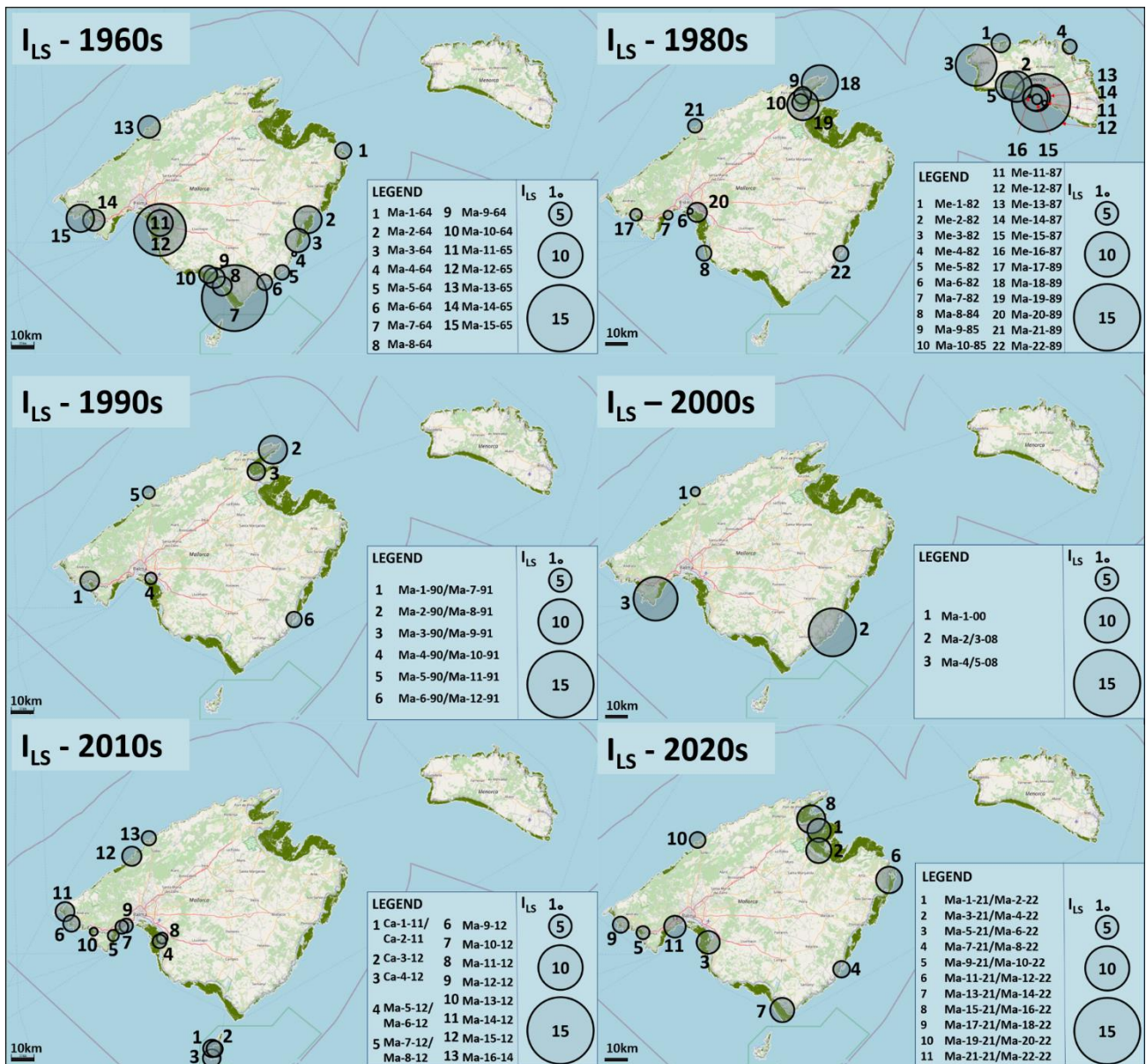


FIGURE 37. Maps of the Balearic Islands (Mallorca, Menorca, and Cabrera) with sampling locations showing the I_{LS} values during 1960s, 1980s, 1990s, 2000s, 2010s, and 2020s decades. The circle size is proportional to the I_{LS} . The green area represents the *Posidonia oceanica* cover (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer)

Box-plot charts (Fig. 38) represent I_{LS} values for individual sample stations (Cala Blava, Portocolom, Port de Sóller, and Port d'Andratx), along a time series. At Cala Blava (box-plot 1) the highest I_{LS} was obtained in 1965. Then, the I_{LS} value fell sharply in 2012 (1.82-5.04), and in 2021 (4.87) it began to increase, maintaining this trend until the last sampling in 2022 (5.10). In Portocolom (box-plot 2) the I_{LS} value was very low (0.81) in 1964, but increased in 1989 to 4.36, and remained at about the same level for the next two years. In Port de Sóller (box-plot 3) eight samples from 1965 to 2022 were analyzed, showing a decreasing trend from 1965 (4.76) to 1991 (0.64), and the subsequent increase to equivalent levels in 2022 (3.95). In Port d'Andratx (box-plot 4), statistical analysis shows that I_{LS} values do not vary significantly between 2012 and 2022, fluctuating between 4.22 and 3.62 respectively.

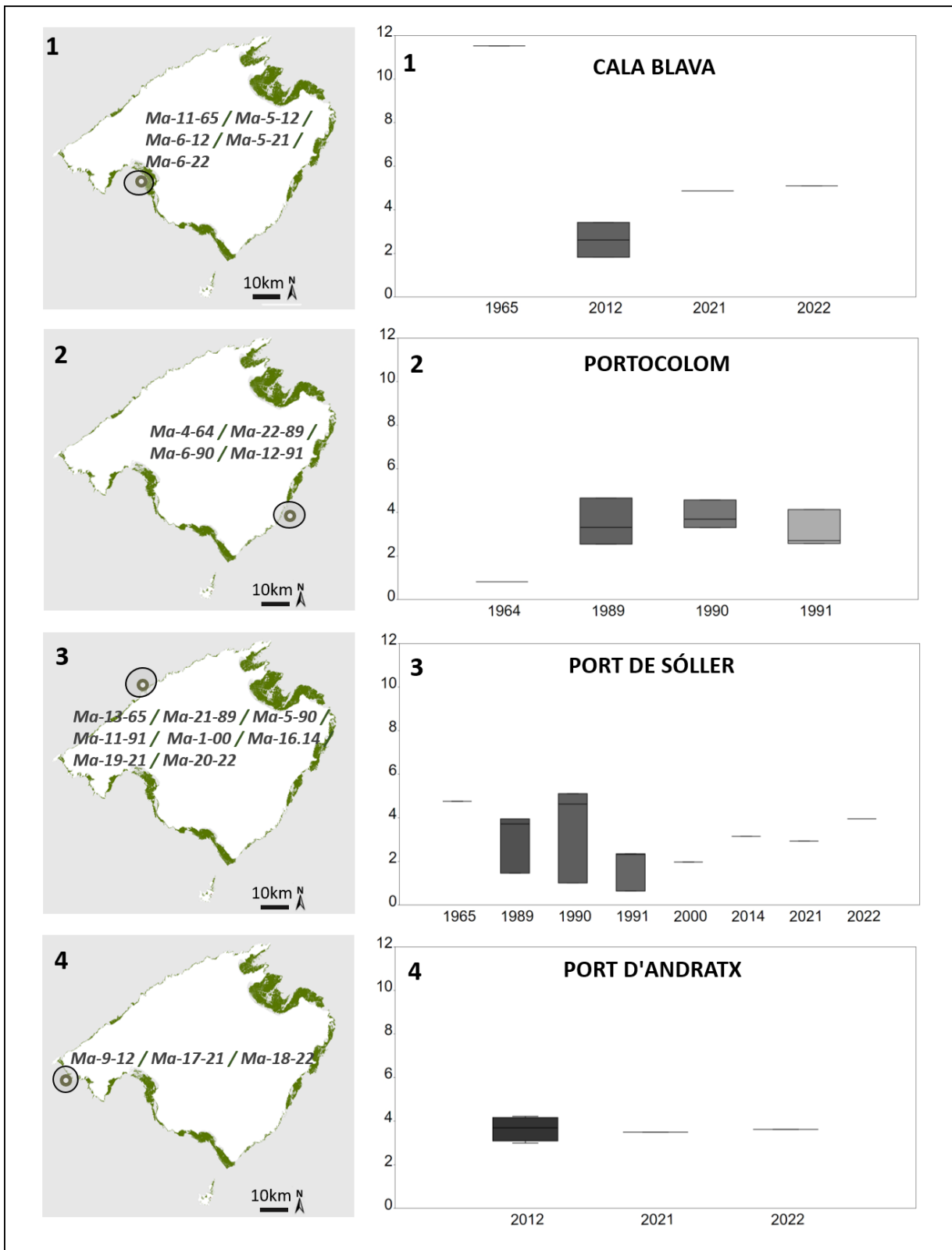


FIGURE 38. ANOVA Model Box-plot of ecological index values (I_{L5}) along a time series. Box-plot charts corresponding to a single sampling site are: 1) Cala Blava; 2) Portocolom; 3) Port de Sóller; 4) Port d'Andratx. Samplin localities are indicated by white dots within a grey ellipse. The green area represents *Posidonia oceanica* cover (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

The box-plot analysis (Fig. 39) was performed with data of eleven sampling sites, grouped in four zones according to geographical proximity and similar characteristics. Each zone is marked by a circle on the map of Mallorca and is labelled with the number of the corresponding box-plot. Each plot represents the I_{LS} values for each year of sampling in that zone. At Santa Ponça and Cala de Santa Ponça zone (box-plot 1) the I_{LS} value was high for the first sample, obtained in 1964, but rapidly decreased to a minimum in 2012, followed by a moderate increase in 2021 and 2022 samples. At Ses Illetes, Portals Nous and Magaluf zone (box-plot 2) the I_{LS} value has been continuously rising throughout the period from 1982 to 2022. At S'Arenal – Es Carnatge – Ciutat Jardí area (box-plot 3), the I_{LS} value was high for the first sample, obtained in 1965, but decreased in 1982 to minimum values (1.05), increase in 1989 with very variable values (2.23–5.98), and stabilizes later around 2.52 in 1990 and 1991. At Marina de Bonaire – Es Barcarès – Badia de Pollença area (box-plot 4), I_{LS} values reached a peak of 3.74 in 1985 and rose to 10.87 in 1989, then decreased in 1990 (3.61–6.62) and 1991 (1.91–3.54), and increased again in 2021 (5.58) and 2022 (6.83).

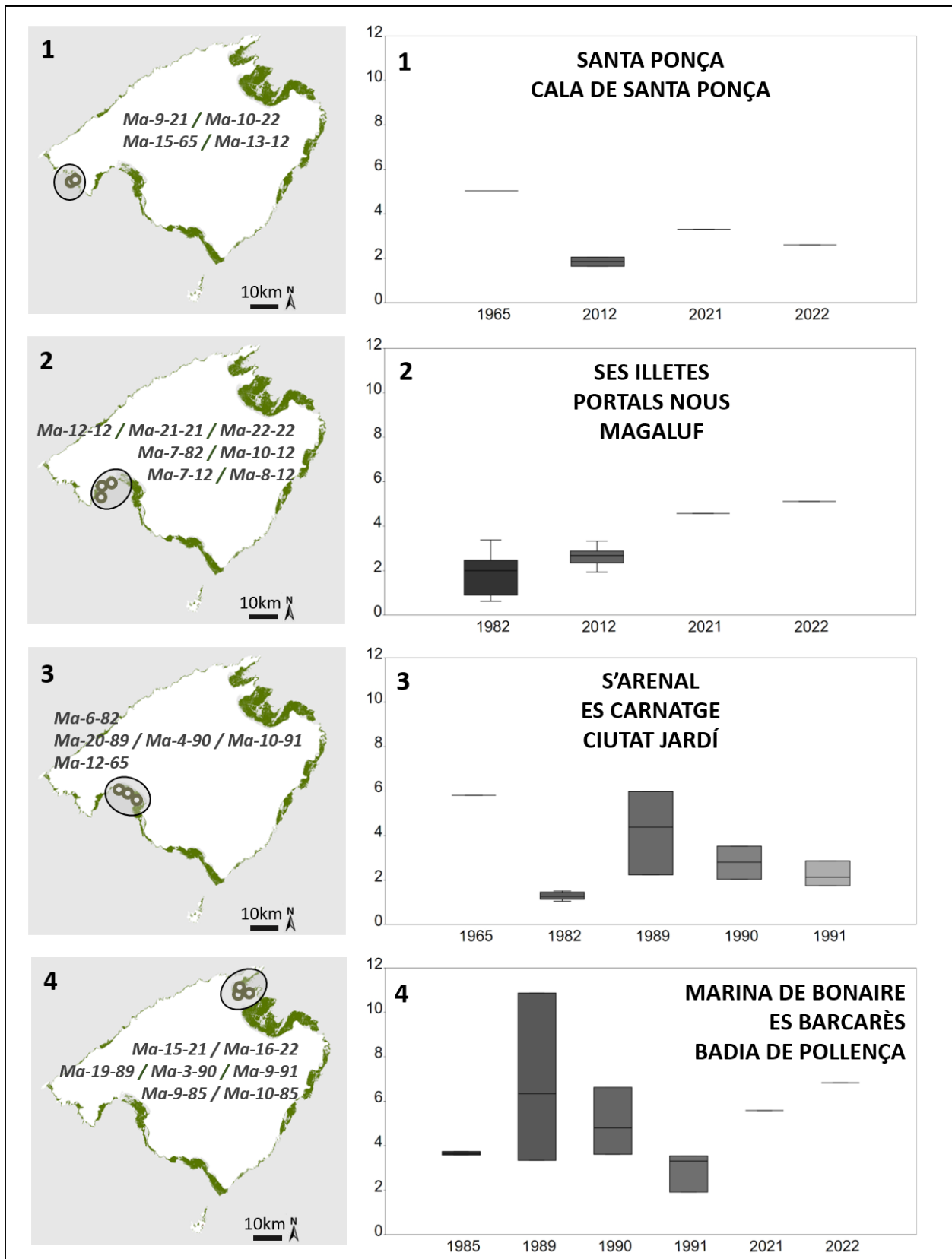


FIGURE 39. ANOVA Model Box-plot of ecological index values (I_{LS}) along a time series. Different samples geographically close are grouped: 1) Santa Ponça and Cala de Santa Ponça; 2) Ses Illetes, Portals Nous, and Magaluf; 3) S'Arenal, Es Carnatge, and Ciutat Jardí; 4) Marina de Bonaire, Es Barcarès, and Badia de Pollença. The green area represents *Posidonia oceanica* cover (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project (actualized in 09/03/2022)) from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_Posidonia_IB/MapServer).

CHAPTER 4

DISCUSSION

Based on the strong correspondence between *P. oceanica* and its associated foraminiferal assemblage, Khokhlova (2013) and Mateu-Vicens et al. (2014) developed bioindicator indices specific for this type of seagrass meadows. Thus, a modified FORAM Index (FI') was used to evaluate *P. oceanica* meadows at warm temperate latitudes in the Western Mediterranean. Further, a new life-span index (I_{LS}) was created to clarify distribution patterns, highlighting the differences between long and short-lived groups with opposite ecological requirements and disregards less ecologically significant morphotypes. The validity of these indices depends on the robustness of the ecological groups on which they are based, the Langer (1993) morphotypes modified by Khokhlova (2013) and Mateu-Vicens et al. (2014). To verify independently that each morphotype represents its own and differential ecological characteristics, its position in the trophic web has been analyzed by analyzing the stable isotopes of C and N (objective 1).

Both, the FI' and I_{LS} indices, are very highly correlated and, along with shoot density and canopy measurements, were regarded as reference parameters for quality assessment and preservation of *P. oceanica* meadows. In addition, the use of these indices in combination with other types of indicators such as the percentage of aberrant forms (FAI) or the analysis of the composition of the shell, allows the detection of different types of stress, which include the sudden alteration of the conditions of the seagrass meadows, and prolonged, albeit low-intensity, exposure to pollutants such as heavy metals (objective 2). Finally, due to their simplicity and ease of application, FI' and I_{LS} can be calculated from historical information to show trends in the health of *P. oceanica* meadows over time (objective 3).

Below are discussed in detail the three objectives stated in this thesis.

4.1. OBJECTIVE 1: N AND C ISOTOPIC ANALYSIS TO VALIDATE KHOKHLOVA'S (2013) ECOLOGICAL GROUPS ON WHICH THE BIOINDICATOR INDICES ARE BASED.

4.1.1. Quantitative Analysis by Morphotype

The relative distribution of the different morphotypes and their generic composition in the *Posidonia oceanica* meadows of Mallorca are similar to those reported previously in studies performed on living (Langer, 1993; Abril, 1993; Ribes et al., 2000) and dead foraminiferal assemblages (Mateu-Vicens, et al., 2010; Frezza et al., 2011; Khokhlova, 2013; Mateu-Vicens et al., 2014). However, there are substantial differences between the foraminiferal assemblages of *P. oceanica* and the multispecific meadows of Madagascar, where large miliolid forms (LM morphotype) are more

abundant (up to 7%, including *Peneroplis* and *Marginopora*) and the large rotaliid genus *Amphistegina* (LR morphotype) occurs (7%).

The abundance of LM and LR morphotypes in Madagascar is clearly associated with the warm temperatures (mean annual surface temperature is 28.5°C; Tomaczak and Godfrey, 1994). The range of temperature for *Peneroplis* is 18–27°C and for *Marginopora* is 18–26°C (Murray, 2006). *Amphistegina* has a minimum thermal threshold at 15°C in winter, however it clearly prefers temperatures >20°C (Murray, 2006). In the Mediterranean, this genus is restricted to the eastern side, and has not been reported in modern foraminiferal assemblages of the Balearic Islands, where temperature ranges between 16°C and 31°C (Fernández de Puellas, 2007), which, in turn, probably limits the abundances of the few symbiont-bearing porcelaneous genera that are found (i.e., *Peneroplis* and *Sorites*).

Another important difference between the Mallorca and Madagascar assemblages is the abundance of the AF* morphotype in the former. As mentioned above, Mediterranean seagrass meadows are dominated by *P. oceanica* whereas, in Madagascar, meadows are multispecific and no species is clearly dominant. *Posidonia oceanica* possesses leaves almost one cm wide with relatively low mortality rates (0.06 year⁻¹; Duarte et al., 2006), which makes them a suitable substrate for long-lived, encrusting taxa that form the AF* morphotype (i.e., *Planorbulina*, *Nubecularia*, and *Cibicidella*). In contrast, phanerogams in the meadows of Madagascar, such as *Syringodium* and *Halodule*, have very narrow leaves, which hinders the adherence of the encrusting forms and limits the size to which they can grow. Moreover, the leaf-longevity of the Madagascar phanerogam species is shorter than that of *P. oceanica*. The genus *Cymodocea* might provide a foliar surface suitable for encrusting foraminiferal colonization. However, its high mortality rates (4.47 year⁻¹; Duarte et al., 2006) are not compatible with the long life-cycles of the genera forming the AF* morphotype.

4.1.2. Stable Isotopes Signatures of the Foraminiferal Morphotypes

The $\delta^{13}\text{C}$ of an organism tends to be similar to the $\delta^{13}\text{C}$ of its diet (McCutchen et al., 2003). However, the carbon isotopic ratio may change depending on the metabolism of the organism, the utilization of metabolic CO₂ during the shell formation, the algal-symbiont photosynthetic activity, growth rate, and the carbon concentration (Rohling and Cooke, 2002; Murray, 2006). In our study, the graphic representations (Figs. 30, 31) and the statistical analyses (Table 14) of stable isotopic values for the foraminiferal morphotypes and locality revealed that the only significant differences were in $\delta^{13}\text{C}$ values by morphotypes.

To provide a framework to compare our results, isotopic data of epiphytic organisms from *P. oceanica* meadows from Mallorca and other seagrass species from tropical settings, and values corresponding to benthic, deposit-feeding foraminifera have been gathered from literature (Fig. 40). For the analysis of the effect of mixotrophy in symbiont-bearing foraminifera, non-zooxanthellate and zooxanthellate corals have been included. Moreover, isotopic data of cyanobacteria and diatoms are also provided, as these organisms represent an important food source for many filter-feeders and foraminifera. Unfortunately, we have not found information of other studies on isotopic analysis of epiphytic foraminifera.

FIGURE 40. Dual isotope scatter plot ($\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$) of the mean values for each morphotype at each sampling locality and data obtained from literature corresponding to opportunistic foraminifera, filter-feeders, non-zooxanthellate and zooxanthellate corals, and cyanobacteria (Yamamuro, 1999; Muscatine et al., 2005; Carlier et al., 2007; Nomaki et al., 2008; Deudero et al., 2011; Lebreton et al., 2011; Wada et al., 2012; Jeffreys et al., 2015). Four shaded areas are defined corresponding to groups of organisms with different ecological significance. Notice also the different sizes of the symbols and letters. Larger symbols and names in bold correspond to our data and data from literature that are more relevant for the analysis of our results. **Opportunistic deposit-feeding foraminifera:** Allog: Allogromiidae, Astr: Astrorhizidae, Bagg: Bagginidae, Bol: Boliviniidae, Bul: Buliminidae, Chil: Chilostomellidae, Hapl: Haplophragmoididae, Non: Nonionidae, Plan: Planulinidae, Rhab: Rhabdaminidae, Uvig: Uvigerinidae, Val: Valvulinidae. **Filter-feeding metazoans:** Biv: Bivalves, Cor: Non-zooxanthellate corals, Pol: Polychaetes, Spo: Sponges. **Zooxanthellate corals:** Z-cor. **Seagrass-related organisms.** Epiphytic foraminifera morphotypes: AF, AR, B*, C, D*, LM, LR; SG: Seagrass (including *P. oceanica* and genera *Cymodocea*, *Halodule*, *Syringodium*, *Thalassia*), Asc: Epiphytic ascidian, Bry: Epiphytic bryozoan. **Diatoms:** Diat. **Cyanobacteria:** Cyan. **Other.** Ser: Serpulid, Sed: Sediment from *P. oceanica* meadows from Mallorca, OM: Organic matter from *P. oceanica* meadows from Mallorca.

Figure 40 shows three main groups of organisms according to their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values: 1) opportunistic foraminifera and filter-feeders, 2) zooxanthellate corals and 3) seagrass-related organisms. Most of the opportunistic foraminifera and filter-feeding biota present $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranging between -26‰ and -16‰, and between 5‰ and 17‰, respectively, which is consistent with their feeding mode that is largely based on organic matter (phytodetritus from various phytoplanktonic species) and benthic diatoms (Nomaki et al., 2008; Lebreton et al., 2011; Jeffreys et al., 2015).

Isotopic signals differ considerably between non-zooxanthellate corals (without algal symbionts) and zooxanthellate corals (with algal symbionts). Whilst, $\delta^{13}\text{C}$ is similar in both types of corals, zooxanthellate taxa are much more depleted in ^{15}N . Non-symbiotic corals are purely heterotrophic and their isotopic signal is consistent with that of other filter-feeding biota. In contrast, the zooxanthellate ones combine heterotrophy and autotrophy. Moreover, some zooxanthellate corals have established another symbiosis with cyanobacteria that fix atmospheric nitrogen ($\delta^{15}\text{N} \approx 0\text{‰}$), providing ^{15}N -depleted ammonium nitrogen to the host, which is consistent with the observed $\delta^{15}\text{N}$ values in symbiont-bearing corals, much lower than in the non-symbiotic taxa (Muscatine et al., 2005).

In seagrass meadows, $\delta^{13}\text{C}$ data indicate that microphytobenthos, largely constituted by diatoms living in the uppermost few millimeters of the sediment, is the main food source followed by epiphytic diatoms, for grazers, deposit feeders and suspension feeders (Lebreton et al., 2011). Although still an open question, there is evidence that seagrass leaves and detritus do not constitute an important food source for many consumers (Yamamuro, 1999; Lebreton et al., 2011).

Seagrass isotopic signatures are characterized by relatively high $\delta^{13}\text{C}$ and relatively low $\delta^{15}\text{N}$ values. As mentioned above, low $\delta^{15}\text{N}$ signatures can be related to the direct fixation of atmospheric nitrogen by cyanobacteria that generate ^{15}N -depleted compounds. Indeed, Yamamuro (1999) reported the lowest $\delta^{15}\text{N}$ values for any seagrass leaves (*Syringodium isoetifolium* (Ascherson) Dandy, 1939 and *Halodule uninervis* (Forsskål) Ascherson, 1882), and attributed these results to epiphytic cyanobacteria.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values reported in epiphytic ascidians (Yamamuro, 1999), bryozoans (Deudero et al., 2011) and those obtained from the epiphytic foraminifera of the present study fit within the numeric range of isotopic signatures reported in seagrass leaves and rhizomes. Interestingly, most $\delta^{15}\text{N}$ values of the epiphytic biota range between 0‰ and 3.5‰, which compared

with those corresponding to cyanobacteria (-1.5‰ to -3.0‰, Wada et al., 2012) represents an enrichment (3.5‰) equivalent to increase of one a trophic level.

Moreover, $\delta^{13}\text{C}$ values reported in cyanobacteria are variable from depleted (-25‰) to relatively enriched (-3‰) signatures. The latter correspond to algal mats with limited CO_2 supply during growth (Wada et al., 2012) and include species (*Oscillatoria* spp.) common in the Mediterranean seagrass beds (Mabrouk et al., 2014). Comparably high $\delta^{13}\text{C}$ signatures are reported in epiphytic foraminifera from the studied localities, regardless of morphotype. Thus, cyanobacteria seem to represent a major food source for seagrass epibionts, including epiphytic foraminifera.

Although the isotopic signatures are relatively homogeneous for the epiphytic biota, some differences occur among the epiphytic foraminiferal morphotypes. The AF* and AR* morphotypes are assumed to feed on diatoms, bacteria and fungi from the surface of *Posidonia* leaves (Langer, 1993; Langer and Gehring, 1993). The $\delta^{13}\text{C}$ values reported in epiphytic diatoms are approximately -12‰ (Carlier et al., 2009; Lebreton et al., 2011); grazers feeding on diatoms show slightly-enriched values (-9.7‰, Lebreton et al., 2011). Nevertheless, AF* and AR* from Sa Foradada and Sant Elm were more $\delta^{13}\text{C}$ -enriched (-8‰ to -2‰) than other epiphytic foraminifera and typical diatom grazers, which indicates that these morphotypes feed on a more-enriched ^{13}C sources such as cyanobacteria. This food source is consistent with their sessile life mode, which requires the attachment to the plant by glycosaminoglycan "glues" that, in turn, provide a substratum for bacterial growth (Langer and Gehring, 1993). This process is known as "farming" (Hallock, 2002; Pomar and Hallock, 2008).

Both B and D* morphotypes present a wide range of $\delta^{13}\text{C}$ values compared with other morphotypes (Figs. 30, 31). In Sant Elm, the B and D* morphotypes have the most depleted values, suggesting that the food source, besides cyanobacteria, likely includes microphytobenthos (including diatoms) and seagrass phytodetritus. These morphotypes are relatively motile and dwell on both the leaves and rhizomes of *Posidonia* (Lipps, 1975; Langer, 1993), which provides the possibility for greater selectivity of food sources than for sessile morphotypes. The D* morphotypes include opportunistic taxa that dwell in the rhizomes and feed on a variety of organic matter and particles (Lipps, 1975).

Depleted $\delta^{13}\text{C}$ values (-20‰ - Deudero et al., 2010; -22‰ - Lebreton et al., 2011) are common for particulate organic matter. Deudero et al. (2010) reported depleted $\delta^{13}\text{C}$ (-19.30‰) in the bryozoan *Reteporella grimaldii* (Jullien, 1903) that colonizes the *P. oceanica* rhizomes. Such a coincidence in organisms occupying a similar biotope may be indicative of a factor controlling the type of carbon entering in the ecosystem. Terrestrial inputs of plant organic-matter, transported by rivers, can deplete the carbon isotope values of the dissolved organic matter compared to the seawater (Peterson, 1999). In Sant Elm, the occurrence of two creeks, active during rainy periods, can be related to the introduction of plant organic-matter used as C source for bacteria and phytoplankton depleting, in consequence, the carbon signature.

The symbiont-bearing foraminifera (LM and LR) with mixotrophic feeding strategies have two sources of organic carbon. The foraminifera use the energy from symbiont photosynthesis for their respiration. The CO_2 produced by the holobiont respiration is, in turn, reused as substrate by the symbiont photosynthesis (Ter Kuile and Erez, 1991; Hallock, 2002). Photosynthesis strongly discriminates in favour of ^{12}C (Rohling and Cooke, 2002; Murray, 2006). Therefore, the light isotope is reused within the photosynthesis-respiration cycle and the heavy isotope precipitates as calcium carbonate to form the shell (Murray, 2006). On the other hand, these foraminifera may feed on free-living diatoms, bacteria (including cyanobacteria) and fungi, which they use for growth and

reproduction. Associated with the mixotrophic strategy, more-depleted $\delta^{13}\text{C}$ values than those observed for LM (-1.8 to -0.9‰) and LR (-2.9‰) morphotypes were expected. High $\delta^{13}\text{C}$ values may represent the cyanobacterial component of their diets. However, the $\delta^{13}\text{C}$ values reported for the symbiont-bearing morphotypes are similar to those found in tests of *Marginopora* (-1.2‰) and *Amphistegina* (-3.8‰) in other localities of the Indian Ocean (Saraswati, 2007). Thus, the enriched values could indicate that, during the acidification, some residues of shell remained in the samples. This hypothesis was confirmed after reviewing the acidified samples of LM and LR of Madagascar, where shell fragments were observed (Fig. 41). In future studies, the acidification treatment should be repeated several times with the same HCl concentration. Increasing the concentration is not considered an option to avoid cellular damage.

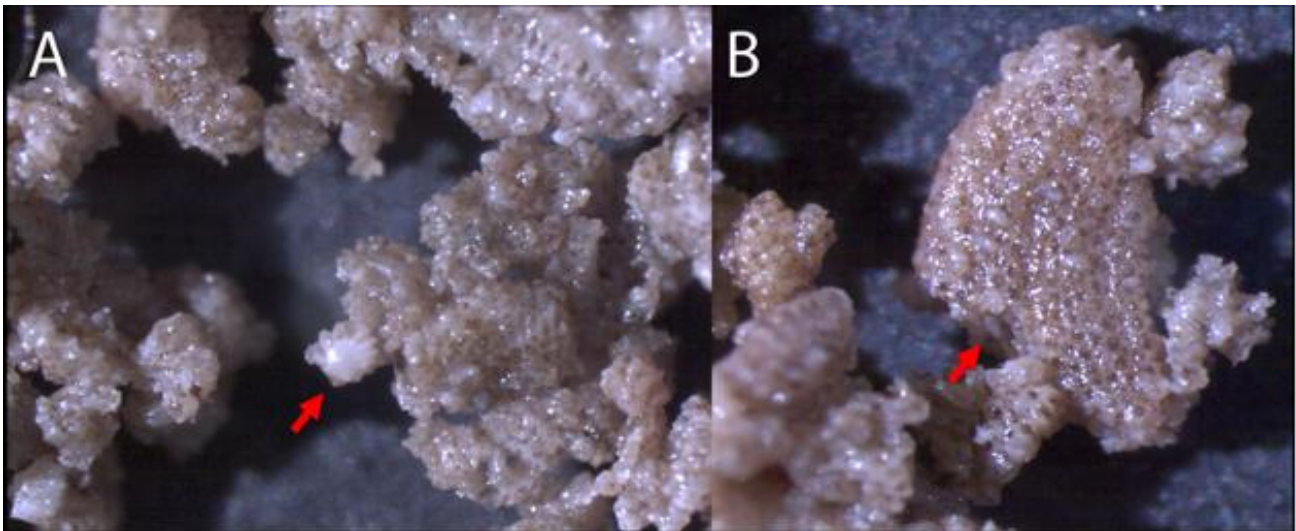


FIGURE 41. Fragments of non-dissolved shells of LM morphotype, A) Well-preserved *Peneroplis* (40x) and B) fragment of *Marginopora* (40x).

Regarding $\delta^{15}\text{N}$ signature of the different morphotypes, despite finding no statistically significant differences, probably due to the low sample size, some trends can be mentioned. Across the different morphotypes, the $\delta^{15}\text{N}$ values ranged between 0.5 and 3‰, except for the AF* morphotype from Madagascar, which was more enriched ($\delta^{15}\text{N} = 5‰$). Values reported for the AF* and AR* morphotypes are comparable to those found in epiphytic, filter-feeding, bryozoans ($\delta^{15}\text{N} = 2.46‰, 3.47‰$) on *P. oceanica* (Deudero et al. 2010; 2011), indicating likely similar food sources, including cyanobacteria. Observed $\delta^{15}\text{N}$ values in AF*, AR* and B morphotypes can be related to the depleted $\delta^{15}\text{N}$ values of the cyanobacteria (-1.5‰ to -3.0‰), associated with nitrogen fixation (Wada et al., 2012), which is consistent with enrichment reported in other epiphytic sessile consumers.

The $\delta^{15}\text{N}$ values of C morphotype in the two localities are very similar, which indicates similar food sources as suspension feeders. These foraminifera extend a pseudopodial network between the leaves of seagrass or algae, being an efficient gatherer of suspended particles (Langer, 1993), which mostly consist of diatoms and detritus, forming most of their diet (Lipps, 1975; Langer, 1993; Ribes, 1998). Morphotype C does not secrete the glycosaminoglycans that support the cyanobacterial farming, which might explain the relatively high $\delta^{15}\text{N}$ values of this morphotype with respect to the others. SIMPER analysis reflects that those closest to C are B, D* and AF*, and the most distant ones are LM and LR. The former group includes sessile taxa with heterotrophic strategies. In contrast, LM

and LR host algal symbionts that determine a totally different life mode. However, in these two groups cyanobacteria play an important role as food source.

Both symbiont-bearing morphotypes, and LR, show similar isotopic contributions, as indicated by the shortest distances among all LM morphotypes in the SIMPER analysis (3.75). The LM morphotype is formed by two genera: *Peneroplis*, which host rhodophycean algae as symbionts (*Phorphyridium purpureum*; Lee, 1990), and *Marginopora*, with dinoflagellate (various species of *Symbiodinium*; Momigliano and Uthicke, 2013) symbionts. The LR morphotype in this study included only *Amphistegina*, which hosts diatoms (Lee, 2006). The occurrence of symbionts seems to exert some influence on the N metabolism. Thus, association between algae (e.g., *Symbiodinium*) and nitrogen-fixing bacteria (Silverstein et al., 2012) and cyanobacteria (Muscatine et al., 2005), along with the bacterial and fungal farming, could explain depleted $\delta^{15}\text{N}$ values in all symbiont-bearing morphotypes. This is consistent with the observations reported in other mixotrophic organisms such as zooxanthellate corals with symbiont cyanobacteria (Muscatine et al., 2005).

As mentioned above, former morphotype classification (Langer, 1993) distributed the symbiont-bearing taxa within A and D morphotypes. A morphotype includes also the AF* and AR* category of Khokhlova (2013) (Mateu-Vicens et al., 2014). The SIMPER analysis, based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, shows great distances between the symbiont-bearing morphotypes (LM and LR) and AF*, which in turn reflects the exploitation of different trophic resources. This result is not consistent with the premise that morphotypes group taxa with similar trophic strategies. Thus, the modified classification by Mateu-Vicens et al. (2014) appears to be more suitable for the classification of the epiphytic foraminifera. However, the nitrogen isotope values should be interpreted with care, as most foraminiferal species contain relatively little protoplasm (used for analysis) compared to the whole biomass, which mostly corresponds to the shell. Indeed, Iken et al. (2001) pointed out that the occurrence of vacuoles with waste substances might modify the nitrogen isotopic signal.

4.2. OBJECTIVE 2: COMPARATIVE ANALYSIS OF THE ENVIRONMENTAL CONDITIONS OF *P. OCEANICA* MEADOWS USING 1) BIOINDICATOR INDICES BASED ON EPIPHYTIC FORAMINIFERA, 2) THE FREQUENCY OF ABNORMAL-GROWTH FORMS AND 3) THE TRACE-ELEMENTAL CONCENTRATIONS WITHIN THEIR TESTS.

4.2.1. Use of Foraminiferal Bioindicator Indices to Evaluate Ecological Conditions of *P. oceanica* Meadows

Foraminiferal bioindicator indices were applied to evaluate ecological conditions in selected *P. oceanica* meadows from the Balearic Islands, affected by different types of anthropogenic impacts. The indices FI', I_{LS} and FSI from Port d'Andratx, Magaluf and the two sampling sites from Cabrera were very similar, contrasting with the significant quantitative differences of the deformed specimens, especially between Cabrera1 and the rest of the sampling stations. The FI' and I_{LS} indices characterized by the high value for all sampling sites strongly correlated to good conditions of *P. oceanica* meadows in Port d'Andratx and Magaluf (touristic areas under anthropogenic influence), and very good conditions in Santa Maria Bay (Cabrera1 in particular) according to the values of the Water Framework Directive in the Balearic Islands (Barón et al., 2011). The FSI values were equally

high in all samples and indicated a dominance of stress-sensitive species, typical of oligotrophic waters with no significant pollution factors (Dimiza et al., 2016). Also, the diversity (H') reported in the different sampling sites corresponds to foraminiferal assemblages from other *Posidonia oceanica* meadows mentioned by Frezza et al. (2011) and Mateu-Vicens et al. (2014). In consequence, all these indices, along with another ecological parameter such as shoot density and canopy measurements (Mateu-Vicens et al., 2014) agree with well-preserved *P. oceanica* meadows.

The contrasting results between high values of the applied indices and the abundance of aberrant foraminiferal shells, especially for Cabrera1, indicate that both proxies do not reflect the same environmental impacts. One might hypothesize that, while the foraminifera-based indices were very sensitive to sudden and intense environmental changes, that may induce drastic compositional changes in the foraminiferal assemblages, the occurrence of deformed individuals might reflect sublethal exposures to low, but persistent, concentrations of accumulative pollutants. In this case, foraminiferal assemblages would remain unaltered and the bioindicator indices would show high values corresponding to good ecological states. However, the prolonged exposure to this type of pollutant could interfere with biological processes that would result in viable forms but with very easily detectable distinctive aberrant features.

The direct correspondence between the type and degree of pollution, and the type of morphological deformity on foraminiferal species is controversial as the effect of heavy metals is modulated by environmental factors (Sharifi et al. 1991; Yanko et al., 1998; Samir and El-Din 2001; Polovodova and Schönfeld, 2008), which are in agreement for the most frequent deformities (abnormal coiling and occurrence of aberrant shaped and sized chambers) in all sites of this study. However, other, less common deformities (protuberances in the shell and growth of supernumerary chambers) that consistently occurred in the different localities were strongly correlated with the presence of heavy metals, especially Zn, Ni and As.

4.2.2. Heavy-Metal Analysis

Foraminifera may incorporate, naturally, in their tests, metals such as Zn, Cu and Cd in concentrations of $1.5\text{--}6 \times 10^{-6}$ mol/mol Ca (0.98–3.92 ppm), $<0.2 \times 10^{-6}$ mol/mol Ca (< 0.13 ppm), and $0.02\text{--}0.25 \times 10^{-6}$ mol/mol Ca (0.02–0.28 ppm), respectively (Lea, 1999). However, the analysed foraminiferal tests from all of the sampling sites, including the non-deformed control specimens showed higher heavy-metal abundances than those from natural conditions. Moreover, along with Zn, Cu and Cd, other metals, such as Pb, Co, Ni, As and Sn, that are not normally incorporated in natural conditions were measured, which is indicative of some form of pollution.

Little information is available in the literature for the study area (Table 19), corresponding to heavy-metal concentrations from Santa Maria bay (Cabrera) and Magaluf but not Port d'Andratx (Albertí et al., 2010). Nevertheless, our results were consistent with independent pieces of evidence based on the heavy-metal concentrations in tissues of *Pinna nobilis* (a filter-feeding fan mussel) specimens, sampled from the same places and at the same time as sediment samples of the present work (Vázquez-Luis et al., 2016) (Table 20). Despite *P. nobilis* forms a calcareous shell that may incorporate heavy metals from the milieu, there are significant differences among their uptake rates. This prevents the metal content in the shell to be a reliable indicator, making soft tissues more representative of the surrounding environment (Montroni et al., 2021).

TABLE 19. Heavy-metal concentrations of Cd, Ni, Pb, and Hg (ppm) in sediment from Santa Maria bay (Cabrera) and Magaluf (Albertí et al., 2010). In Port d'Andratx no data available.

	Cd (ppm)	Ni (ppm)	Pb (ppm)	Hg (ppm)
CABRERA (SANTA MARÍA)	0.07	4	0.29	0.01
MAGALUF	0.185	2	0.19	0.01
PORT D'ANDRATX	NOT AVAILABLE			

TABLE 20. The average concentrations with standard deviations (\pm SD) of heavy metal such as Zn, Cu, Cd, Hg, Pb (ppm dry wt. soft tissues) and the Metal Pollution Index (MPI) in the *P. nobilis* (Vázquez-Luis et al., 2016) from Cabrera (Cabrera1), Port d'Andratx and Magaluf. Minimum and maximum values are given in parenthesis. The highest values are marked in bold. In Cabrera2 no data available.

LOCATION	Zn (\pm SD) (min.-max.)	Cu (\pm SD) (min.-max.)	Cd (\pm SD) (min.-max.)	Hg (\pm SD) (min.-max.)	Pb (\pm SD) (min.-max.)	Average value of MPI for all metals (\pm SD)
PORT D'ANDRATX	4034.25\pm358.7 (3670-4529)	6.05 \pm 2.21 (4.1-9.2)	1.61 \pm 0.61 (1.21-2.5)	0.3 \pm 0.04 (0.25-0.35)	0.9 \pm 0.2 (0.6-1)	6.22 \pm 0.53
MAGALUF	1763 \pm 607.88 (1244-2631)	197.85\pm88.08 (127.1-319.2)	8.92 \pm 3.01 (5.82-13.03)	0.8 \pm 0.22 (0.56-1.09)	20 \pm 6.3 (12-27)	34.45 \pm 11.55
CABRERA1	2708.33 \pm 347.76 (2364-3224)	57.25 \pm 30.41 (20.9-96.5)	26.44\pm6.97 (15.03-33.1)	0.96\pm0.13 (0.82-1.16)	22.7\pm5.2 (15-29)	38.17\pm8.2
CABRERA2	NOT SAMPLED IN Vázquez-Luis et al., 2016					

4.2.3. Origin of the Pollutants

The studied localities correspond to three different environmental scenarios. The data for the various indicators were analysed particularly for each site, offering a plausible explanation for the origin of the ecological impacts.

4.2.3.1. Port d'Andratx

The percentage of deformities (6% on average, Table 15) recorded in Port d'Andratx was consistent with those observed in heavily polluted areas in Norway (Alve, 1991) and in the Adriatic Sea (Coccioni, 2000). The most dominant heavy metals reported in the deformed foraminiferal tests in this study were Zn (36.13 \pm 23.65 ppm), Cu (10.23 \pm 10.00 ppm) and Pb (22.71 \pm 15.88 ppm). Vázquez-Luis et al. (2016) also detected elevated MPI values (6.22 \pm 0.53 ppm) in Port d'Andratx, mostly associated with high Zn levels in the tissues of the fan mussel *P. nobilis* (Table 20). Zn is a trace metal that is easily incorporated into foraminiferal tests (Elberling et al., 2003) with marine sources associated with aerosol deposition (Tovar-Sánchez et al., 2010), household detergent products (Samir and El-Din, 2001), and urban wastewaters (Rumolo et al., 2009). Sources of Zn may also be related to antifouling coatings that protect boat hulls from the colonization of encrusting biota (Paradas and Amado Filho, 2007). Although the use of antifouling products without heavy-metal-based biocides is becoming widely practiced (Candelas-Corrales, 2018), Paradas and Amado Filho (2007) claimed that antifouling paints are the primary source of Zn concentrations in marina and yacht club areas, as Cu and Zn are the most abundant metal elements presented in that type of paint.

The Port d'Andratx, with a marina of ~500 moorings for recreational boats (<https://www.andratx.cat>) and a professional fleet of 10–14 ships, consistent throughout the last decade (ibestat), constitutes an area of high boat concentration. Consequently, the use of antifouling coatings rich in Zn and Cu during vessel maintenance, and/or the paint degradation of their hulls may have represented a long-term input of heavy metals, resulting in prolonged exposure to pollutants for the foraminiferal assemblages. In addition, the source of Pb and minor amounts of Ni and As incorporated in the foraminiferal tests (Table 17), may have resulted from the combustion of fuel from the recreational and professional fleet and/or from anti-fouling paints or anti-corrosive coatings (Martínez-Colón et al., 2009).

4.2.3.2. Magaluf

In Magaluf, the FAI (4% on average, Table 15) was much lower than in Cabrera1 (23% on average), but similar to Port d'Andratx (6% on average) and Cabrera2 (4% on average), and comparable to the data documented in areas with variable sources of pollutants, from urban sewage (Caruso et al., 2011) to industrial waste (Alve, 1991; Yanko et al., 1998; Romano et al., 2008; 2009; 2013; Frontalini and Coccioni, 2008; Frontalini et al., 2009). The dominant heavy metals in aberrant tests were partially coincident with those found in sediment samples (Albertí et al., 2010) and *P. nobilis* tissues (Vázquez-Luis et al., 2016). Thus, Pb and Zn were the most abundant heavy metals in the abnormal foraminiferal tests; Ni, Pb and Cd dominated in sediments, and Zn and Cu in *P. nobilis* tissues. Lead, along with other metals such as Hg and As, has been part of the composition of fuels used over decades by boats and ships and released by exhaust systems (Polovodova and Schönfeld, 2008). Moreover, Pb and Zn are components in the pigment base of anticorrosive and primer paints extensively used in different types of vessels (Polovodova and Schönfeld, 2008). However, despite the limitations of foraminifera to absorb Pb (Frontalini et al., 2010) and the competition between Pb and Zn (Elberling et al., 2003), Pb was one of the most abundant metal in foraminiferal tests of Magaluf.

The third most abundant heavy metal in the foraminiferal tests was Cu. The toxic effect on foraminifera at different concentrations of Cu documented by Alve and Olsgard (1999) revealed that the presence of Cu in low concentrations may disrupt the ability of foraminifera to build new chambers and cause reproductive cycle disorders. However, the high concentrations of Cu recorded in Magaluf tests are not associated with high values of FAI, but to small-sized individuals. Accordingly, Martínez-Colón et al. (2017) also noticed that despite its high toxicity, total Cu concentration in sediment was not associated with high abundances of aberrant foraminifera and Le Cadre and Debenay (2006) related the presence of this metal with dwarf specimens of the genus *Ammonia*. Ni, in contrast to the other heavy metals, showed a higher concentration in sediments than in the foraminiferal tests. Observations consistent with this result are reported for *A. tepida*, which reduced the incorporation of this metal into the test, when cultured at high Ni concentrations in water (Munsel et al., 2010). In the study area, Ni and As may have a similar origin as in Port d'Andratx. Besides heavy metals, other sources of pollution that might have affected the abnormal foraminiferal growth should be taken into consideration. Thus, Sureda et al. (2013) noticed that coastal waters around Magaluf contained elevated levels of dissolved inorganic nitrogen caused by anthropogenically enriched groundwater discharges. Therefore, in this locality, it is difficult to assign a specific source of pollution over other causes that may have modulated foraminiferal test development.

4.2.3.3. Cabrera (Cala Santa Maria)

The most abundant heavy metals in sediments from Cala Santa Maria were Ni and Pb (Albertí et al., 2010). However, different sampling sites (Cabrera1 and Cabrera2) within the area, showed significant differences in the heavy-metal content of the foraminiferal tests. Abnormal foraminiferal tests from Cabrera1, consistent with the highest heavy-metal concentration in *P. nobilis* from Cabrera1 (Vázquez-Luis et al., 2016), were far more abundant and contained much higher concentrations of Zn and Pb than in Cabrera2. Nevertheless, Cu, Ni and As concentrations in foraminiferal tests were comparable at both sites (Table 20). As observed in Magaluf, in Cabrera1 and Cabrera2, all heavy metals but Ni showed higher concentrations in the foraminiferal tests than in the sediments. Vázquez-Luis et al. (2016) stated the absence of an evident heavy metal source in the marine protected area. In contrast, Tovar-Sánchez et al. (2010) cited natural and anthropogenically influenced aerosols as a primary source of heavy-metal accumulation in *P. oceanica* rhizomes in Cabrera. However, if atmospheric aerosols are the primary means of introduction of heavy metals, this process would lead to a more or less homogeneous distribution of the toxic elements on an area comprising both sampling sites, separated < 1 km, and the subsequent effects on the organisms (bioaccumulation in *P. nobilis* tissues and occurrence of abnormal foraminiferal tests) should not present significant differences between Cabrera1 and Cabrera2. Conversely, the significant differences reported by Vázquez-Luis et al. (2016) and the observations of the present study indicate that other pollution sources should be considered, at least on a small spatial scale. As previously explained, this study area was a shooting range and the scenario of war games of the Spanish army until late 80s of the 20th century. Plausibly, the projectile impact points in the seagrass meadows contain ammunition fragments that, according to Zwijnenburg and te Pas (2015), continuously release heavy metals such as Cd, Pb, Zn, Co and Ni. Similar observations were performed by Jung et al. (2010), who documented that ammunition was the source of Cd, Pb, Cu, and Zn contamination in sediments from a former shooting range (Asan Bay, South Korea). In agreement with this interpretation, the occurrence of high concentrations of Hg in *P. nobilis* from Cabrera (Vázquez-Luis et al., 2016) can also be related to the long military past of the island as there were not alternative sources such as urban sewage waters. Indeed, Gębka et al. (2016) analysed the impact of military activities on the Baltic Sea and suggested that the projectiles could be a potential source of Hg in both terrestrial and marine environments, as Hg had been part of the fulminate content in blasting caps of different types of ammunition.

Despite the absence of data for heavy-metal concentrations in *P. nobilis* for Cabrera2, the significantly different abundances of deformed foraminifera, and the Zn and Pb content in the foraminiferal tests, between Cabrera1 and Cabrera2, might indicate that the exposure to the pollutants is locally variable and randomly occurring. This irregular pattern is consistent with the distribution of the impact points where small projectile fragments might remain after the historical military exercises. Comparably, in the Baltic Sea, World War II impact zones presented elevated heavy-metal levels in sediments with respect to surrounding areas (Gębka et al., 2016). Both, Cabrera and the Baltic scenarios might represent a similar situation, in which small amounts of heavy metals were being continuously released, forming a persistent pollution source with a chronic effect on the biota.

Thus, as explained above, these long-term exposures to small amounts of persistent pollutants would not affect the composition of the foraminiferal assemblage in terms of diversity nor in the abundance of the more sensitive forms (A* and SB morphotypes) as indicated by the foraminiferal-based indices, but the high number of tests with abnormal growth reflects the effect

of the heavy metals on biological processes. Consistently, during their study, Vázquez-Luis et al. (2016) reported very high densities of *P. nobilis* populations including numerous individuals with accumulated heavy metals in their tissues. Unfortunately, during the last four to five years these populations have disappeared almost completely (Vázquez-Luis et al., 2017) from the effects of a protozoan parasite (*Haplosporidium pinnae*) that has caused considerably worse damage than the heavy metal pollution, which heavily compromises these studies.

4.2.3.4. Control

Control specimens correspond to non-deformed individuals from the different sampling sites. These foraminifera presented heavy-metal concentrations lower than those corresponding to aberrant tests from the more anthropized sampling sites (Port d'Andratx and Magaluf) but are comparable to Cabrera stations, specifically Cabrera1. This observation supports the likelihood that the occurrence of aberrant individuals is related to other environmental parameters (temperature, salinity, food supply, dissolved oxygen, and pH) besides heavy-metal pollution (Martínez-Colón et al., 2009). However, the importance of trace elements on the morphological response is highlighted, as the deformed tests contained higher heavy-metal concentrations. Consistently, in more-or-less pristine areas, very localized but chronic impacts may induce intense but spatially-reduced responses, reflected in the abundance of abnormally-grown specimens.

4.2.4. Abnormal Growth Patterns and Pollutants: Comparison with Other Study Cases Worldwide

The percentage of abnormalities (FAI) in the studied samples varied greatly among the different locations. The lowest and highest FAI values were reported in Cabrera. While in Cabrera2, the abundance of abnormal tests (FAI) was < 5%, significantly higher values (22%–26%) were found in Cabrera1, although both sampling sites are located within a marine protected area with minimal human impacts over the past three decades. Amounts of aberrant foraminifera similar to those from Cabrera1 were reported in Southampton Water (England), an estuarine system polluted with hydrocarbons and heavy metals from an oil refinery, with up to 20% of deformed tests (Sharifi et al., 1991). This study was based on the analysis of three stress-tolerant species, *Ammonia beccarii*, *Elphidium excavatum* and *Haynesina germanica*, which constituted over 85% of the total foraminiferal population (live + dead). In contrast, in Cabrera foraminiferal diversity was high (Table 21) and matched the taxonomic composition of the foraminiferal assemblages from well-preserved *P. oceanica* meadows (Mateu-Vicens et al., 2014). However, in the sampling sites in Mallorca, the FAI was lower than in Cabrera1, with up to 11% of deformation in Port d'Andratx and up to 7% in Magaluf. Both localities, Port d'Andratx and Magaluf, are affected by discharge-treated sewage, mostly from domestic origin (Vázquez-Luis et al., 2016), compared to the Cabrera sites, where the anthropogenic impact is minimal.

In the Mediterranean, most studies have been conducted in coastal lagoons and restricted areas from both the western (Coccioni, 2000; Romano et al., 2008; 2009; 2016; Frontalini et al., 2010; Bergamin et al., 2019) and eastern (Yanko et al., 1998; Samir, 2000) basins, showing FAI values within the ranges of Cabrera2, Port d'Andratx and Magaluf localities. However, very few studies on the occurrence of aberrant forms related to pollution have been performed in seagrass foraminiferal assemblages and none of them showed abundances of deformed tests as high as those reported in

Cabrera1. Accordingly, Caruso et al. (2011) studied epiphytic foraminifera in *P. oceanica* meadows from Sicily (Italy) and exclusively analysed the amount of abnormal specimens of *Lobatula lobatula*, a low-Mg calcite species, less suitable to uptake external heavy metals than porcelaneous taxa such as *Sorites* spp. and *Peneroplis* spp. (Bergamin et al., 2019). These authors found that in the heavily-polluted area of Palermo Harbour, which is influenced by intense sea traffic, dockyard activities, untreated urban sewage, industrial discharge and goldsmith pollution, up to 12.7% of foraminiferal tests were deformed (Table 22). In contrast, in other areas with lower anthropogenic impacts (e.g., Gulf of Termini, Sicily) the percentage of abnormal foraminifera diminished considerably (Table 22). Indeed, in Lampedusa island, an area with little anthropogenic impact (except for the harbour that hosts small fishing boats and ferries), the percentage of deformation was up to 1.5% and only reached up 9.18% inside the port (Table 22).

TABLE 21. Relative abundance of foraminiferal species from each sample (Morphotypes according to Mateu-Vicens et al., 2014; test types are H – hyaline, P – porcelaneous, and A – agglutinated respectively)

	Test type	Morph	SMRA-1	SMRA-2	SMRA-3	SMRA-4	SMRA-5	SMRA-6	SMRA-7	ADTX-1	ADTX-2	ADTX-3	ADTX-4	MGLF-1	MGLF-2	MGLF-3	MGLF-4
<i>Adelosina cliarensis</i>	P	D	0.0	0.0	0.7	1.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
<i>Adelosina dubia</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	1.3
<i>Adelosina ferrusaci</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Adelosina laevigata</i>	P	D	0.0	0.0	0.7	0.0	0.0	0.0	0.0	1.3	0.7	1.3	1.3	0.0	0.0	1.3	2.7
<i>Adelosina mediterraneensis</i>	P	D	0.7	1.3	0.0	0.7	4.7	2.7	2.0	4.7	1.3	3.3	4.0	2.7	0.0	2.0	0.7
<i>Adelosina pulchella</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.7	0.7	0.7	0.0
<i>Adelosina</i> sp.	P	D	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Affinetrina planciana</i>	P	D	0.0	0.0	1.3	0.0	1.3	2.0	0.7	0.0	0.7	0.0	0.7	0.0	0.0	0.0	0.0
<i>Ammonia beccarii</i>	H	B	0.7	2.0	0.0	0.7	0.0	0.7	1.3	0.7	0.0	0.0	0.0	0.7	0.0	0.0	1.3
<i>Ammonia beccarii</i> var. <i>ammoniformis</i>	H	B	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ammonia beccarii</i> var. <i>inflata</i>	H	B	0.0	0.0	0.0	0.7	1.3	1.3	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.7	1.3
<i>Ammonia parkinsoniana</i>	H	B	0.0	0.7	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Asterigerinata mamilla</i>	H	B	0.0	2.0	4.7	0.7	0.0	0.0	1.3	0.0	1.3	1.3	1.3	1.3	0.0	0.0	0.7
<i>Astrononion stelligerum</i>	H	C	2.7	2.0	2.0	3.3	4.7	6.0	0.7	3.3	5.3	3.3	0.0	4.7	8.7	6.7	6.0
<i>Bolivina beyrichi</i>	H		0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bolivina difformis</i>	H		0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bolivina dilatata</i>	H		0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.0
<i>Bolivina</i> sp.	H		0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bolivina spathulata</i>	H		0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bolivinita quadrilatera</i>	H		0.0	0.0	1.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
<i>Cibicides variabilis</i>	H	B	2.0	1.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cibicides refulgens</i>	H	B	0.7	0.7	0.7	4.0	2.0	2.0	2.7	2.7	4.7	6.0	6.0	5.3	4.0	4.7	5.3
<i>Cornuspira carinata</i>	P	D	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.0
<i>Cyclocibicides vermiculata</i>	H	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.7	0.7	1.3
<i>Cycloforina colomi</i>	P	D	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cycloforina contorta</i>	P	D	0.0	1.3	0.0	0.7	0.0	0.7	0.0	0.0	1.3	2.7	0.7	0.0	0.0	0.7	2.0
<i>Cycloforina rugosa</i>	P	D	0.0	0.7	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
<i>Cycloforina</i> sp.	P	D	0.7	0.0	0.0	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cycloforina tenuicollis</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Discorbis mira</i>	H	B	1.3	2.0	0.7	1.3	0.7	1.3	1.3	0.0	2.0	0.0	0.0	1.3	2.7	2.7	2.7
<i>Dyocibicides biserialis</i>	H		0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Elphidium aculeatum</i>	H	C	0.7	0.0	0.0	2.7	2.0	2.7	4.0	0.7	0.0	0.7	0.0	0.7	0.7	0.7	0.7

<i>Elphidium complanatum</i>	H	C	0.0	1.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Elphidium crispum</i>	H	C	2.7	1.3	1.3	0.7	0.7	1.3	2.0	1.3	2.0	1.3	0.0	4.0	2.0	1.3	1.3
<i>Elphidium macellum</i>	H	C	0.7	1.3	0.0	3.3	2.0	0.0	0.7	2.7	0.7	2.0	0.7	1.3	0.7	0.0	0.7
<i>Elphidium maioricense</i>	H	C	0.7	0.7	1.3	0.0	3.3	3.3	2.7	2.7	4.7	3.3	1.3	1.3	0.7	0.7	0.0
<i>Elphidium</i> sp.	H	C	0.0	2.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Fissrina</i> sp.	H		0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Fursenkoina tenuis</i>	H		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Glabratella hexacamerata</i>	H		0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Glabratella patelliformis</i>	H		0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Globulina gibba</i>	H		0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0
<i>Guttulina earlandi</i>	H		0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.7	0.0
<i>Hoeglundina elegans</i>	H		0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Lachlanella variolata</i>	P	D	0.7	0.0	1.3	1.3	1.3	1.3	0.7	0.0	2.0	0.7	2.0	0.0	0.7	0.0	0.0
<i>Laevipeneroplis proteus</i>	P	SB	0.0	0.7	2.0	1.3	0.0	1.3	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.7
<i>Lobatula lobatula</i>	H	B	4.0	1.3	4.7	4.7	4.7	3.3	5.3	7.3	4.7	5.3	7.3	4.7	6.7	6.7	4.0
<i>Massilina secans</i>	P	D	0.7	3.3	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Miliolinella grata</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
<i>Miliolinella labiosa</i>	P	D	0.7	0.7	0.0	1.3	0.0	0.7	0.7	1.3	0.7	0.0	0.7	0.0	0.0	0.7	0.0
<i>Miliolinella semicostata</i>	P	D	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
<i>Miliolinella webbiana</i>	P	D	0.0	0.0	0.7	0.7	0.0	0.0	0.0	0.7	0.0	0.7	0.7	0.7	0.0	0.0	0.0
<i>Miniacina miniacea</i>	H	A	0.0	3.3	2.0	2.0	0.0	1.3	0.0	3.3	2.0	0.7	2.0	0.0	0.7	0.7	0.7
<i>Nubecularia lucifuga</i>	P	A	6.7	7.3	4.0	9.3	5.3	8.0	4.7	11.3	6.7	4.7	7.3	6.7	8.0	5.3	11.3
<i>Nubecularia massutiniana</i>	P	A	3.3	2.7	2.7	0.0	1.3	0.7	0.0	1.3	0.0	3.3	2.0	0.7	0.7	1.3	1.3
<i>Peneroplis arietinus</i>	P	SB	0.0	0.0	0.0	0.0	3.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Peneroplis pertusus</i>	P	SB	9.3	5.3	8.7	4.0	0.7	4.0	3.3	4.7	10.0	3.3	4.7	4.0	2.0	3.3	2.0
<i>Peneroplis planatus</i>	P	SB	12.0	6.0	10.0	6.0	4.7	4.0	5.3	3.3	1.3	1.3	7.3	3.3	2.0	1.3	3.3
<i>Planorbulina (Cibicidella) variabilis</i>	H	A	0.0	0.0	0.0	2.0	5.3	3.3	2.0	3.3	2.0	1.3	3.3	0.7	0.0	1.3	0.0
<i>Planorbulina mediterraneensis</i>	H	A	5.3	6.7	5.3	8.7	2.7	7.3	10.7	8.7	6.7	11.3	9.3	9.3	14.0	6.7	6.0
<i>Pseudotriloculina cuneata</i>	P	D	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudotriloculina laevigata</i>	P	D	0.7	0.7	0.0	0.0	0.0	0.7	0.7	0.0	0.0	0.7	0.7	0.0	0.0	0.0	0.0
<i>Pseudotriloculina oblonga</i>	P	D	0.0	0.7	0.7	0.0	0.7	0.7	0.0	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0
<i>Pseudotriloculina rotunda</i>	P	D	0.7	0.0	1.3	1.3	0.7	0.0	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.7	1.3
<i>Pseudotriloculina sidebottoni</i>	P	D	0.0	0.7	0.7	0.7	4.0	0.0	0.0	2.0	0.0	0.0	0.0	0.7	0.0	0.0	0.7
<i>Pyrgo elongata (williamsoni)</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
<i>Quinqueloculina annectens</i>	P	D	0.7	0.0	0.0	0.7	0.0	1.3	2.0	0.0	0.7	0.7	0.0	0.0	0.7	0.0	0.0
<i>Quinqueloculina berthelotiana</i>	P	D	1.3	1.3	2.7	2.7	4.7	5.3	6.7	3.3	2.7	1.3	2.7	2.7	2.7	2.7	3.3
<i>Quinqueloculina costata</i>	P	D	0.0	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.7	0.7	2.0	0.0	2.0	0.0
<i>Quinqueloculina disparilis</i>	P	D	0.7	0.7	0.7	0.0	0.7	1.3	1.3	0.7	2.0	4.0	1.3	0.7	4.0	0.0	1.3
<i>Quinqueloculina irregularis</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.7	1.3	0.0	0.0
<i>Quinqueloculina jugosa</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Quinqueloculina laevigata</i>	P	D	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.7	0.0	0.7	0.7	1.3	0.0	0.0	1.3
<i>Quinqueloculina lamarckiana</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Quinqueloculina limbata</i>	P	D	1.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0
<i>Quinqueloculina nodulosa</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Quinqueloculina parvula</i>	P	D	0.0	0.0	0.7	0.7	2.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.7	0.7
<i>Quinqueloculina schlumbergeri</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	1.3
<i>Quinqueloculina seminula</i>	P	D	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	1.3	0.7	0.7	0.0
<i>Quinqueloculina sidebottomi</i>	P	D	0.0	0.7	0.7	0.0	0.0	1.3	2.7	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0
<i>Quinqueloculina</i> sp.	P	D	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
<i>Quinqueloculina stelligera</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<i>Quinqueloculina undulata</i>	P	D	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Quinqueloculina ungeriana</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.3	0.0	0.7	0.0
<i>Quinqueloculina vulgaris</i>	P	D	0.7	0.7	0.7	0.0	0.7	0.7	0.0	1.3	1.3	1.3	2.0	4.7	2.0	8.0	4.0
<i>Rosalina bradyi</i>	H	B	2.7	2.0	2.0	2.7	3.3	2.7	2.0	3.3	7.3	6.0	5.3	4.7	3.3	4.7	6.0
<i>Rosalina globularis</i>	H	B	1.3	1.3	1.3	1.3	0.0	6.7	2.0	6.7	2.7	6.0	3.3	2.0	2.7	2.0	0.7
<i>Rosalina globularis</i> var. <i>anglica</i>	H	B	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rosalina macropora</i>	H	B	0.0	0.7	2.7	1.3	2.0	1.3	3.3	0.7	3.3	2.0	1.3	1.3	1.3	4.0	2.7
<i>Rosalina mediterraneensis</i>	H	B	0.7	0.7	0.7	3.3	2.0	0.0	1.3	0.0	0.0	0.7	0.0	0.0	2.0	0.7	2.7
<i>Rosalina posidonicola</i>	H	B	0.7	3.3	1.3	1.3	2.0	1.3	1.3	0.0	2.0	0.7	2.0	0.0	1.3	1.3	1.3
<i>Rosalina</i> sp.	H	B	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sigmoilinita costata</i>	P	D	0.7	0.7	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	2.7	5.3	0.7	0.0
<i>Siphonaperta agglutinans</i>	P	D	1.3	1.3	1.3	0.7	0.0	0.0	1.3	2.0	0.0	2.0	0.0	2.0	0.7	1.3	2.0
<i>Siphonaperta aspera</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Siphonaperta dilatata</i>	P	D	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0	1.3	0.0	1.3	1.3	0.0	0.7	0.0
<i>Siphonaperta irregularis</i>	P	D	1.3	0.7	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
<i>Siphonaperta quadrata</i>	P	D	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sorites orbiculus</i>	P	SB	11.3	4.7	7.3	6.7	6.0	4.7	5.3	2.0	2.7	2.7	4.7	0.0	0.0	0.7	2.0
<i>Spirillina vivipara</i>	H	A	3.3	2.0	1.3	0.0	0.0	0.0	0.7	0.0	0.0	0.7	0.0	1.3	1.3	1.3	0.0
<i>Spiroloculina depressa</i>	P	D	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
<i>Spiroloculina nitida</i>	P	D	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.7	2.0	0.0
<i>Spiroloculina ornata</i>	P	D	1.3	0.7	1.3	2.0	2.7	0.7	2.7	2.7	1.3	1.3	1.3	0.0	3.3	2.7	2.0
<i>Spiroloculina ornata</i> var. <i>tricarinata</i>	P	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.7	2.7	2.0	0.0
<i>Textularia agglutinans</i>	A	D	0.7	0.0	0.0	0.0	0.0	0.0	1.3	0.0	1.3	0.0	0.0	3.3	2.0	1.3	3.3
<i>Textularia candeiana</i>	A	D	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
<i>Textularia pseudoturris</i>	A	D	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.0	0.0	0.7
<i>Textularia</i> sp.	A	D	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tretomphalus bulloides</i>	H	B	0.0	1.3	0.0	0.0	1.3	0.7	0.0	0.0	0.0	0.0	0.7	1.3	2.0	0.7	0.7
<i>Triloculina affinis</i>	P	D	0.0	0.0	0.0	0.7	0.7	0.0	0.7	0.7	0.0	0.7	0.7	1.3	1.3	0.0	0.0
<i>Triloculina marioni</i>	P	D	0.0	0.7	0.7	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.7	1.3	0.7
<i>Triloculina oblonga</i>	P	D	0.0	0.0	0.7	0.0	0.0	0.0	0.7	0.7	0.0	0.0	0.0	0.7	0.0	1.3	0.0
<i>Triloculina plicata</i>	P	D	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Triloculina schreiberiana</i>	P	D	1.3	1.3	0.0	1.3	0.7	0.0	1.3	0.0	0.0	2.0	0.0	0.7	0.7	0.0	0.0
<i>Triloculina</i> sp.	P	D	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Triloculina trigonula</i>	P	D	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Vertebralina striata</i>	P	D	2.0	3.3	2.0	5.3	4.0	4.7	3.3	1.3	2.0	2.7	1.3	0.0	0.7	1.3	2.7
<i>Wellmanellinella striata</i>	P	D	0.7	2.7	0.7	3.3	3.3	0.7	1.3	2.7	0.7	2.0	0.7	1.3	0.0	1.3	0.7

Table 22. Summary of some published work dealing with evaluation of percentages of deformed foraminifera reported in the present study compared with other shallow-water areas under different anthropogenic impacts, including heavy metals. Scientific nomenclature is as in the original references.

Study	Percentage of deformed foraminifera (FAI sensu Coccioni et al., 2003; 2005)	Locality and environmental conditions	Foraminiferal taxa
Sharifi et al. (1991)	10 to 20	Southampton Water (England): estuarine system polluted with hydrocarbons and heavy metals by oil industry (refinery).	2 species: <i>Ammonia beccarii</i> , <i>Haynesina germanica</i>

Yanko et al. (1998)	2 to 3	Haifa Bay and Atlit Bay (Israel): industrial waste (sewage and oil refinery) and chemical pollution from rivers	27 species: <i>Adelosina cliarensis</i> , <i>Adelosina elegans</i> , <i>Adelosina intricata</i> , <i>Adelosina phoenicia</i> , <i>Adelosina pulchella</i> , <i>Ammonia batava</i> , <i>Ammonia tepida</i> , <i>Amphistegina lobfera</i> , <i>Asterigerinata mamilla</i> , <i>Bolivina spathulata</i> , <i>Challengerella bradyi</i> , <i>Criboelphidium poeyanum</i> , <i>Eggerelloides advenus</i> , <i>Haynesina depressula</i> , <i>Miliolinella subrotunda</i> , <i>Pararotalia spinigera</i> , <i>Parrina bradyi</i> , <i>Peneroplis pertusus</i> , <i>Peneroplis planatus</i> , <i>Pseudohauerina diversa</i> , <i>Pseudononion japonicum</i> , <i>Pseudotriloculina subgranulata</i> , <i>Quinqueloculina disparilis</i> , <i>Sigmoilina costata</i> , <i>Triloculina schreiberiana</i> , <i>Triloculina earlandi</i> , <i>Triloculina marioni</i>
Coccioni, 2000	> 10	Goro Lagoon (Adriatic Sea, Italy): polluted waters from industrialized and crop-cultivated areas through the Po river.	6 species: <i>Ammonia parkinsoniana</i> , <i>Ammonia tepida</i> , <i>Cassidulina carinata</i> , <i>Haynesina germanica</i> , <i>Quinqueloculina seminula</i>
Samir, 2000	up to 11	Manzalah Lagoon (Nile Delta, Egypt): urban sewage and industrial waste water with heavy metals	4 species: <i>Ammonia beccarii</i> , <i>Ammonia tepida</i> , <i>Ammonia parkinsoniana</i> , <i>Criboelphidium gunteri</i>
Caruso et al., 2011	up to 12.7	<i>Posidonia oceanica</i> meadows (Gulf of Palermo, Sicily, Italy): heavily polluted area submitted to untreated urban sewage, industrial discharge, goldsmiths pollution	only studied <i>Lobatula lobatula</i>
	up to 4.8	<i>Posidonia oceanica</i> meadows (Gulf of Termini, Sicily, Italy): moderately polluted area submitted to untreated urban sewage	only studied <i>Lobatula lobatula</i>
	up to 1.5 (exceptionally in the harbour 9.18)	<i>Posidonia oceanica</i> meadows (Lampedusa island, Sicily, Italy): scarcely polluted area (little anthropogenic impact, with the exception of the harbour that hosts small fishing boats and ferries)	only studied <i>Lobatula lobatula</i>
Romano et al., 2008	< 5.5	Bagnoli (Gulf of Naples, Italy): Industrial plant.	Mostly <i>Miliolinella subrotunda</i> and <i>Elphidium advenum</i>

Romano et al., 2009	< 5	Bagnoli (Gulf of Naples, Italy): Industrial plant.	41 species: mostly <i>Miliolinella subrotunda</i> and <i>Elphidium advenum</i>
Frontalini et al., 2010	4.9 to 12.9 2.5 to 6.1	Ortobello lagoon (Tyrrhenian Sea, Italy): eutrophication due to agriculture, aquaculture and urban activities. Lesina lagoon (Adriatic Sea, Italy): eutrophication	Most abundant: <i>Ammonia tepida</i> , <i>Haynesina germanica</i> , <i>Criboelphidium oceanensis</i> , <i>Rosalina globularis</i> , <i>Quinqueloculina costata</i> , <i>Quinqueloculina seminula</i> , <i>Bolivina striatula</i> , <i>Trichoyalus lacunae</i>
Romano et al., 2013	Up to 2	Augusta Harbour (Sicily, Italy): chemical and petrochemical pollution.	<i>Miliolinella subrotunda</i> , <i>Sigmoilinita costata</i> , <i>Bulimina marginata</i> , <i>Rosalina bradyi</i> , <i>Quinqueloculina lata</i> , <i>Ammonia tepida</i>
Bergamin et al., 2019	1 to 8	Eastern Ligurian Sea (Italy): natural source of metals from Ophiolites.	<i>Ammonia beccarii</i> , <i>Bulimina seminuda</i> , <i>Elphidium advenum</i> , <i>Elphidium crispum</i> , <i>Elphidium pulvereum</i> , <i>Haynesina depressula</i> , <i>Peneroplis pertusus</i> , <i>Quinqueloculina parvula</i> , <i>Quinqueloculina seminula</i> , <i>Triloculina schreberiana</i> , <i>Miliolinella subrotunda</i> , <i>Siphonaperta aspera</i> , <i>Bolivina striatula</i> , <i>Eggerelloides scaber</i>
Present study	22 to 26	<i>Posidonia oceanica</i> meadows (Cala Santa Maria (Cabrer1), Cabrera, Balearic Islands, Spain): Marine Protected Area since 1991 till now (forbidden are all direct exploitation of natural resources and scuba diving without a special permission; navigation around the island is limited), old military zone since 1916 to 1991	11 species (from 27): <i>Elphidium complanatum</i> , <i>Elphidium maioricense</i> , <i>Lobatula lobatula</i> , <i>Massilina secans</i> , <i>Miliolinella labiosa</i> , <i>Peneroplis pertusus</i> , <i>Peneroplis planatus</i> , <i>Planorbulina mediterraneensis</i> , <i>Quinqueloculina berthelotiana</i> , <i>Rosalina bradyi</i> , <i>Sorites orbiculus</i>
	1 to 6	<i>Posidonia oceanica</i> meadows (Cala Santa Maria (Cabrer2), Cabrera, Balearic Islands, Spain): Marine Protected Area since 1991 till now (forbidden are all direct exploitation of natural resources and scuba diving without a special permission; navigation around the island is limited), old military zone since 1916 to 1991	11 species (from 27): <i>Astrononion stelligerum</i> , <i>Cibicides refulgens</i> , <i>Elphidium maioricense</i> , <i>Lobatula lobatula</i> , <i>Peneroplis pertusus</i> , <i>Peneroplis planatus</i> , <i>Planorbulina mediterraneensis</i> , <i>Sorites orbiculus</i> , <i>Spiroloculina ornata</i> , <i>Vertebralina striata</i> , <i>Wellmanellinella striata</i>

	2 to 10	<i>Posidonia oceanica</i> meadows (Port d'Andratx, Mallorca, Balearic Islands, Spain): recreational harbour with high anthropogenic influence	13 species (from 27): <i>Astrononion stelligerum</i> , <i>Cibicides refulgens</i> , <i>Lachlanella variolata</i> , <i>Lobatula lobatula</i> , <i>Miliolinella webbiana</i> , <i>Peneroplis pertusus</i> , <i>Peneroplis planatus</i> , <i>Planorbulina mediterraneensis</i> , <i>Quinqueloculina berthelotiana</i> , <i>Rosalina globularis</i> , <i>Rosalina macropora</i> , <i>Sorites orbiculus</i> , <i>Spiroloculina ornata</i>
	3 to 7	<i>Posidonia oceanica</i> meadows (Magaluf, Mallorca, Balearic Islands, Spain): with elevated level of dissolved inorganic nitrogen caused by anthropogenically enriched groundwater discharges	7 species (from 27): <i>Astrononion stelligerum</i> , <i>Cibicides refulgens</i> , <i>Lachlanella variolata</i> , <i>Lobatula lobatula</i> , <i>Peneroplis planatus</i> , <i>Planorbulina mediterraneensis</i> , <i>Wellmanellinella striata</i>

4.3. OBJECTIVE 3. APPLICATION OF THE BIOINDICATOR INDICES TO HISTORICAL DATA SERIES BASED ON INFORMATION FROM LOCALITIES OF THE BALEARIC ISLANDS (MALLORCA, MENORCA, AND CABRERA)

4.3.1. Bibliographic Data Analysis

4.3.1.1. Urban transformation of the coastal area of the Balearic Islands (1960s–2020s).

During the early 1960s, the Balearic Islands emerged as a significant tourist destination in the Mediterranean and played a pioneering role in the development of mass tourism worldwide (Hof and Schmitt, 2011; Hof and Blázquez-Salom, 2013). Several factors, including the islands' favorable geographic location, abundant sandy beaches, and the Mediterranean Sea, contributed to this growth. The islands' tourism policy prioritized tourism as the main driver of economic development, as evidenced by the 1958 Financial Stabilization Plan's approval, which favored foreign real estate companies (Horrach, 2009). In 1960, two years later, Son Sant Joan Airport opened, accommodating domestic and international traffic (AENA, 2023). The coastal area surrounding Mallorca's capital, Palma, and close to the airport witnessed large-scale building constructions (Pons et al., 2014). Furthermore, the establishment of a tour operator and the introduction of charter flights in 1967 (Horrach, 2009) marked the emergence of mass tourism.

In a similar vein, the tourism development of Menorca and Mallorca began near their respective capitals, particularly within the first kilometer of the coastal zone. While Mallorca's tourist zone expanded to smaller coast-side towns, Menorca focused on two primary cities, Maó and Ciutadella (Pons et al., 2014). Due to the surging tourist demand, construction proceeded at an alarming pace, with little regard for the destruction of unique natural complexes. The construction of hotels in other coastal areas of the island only became possible after the construction of various

freeways. In 1956, only 3.33% of the urban zone in the first kilometer of the shoreline on Mallorca was urbanized, whereas by 2006, it had increased to 22.16 percent (Pons and Rullan, 2014). In 1965, Mallorca had 1284 hotels and pensions, compared to 563 in 1961 (Vives, 2011), even though no new urban infrastructures were created to support the development of hotels. Consequently, hotels formed a continuous seafront, resulting in the destruction of the landscape and the uncontrollable pollution of such areas with household waste directly dumped into the sea until the publication of Royal Decree 356/1985 in 1985. Thus, any discharges into the sea, whether liquid or solid, direct or through outfalls, required authorization from the competent administration (Comunitat Autònoma de les Illes Balears (CAIB)).

The economic crisis of 1973–1979, which resulted in changes in the average tourist's needs due to problems related to infrastructure, accommodation services, and the negative impact on the environment, necessitated the enactment of the law. However, despite the economic downturn of the 1970s, urbanization continued on the islands until 1995 (Pons and Rullan, 2014). The first coastal vacation centers emerged around former settlements, such as Colònia de Sant Jordi, and others originated from fishing villages, such as Port de Pollença or Port d'Alcúdia (Horrach, 2009). In an effort to make it easier to access Mallorca's holiday resorts, multi-storey structures are being built along the coastline, exacerbating negative anthropogenic pressure on the area (Horrach, 2009).

Magaluf, the most popular resort on Mallorca, was created by mass tourism and had the highest tourist bed densities after Palma. It gained global attention as a famous example of poorly conceived construction. In the late 1960s and early 1970s, Magaluf was built on the coastline (Fig. 42), sometimes almost in the sea, despite being located in the bay of Palma (Manchado, 2009; Pons et al., 2014).

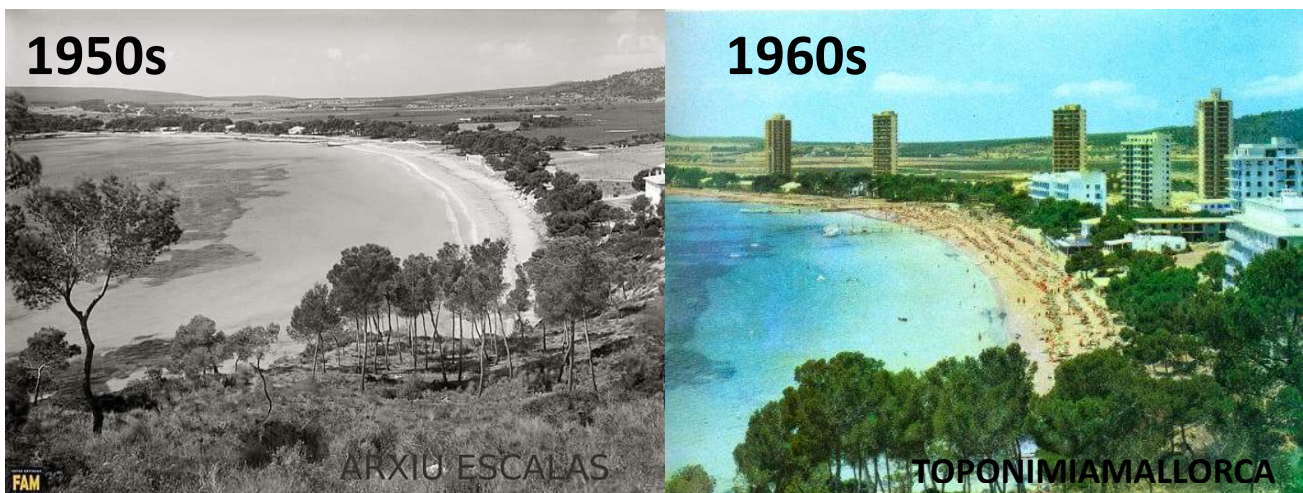


FIGURE 42. The transformation of shoreline of Magaluf from 1950s to 1960s.

Between 1985 and 1988, the Balearic Islands experienced a second tourism boom, which coincided with Spain's integration into the European Union in 1986. This led to a 60% increase in tourist accommodation compared to the early 1980s (Vives, 2011; Pons et al., 2014). Overall, between 1956 and 2006, the coastal urbanization saw remarkable growth (Pons and Rullan, 2014). According to Hof and Schmitt (2011), the urbanized land on the first kilometer from the shoreline of the Balearic Islands was 32.3% in 1956, increasing to 43% in 2006.

After 20 years of unchecked land use that resulted in environmental damage, the third tourism boom, known as "quality" tourism (Bardolet and Sheldon, 2008), coincided with the enactment of laws to regulate resource use and prevent environmental pollution. In 1984, the government issued a decree to protect the natural areas of special interest (ANEIS) in the Balearic Islands, followed by zoning strategies and land usage regulations (Bardolet and Sheldon, 2008). Spain passed a Coastal Law in 1988, establishing a nature buffer zone, and since 1991 (LEN Law 1991), 39% of the Balearic Islands have been protected. In 1995, POOT approved a regulation for coastal areas that superseded municipal authorities' sovereignty. Finally, the PTI laws (Insular Territorial Plans) in 2004 (Law 8/2003) limited the speed of urban land development for accommodation.

Changes in wastewater management were also significant, as summarized by Vaquer-Sunyer et al. (2021). The treatment of urban wastewater was the focus of Directive 91/271/CEE, followed by Directive 2000/60/EC of the European Parliament and the Council in 2000 and Directive 2008/56/EC of the European Parliament and the Council in 2008, which established a framework for community action for marine environment policy, among others. Decree 49/2003 declared sensitive areas in the Balearic Islands, and the Hydrological Plan of the Balearic Islands, approved in 2001 (Royal Decree 378/2001), established the standards for urban wastewater treatment.

4.3.1.2. *The series data analysis of the I_{LS} index by decades.*

The limited availability of data series biases the analysis of trends presented by different indices. Some localities are repeated in multiple studies with higher sampling frequencies, allowing for the establishment of long-term series reflecting the health status of the *P. oceanica* meadow over the last 50–60 years. However, other sample points only appear in one study, representing only one point on the timeline. Additionally, the sampling methodologies used in different works are not always consistent. Some studies rely on living foraminifera associations, while others consider dead foraminifera associations. Nonetheless, both types of information have been shown to be comparable (Samir and El-Din, 2001; Murray, 2006).

The heterogeneity in sampling methodology and temporal variation across case studies limits the application of statistical analysis. Therefore, this thesis section is descriptive, attempting to associate the temporal evolution of the indices and their spatial variability with elements related to anthropic activities such as tourism increase, land use, sewage treatment plant installations, and more.

Since the FSI index shows little variability over time and between different locations and the FI' and I_{LS} indices are highly correlated, the discussion primarily focuses on the I_{LS} . This index amplifies the differences between the environmental health states of the studied meadows, as evidenced by Khokhlova (2013) and Mateu-Vicens et al. (2014).

Upon analyzing the I_{LS} index evolution from the 1960s to the 2020s by decades (Table 18 and Fig. 37), it becomes evident that the 1970s lack sufficient data due to inadequate sampling efforts. Moreover, the sampling frequency has not been uniform over time, with the fewest studies conducted in the 1990s and 2000s. The I_{LS} values obtained in the 1960s, although the highest recorded, vary greatly, ranging from 14.65 to 0.81. In the 1980s, the majority of I_{LS} values were similar to those of the previously sampled localities, with some even overlapping. However, data from the northeast of Mallorca and various stations on the island of Menorca were added. A general decrease in the I_{LS} index is observed, with only Badia d'Alcúdia and the south and west of Menorca recording

values comparable to those in the 1960s. In the 1990s, I_{LS} values remained stable or slightly decreased despite a lower number of locations studied than the previous decade. However, in the 2000s, the index rose considerably in two of the three sampled locations, with values significantly exceeding the previous trend, indicating a potential bias in specimen selection. In contrast, the third location (Port de Sóller) maintained consistent values with previous studies. In the 2010s, I_{LS} values were lower than those recorded in the 2000s and aligned with studies from the 1960s-1990s. The data collected encompassed not only the island of Mallorca but also the Cabrera Archipelago National Park. I_{LS} ranges for the island of Mallorca were between 1.82 and 4.36, while for Cabrera, values ranged from 3.52 to 4.68. Finally, in the 2020s, a new study focused on applying these indices to different locations along the Mallorcan coast, indicating a tendency to increase compared to previous data. Points not previously sampled had values exceeding 5.0 in almost all cases, with the locality of Es Caragol, which recorded the highest values in the 1960s (14.65), falling to 5.52 in the 2020s despite being a pristine area. During the summer months, this site faces intense anthropic pressure caused by the large number of daily boats anchoring on the *P. oceanica* meadow until 2019, the enforcement of current regulations became more stringent.

4.3.1.3. Time evolution of the I_{LS} index

The analysis of the I_{LS} index has focused on locations with data from at least three different decades, including Cala Blava, Port de Sóller, Portocolom, and Port d'Andratx (Fig. 38 (1-4)). When exploration points from different sources were geographically close but did not exactly coincide, they were grouped into zones (Santa Ponça – Cala de Santa Ponça, Ses Illetes – Portals Nous – Magaluf, S'Arenal – Es Carnatge – Ciutat Jardí, Marina de Bonaire – Es Barcarès – Badia de Pollença) to evaluate the I_{LS} trend (Fig. 39 (1-4)). This approach allowed for a fairly homogeneous assessment of the island of Mallorca's coast.

The I_{LS} values for Cala Blava showed the highest recorded value of 11.52 in 1965, decreasing to 3.21 in 2012, and subsequently increasing to 4.87 in 2021 and 5.10 in 2022. Cala Blava is located within the Badia de Palma Marine Reserve, which was established in 1982 but did not become effective until 1999 when the monitoring by Directorate of Fisheries and the Marine Environment of the Government of the Balearic Islands began (Morey et al., 2021). The implementation of protective measures has had a positive impact on the *P. oceanica* meadows, evident by the increased I_{LS} values.

Port de Sóller has the longest time series, including the years 1965, 1989, 1990, 1991, 2000, 2014, 2021, and 2022. The I_{LS} value in 1965 was 4.76 before experiencing the intense tourist development of subsequent decades. I_{LS} fluctuated strongly during the years 1989, 1990, and 1991, showing a downward trend. From 2000, I_{LS} increased until reaching values similar to 1965 in 2022. Although Port de Sóller is a sheltered port with many vessels anchored in the summer season, it lacked a wastewater purification system until 1992, which severely impacted the *P. oceanica* meadow. The quality of water has improved, and mooring regulations have led to a better state of health for the meadow, although it remains inferior to that of the 1960s.

The Portocolom time series covers the years 1964, 1989, 1990, and 1991. Unlike other analyzed areas, I_{LS} had very low values in the 1960s (0.81) but increased and stabilized during the 1980s (2.54-4.66) and 1990s (3.30-4.57). In Port d'Andratx, the time series covers the years 2012, 2021, and 2022, with I_{LS} values of 3.65, 3.49, and 3.62, respectively. The homogeneous results suggest that there have been no significant recent variations in the health status of *P. oceanica* meadows. The meadows continue to suffer from anthropogenic impacts due to a large recreational fleet and

intensive urban development, particularly since the late 1950s, 1980s, and 1990s (Hof and Schmitt, 2011; Pons and Rullan, 2014).

In the Santa Ponça – Cala de Santa Ponça area, the time series includes samples from 1965, 2012, 2021, and 2022. The I_{LS} values were high in 1965 (5.03) and decreased in 2012 (1.64–2.05), but increased slightly in 2021–2022 (3.30–2.60). This evolution reflects the impact of tourism development in the area, which began in Mallorca in the late 1960s, with a period of intense and disorderly growth during the 1970s–1990s (Hof and Schmitt, 2011; Pons and Rullan, 2014).

The Ses Illetes – Portals Nous – Magaluf area has been a significant tourist destination since the 1960s–70s (Vives, 2011; Pons et al., 2014), and the analyzed time series begins in 1982 and includes the years 2012, 2021, and 2022. The trend of the I_{LS} index clearly shows an increase from 1982 (0.62–3.38) to 2022 (5.11). The improvement in environmental conditions can be attributed to stricter environmental regulations since the implementation of the Royal Decree 356/1985 in 1985, which requires the treatment of wastewater before being discharged into the sea.

In the S'Arenal – Es Carnatge – Ciutat Jardí area, the time series covers the years 1965, 1982, 1989, 1990, and 1991. The I_{LS} reaches its maximum value (5.81) at the beginning of the series, then decreases in 1982 to minimum values (1.05), increases in 1989 with very variable values (2.23–5.98), and stabilizes later around 2.52 in 1990 and 1991. The significant decrease from 1965 can be attributed to the strong tourist development that led to the destruction of extensive dune fields in the Badia de Palma area, which are now occupied by large hotel complexes hosting a large number of tourists (Pons and Rullan, 2014). The slight increase in I_{LS} from 1989 probably reflects the improvement in water quality after the treatment plant was put into operation in 1987 (EMAYA, 2008).

The I_{LS} index in the northeast of Mallorca, covering the Marina de Bonaire – Es Barcarès – Badia de Pollença area, has shown significant fluctuations over the years. The values reached a peak of 3.74 in 1985 and rose to 10.87 in 1989, but fluctuated between 3.34 and 10.87 during this period. The values then decreased in 1990 (3.61–6.62) and 1991 (1.91–3.54), reflecting the disorderly development in the area. However, the I_{LS} index has increased again in 2021 (5.58) and 2022 (6.83), suggesting an improvement in environmental conditions. These fluctuations likely reflect the pre-tourism situation in the area and the subsequent disorderly development until the late 1980s and 1990s. However, the recent increase in the I_{LS} index indicates the effectiveness of stricter regulations in protecting the marine environment.

The recent increase in the I_{LS} index might indicate that stricter regulations are effective in protecting the marine environment. The European Union has enforced the protection of *P. oceanica* meadows through the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategies Framework Directive (MSFD, 2008/56/EC). Meanwhile, at the national and regional level, the legal framework is based on Royal Decree 139/2011 and Decree 25/2018, respectively. Regional authorities have used the latter to regulate boat anchoring, pursuing those who anchor in the meadow rather than on the rocky bottom or buoys installed to reduce pressure on *P. oceanica*.

CHAPTER 5

CONCLUSION

5.1. OBJECTIVE 1: N AND C ISOTOPIC ANALYSIS TO VALIDATE KHOKHLOVA'S (2013) ECOLOGICAL GROUPS ON WHICH THE BIOINDICATOR INDICES ARE BASED.

Genera of epiphytic foraminifera from two localities of Mallorca, Sa Foradada and Sant Elm, show similar relative abundances, and are dominated by encrusting and smaller rotalids. In Madagascar the seagrass foraminiferal assemblages are dominated by smaller miliolids, and larger miliolids and rotalids are common, associated with year-round temperatures warmer than those of the Mediterranean.

Although the proportions of the genera forming the morphotypes differ among the three sampling sites, statistical analyses revealed no differences in stable C and N composition with locality.

The $\delta^{15}\text{N}$ values ranged from 0.26–5.14‰, no significant differences in $\delta^{15}\text{N}$ were found among morphotypes. These low values are indicative of cyanobacteria as an important food source for epiphytic foraminifera. This observation is consistent with those of sessile, filter-feeding, epiphytic metazoans that also feed, at least partly, on cyanobacteria.

The $\delta^{13}\text{C}$ ratios exhibited substantial, statistically significant variability (-18.26 – -0.90) across morphotypes.

The sessile morphotypes (AF* and AR*) revealed similar values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, indicating similar trophic strategies, largely based on cyanobacteria farming. Therefore, the two morphotype can be unified in the same morphotype A*.

The morphotypes that present more variability in $\delta^{13}\text{C}$ are B and D*, which includes many motile smaller rotalids and miliolids, possibly allowing them to feed on a wider spectrum of sources (microalgae, cyanobacteria, phytodetritus).

Though not statistically significant, possibly because of small sample size, $\delta^{15}\text{N}$ values of symbiont-bearing morphotypes (LM and LR) were more depleted than the other morphotypes. In contrast, the $\delta^{13}\text{C}$ values were more enriched than in the other morphotypes. This paradoxical result is explained by the contamination of the samples with foraminiferal-test remains. In future studies the hydrochloric acid (HCl) treatment should be repeated to dissolve the test completely.

The isotopic results support the modification of the Langer (1993) classification by separating the symbiont-bearing forms from other morphotypes. Morphotypes hosting symbiotic algae (LR and LM) are clearly distinct from the other morphotypes and very similar to each other.

Therefore, the modified epiphytic morphotypes represent objective ecological differences that provide robustness the FI' and I_{LS} bioindicator indices, specific to evaluate the health state of *P. oceanica* seagrass meadows.

5.2. OBJECTIVE 2: COMPARATIVE ANALYSIS OF THE ENVIRONMENTAL CONDITIONS OF *P. OCEANICA* MEADOWS USING 1) BIOINDICATOR INDICES BASED ON EPIPHYTIC FORAMINIFERA, 2) THE FREQUENCY OF ABNORMAL-GROWTH FORMS AND 3) THE TRACE-ELEMENTAL CONCENTRATIONS WITHIN THEIR TESTS.

The use of foraminifera as bioindicators, combining different approaches such as ecological indices, quantification of abnormal growth patterns and geochemical analysis of their tests, are very helpful in determining the health of seagrass meadows ecosystems which are under constant negative influence of some stress in areas that have no obvious symptoms of degradation.

The benefit of using foraminifera is that they provide cost-effective, both short- and long-term analyses that do not require damaging any biota as for example, protected organisms such as *P. nobilis* and *P. oceanica*. Since *P. nobilis* has almost disappeared from its natural habitat, the use of foraminifera as bioindicators may gain relevance in the analysis of the water quality in *P. oceanica* ecosystems.

The foraminiferal (FI' , I_{LS} , and FSI) and biodiversity (H') indices indicated a good ecological status of the seagrass meadows; however, the morphological patterns (deformations of foraminiferal tests) and test compositions may reveal different pollution impacts, including the presence of heavy metals. Although, the apparent contradictory results respond to differences on spatial scales. FI' , I_{LS} , FSI and H' reflect conditions over a large area, whereas the morphological and geochemical analyses of foraminiferal tests shows very localized but long-lasting impacts.

Although there was not a straightforward relationship between the quantity of aberrant foraminiferal tests and the impact of different pollutants, heavy metals were present in higher concentrations in deformed tests. Moreover, in undisturbed areas where impacts were very localized and persistent, there was a significant correlation between heavy-metal content in the foraminiferal tests and the percentage of deformed individuals.

Despite the fact that the most frequent deformities in all sites (abnormal coiling and occurrence of aberrant shaped and sized chambers) cannot be associated to any particular heavy metals, other less common abnormalities (protuberances in the test and growth of supernumerary chambers) have been strongly correlated with the presence of heavy metals (Zn, Ni and As). However, more research is necessary to establish univocal cause-effect patterns between the abundance and type of aberrant growth forms and the uptake of specific heavy metals.

Therefore, besides performing the analysis of FI' , I_{LS} , FSI and H' to assess the conservation state of the *P. oceanica* meadows, other parameters such as the composition of the foraminiferal tests should be considered, especially when the percentage of abnormal forms rises above the expected in natural conditions (1%).

5.3. OBJECTIVE 3. APPLICATION OF THE BIOINDICATOR INDICES TO HISTORICAL DATA SERIES BASED ON INFORMATION FROM LOCALITIES OF THE BALEARIC ISLANDS (MALLORCA, MENORCA, AND CABRERA)

Due to the variance in sampling techniques and frequency utilized in different case studies, statistical analysis is constrained. Therefore, the historical data series approach primarily focuses on providing descriptive associations between the temporal changes of bioindicator indices and their spatial variability with anthropic activities, such as the rise in tourism over the last 60 years and the resulting utilization of land and water resources.

The I_{LS} index is preferred over FSI and FI' for analyzing *P. oceanica* meadows due to its superior resolution. In the 1960s, the highest recorded I_{LS} values varied greatly, ranging from 14.65 to 0.81. Although the majority of I_{LS} values in the 1980s were similar to those from previous studies, a general decline in the I_{LS} index was observed, with only Badia d'Alcúdia and the south and west of Menorca showing values comparable to those in the 1960s. During the 1990s, fewer locations were studied than in the previous decade, and I_{LS} values either remained stable or slightly decreased. However, in the 2000s, the index increased significantly in two of the three sampled locations. In the 2010s, data collection expanded to include not only the island of Mallorca but also the Cabrera Archipelago National Park, and I_{LS} values were lower than those recorded in the 2000s but consistent with studies from the 1960s to the 1990s. As of the 2020s, there appears to be a tendency towards an increase in I_{LS} values compared to previous data.

A general decrease in I_{LS} values can be observed in the studied time series from the 1960s to the late 1980s – early 1990s, which coincides with the exponential growth of the tourism industry characterized by disorderly development during that period. However, an improvement in I_{LS} values in Port de Sóller and sites along the Badia de Palma was observed after the implementation of Royal Decree 356/1985 in 1985, which mandates the treatment of wastewater prior to discharge into the sea. Furthermore, the incorporation of Cala Blava into the Badia de Palma Marine Reserve in 1982, which became effective in 1999, is reflected in the I_{LS} values in that area.

The recent increase in the I_{LS} index might indicate that stricter regulations by European, national and regional authorities are effective in protecting the *P. oceanica* ecosystem. Regional authorities enacted the Decree 25/2018 that, among other aspects, regulates boat anchoring, pursuing those who anchor in the meadow rather than on the rocky bottom or buoys installed to reduce pressure on *P. oceanica*.

FINAL CONSIDERATION

This Ph.D. research confirms the usefulness of bioindicator indices specifically designed for *P. oceanica* meadows. The selection of ecological categories (morphotypes) to develop these indices has been demonstrated to be robust, as supported by independent evidence from the analysis of C and N stable isotopes to determine the morphotypes' trophic position in the food web. By combining the FI' and I_{LS} indices with other indicators (FAI, heavy metal content in foraminiferal tests), environmental conditions can be characterized for short and long-term exposures to various stressors, including pollutants such as heavy metals.

The simplicity of the FI' and I_{LS} indices allows for their easy application to a wide range of databases, making them a useful tool for analyzing trends in environmental variability over time. In the case of the Balearic Islands, where data series have been documented since the 1960s in various publications and theses, these indices can provide valuable insights into the changing environmental conditions.

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Chapter 3. Supplementary Material

SM 1. Morphotype (A*, B, C, D*, SB) relative abundances and values of I_{LS} , FI', FSI for all sampling stations of Mallorca, Menorca, and Cabrera Islands grouped for decades. ND – no data. 1 - Mateu, 1970, 2 - Gazá, 1988, 3 - Moreiro, 1993, 4 - Abril, 1993, 5 - Mateu et al., 2001, 6 - Mateu-Vicenc et al., 2010, 7 - Khokhlova, 2013 and Mateu-Vicens et al., 2014, 8 - Racis, 2015, 9 - Mateu-Vicens et al., 2016, 10 - Vaquer, 2016, 11 - Khokhlova et al., 2022, 12 - Khokhlova and Mateu-Vicens, 2023, 13 - Florit, 1983.

MALLORCA AND CABRERA												
LOCALITY	SAMPLING NAME	DATE	DEPTH (m)	A*	B	C	D*	SB	I_{LS}	FI'	FSI	
1	Cala Agulla	Ma-1-64	11/10/1964	0	0.19	0.29	0.16	0.19	0.08	3.69	3.81	8.21
	Cala Mendia	Ma-2-64	12/10/1964	0	0.39	0.17	0.06	0.22	0.14	6.17	6.05	9.65
	Cala Murada	Ma-3-64	12/10/1964	0	0.47	0.16	0.04	0.26	0.06	5.39	6.00	10.00
	Portocolom	Ma-4-64	11/10/1964	0	0.10	0.00	0.20	0.50	0.01	0.81	1.98	8.22
	Porto Petro	Ma-5-64	11/10/1964	0	0.14	0.05	0.14	0.19	0.10	3.21	2.95	6.57
	Cala Santanyí	Ma-6-64	20/12/1964	0	0.31	0.06	0.08	0.39	0.14	3.39	5.11	9.75
	Es Caragol	Ma-7-64	20/12/1964	0	0.53	0.00	0.00	0.12	0.35	14.65	8.94	10.00
	Colònia de Sant Jordi	Ma-8-64	20/12/1964	0	0.19	0.11	0.06	0.32	0.28	4.21	5.42	9.65
	Ses Covetes	Ma-9-64	20/12/1964	0	0.23	0.09	0.06	0.29	0.24	4.53	5.35	9.22
	Sa Ràpita	Ma-10-64	20/12/1964	0	0.22	0.15	0.10	0.30	0.21	4.03	5.12	9.60
	Cala Blava	Ma-11-65	03/01/1965	0	0.32	0.00	0.14	0.07	0.21	11.52	5.71	7.75
	S'Arenal	Ma-12-65	03/01/1965	0	0.24	0.04	0.04	0.29	0.37	5.81	6.57	9.82
	Port de Sóller	Ma-13-65	03/01/1965	0	0.34	0.10	0.09	0.30	0.17	4.76	5.74	9.91
	Camp de Mar	Ma-14-65	03/01/1965	0	0.32	0.09	0.15	0.19	0.16	6.15	5.42	9.16
	Santa Ponça	Ma-15-65	03/01/1965	0	0.19	0.14	0.04	0.27	0.31	5.03	5.70	9.64
2	Ciutat Jardí	Ma-6.1-82	11/1982	20	0.10	0.49	0.11	0.24	0.00	1.32	2.47	9.49
	Ciutat Jardí	Ma-6.2-82	11/1982	20	0.09	0.52	0.11	0.23	0.00	1.21	2.35	9.39
	Ciutat Jardí	Ma-6.3-82	11/1982	20	0.16	0.21	0.20	0.36	0.00	1.44	2.78	9.17
	Ciutat Jardí	Ma-6.4-82	11/1982	20	0.05	0.64	0.10	0.17	0.00	1.05	2.16	9.50
	Ciutat Jardí	Ma-6.5-82	11/1982	20	0.09	0.46	0.15	0.25	0.00	1.16	2.34	9.33
	Ciutat Jardí	Ma-6.6-82	11/1982	20	0.14	0.37	0.16	0.29	0.00	1.51	2.73	9.37
	Portals Nous	Ma-7.1-82	11/1982	22	0.11	0.03	0.17	0.68	0.00	0.62	2.17	9.74
	Portals Nous	Ma-7.2-82	11/1984	22	0.16	0.04	0.15	0.63	0.00	0.90	2.60	9.52
	Portals Nous	Ma-7.3-82	11/1984	22	0.27	0.17	0.10	0.44	0.00	1.93	3.66	9.46
	Portals Nous	Ma-7.4-82	11/1984	22	0.28	0.29	0.08	0.33	0.00	2.47	3.84	9.49
	Portals Nous	Ma-7.5-82	11/1984	22	0.35	0.28	0.07	0.29	0.00	3.38	4.49	9.63

	Portals Nous	Ma-7.6-82	11/1984	22	0.29	0.24	0.08	0.37	0.00	2.34	3.89	9.57
	Portals Nous	Ma-7.7-82	11/1984	22	0.28	0.18	0.08	0.44	0.00	2.00	3.74	9.46
	Cap de Regana	Ma-8.1-84	04/1982	20	0.04	0.19	0.11	0.31	0.29	3.07	4.25	9.24
	Cap de Regana	Ma-8.2-84	04/1982	20	0.02	0.25	0.09	0.25	0.35	4.05	4.66	9.38
	Cap de Regana	Ma-8.3-84	04/1982	20	0.03	0.17	0.12	0.32	0.31	3.00	4.23	9.15
	Cap de Regana	Ma-8.4-84	04/1982	20	0.02	0.16	0.07	0.33	0.37	3.34	4.63	9.29
	Cap de Regana	Ma-8.5-84	04/1982	20	0.02	0.13	0.17	0.32	0.28	2.72	3.90	9.06
	Cap de Regana	Ma-8.6-84	04/1982	20	0.01	0.16	0.04	0.33	0.37	3.35	4.59	8.93
3	Badia de Pollença	Ma-9-85	06/1985	20	0.21	0.19	0.03	0.33	0.22	3.74	5.06	9.54
	Badia de Pollença	Ma-10-85	06/1985	7	0.51	0.04	0.01	0.43	0.00	3.58	5.62	9.83
4	Cala Fornells	Ma-17.1-89	1989	3-6	0.20	0.30	0.11	0.28	0.11	3.06	4.12	9.73
	Cala Fornells	Ma-17.2-89	1989	3-6	0.13	0.43	0.06	0.25	0.11	2.74	3.68	9.34
	Cala Fornells	Ma-17.3-89	1989	3-6	0.02	0.25	0.12	0.35	0.21	2.01	3.37	9.24
	Port de Sóller	Ma-18.1-89	1989	3-6	0.22	0.56	0.01	0.13	0.00	9.73	5.06	9.62
	Port de Sóller	Ma-18.2-89	1989	3-6	0.22	0.52	0.03	0.13	0.02	8.91	5.46	9.25
	Port de Sóller	Ma-18.3-89	1989	3-6	0.17	0.17	0.03	0.39	0.01	5.27	4.96	9.68
	Es Barcarès	Ma-19.1-89	1989	3-6	0.26	0.36	0.01	0.08	0.27	10.87	6.18	9.90
	Es Barcarès	Ma-19.2-89	1989	3-6	0.17	0.46	0.03	0.12	0.19	6.34	4.76	9.73
	Es Barcarès	Ma-19.3-89	1989	3-6	0.11	0.25	0.05	0.31	0.25	3.34	4.55	9.81
	Portocolom	Ma-20.1-89	1989	3-6	0.15	0.54	0.04	0.17	0.08	5.98	4.36	9.62
	Portocolom	Ma-20.2-89	1989	3-6	0.14	0.53	0.03	0.13	0.14	4.38	3.63	9.54
	Portocolom	Ma-20.3-89	1989	3-6	0.08	0.47	0.07	0.22	0.13	2.23	2.96	9.37
	Es Carnatge	Ma-21.1-89	1989	3-6	0.19	0.54	0.02	0.10	0.12	3.72	3.47	9.06
	Es Carnatge	Ma-21.2-89	1989	3-6	0.13	0.62	0.02	0.10	0.10	3.95	3.59	9.16
	Es Carnatge	Ma-21.3-89	1989	3-6	0.09	0.47	0.13	0.19	0.07	1.46	2.55	7.47
	Cala Murta	Ma-22.1-89	1989	3-6	0.38	0.50	0.01	0.05	0.02	3.31	3.65	9.87
	Cala Murta	Ma-22.2-89	1989	3-6	0.46	0.33	0.03	0.09	0.01	4.66	4.02	9.65
	Cala Murta	Ma-22.3-89	1989	3-6	0.32	0.33	0.04	0.19	0.08	2.54	3.38	9.76
	Cala Fornells	Ma-1.1-90	1990	3-6	0.09	0.48	0.15	0.15	0.12	3.36	3.49	9.63
	Cala Fornells	Ma-1.2-90	1990	3-6	0.16	0.42	0.07	0.18	0.13	3.87	4.01	9.26
	Cala Fornells	Ma-1.3-90	1990	3-6	0.02	0.25	0.11	0.38	0.20	1.75	3.24	9.63
	Cala Murta	Ma-2.1-90	1990	3-6	0.34	0.49	0.01	0.10	0.02	6.94	4.73	9.56
	Cala Murta	Ma-2.2-90	1990	3-6	0.42	0.35	0.03	0.12	0.01	7.16	5.18	9.41
	Cala Murta	Ma-2.3-90	1990	3-6	0.42	0.29	0.05	0.14	0.07	7.51	5.76	9.80
	Es Barcarès	Ma-3.1-90	1990	3-6	0.29	0.36	0.00	0.15	0.16	6.62	5.38	9.68

Es Barcarès	Ma-3.2-90	1990	3-6	0.24	0.43	0.02	0.16	0.10	4.80	4.39	9.54	
Es Barcarès	Ma-3.3-90	1990	3-6	0.16	0.20	0.04	0.33	0.25	3.61	4.92	9.83	
Es Carnatge	Ma-4.1-90	1990	3-6	0.20	0.41	0.02	0.27	0.06	2.80	3.77	8.79	
Es Carnatge	Ma-4.2-90	1990	3-6	0.20	0.47	0.06	0.18	0.06	3.52	3.78	9.51	
Es Carnatge	Ma-4.3-90	1990	3-6	0.15	0.26	0.16	0.30	0.06	2.03	3.20	9.10	
Port de Sóller	Ma-5.1-90	1990	3-6	0.29	0.53	0.02	0.12	0.01	5.11	4.14	9.53	
Port de Sóller	Ma-5.2-90	1990	3-6	0.20	0.59	0.05	0.08	0.00	4.63	3.39	9.16	
Port de Sóller	Ma-5.3-90	1990	3-6	0.11	0.17	0.02	0.46	0.02	1.01	2.16	7.47	
Portocolom	Ma-6.1-90	1990	3-6	0.20	0.51	0.03	0.15	0.10	4.57	4.22	9.77	
Portocolom	Ma-6.2-90	1990	3-6	0.17	0.50	0.04	0.18	0.10	3.69	3.87	9.78	
Portocolom	Ma-6.3-90	1990	3-6	0.14	0.45	0.06	0.21	0.12	3.30	3.89	9.90	
Cala Fornells	Ma-7.1-91	1991	3-6	0.03	0.23	0.54	0.11	0.08	2.36	2.75	9.77	
Cala Fornells	Ma-7.2-91	1991	3-6	0.11	0.33	0.16	0.18	0.20	4.29	4.24	9.68	
Cala Fornells	Ma-7.3-91	1991	3-6	0.06	0.23	0.13	0.27	0.22	2.96	3.78	8.98	
Cala Murta	Ma-8.1-91	1991	3-6	0.32	0.50	0.01	0.14	0.01	5.22	4.40	9.74	
Cala Murta	Ma-8.2-91	1991	3-6	0.36	0.33	0.03	0.22	0.01	4.25	4.57	9.48	
Cala Murta	Ma-8.3-91	1991	3-6	0.33	0.34	0.05	0.15	0.10	6.47	5.21	9.64	
Es Barcarès	Ma-9.1-91	1991	3-6	0.13	0.50	0.01	0.20	0.15	3.54	3.99	9.86	
Es Barcarès	Ma-9.2-91	1991	3-6	0.10	0.32	0.04	0.37	0.13	1.91	3.38	9.69	
Es Barcarès	Ma-9.3-91	1991	3-6	0.07	0.20	0.05	0.34	0.32	3.31	4.73	9.80	
Es Carnatge	Ma-10.1-91	1991	3-6	0.08	0.47	0.06	0.19	0.13	2.86	3.37	9.38	
Es Carnatge	Ma-10.2-91	1991	3-6	0.10	0.49	0.06	0.24	0.08	2.13	3.12	9.51	
Es Carnatge	Ma-10.3-91	1991	3-6	0.07	0.35	0.16	0.26	0.08	1.74	2.79	9.25	
Port de Sóller	Ma-11.1-91	1991	3-6	0.20	0.44	0.03	0.26	0.01	2.32	3.29	9.36	
Port de Sóller	Ma-11.2-91	1991	3-6	0.17	0.43	0.05	0.23	0.02	2.35	3.11	9.03	
Port de Sóller	Ma-11.3-91	1991	3-6	0.06	0.16	0.02	0.45	0.01	0.64	1.51	7.00	
Portocolom	Ma-12.1-91	1991	3-6	0.08	0.56	0.04	0.14	0.18	4.13	3.84	9.84	
Portocolom	Ma-12.2-91	1991	3-6	0.06	0.56	0.05	0.18	0.12	2.57	3.17	9.69	
Portocolom	Ma-12.3-91	1991	3-6	0.08	0.55	0.06	0.18	0.10	2.70	3.26	9.82	
5	Port de Sóller	Ma-1-00	2000	12	0.04	0.78	0.03	0.08	0.03	1.97	2.42	9.69
6	Cala d'Or	Ma-2-08	2008-2009	6-8	0.43	0.28	0.05	0.12	0.12	9.27	6.32	10.00
	Cala d'Or	Ma-3-08	2008-2009	6-8	0.54	0.20	0.04	0.10	0.12	12.05	7.18	10.00
	Portals Vells	Ma-4-08	2008-2009	6-8	0.48	0.24	0.04	0.11	0.12	10.43	6.72	10.00
	Portals Vells	Ma-5-08	2008-2009	6-8	0.46	0.32	0.05	0.11	0.06	9.22	6.01	9.94
7	Els Estels	Ca-4.1-12	2012	10-20	0.35	0.22	0.07	0.25	0.13	4.99	5.51	9.96

	Els Estels	Ca-4.2-12	2012	10-20	0.20	0.29	0.06	0.31	0.14	3.10	4.35	9.73
	Cala Santa Maria	Ca-2.1-11	2012	10	0.21	0.14	0.09	0.35	0.20	3.41	4.90	9.82
	Cala Santa Maria	Ca-2.2-11	2012	10	0.22	0.24	0.07	0.32	0.13	3.15	4.38	9.28
	Cala Santa Maria	Ca-2.3-11	2012	10	0.14	0.23	0.07	0.34	0.24	3.21	4.63	9.82
	Ses Illetes	Ma-12.1-12	2012	4-5	0.15	0.23	0.12	0.32	0.15	2.70	3.96	9.28
	Ses Illetes	Ma-12.2-12	2012	4-5	0.13	0.24	0.14	0.28	0.16	2.89	3.89	9.46
	Ses Illetes	Ma-12.3-12	2012	4-5	0.10	0.25	0.14	0.23	0.19	3.33	3.85	8.97
	Santa Ponça	Ma-13.1-12	2012	4-5	0.21	0.19	0.11	0.38	0.04	2.05	3.48	9.15
	Santa Ponça	Ma-13.2-12	2012	4-5	0.19	0.18	0.08	0.40	0.05	1.85	3.27	8.97
	Santa Ponça	Ma-13.3-12	2012	4-5	0.17	0.15	0.08	0.44	0.06	1.64	3.14	9.01
	Cala Blava	Ma-5-12	2012	5-6	0.12	0.19	0.10	0.44	0.14	1.82	3.50	9.69
	Portals Nous	Ma-10-12	2012	6-8	0.27	0.27	0.14	0.30	0.03	2.87	4.06	9.78
	Son Verí	Ma-11-12	2012	6-7	0.20	0.20	0.11	0.37	0.12	2.56	4.13	9.78
	Magaluf	Ma-8-12	2012	6-7	0.21	0.22	0.11	0.36	0.09	2.49	3.95	9.64
8	Cala Blava	Ma-6.1-12	2012	5-6	0.24	0.10	0.05	0.38	0.20	3.42	5.12	9.87
	Cala Blava	Ma-6.2-12	2012	5-6	0.29	0.16	0.05	0.27	0.21	5.04	5.66	9.85
9	Sa Foradada	Ma-14.1-12	11/2012	15	0.33	0.46	0.03	0.16	0.02	5.84	3.84	9.98
	Sa Foradada	Ma-14.2-12	11/2012	15	0.30	0.46	0.03	0.19	0.03	3.79	3.31	10.00
	Sa Foradada	Ma-15.1-12	11/2012	15	0.33	0.38	0.03	0.25	0.02	5.06	4.60	9.99
	Sant Elm	Ma-15.2-12	11/2012	15	0.23	0.69	0.01	0.06	0.01	4.33	4.42	9.99
	Sant Elm	Ma-15.3-12	11/2012	15	0.17	0.72	0.01	0.09	0.00	3.69	4.50	9.96
10	Port de Sóller	Ma-16-14	10/2014	4-7	0.36	0.03	0.15	0.06	0.40	3.15	2.42	ND
11	Cala Santa Maria	Ca-1.1-11	25/11/2011	8	0.21	0.11	0.07	0.25	0.33	5.63	6.03	9.70
	Cala Santa Maria	Ca-1.2-11	25/11/2011	8	0.27	0.18	0.09	0.27	0.17	4.33	5.14	9.64
	Cala Santa Maria	Ca-1.3-11	25/11/2011	8	0.17	0.19	0.06	0.31	0.28	4.09	5.27	9.76
	Cala Santa Maria	Ca-3.1-12	23/05/2012	8-12	0.25	0.21	0.10	0.24	0.18	4.75	5.19	9.82
	Cala Santa Maria	Ca-3.2-12	23/05/2012	8-12	0.18	0.18	0.13	0.35	0.15	2.78	4.23	9.76
	Cala Santa Maria	Ca-3.3-12	23/05/2012	8-12	0.21	0.19	0.13	0.28	0.16	3.70	4.67	9.82
	Cala Santa Maria	Ca-3.4-12	23/05/2012	8-12	0.19	0.21	0.10	0.35	0.14	2.84	4.29	9.70
	Port d'Andratx	Ma-9.1-12	15/05/2012	8-12	0.31	0.21	0.11	0.27	0.10	4.22	5.03	9.94
	Port d'Andratx	Ma-9.2-12	15/05/2012	8-12	0.18	0.28	0.13	0.27	0.15	3.39	4.35	9.88
	Port d'Andratx	Ma-9.3-12	15/05/2012	8-12	0.24	0.28	0.11	0.30	0.07	2.99	4.21	10.00
	Port d'Andratx	Ma-9.4-12	15/05/2012	8-12	0.25	0.27	0.02	0.29	0.17	4.00	5.01	9.94
	Magaluf	Ma-7.1-12	16/05/2012	6-7	0.20	0.22	0.12	0.38	0.07	2.20	3.79	9.52
	Magaluf	Ma-7.2-12	16/05/2012	6-7	0.25	0.26	0.13	0.32	0.04	2.68	4.03	9.76

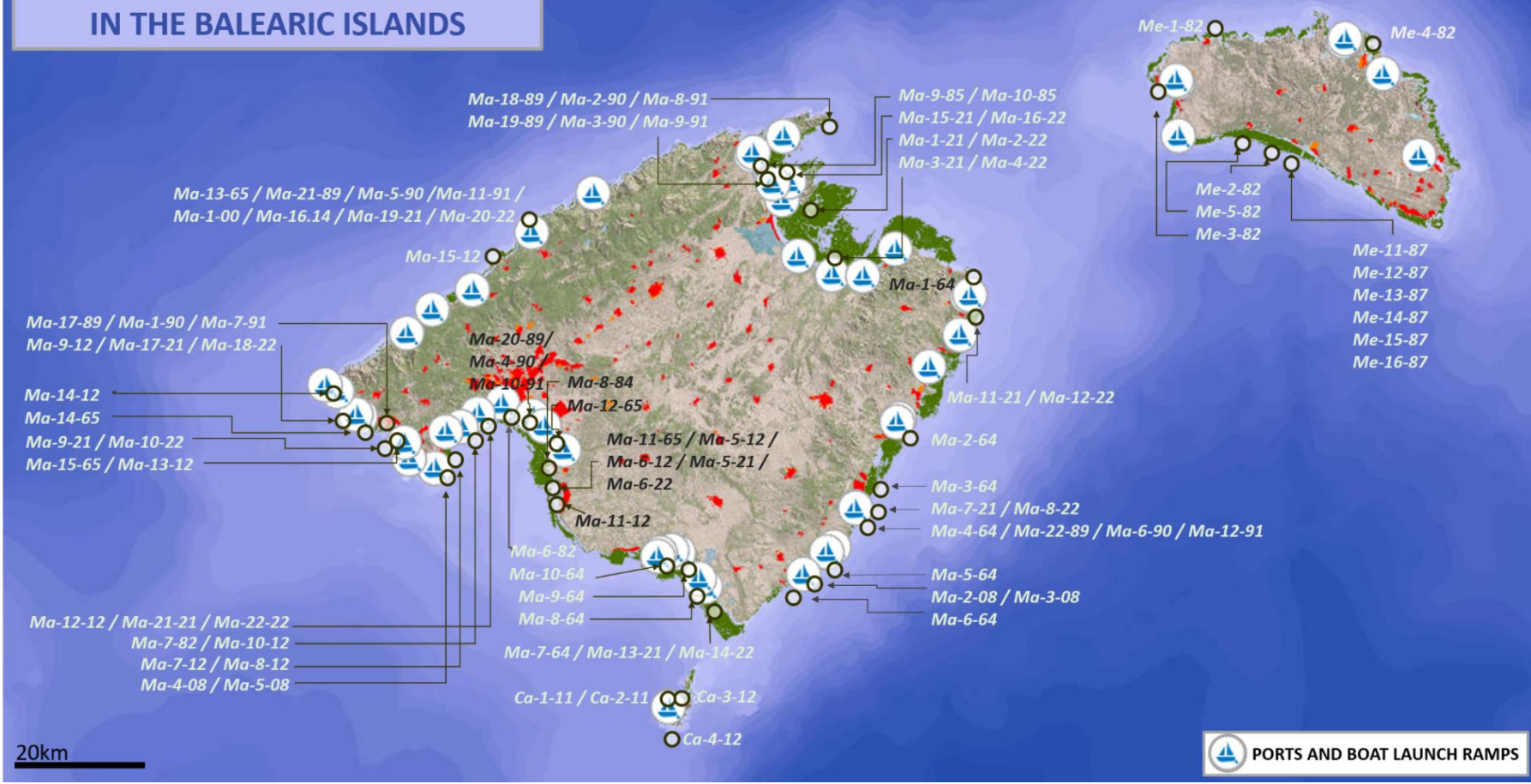
	Magaluf	Ma-7.3-12	16/05/2012	6-7	0.19	0.27	0.09	0.39	0.05	1.93	3.52	9.76
	Magaluf	Ma-7.4-12	16/05/2012	6-7	0.21	0.27	0.09	0.33	0.08	2.64	3.97	9.46
12	Alcanada	Ma-1-21	08/2021	2-4	0.26	0.13	0.06	0.28	0.26	5.04	5.91	ND
	Alcanada	Ma-2-22	01/2022	2-4	0.18	0.14	0.03	0.28	0.37	5.30	6.11	ND
	Ca'n Picafort	Ma-3-21	08/2021	2-4	0.21	0.30	0.12	0.18	0.19	5.42	5.04	ND
	Ca'n Picafort	Ma-4-22	01/2022	2-4	0.21	0.25	0.18	0.16	0.20	5.92	5.13	ND
	Cala Blava	Ma-5-21	08/2021	2-4	0.10	0.23	0.11	0.23	0.32	4.87	5.18	ND
	Cala Blava	Ma-6-22	01/2022	2-4	0.14	0.26	0.07	0.23	0.30	5.10	5.33	ND
	Cala Brafí	Ma-7-21	08/2021	2-4	0.17	0.26	0.10	0.30	0.17	3.22	4.40	ND
	Cala Brafí	Ma-8-22	01/2022	2-4	0.14	0.29	0.14	0.22	0.22	4.23	4.63	ND
	Cala de Santa Ponça	Ma-9-21	08/2021	2-4	0.34	0.28	0.06	0.30	0.01	3.30	4.51	ND
	Cala de Santa Ponça	Ma-10-22	01/2022	2-4	0.26	0.29	0.09	0.33	0.02	2.60	3.97	ND
	Capdepera	Ma-11-21	08/2021	2-4	0.22	0.27	0.12	0.18	0.21	5.80	5.26	ND
	Capdepera	Ma-12-22	01/2022	2-4	0.23	0.26	0.09	0.18	0.24	6.18	5.55	ND
	Es Caragol	Ma-13-21	08/2021	2-4	0.14	0.25	0.12	0.21	0.28	4.96	5.13	ND
	Es Caragol	Ma-14-22	01/2022	2-4	0.38	0.07	0.08	0.26	0.21	6.07	6.47	ND
	Marina de Bonaire	Ma-15-21	08/2021	2-4	0.22	0.25	0.14	0.18	0.21	5.58	5.21	ND
	Marina de Bonaire	Ma-16-22	01/2022	2-4	0.21	0.24	0.09	0.17	0.29	6.83	5.86	ND
	Port d'Andratx	Ma-17-21	08/2021	2-4	0.34	0.27	0.05	0.30	0.04	3.49	4.67	ND
	Port d'Andratx	Ma-18-22	01/2022	2-4	0.25	0.38	0.12	0.21	0.04	3.62	4.10	ND
	Port de Sóller	Ma-19-21	08/2021	2-4	0.23	0.26	0.07	0.33	0.10	2.93	4.32	ND
	Port de Sóller	Ma-20-22	01/2022	2-4	0.24	0.20	0.11	0.29	0.17	3.95	4.97	ND
	Ses Illetes	Ma-21-21	08/2021	2-4	0.16	0.30	0.12	0.21	0.21	4.57	4.78	ND
	Ses Illetes	Ma-22-22	01/2022	2-4	0.16	0.31	0.08	0.20	0.25	5.11	5.07	ND

MENORCA

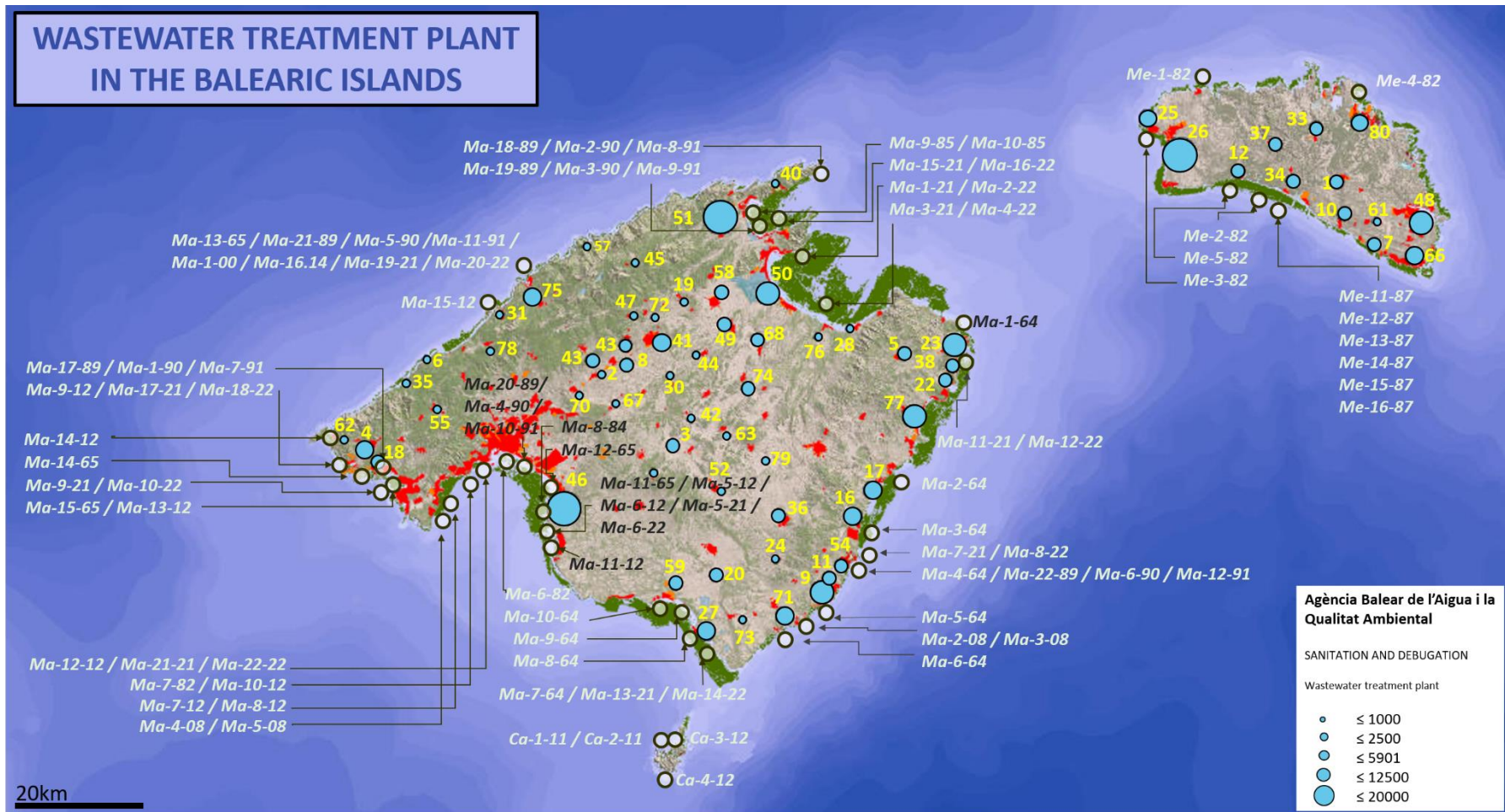
	LOCALITY	SAMPLING NAME	DATE	DEPTH (m)	A*	B	C	D*	SB	I _{LS}	FI'	FSI
3	South Menorca	Me-11-87	06/1987	29	0.01	0.49	0.20	0.14	0.03	1.10	2.01	9.02
	South Menorca	Me-12-87	06/1987	19	0.67	0.07	0.01	0.12	0.13	12.99	8.30	10.00
	South Menorca	Me-13-87	06/1987	19	0.31	0.15	0.03	0.21	0.14	5.47	5.09	9.78
	South Menorca	Me-14-87	06/1987	7	0.24	0.39	0.05	0.16	0.16	5.78	5.00	9.94
	South Menorca	Me-15-87	06/1987	29	0.30	0.23	0.03	0.42	0.01	2.30	4.05	9.72
	South Menorca	Me-16-87	06/1987	30	0.04	0.11	0.11	0.59	0.00	0.38	1.47	8.55
13	Ses Fontanelles	Me-1.1-82	19/09/1982	3-5	0.17	0.46	0.02	0.25	0.09	2.92	3.81	ND
	Ses Fontanelles	Me-1.2-82	19/09/1982	3-5	0.33	0.33	0.02	0.20	0.10	5.31	5.21	ND

Cala Mitjana	Me-2.1-82	05/08/1982	3-5	0.28	0.57	0.03	0.06	0.03	7.33	4.32	ND
Cala Mitjana	Me-2.2-82	05/08/1982	3-5	0.27	0.57	0.02	0.07	0.02	6.39	4.16	ND
Cala En Blanes	Me-3.1-82	27/09/1982	3-5	0.72	0.18	0.00	0.10	0.00	13.15	7.65	ND
Cala En Blanes	Me-3.2-82	27/09/1982	3-5	0.24	0.44	0.01	0.16	0.11	5.03	4.54	ND
Arenal d'en Castell	Me-4.1-82	13/09/1982	3-5	0.29	0.39	0.00	0.30	0.02	3.00	4.17	ND
Arenal d'en Castell	Me-4.2-82	13/09/1982	3-5	0.15	0.38	0.02	0.22	0.12	3.24	3.74	ND
Cala Galdana	Me-5.1-82	16/08/1982	3-5	0.40	0.42	0.00	0.13	0.02	6.95	5.24	ND
Cala Galdana	Me-5.2-82	16/08/1982	3-5	0.29	0.36	0.00	0.19	0.14	5.60	5.20	ND

PORTS AND BOAT LAUNCH RAMPS IN THE BALEARIC ISLANDS



SM 2. Map of the Balearic Islands showing the sample distribution corresponding to data analyzed in objective 3, the *P. oceanica* coverage (in green), and urban planning of all the municipalities (in red - urban land, in orange - developable land (actualized on 01/04/2022)), showing the location of ports and boat launch ramps, actualized on 25/01/2023 (from https://ideib.caib.es/geoserveis/rest/services/public/GOIB_SUPE_IB/MapServer) with layers: GOIB_MUIB, and GOIB_Posidonia_IB (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project) actualized in 09/03/2022)).



SM 3. Map of the Balearic Islands showing the sample distribution corresponding to data analyzed in objective 3, the *P. oceanica* coverage (in green), and urban planning of all the municipalities (in red - urban land, in orange - developable land (actualized on 01/04/2022)), showing location of treatment station (blue circles) (actualized on 26/01/2023) (from: https://ideib.caib.es/geoserveis/rest/services/public/GOIB_ABAQUA_IB/MapServer, with layers: GOIB_ABAQUA_IB, GOIB_MUIB, and GOIB_Posidonia_IB (Map Service conservation of *Posidonia oceanica* datasets (25/2018 Decree and LIFE Posidonia project) actualized in 09/03/2022)).

